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29th February 2024

Organized By

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Department of Forensic Biology
Government Institute of Forensic Science,
Chhatrapati, Sambhajinagar, Maharashtra, India

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under Research Development Cell,
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OBJECTIVES OF THE CONFERENCE

- Enable opportunities for collaboration, and the exchange of latest ideas in chemical and biological sciences.
- Discuss the challenges and explore opportunities in forensic investigations and scientific research

THEME OF THE CONFERENCE

- Forensic Medicine, Forensic Chemistry, Forensic Toxicology and Pharmacology
- Forensic Biology, Forensic Serology, Forensic DNA Analysis
- Related Chemical and Biological Sciences
- Crime Scene Management
- Nanomaterials in forensics
- Organic synthesis, synthesis of bioactive heterocycles, coordination chemistry
- Examination Forensic Exhibits
- Material science
- Interdisciplinary research

- Green Chemistry

ABOUT THE DEPARTMENT OF FORENSIC CHEMISTRY

Department of Forensic Chemistry runs a PG Specialization in Forensic Chemistry and Toxicology. The laboratory is equipped with sophisticated equipment. The department has collaborated with various national institutions and universities and produced more than 100 research papers of international repute along with two Indian patents published. Faculty members are engaged in research work on several domains of forensic chemistry and toxicology like the development of colorimetric chemosensors for detection of heavy metal ions, and pesticides, and the development of novel methods for the examination of forensic-related exhibits.

ABOUT THE DEPARTMENT OF FORENSIC BIOLOGY

The department runs a specialization in Forensic Biology, Serology, and DNA Fingerprinting. The laboratory is equipped with sophisticated equipment. DNA extraction, amplification, and identification from various sources. The department also works on biological fluids examination, serological testing, and enzymatic studies. Entomological studies, microbiological studies, diatoms studies, and wildlife forensics are also carried out in the department

ABOUT INSTITUTE

Taking a visionary step, the Department of Higher and Technical Education, Government of Maharashtra started an aided and fully dedicated Forensic Science Institute in the state on 17th August 2009 at Chhatrapati Sambhajinagar. The objective of starting such an Institution was to generate skilled manpower in the field of forensic sciences to cater needs of forensic science laboratories and law enforcement agencies in India and globally. The institute is dedicated

to forensic science studies and is the first of its kind in the country. Presently, we are offering one undergraduate, one postgraduate, and two PG diploma courses in forensic science and allied disciplines. The Institute has seven departments covering forensic sciences and allied disciplines. The laboratories in these departments have both basic and state-of-the-art instrumentation facilities.
Website: https://gifsa.ac.in

EDITORS MESSAGE

Dr. Devidas S. Bhagat
Convener & Director of Research and Development Cell

As the convener, I am delighted to extend heartfelt thanks to all the delegates attending this national conference. I strongly believe that this academic event provided valuable opportunities to students and budding scientists. The event helped the participants significantly in enhancing their overall attitude toward the research process.

The Department of Forensic Chemistry and Forensic Biology in the Government Institute of Forensic Science Chhatrapati Sambhajinagar and research development center organized a symposium, titled "Exploring New Horizons in Forensic Chemical and Biological Sciences (ENHFCBS 2024)," has facilitated fruitful discussions on a thematic platform.

We have received several full-length articles on diverse topics in forensic sciences relating to the conference theme, after rigorous peer review out of which the following 35 were recommended by the subject experts.

RESOURCE PERSON

		Topic
Dr. B. B. Shingate Professor, Dept. of Chemistry, Dr. BAMU, Chhatrapati Sambhajinagar		***Chemistry and Biology Interface***
Dr. Manoj B. Gawande Scientist & Professor, Dept. of Industrial and Eng. Chem., IICT- Jalna		***The Rise of Chemical Sciences and Nanomaterials for Advanced Applications***
Dr. Santosh R. Kote Assistant Chemical Analyzer, DFSL, Chhatrapati Sambhajinagar		***Role of Forensic Science in Career, Evidence and Justice System.***
Dr. G. D. Khedkar Professor & Director, Paul Hebert Centre for DNA Barcoding and Biodiversity Studies, Dr. BAMU, Chhatrapati Sambhajinagar		***Wildlife Forensic and DNA Forensic***

CONTENT

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Spathodea Campanulata Flower Extract Mediated Synthesis of CuO Nanoparticles and Study of its Antimicrobial Activity

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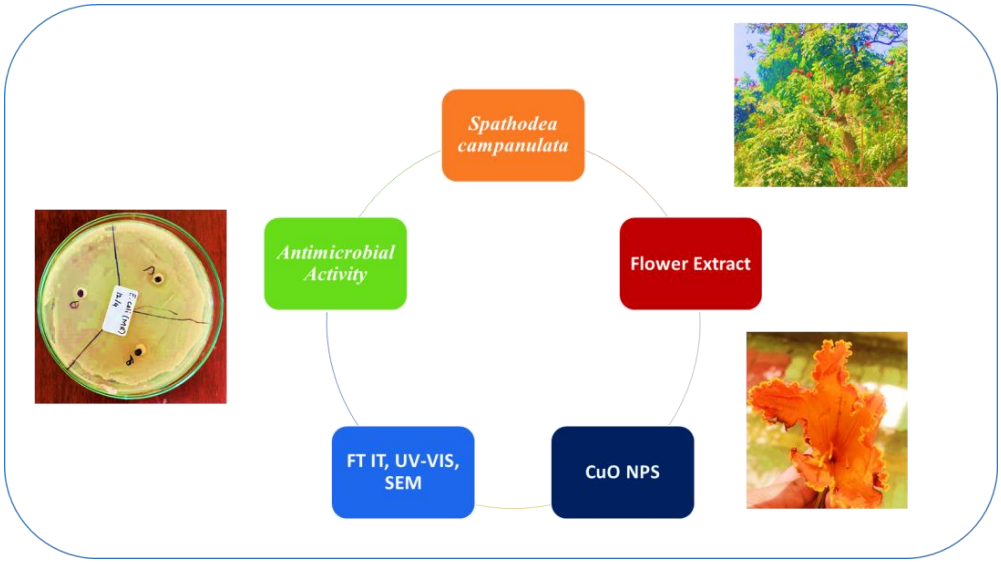
ABSTRACT

Metal-based nanoparticles have become quite popular due to their fascinating optical, magnetic, biological, and physical characteristics. There are numerous physical, chemical, and biological methods for creating these nanoparticles. Since scientist emphasis on cheap, eco-friendly, and environmentally safe method for producing nanoparticles, biological methods are generally chosen. In present study, we demonstrated greener synthesis of copper oxide (CuO) nanoparticles using an aqueous extract of Spathodea campanulata of flowers as a reducing, capping, and stabilizing agent. The nanoparticles were characterized using UV-VIS spectroscopy, and Fourier-transform infrared spectroscopy (FT-IR), and TLC. The antimicrobial activity of the synthesized CuO nanoparticles was assessed against Escherichia coli, Escherichia coli (Multiple Drug Resist), Bacillus Subtilis using standard microbiological techniques. This study highlights the feasibility of utilizing plant extracts for the eco-friendly synthesis of metal nanoparticles and underscores the antimicrobial efficacy of CuO nanoparticles synthesized via this green route. We used ultra-sonication for synthesis of CuO NPs. Ultrasound wave facilited greener methodology for synthesis of CuO NPs.

Keywords: Spathodea campanulata, CuO Nanoparticles, Green Synthesis, Antimicrobial Activity



Graphical Abstract

I. INTRODUCTION

Scientists' main focus these days is developing effective green chemistry techniques to synthesize metal nanoparticles. They have looked into ways to produce well-characterized nanoparticles in an environmentally sustainable manner. The use of living things to produce metal nanoparticles is one of the most widely explored techniques. Plants appear to be the best options among these creatures and are appropriate for the large-scale production of nanoparticles. In the past, chemical and physical processes were used to create nanoparticles [1]. Physical and chemical procedures have numerous disadvantages, such as the utilization of harmful byproducts, excessive energy consumption, and the usage of poisonous solvents. As a result, scientists have been concentrating increasingly in the past few years on creating effective techniques for green synthesis [2]. Using different plant extracts to produce well-characterized nanoparticles is a speedier, more environmentally friendly, and more sustainable method. The functional capabilities of nanoparticles can be significantly altered by varying their size and shape. As a result, there are numerous uses for green synthesis in the production of nanoparticles [3].

The utilization of plant extracts in the green and eco-friendly synthesis of copper oxide (CuO) nanoparticles represents a burgeoning area of research with significant potential benefits. This approach leverages the inherent reducing and stabilizing properties of phytochemicals present in various plant extracts, thereby eliminating the need for hazardous chemicals and energy-intensive processes typically associated with conventional nanoparticle synthesis methods [4]. The green synthesis route offers several advantages, including environmental sustainability, cost-effectiveness, scalability, and reduced toxicity compared to traditional chemical methods. Plant extracts serve as natural sources of bioactive compounds such as phenols, flavonoids, alkaloids, and terpenoids, which act as reducing agents to convert metal ions into nanoparticles [5]. Moreover, these phytochemicals also play a crucial role in controlling the size, shape, and stability of the synthesized nanoparticles. The eco-friendly nature of plant extract-mediated synthesis aligns with the principles of green chemistry, promoting cleaner production processes and minimizing environmental impact [6]. Additionally, the biocompatibility of plant-derived nanoparticles makes them promising candidates for various biomedical applications, including drug delivery, imaging, and antimicrobial formulations [7].

Research on the antimicrobial activity of plant extract-mediated nanoparticles has gained significant attention due to their potential in combating microbial infections [8]. These nanoparticles, synthesized using plant extracts as reducing and

stabilizing agents, exhibit enhanced antimicrobial properties compared to conventional antibiotics. They offer several advantages such as biocompatibility, eco-friendliness, and reduced toxicity [9]. Various studies have reported the effectiveness of these nanoparticles against a wide range of pathogens including bacteria, fungi, and viruses [10].

Spathodea campanulata extract shown broad spectrum of biological activity includes Methanol extract of *Spathodea campanulata P.* (Beauv.) leaves demonstrate sedative and anxiolytic like actions [11], Three cancer cell lines were tested: Hs683 (human oligodendroglioma), MCF7 (human breast carcinoma), and murine B16F10 (mouse melanoma) for antiproliferative activity, and the extract from *Spathodea campanulata* flowers showed antimalarial, antiplasmodial, and *in vivo* acute oral toxicity against *Plasmodium falciparum* strain 3D7 [12], antioxidant activity [13], and anti-HIV, anti-complement, and hypoglycemic properties of the stem bark of *Spathodea campanulata* [14].

Their mode of action involves disrupting microbial cell membranes, inhibiting vital enzymes, and interfering with cellular processes, leading to microbial death or growth inhibition. Additionally, their ability to overcome drug resistance in pathogens makes them promising candidates for future antimicrobial therapies. Further research is needed to optimize their synthesis methods, understand their mechanism of action, and evaluate their safety for clinical applications. Overall, plant extract-mediated nanoparticles represent a promising avenue in the development of novel antimicrobial agents.

II. MATERIAL AND METHODS

Deionized water and copper sulfate ($CuSO_4.7H_2O$) purchase from local vendor.

2.1 Preparation of flower extract

One gram of dried flower powder and 50 mL of deionised water were added in conical flask. This mixture was stirred for 20 min at 70°- 80° C on hot plate with magnetic stirring. The light yellow- brown color solution was obtained and it was filtered with Whatman filter paper. The extract was stored at 4° C.

2.2 Preparation of CuO nanoparticles

40 mL of 0.01 M Cupric Sulphate ($CuSO_4.5H_2O$) solution was prepared and 20 mLof flower extract were taken in conical flask. Mixture kept in ultrasonic bath at room temperature for 1 hour color changed from greenish to brownish light color.

2.3 Isolation of CuO Nanoparticles

Synthesized CuO NPs solution was centrifuged at 8500 rpm for 30 minutes. The supernatant was collected in beaker. The brownish black colored settled CuO crystals were gently scratched using forceps collected into sample vials.

III.RESULT AND DISCUSSION

The *Spathodea campanulata* flowers were collected from the campus of Government Institute of Forensic Science Chhatrapati Sambhajinagar. The surface sterilization was done using 0.1 % mercury chloride ($HgCl_2$) for 5 minutes and then washed thrice with doubled-distilled sterile water and the flowers were subject to air drying in sunlight for two days. The grinding of flowers was done by an electrical grinding mill. The fine powder of flowers was added into deionized water containing Elimeyer flak. The ultrasonic wave increase facilitated the rapid extraction of active constituents from the powder of flowers by the cavitation phenomenon.

The ultraviolet-visible (figure 1) spectral analysis of flower extract-mediated synthesized CuO nanoparticles showed lambda max at 375 nm in deionized water as solvent. The *Spathodea campanulata* flowers extract mediated synthesized CuO NPs were subject for Fourier transform infrared spectral analysis (figure 2); shows stretching frequencies (ν) at 933 cm^{-1}, 1094 cm^{-1} (C-C, C-N and C-O stretching), 1472 cm^{-1} (N=O stretching), 2968 cm^{-1}, 3037 cm^{-1} (C-H stretching) and 3248 cm^{-1}, 3295 cm^{-1}, 3378 cm^{-1} (broad peak of O-H, N-H).

Figure 1: UV-VIS spectra of *Spathodea campanulata* flowers synthesized CuO NPS

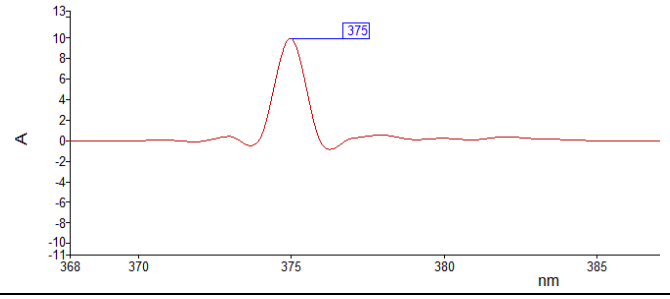
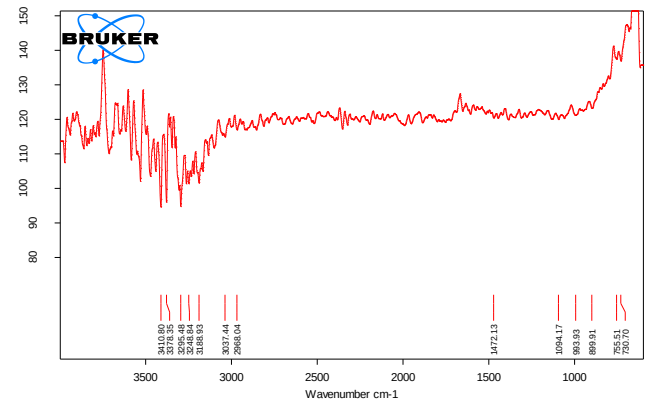


Figure 2: FT-IR spectra of *Spathodea campanulata* flowers synthesized CuO NPS



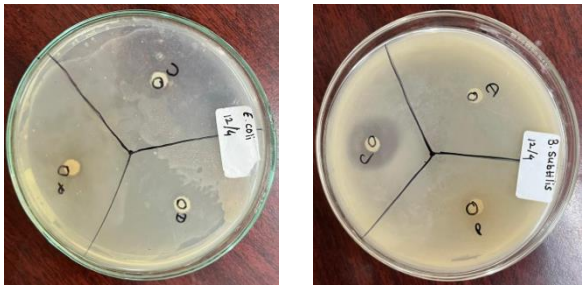
One mL of nutrient broth was inoculated with a loop-ful culture of respective bacteria *E. Coli, E. Coli (Multiple Drug Resist),* and *Bacillus Subtilis.* It was incubated at room temperature for 24 hrs. The next day nutrient agar plates were prepared and allowed to solidify. 0.1mL of respective culture was spread on the Nutrient Agar plate by spread plate method. Then 50 µL of sterile distilled water, plant extract and CuO nanoparticle solution were added on the sterile disc. The disc was placed on Nutrient agar plates containing the respective cultures. The Petri plate labeled as control was kept as it is i.e. without any kind of additions. All plates were incubated at a lower temperature for 10 minutes for diffusion of extract CuO nanoparticle solution. After 10 minutes, it was incubated at room temperature for 24 hours. The next day zone of microbial growth inhibition was observed and measured.

CuO NPs were subject to antimicrobial assay against *E. Coli, E. Coli (Multiple Drug Resist),* and *Bacillus Subtilis*. In figure 3; in this antimicrobial assay against *E. Coli* bacteria zone of inhibition of CuO NPs, flower extract and distilled water were observed upto 9 mm, 7 mm and 0 mm respectively. Whereas, antimicrobial assay against *E. Coli (Multiple Drug Resist)* bacteria zone of inhibition of CuO NPs, flower extract and distilled water observed upto 11 mm, 8 mm and 0 mm respectively. Similarly, antimicrobial assay against *Bacillus Subtilis* bacteria zone of inhibition of CuO NPs, flower extract and distilled water observed upto 23 mm, 6 mm and 0 mm respectively. Synthesized flower extract capped CuO NPs shows more antimicrobial activity as compared to flower extract of *Spathodea campanulata*. In antimicrobial assay against *Bacillus Subtilis* more activity (observed more zone of inhibition) as compared to antimicrobial assay against *E. Coli, E. Coli (Multiple Drug Resist)*. All observed results summarized in table no 1.

Table 1: Observation of bacterial assay (zone of inhibition) of CuO NPs

Entry	Bacteria Name	CuO NPs	Flower extract	Distilled Water
1	*E. Coli*	9 mm	7 mm	0 mm
2	*E. Coli (Multiple Drug Resist)*	11 mm	8 mm	0 mm
3	*Bacillus Subtilis*	23 mm	6 mm	0 mm

Figure 3: CuO nanoparticles against *E. Coli, E. Coli (Multiple Drug Resist) Bacillus Subtilis*



E. Coli *Bacillus Subtilis*

E. Coli (Multiple Drug Resist)

IV. CONCLUSION

In conclusion, the study investigated the synthesis of CuO nanoparticles using *Spathodea campanulata* plant extract as a reducing and capping agent. *Spathodea campanulata* of flower and CuO nanoparticles shown excellent antimicrobial activity against *E. Coli, E. Coli (Multiple Drug Resist), and Bacillus Subtilis.* Synthesized flower extract capped CuO NPs shows more antimicrobial activity as compared to flower extract of *Spathodea campanulata*. In antimicrobial assay against *Bacillus Subtilis* more activity (observed more zone of inhibition) as compared to antimicrobial assay against *E. Coli, E. Coli (Multiple Drug Resist)*. This has several benefits, including being easy to use, quick, economical, and environmentally beneficial. Overall, this study contributes to the growing body of knowledge on green nanotechnology and its potential impact on addressing global challenges in healthcare and environmental sustainability.

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Exploring the Applications of Diatoms

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ABSTRACT

Diatoms are unicellular eukaryotic microalgae having frustules, with ecological significance, and diversity. They are cosmopolitan, generally inhabiting fresh and marine habitats. There are over 2,00,000 diatom taxa throughout the universe and 14,700 taxa have been reported in India from fresh and marine aquatic habitats. They are the key player in the global carbon cycle and the major source of atmospheric oxygen. Diatoms have potential applications across various fields such as oil exploration, Pre- and Post-mortem drowning investigation, acts as an environmental indicator, pharmaceutical use, Waste degradation, Bioremediation, Biosensors, Bio silica pattern generation, Building Materials and fillers, Abrasives, jewellery design, toxicity testing, and eutrophication of aqueous ecosystems. This article provides an outline of the present status of diatoms and highlights their wide-ranging applications. Sustained study in this area promises to unlock their full potential and harness their benefits for the development of society and the environment.

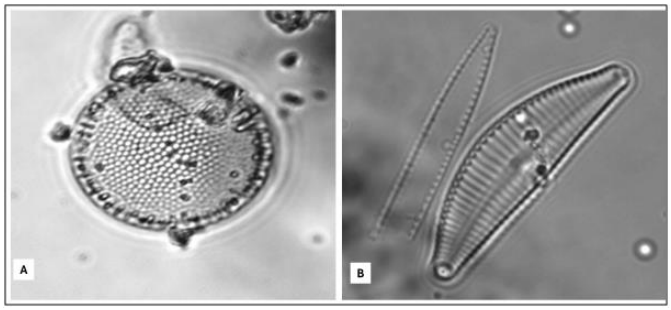
Keywords: Diatom; Diatomaceous earth; Environmental Indicator; Pre-mortem drowning; Post-mortem drowning.

I. INTRODUCTION

Diatoms belong to the group of microalgae ranging in size from 2-500µm. Diatoms are widely distributed globally in almost every water bodies either single or in colonial forms. Their distribution can also be observed over moist surfaces. They possess a transparent cell wall having a nanostructure composed of hydrogenated amorphous silica which allows them to carry out photosynthesis efficiently. This nanostructure is called frustule. Frustule consists of two halves, epivalve and hypovalve respectively, ornamented with different patterns. The lining associated with the two valves is the cingulum. They

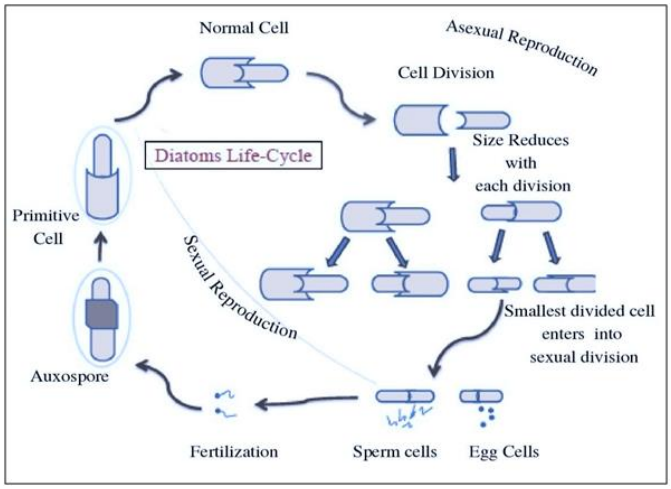
are the key player in the global carbon cycle and the major source of atmospheric oxygen [1]. Diatoms are mostly non-motile. Some pennate diatoms tend to move by secreting viscid material through raphe.

Diatoms are generally found in two shapes based on symmetry, one is centric with a centrally symmetrical structure (Figure (a)) and another pennate with a bilaterally symmetrical structure along the long axis (Figure (b)).



1. Showing A. Centric diatoms and B. Pennate diatoms

Diatoms reproduce by both sexual and asexual means but vegetative form is dominant [2]. Through each division of diatom, the size of the cell decreases. Cell enters sexual reproduction once the cell size is reduced to its smallest size. In favorable conditions, the entire cell cycle of Diatoms is completed within 24 hours.



2. Typical Life Cycle of Diatoms

Diatoms have chlorophyl-a and -c that converts solar energy into chemical energy through photosynthesis. As per the literature, diatoms contribute about 25% of environmental oxygen and about 40% of ocean oxygen. They are the major source of food for aquatic microorganisms.

History: -

The first observation of diatoms recorded by an English man in 17th century using a simple microscope, are published in the *Philosophical Transactions* by Royal Society of London. In the late 18th century, classification of the diatoms and maintenance of their records started by the 19th century, with the advent of advanced microscopes, in-depth study of the silica structure, patterns and varieties.

Initially, there was a dispute regarding the classification of diatoms as whether to classify diatoms as plants or animals. However, the monograph published by Kutzing in the year 1844 classified all diatoms as algae. After this the difference of opinion on classification disappeared and the scientific community accepted Diatoms classified as algae.

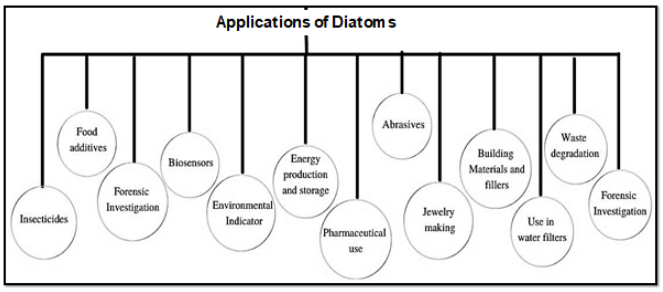
Recent Trends in Diatoms: -

There are more than 30 thousand species of diatoms known till date. Each year many new species are identified and submitted by the researchers due to their extensive work. There have been several advancements made in the field in the past few decades.

Diatoms due to their unique characteristics and wide availability have a broad range of applications [3]. Understanding the possible applications of diatoms will give insight into future work to be carried out

Applications: -

Diatoms by altering surface can be used for many applications. Some of them are as;



- **Forensic Investigation**

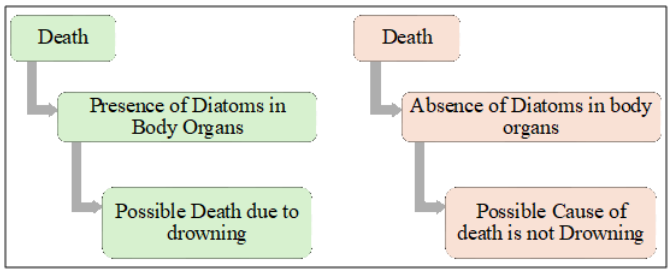
Diatoms plays catalytic role in drowning cases, discriminating easily between pre-mortem and post-mortem drowning. This test is regarded gold standard

because upon drowning the vital clues/ signs, that helps in understanding the reason for death generally disappears, on the onset of putrefactive changes and here the diatom test proves to be reliable to conclude whether the victim died of drowning or not [4], [5], [6].

When the person is submerged in water, respiration is increased due to asphyxia which causes water to make its way into the lungs, brain, spleen, femur, etc. In the case of Dead drowning, no diatom population can be noticed as there is a lack of circulation. However, the concentration of the diatoms also matters in the process of making assertions.

Diatoms over the clothes and other belongings of the victim are also used to link the site of drowning.

Since there is large variability in the diatom species and noticeable changes in temperature, pH, locations, etc. they can be used for determining geographical location. Diatoms play a cardinal role in Investigation [7].



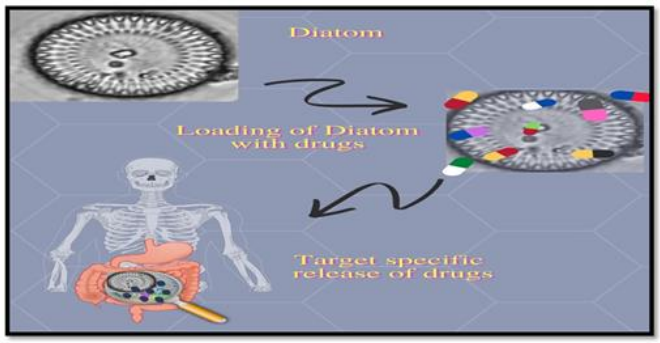
- **Pharmaceutical use**

It has been always seen that for effective Drug delivery, the carrier should have certain characteristics such as the ability to control the release of drugs, they should be biodegradable; they should be water insoluble, etc. These properties can be developed by modification of certain materials with chemical treatment. But the drawback is that the processing and the cost both increases. Also, there are hazardous effects of harmful chemicals used in processing.

Diatom is a Nano-structured algae which are well-suited choice for drug delivery [8]. Diatom frustules provide more surface area due to the symmetric arrangement of pores and thus increase the drug-holding capacity [9]. Diatoms are biodegradable and as carriers have controlled drug release ability. Using diatom frustule as a drug delivery system allows target-specific release of drugs. Also, it overcomes the drawback of the drug delivery system being getting solubilized.

Another advantage of using diatom frustule enables the slow and sustainable release of drugs to the targeted site.

The diatom metabolites are used as anti-cancer and antibacterial agents[10]. Besides this, these bioactive metabolites can also be used as antioxidants. Apart from this, Diatoms also have applications in tissue engineering and preparation of hemorrhage control tapes.



3. Diatoms drug loading and its release at targeted sites.

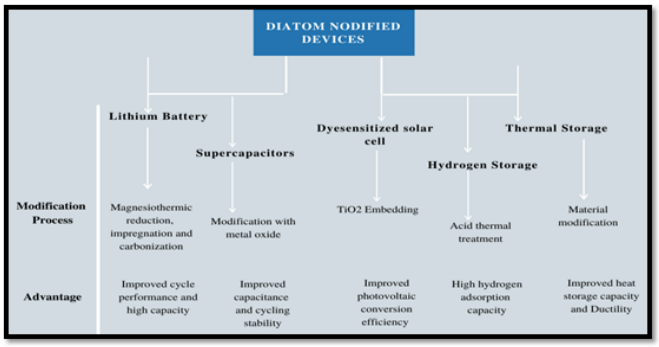
Diatoms have a drawback that is not readily soluble in biological fluid. This may result in the accumulation of silica particles in the body and have other negative health impact.

- **Energy production and storage:**

Diatoms having unique three-dimensional features and favorable surface area makes it suitable to be modified into conductive as well as semi-conductive material without changing the morphology. Natural siliceous diatoms act as a suitable raw material for lithium-ion batteries which are reduced with a magnesio-thermic process followed by carbonization. Other modification with magnesium oxide or titanium oxide makes this raw material suitable as super-capacitors and solar cells [11]. Other modifications can also be carried out to use these

porous materials as hydrogen storage properties and thermal energy storage.

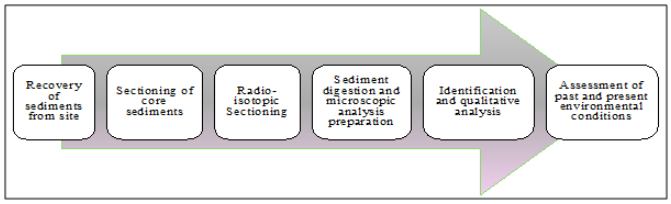
This naturally available material reduces the problem of environmental pollution which is generally the major drawback in the case of other synthetically prepared materials through various chemical processing methods.



3. Diatoms modified in different energy storage devices.

- **Environmental Indicator: -**

Diatoms play an important role in long-term and short-term assessment of environmental change. They are a group of bio-organisms with a high degree of versatility and strong ecological indicators. Their rate of growth is impacted due to changes in aquatic conditions. Also, in some Diatoms certain deformities like the development of a single valve, etc. can be seen due to changes in environmental conditions. Diatoms respond instantly to many ecological changes[12]. They help in addressing environmental conditions such as Eutrophication, Acidification, and majorly, climate change[13]. Since diatoms have a limited lifespan, Diatomite analysis can be useful in the assessment of long-term climatic variability[14].



4. Process of Diatoms analysis for environmental indicator

- **Use in filters:**

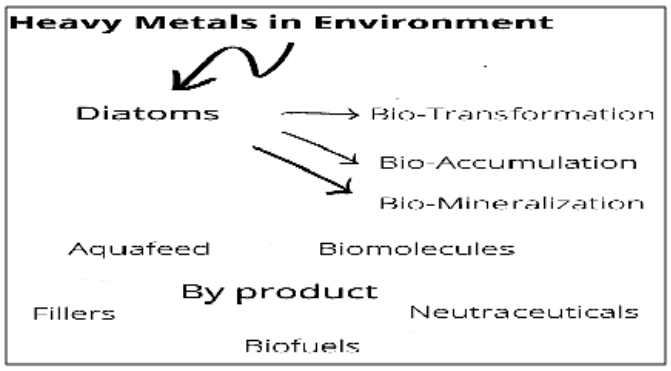
Diatomaceous earth, the skeletonized part of diatoms makes the best choice for filtration due to its distinctive physical properties[8], [12]. The microstructure of diatomite filters the particulate forms present in the water. The diatomite layer is formed over a permeable porous filter called a septum. The filtration is carried out in two forms which include surface filtration and deep filtration. In surface filtration, the pore size is decreased such that the contaminant to be separated has a larger diameter than the pore. Where the contaminant does not pass through the pore and is left behind thereby separating it from pure liquid. In deep filtration, the pores are large enough to trap contaminants. where the undesired particles penetrate the filter medium up to a certain point thereby resulting in the pure substance eluting out of the filtration assembly.

Deep filtration has more capacity than that of surface filtration. Also is more effective and efficient. The inert nature of diatomaceous earth is safe for using it in the filtration of liquid that can be used for consumption. It is used widely for water filtration, beer filtration, Oil filtration, etc. They are effective in filtering algae, cysts, and any other particulate matter present.

- **Waste degradation:**

Diatoms are found abundant in water bodies. They possess unique photosynthetic, metabolic, and cellular characteristics that play a pivotal role in the removal of pollutants that stem from industries, agricultural activities, or any other anthropogenic activity. The metabolic potential of diatoms makes them suitable agent for bioremediation and wastewater treatment[15]. They play a fundamental role in metabolization of pollutants such as nitrates, phosphates, lead, iron, chromium, and other heavy metals[16].

The capture of carbon dioxide by diatoms results in valuable end products such as biofuels, Biofertilizers, and Food supplements with high nutritional value to other organisms in water-bodies.

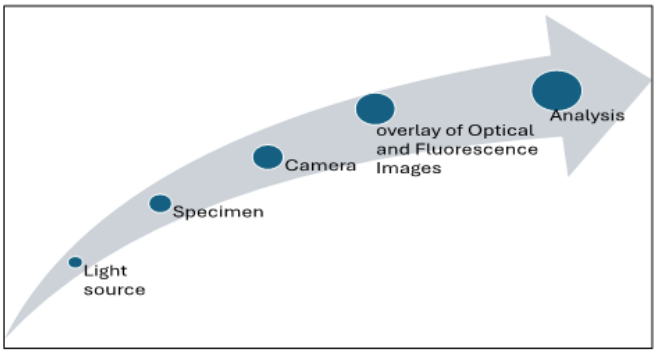


5. Transformation of contaminants and By-products.

- **Biosensors:**

Diatom frustules have photo-luminescent properties which makes them suitable as biosensors[8], [17]. This photoluminescence emitting property is due to the presence of silanol (Si-OH) and siloxane (Si-O-Si) groups present over the frustule. Frustules are highly sensitive to change. The electron from the silica shell is attracted by electrophilic substances which gives photoluminescence. However certain basic modifications have to be done for more precision.

They do not require any expensive processing to be done for use and can be made to order easily. Biosensors made out of diatoms assure low cost due to wide availability. Photoluminescence-based studies of the immune complex have attracted immense attention. Diatomaceous earth can also function as DNA-based biosensors[18]. Silica-based biosensors show high performance compared to other metallic biosensors.

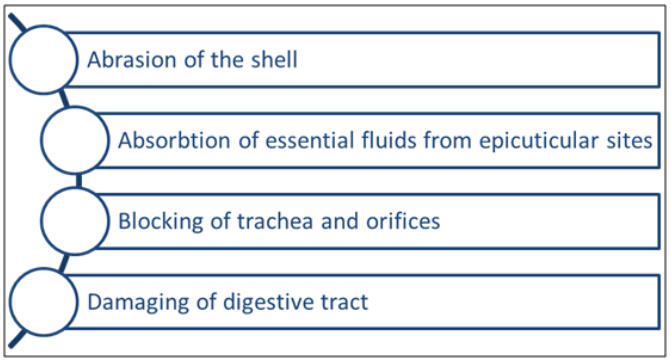


7. Experimental Setup For Analysis

- **Insecticides:**

Diatoms possess unique silica wall that settles down on the waterbed known as diatomaceous earth. Diatomaceous earth has an abrasive property that causes the insect's exoskeleton to be damaged [19]. Diatomite has an absorbing property that absorbs the fluids from the exoskeleton of insects, living the dry remains.

Using diatomaceous earth does not affect the quality of grains if used as grain protectants and is even non-toxic for humans. It is also used by farmers and gardeners for controlling insects and pests over crops. It is a highly potent insecticide in dried conditions.



- **Building Materials and fillers:**

The use of Diatoms for construction has been evident since the ancient Egyptian era. Diatoms used in the bricks ensure light and long-lasting building materials. But it is inoperative using purely diatoms with other supporting materials for modern highly durable construction. However, mixing diatomite with certain suitable materials can be used for fabricating modern constructions.

Diatoms used in construction act as water repellants that lead to long durability. The fabricating material made using Diatomaceous earth also corroborates about 55% of low heat conductance. On the other hand, Diatoms are also used as fillers in paper industries to increase the life of paper and have high-quality printing papers, in Rubber factories, and in paints for increasing antimicrobial activity of surface and also increasing life and in certain asphalts. The Diatomaceous earth extract after other applications can be reused in tiles manufacturing, pottery, and other artifacts.

- **Jewelry making:**

Diatoms are ornamented with different patterns and designs of holes and pores. Inspired by these designs, Diatoms are used in jewelry making. Various companies are making handmade jewelry by utilizing natural structures and patterns of diatoms.

- **Food additives:**

Diatoms are classified as food-grade and non-food-grade diatoms. Food-grade diatoms have less than 2% crystalline silica whereas non-food grade have a higher percentage of crystalline silica which is often toxic to humans and animals and therefore it does not have its application in food industries.

Food grade diatoms are used in Food industries as pesticides and anticaking agents. Recently researchers have come up with Food additives that promote healthy growth and treat certain intestinal parasites. Diatomaceous earth also has these properties of food additives and therefore it is used as Food additives for farm animals[12], [20], [21]. It has been also theorized that food-grade diatoms can boost the immune system. However, there is no scientific basis for the theory.

- **Diatoms as abrasives: -**

Diatoms' rough surface facilitates its use as abrasive material[22]. It is used for polishing metallic surfaces and other soft solids.

Diatomites are used as abrasives in cleansing skin. It is sometimes used in soaps and other bathing materials. Diatoms are also used largely in toothpaste as a fine abrasive for cleaning teeth. Diatoms also have applications in beauty products such as facial scrubs, Anti-pimple talcum powders, and face packs, etc.

- **Future prospective: -**

Research has been going on to explore more possible applications of these tiny silica glasses. Researchers are exploring the use of diatoms in spacecraft building as it provides light and high tensile strength material.

There is also an attempt made by NASA to understand the effect of microgravity on diatoms whether they behave similarly in microgravity, can they have the same morphogenesis under microgravity, etc.

Applications in all possible fields should be explored to increase the use of naturally occurring materials rather than using chemically treated and synthesized products. Using naturally found materials such as diatom can result in less pollution as they are

II. CONCLUSION

Diatoms are the ones which existed for a very long on Earth and their presence can be traced as long as 200 million years ago but their use and applications have gained attention in the last few decades. Being present widely, Diatoms have numerous applications ranging from household to commercial. Diatom acts as selective to the stimuli and its porosity and optical transparency are advantages. High porosity provides more surface area and pore volume which is needed in case of drug delivery while optical transparency ensures optical detection. Diatoms prove to be the best and most economical choice. Another advantage is that no negative impact on humans has been reported to date. However, inhaling the diatomaceous earth (which is typically in powdered form) can cause respiratory disease and in rare cases lung cancer. These problems are more often reported in those working in diatomite production areas.

Exploring new domains of application of Diatoms can lead to the replacement of most synthetic materials causing more pollution and having less advantage shortly.

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Declarations:

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Conflicts of interest/Competing interests:

The authors declare that they have no competing interests

Availability of data and material:

All data generated or analysed during this study are included in this published article.

Code availability:

Not applicable

Authors' contributions:

AG/Corresponding author conceived the idea of manuscript preparation, design, data analysis, interpretation, and critical revision of the article. SS, SG and MS helped in the design, data analysis and gave valuable inputs in manuscript writing. All the authors read, discussed the results, and gave approval for submission.

Ethics approval:

Not applicable

Consent to participate

Not applicable

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Emotion detection by Deep Face Library using Python

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ABSTRACT

Emotion detection is a popular application of affective computing, which aims to enable machines to recognize and interpret human emotions. Emotion detection has various real-world applications, such as in healthcare, education, marketing research, and human-computer interaction. With the advancement of deep learning techniques, deep neural networks have been employed to classify emotions from various types of input data, such as images, audio, and text. In this research project, we aim to develop an emotion detection system using DeepFace, a state-of-the-art deep learning library that can detect facial expressions with high accuracy. Additionally, we have used the K-Nearest Neighbours (KNN) algorithm to classify the emotions detected by DeepFace. We have collected the database of total 57 images with three basic facial expressions, namely Angry, Happy and Neutral of 19 students. Using DeepFace library, accuracy of 44% has been achieved whereas using kNN, when the dataset is divided into 80% for training and 20% for testing, we have achieved the highest accuracy of 85% using Minkowski distance metric and the value of k as 6. KNN outperformed the DeepFace library by 41%.

Keywords: Machine learning, DeepFace, kNN, Emotion detection.

I. INTRODUCTION

Emotion detection has become a prominent research topic due to its wide range of applications in various fields such as psychology, marketing, and human-computer interaction. The ability to detect emotions from facial expressions can aid in understanding human behavior and provide valuable insights for decision-making processes.

In this research project, we aim to develop an emotiondetection system using DeepFace, a state-of-the-art deep learning library that can detect facial expressions with highaccuracy.Additionally,we will use the K-Nearest Neighbours (KNN) algorithm to classify the emotions detected by DeepFace. The motivation behind doing an emotion detection project using the DeepFace library in Python is to enable

machines to understand and respond to human emotions more accurately and efficiently.

Emotion detection has various real-world applications, such as in healthcare, education, marketing research, and human-computer interaction. For example, in healthcare, emotion detection can be used to monitor patients' emotional states and provide appropriate interventions, while in education, it can be used to measure students' engagement levels and adjust teaching strategies accordingly. In marketing research, emotion detection can be used to understand consumers' emotional responses to products or advertisements.

By developing an emotion detection model using DeepFace we can contribute to this field and potentially create a useful tool for various industries. Additionally, working on this project can help you gain hands-on experience in deep learning, image processing, and data analysis using Python. The motivation for this project is to explore the potential of DeepFace and KNN in developing an efficient and accurate emotion detection system. This research can contribute to the development of emotion-based technologies that can enhance human-computer interaction and improve decision-making processes.

II. METHODS AND MATERIAL

Materials :-TOOLS, SOFTWARE, PROGRAMMING LANGUAGES AND PYTHON LIBRARIES

Mobile Phone, Jupyter Notebook, Python, Itertools, Keras, TensorFlow, Pandas, NumPy, OpenCV, Sklearn, Matplot, Seaborn, Microsoft Office Picture Manager, Microsoft Excel.

Methodology :-

Collection Of Database:-

The database of digital images with three basic facial expressions Angry, Happy and Neutral were collected from M.Sc. I and M.Sc. II year students of GIFS, Aurangabad using Motorola G72 Smartphone in controlled environment with proper light and seating arrangement. Three folders named Angry, Happy and Neutral were made. Giving names Happy 1, Neutral 2 and Angry 3 for images of happy ,neutral and angry emotions . All these folders were stored in single folder named as 'Image Data'.

The photographs were taken from 19 students showing three emotions .Also there is a one photograph for each emotions, so there are total 3 photographs of each person having the emotion angry, happy and neutral in each photograph. In this way, the dataset consists of 57 images having 19 images for each emotion.

All of these gathered photos were manually cropped to highlight a particular area of interest. Therefore, 57 images in total were manually cropped and stored in a folder for later processing.

Fig.1 (a)Happyemotion (b) Angry emotion (c)Neutral emotion

Use of DeepFace library to extract dominant emotion from facial images:

The deepface library was applied to the digital database in order to extract the dominant emotion shown on the faces. The emotions were extracted from each image after thorough analysis. The accuracy was determined for all the images that were successfully identified for each mood.

Applying kNN algorithm to the dataset:

The K-Nearest Neighbor technique is used to detect emotion after using the deep face library to determine the emotion in the photos. The dataset for this experiment was split into training and testing with different percentages for three experiments. By varying the value of k for each distance metric from 1 to 5, a total of 5 different distance metrics, including Euclidean , cosine, Minkowski, Manhattan, and

Hamming distance metrics, were used. In the first trial, the dataset was divided so that 30% of it was utilised for testing and 70% for training .Then, by altering the value of k for each distance metric, accuracy was examined. A second experiment was run on the database, where training and testing were conducted using 80% and 20% of the whole database, respectively. To determine which distance metric provides greater accuracy, all 5 were used. Similar to the second experiment, the accuracy in the third experiment was measured using all distance metrics by changing the value of k each time, but this time, the dataset was divided into training and testing so that 90% of the training data and 10% of the testing data were used. After the performance of all three experimentation the maximum accuracy was determined for each experiment when the data was split into different percentage in those three experiments.

III.RESULTS AND DISCUSSION

For experiment 1 :

In this project, Deep face library of python is used to determine the facial expression from the images and the dominant emotion was find out from each facial images.

This study analyzed a dataset of 57 facial images, with 19 images for each of the three emotions: angry, happy, and neutral. The identification of emotions in these images was performed using a certain library. The results show that the library correctly identified 1 out of 19 images with angry facial expression, 18 out of 19 images with happy facial expression, and 15 out of 19 images with neutral facial expression.

Table 1: Accuracy achieved using deep face liabrary

Criteria	Total Images	Correctly Identified Images	Accuracy rate in %
Overall images	57	34	44%
Angry Emotion	19	1	4%
Happy emotion	19	18	72%
Neutral emotion	19	15	56%

For experiment 2:

In this kNN algorithm is applied to the dataset .The value of k is taken from 1 to 6 and total 5 distance metrics were used i.e. Cosine, Euclidean , Manhattan, Hamming and Minkowski distance metric. For this the dataset was split into training and testing in three different ways .First the 70% of the dataset was used for training and 30% was used for testing. Similarly, for second experiment 80% of the total dataset was used for training and 20% was used for testing. In third experiment , 90% of the dataset was used for training and 10% was used for testing and the accuracy was calculated using each distance metrics with the value of k from 1 to 6.

The accuracy obtained for each experiment is tabulated as follows :

1) When the dataset is split as 70% for training and 30% for testing purpose , the accuracy achieved for each distance metric by changing the value of k from 1 to 6 is as follows.

Table-2: Accuracy for each distance metric when dataset split between 70% for training and 30% for testing.

Metric \ k	1	2	3	4	5	6
Cosine	63.33	63.33	63.33	63.33	66.66	70
Euclidean	66.66	63.33	63.33	**73.33**	70	70
Manhattan	66.66	63.33	66.66	**73.33**	**73.33**	66.66
Minkowski	66.66	63.33	66.66	**73.33**	70	70
Hamming	26.66	40	26.66	26.66	33.33	33.33

2) When the dataset is split as 80% for training and 20% for testing purpose , the accuracy achieved for each distance metric by changing the value of k from 1 to 6 is as follows.

Table-3: Accuracy for each distance metric when dataset split between 80% for training and 20% for testing.

Metric \ k	1	2	3	4	5	6
Cosine	60	65	70	70	65	70
Euclidean	65	55	65	70	75	75
Manhattan	65	50	65	70	75	75
Minkowski	55	55	55	55	70	**85**
Hamming	45	45	25	25	25	25

3) When the dataset is split as 90% for training and 10% for testing purpose , the accuracy achieved for each distance metric by changing the value of k from 1 to 6 is as follows.

Table-4: Accuracy for each distance metric when dataset split between 90% for training and 10% for testing.

Metric \ k	1	2	3	4	5	6
Cosine	70	70	70	70	**80**	**80**
Euclidean	**80**	60	70	70	**80**	**80**
Manhattan	**80**	50	70	70	**80**	70
Minkowski	**80**	60	70	70	**80**	**80**
Hamming	20	20	20	20	20	20

Based on the data provided, it appears that when the dataset was split into 70% for training and 30% for testing, the highest accuracy achieved was about 73.33%. This accuracy was obtained using Euclidean, Manhattan and Minkowski distance measures when the value of k was set to 4 and 5 .

In second experiment ,when the when the dataset was split between 80% for training and 20% for testing purpose, the maximum accuracy about 85% is obtained with Minkowski distance, when the value of k is 6.

Thirdly when the experiment was performed on the data that was split between 90% for training and 10% for testing , the maximum accuracy about 80% is obtained which is the highest among all three. This was obtained when the value of k is 1, 5 and 6 distance metric was Euclidean, Manhattan and Manhattan.

When the accuracy was checked in all three experiments the maximum accuracy achieved is 85% by using Minkowski distance metric with value of k taken as 6 .When the dataset was split between 80% for training and 20% for testing purpose

IV. CONCLUSION

Using the DeepFace library in Python to detect emotions in facial images has produced promising results. This is because the pre-trained models used in the project were trained on large facial datasets, making them highly accurate in identifying emotions. The library is simple and easy to use. However, the accuracy of the emotion detection algorithm depends on the quality of the input image and the dataset used for training. This model has achieved 46% accuracy. More research could improve the algorithm's performance by incorporating more diverse and balanced datasets. Despite this, DeepFace has demonstrated its potential as a powerful tool in facial analysis, which could be further explored in future studies.

We also discussed the k Nearest Neighbors (kNN) algorithm, which is a simple and effective method for classification and regression tasks. This algorithm can be used in conjunction with DeepFace to detect emotions. While kNN requires all training data during the testing phase, it can be improved by weighting the contributions of the neighbors according to their distance or other criteria, The maximum accuracy achieved is 85% by using Minkowski distance metric with value of k taken as 6 .When the dataset was split between 80% for training and 20% for testing purpose. Overall, our project has shown that using DeepFace and kNN for emotion detection is feasible and has great potential. Our work provides a basis for further exploration and refinement of this technique, which could inspire more research into deep learning and machine learning in facial analysis and recognition. We hope our project inspires new applications and innovations in this exciting field.

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Hand Gesture Recognition Using Simple K-Nearest Neighbor

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ABSTRACT

Hand gesture recognition can be used for identifying the person's gesture made by his/her hand. Applications can be in where people are using sign language for communication, automated systems for converting sign language to text, etc. In this paper we are trying to identify the hand gestures from collected photographs. The dataset is collected from seven students showing three hand gestures namely Victory, Heart and Thumsup. The photographs are collected of both left and right hands. A total of 42 photographs have been collected. Further ORB features have been extracted from the dataset. A simple kNN is used as the classifier. kNN achieved 50% accuracy using minkowski distance metric with value of k=3 to 5, 50% accuracy using hamming and manhattan distance metric with value of k =1 , by using 90% of the dataset for training and 10% for testing. Future work includes usage of different shape features and different classifiers.

Keywords: Hand Gesture, ORB, kNN, Distance metrics.

I. INTRODUCTION

Hand gesture recognitionis technology that uses sensors to read and interpret hand movements as command. Different types of hand gestures include Hi , Thumsup ,Victory , Punch ,Nice , Heart. Gestures are considered as the most natural expressive way for communications between human and computers in virtual system [11].

Applications can be in where people are using sign language for communication, automated systems for converting sign language to text, etc. ..Sign language is a means of communication for people who are hearing and speech impaired. . Hand gesture recognition is useful for the applications that require natural human-computer interaction.

The task of hand gesture recognition is very challenging for following reasons. First, the ability of the system to handle inputs that vary considerably from the input used during the development stage. For hand gesture recognition systems, input that may not be considered during the development stage

includes environmental noise, signers variability, language variability and so on. This is because we will usually apply restrictions on the environment of the signers reduce the problems in the segmentation and tracking process[1].

The hand gesture recognition system can be applied in Gesture- based Gaming control, Gesture control car driving, in communication and also to control the home appliances like Mp3 player ,TV etc.Forensic applicationsof the hand gesture recognitioninvolve deception detection technique.

American sign language is a widely used language for physically. The main model is constructed to recognize sign gesture images of the hand, which utilizes Oriented FAST and Rotated BRIEF (ORB) as a feature detector, having efficacy and performance better than widely used feature detectors such as SIFT and SURF, etc. The model utilizes a k-Nearest Neighbor as a classifiers.The motivation behind this project is to develop the deception detection technique.

II. METHODS AND MATERIAL

Mobile phone –Vivo V29.

Jupyter Notebook-is the Integrated development environment which combine the python with all libraries.

Python-useful for solving statistical problems involving machine learning algorithms.

Pandas

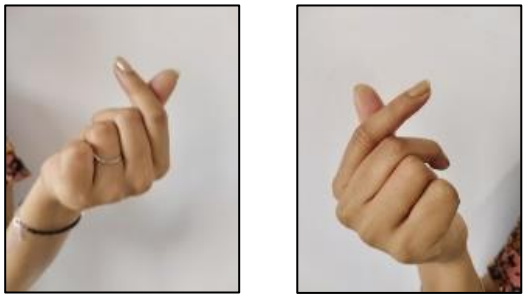
Numpy

OpenCV

Microsoft Excel

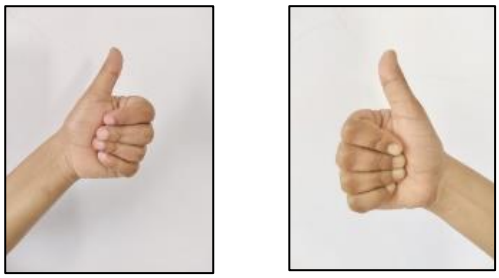
Dataset Used:

A standard dataset has been used; In this paper we are trying to identify the hand gestures from collected photographs. The dataset is collected from seven students showing three hand gestures namely Victory, Heart and Thumsup. The photographs are collected of both left and right hands. A total of 42 photographs have been collected.



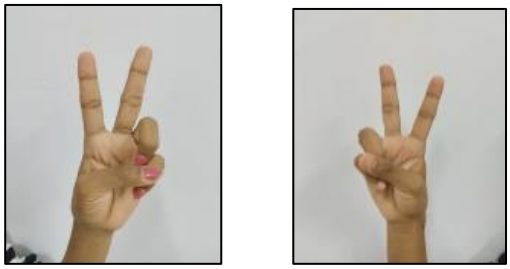
(a)2.3L (b)2.3R

Fig 1:Heart



(a)1.6R (b)1.6L

Fig 2: Thumsup



(a)6.4R (b) 6.4L

Fig3:Victory

R-Right hand

L-Left hand

Feature Extraction:

Feature Detection and Extraction is performed through Oriented Fast and Rotated Brief (ORB). ORB is anefficient feature detection and matching alternative to SIFT or SURF [23]. ORB is made up of two well-knowndescriptors FAST (Features from Accelerated and Segments Test) and BRIEF (Binary Robust IndependentElementary Features) with several modifications to boost the performance.It firstly uses the FAST keypoint detector technique to compute key points along with the orientation whichis calculated by computing the direction of the vector from the located corner point to the intensity weightedcentroid of the patch. The orientation is not

a part of FAST features so ORB uses a multi-scale image pyramid. Thekey points are effectively located at each pyramid level which contains the downsampled version of the image.For computing descriptors, rBRIEF or rotated BRIEF technique is used since BRIEF performs poorly withrotation. In the BRIEF algorithm, the image is smoothened using a Gaussian kernel to reduce noise sensitivity andincreasing the stability of descriptors. A matrix containing coordinates of feature pixels is defined and then using theorientation of patch, it's rotated(steered) matrix is calculated. Considering the key point orientation is consistentenough, the steered matrix can be used to compute essential descriptors with the help of a lookup table comprisingof predetermined BRIEF patterns.ORB feature detector is used to detect patches (as shown in Fig 5) from the image and a 32-dimensional vector for each of the patches generated. Thus, for every image belonging to a set of a single class of sign images, a32-dimensional vector of features is produced.

Classification Model :

The K-Nearest Neighbors (KNN) algorithm is a popular machine learning technique used for classification and regression tasks. It relies on the idea that similar data points tend to have similar labels or values. During the training phase, the KNN algorithm stores the entire training dataset as a reference. When making predictions, it calculates the distance between the input data point and all the training examples, using a chosen distance metric such as Euclidean distance .Next, the algorithm identifies the K nearest neighbors to the input data point based on their distances. In the case of classification, the algorithm assigns the most common class label among the K neighbors as the predicted label for the input data point. Forregression, it calculates the average orweighted average of the target values of the K neighbors to predict the value for the input data point.The KNN algorithm is straightforward and easy to understand, making it a popular choice in various domains. However, its performance can be affected by the choice of K and the distance metric, so careful parameter tuning is necessary for optimal results.

The proposed methodology consists of three major steps – data collection, feature extraction using ORB, classification using k-NN as shown in the flowchart in Fig 4. In this proposed approach, apre-processing technique was used to effectively obtain the feature descriptors in an image. The approach makes useof ORB (Oriented FAST and Rotated BRIEF) feature detection technique and K-nearest neighbor algorithm as a classifier.

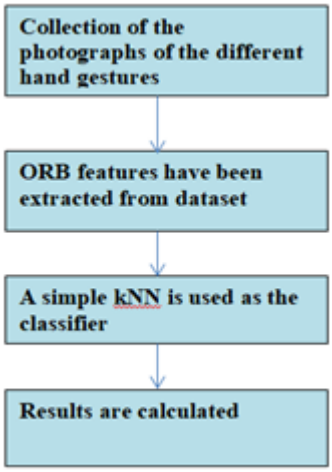


Fig 4: Methodology

III.RESULTS AND DISCUSSION

90% of the dataset for training and 10% for testing.

K	Minkowski	Euclidean	Cosine	Hamming	Manhattan
1	40%	40%	40%	50%	50%
2	30%	30%	10%	20%	20%
3	50%	50%	20%	30%	30%
4	50%	30%	20%	20%	40%
5	50%	20%	20%	20%	30%
6	20%	20%	30%	10%	30%

80% of the dataset for training and 20% for testing

K	Minkowski	Euclidean	Cosine	Hamming	Manhattan
1	35%	35%	25%	30%	35%
2	15%	15%	15%	15%	10%
3	30%	30%	20%	25%	20%
4	20%	20%	20%	15%	20%
5	15%	15%	20%	15%	10%
6	10%	10%	10%	25%	15%

70% of the dataset for training and 30% for testing

K	Minkowski	Euclidean	Cosine	Hamming	Manhattan
1	50%	50%	40%	30%	40%
2	33.33%	33.33%	33.33%	20%	26.66%
3	36%	36%	30%	16.66%	33.33%
4	33.33%	33.33%	30%	30%	33.33%
5	33.33%	33.33%	30%	26.66%	30%
6	26.66%	26.66%	30%	30%	26.66%

IV. CONCLUSION

kNN achieved 50% accuracy using minkowski distance metric with value of k=3 to 5, 50% accuracy using hamming and manhattan distance metric with value of k =1 ,50% accuracy using euclidean distance metric with value of k=3 by using 90% of the dataset for training and 10% for testing. Future work includes usage of different shape features and different classifiers. I would like to explore the factors that govern the relationship between the gestures and deception. And also I would like to study the usefulness of the hand gesture recognition in detecting deception.

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Forensic Bytes: Admissibility and Challenges of Digital Evidence in Legal Proceedings

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ABSTRACT

In today's fast-paced crime landscape, modern legal proceedings have evolved to require an in-depth understanding of the admissibility and court challenges in dealing with digital evidence. The study into the evolution of digital evidence breaks down how it's making its way through the legal system, looking at the admissibilitystandards and weighing the growing challenges that come from technology trumping old-school methods in forensic investigations. The paper conducts an in-depth analysis of the legal framework whichgoverns how digital evidence is ushered into the system, including the criteria for authentication, relevance, and reliability, as well as exploring the potential impacts on the rights of the accused, privacy concerns, and the role of expert witnesses in helping totranslate complex technical information for a legal audience.

Through in-depth case study analysis and examination of jurisprudential developments, this article offers a comprehensive understanding of the current state of admissibility standards and challenges associated with digital evidence in legal proceedings. Doingso is rather pertinent, as theperception of judicial readiness to meet thechallenges that digital evidence presents has implications for many issues of interest to legal practitioners, forensic experts, and policymakers. These include the quality of the forensic science utilized to assist the fact-finder, the privacy rights of individuals who possess or whose lives leave behind digital evidence, and for the future of electronic commerce, not all of which took centre stage in the case of Apple Inc. v. Federal Bureau of Investigation, but are no less important. The present examination of these issues aims to provide a resource to the relevant communities to inform the dialogue needed to address digital evidence challenges in thename of justice.

Keywords: Digital evidence, Admissibility standards, Legal proceedings, Digital forensics, Authentication, Privacy concerns, Expert witnesses

I. INTRODUCTION

In today's world, digital evidence is a very helpful tool in criminal investigations and legal cases. Valuable information about crimes and the behaviour of suspects can be found in digital information from smartphones, computers, cloud storage, and social media. However, legal professionals face many challenges when dealing with digital evidence, such as making sure it can be used in court, that it is real, and that it has not been changed.

In court, the validity of digital evidence is scrutinized through three key aspects: relevancy, authenticity, and integrity. Firstly, the evidence must be pertinent to the case and have a clear connection to the facts being contested. Secondly, it should be authentic,meaning that it has not been altered or manipulated, and originates from a credible source. Lastly, the integrity of the evidence should be intact, indicating that it has remained unchanged since its collection. To safeguard the integrity of digital evidence, strict protocols are implemented. Any unauthorized access, mishandling, or inadequate documentation can jeopardize its integrity, potentially rendering it inadmissible as evidence in court.

With the rapid advancements in technology, the legal framework governing the use and admissibility of digital evidence in legal proceedings must adapt. Balancing the protection of individual rights and the efficiency of criminal investigations presents a global challenge for legal communities. Maintaining confidence in the judicial system depends on legal professionals'capabilityto fairlyaddress cases involvingdigital evidence. This entails a deep understanding of the relationship between technology and the law and a commitment to using best practices in digital forensics and evidence management. [1-4]

"Forensic Bytes: Unveiling Digital Clues in the Courtroom"

Analysing digital evidence in legal settings is an intricate process requiringspecialized knowledge and skills, encompassing data from computers, smartphones, social media, and various electronic devices. The admissibilityof digital evidence is subjectto legal and technical complexities. Ensuring its authenticity, reliability, and integrity is essential for admissibility. Collecting, preserving, and analysing digital evidence requires rigorous protocols and expertise. The use of digital evidence in court has brought up various legal and ethical concerns, including worries about privacy, the possibility of tampering or manipulation, and the need for clear guidelines and standards for collecting and using digital evidence. As technology changes, the legal frame work for allowing and using digital evidence must also change. Striking a balance between safeguarding individual rights and enabling successful criminal investigations remains a significant challenge for legal communities around the world. For the public to trust the judicial system, it is crucial that legalprofessionalsbeabletofairlyand accuratelydecidecasesinvolvingdigitalevidence. To meet the expectation of handling digital evidence effectively, one must comprehensively comprehend the intertwined relationship between technology and the law. Additionally, they must commit to adhering to the best practices in digital forensics and evidence management.[5-9]

In India, examining digital evidence in legal cases presents unique challenges due to the need to

navigate the country's legal framework. The Information Technology Act of 2000 and the Indian Evidence Act of 1872 are the primary laws governing theadmissibility of digital evidence. To address the growing significance of digital data, amendments such as Section 22A were added to accommodate electronic records as admissible evidence in court.[13-14]

In India, when assessing digital evidence in court, the key factors considered are its pertinence to the case, its truthfulness, and its faithfulness to the source. Digital evidence can comprise various forms such as emails, digital photographs, ATM transaction logs, and computer-generated documents. The Supreme Court of India has set the standards for admitting electronic evidence, underscoring the need for genuineness, trustworthiness, and maintaining the original state of the evidence.[10,11]

The recognition of digital evidence in Indian judicial proceedings faces numerous hurdles, including the possibility of manipulation, difficulties in preserving the chain of custody, and the absence of a standardized procedure for verifying the reliability of the information saved in electronic records. Despite these obstacles, the judiciary acknowledges the necessity of digital evidence in preventing cybercrime and has taken steps to update the legal infrastructure to make better use of digital evidence.[12-13]

Effective preservation and handling of digital evidence by law enforcement agencies, investigators, and the public is paramount. Training needs to be provided to enable proper evidence collection and processing, consistently adhering to established standards. Additionally, educating citizens about the proper submission of evidence to authorities is equally vital to ensure the integrity of digital evidence and its effectiveness in supporting investigations.

India's dedication to justice in the modern digital age is evident in its pursuit of a comprehensive legal framework for digital evidence. To keep up with evolving technologies, thecountrycontinuestoadvancedigitalforensicsandlegaleducation,ensuringthatitsjustice systemremainseffectivedespiterapidtechnologicalchanges.Thiscommitmentdemonstrates a balance between technology's realities and the demand for justice in India. In India, thelegal basis for electronic evidence is established bytwo main laws: The Indian Evidence Act, 1872, and The Information Technology (IT) Act, 2000. These laws lay the groundwork forthe acceptance, relevance, and authentication of digital records in Indian courts.[15,16]

The Indian Evidence Act, enacted in 1872, has undergone changes to include provisions specific to electronic records. Some sections of the Act that are particularly relevant to electronic records include Section 65B. This section deals with the admissibilityof electronic records as evidence in court. According to Section 65B, any information stored in an electronic record, whether printed on paper or stored on optical or magnetic media, is considered a valid document.[15]

The Information Technology Act, passed in 2000 and later amended, permits the use of digital evidence in legal proceedings. This law recognizes electronic records and digital signatures as legally valid and outlines how digital certificates and signatures should be managed.Indian courts assess the relevance, truthfulness, and integrity of digital evidence when deciding whether or not to admit it.To be admissible, digital evidence must meet specific criteria set by the Supreme Court of India, including authenticity, reliability, and integrity.[16]

In India, the admissibility of digital evidence is faced with some hindrances. One challenge is the possibility of alteration, which can raise concerns about the reliability of the evidence. Another hurdle is establishing a comprehensive chain of custody, ensuring that the evidencehasnotbeentamperedwiththroughoutitsjourneyfromcollectiontopresentationin court. Furthermore, the absence of standardized procedures for verifying the accuracy of information stored in electronic records poses additional challenges. However, the Indian legalsystemacknowledgesthesignificance

ofdigitalevidence incombatingcybercrimes and has initiated measures to strengthen the legal framework to facilitate its effective utilization.[16.]

Training for law enforcement, investigators, and the public is key to effective handling of digital evidence. Standardized collection, preservation, and presentation of digital evidence are essential. Public education about the proper submission of evidence to authorities is equally crucial.

India's initiative to craft a comprehensive legal landscape for digital evidence exhibits a delicate balance between ensuring justice and acknowledging the challenges posed byever- evolving technology. Sustained progress in digital forensics methodology and training within the legal community will be critical in maintaining the efficiency of the legal system as technology advances rapidly.[17-18]

Requirements for electronic evidence to be admissible in Indian Courts:

In India, Section 65B of the Indian Evidence Act governs the admissibility of electronic evidence in court. This section establishes specific conditions beyond technical aspects that must be met to establish the authenticity of digital records. Before accepting electronic evidence, the court will assess its relevance, truthfulness, and authenticity. The evidence must comply with the legal requirements of authenticity, reliability, and integrity as a whole. In order to prove the authenticity of electronic evidence, a certificate under Section 65B (4) of the Indian Evidence Act is required. To be admissible as evidence, an electronic record must be certified to ensure its authenticity, including details about the computersystem that generated it. If the method of certification doesn't follow the requirements of Section 65B, the electronic evidence will not be allowed in court.[19-21]

In India, specific technical and non-technical conditions need to be met for electronic evidence to be accepted in a court of law. The primary law governing this is Section 65B of the Indian Evidence Act. To be admissible, electronic evidence must be proven genuine. Before admitting digital evidence, the court must determine its relevance, truthfulness, and authenticity. Additionally, the evidence must satisfy three key legal requirements: authenticity, reliability, and integrity. Section 65B (4) of the Indian Evidence Act mandates a certificate for demonstrating the authenticity of electronic evidence. The certificate must confirm that the electronic record is genuine and offer information regarding the computer system used. If the procedural norms specified in Section 65B are not adhered to, the electronic evidence will be rejected.[22-24]

Admissibility of Digital Evidence in Indian Courts

InIndiancourts,digitalevidencemustsatisfycertaintechnicalrequirementstobe considered legally admissible:

1. **Relevancy:** The digital evidence must be directly connected to the facts of the case at hand.
2. **Authenticity:** It must be established that the digital evidence is genuine and has not been tampered with.
3. **Reliability:** The source of the digital evidence must be trust worthy, and appropriate procedures must have been followed during its acquisition and preservation.
4. **Integrity:**Thedigitalevidencemustbeprotectedfromanyunauthorized modifications or corruption throughout the process.

To meet legal requirements for digital evidence in court:

1. **Chain of Custody:** Keep a record of who handled the evidence from when it was seized to when it was presented in court.
2. **Certificate:** Get a certificate signed by a qualified person saying the evidence isreal and hasn't been changed.
3. **Expertise:** Make sure qualified people handle collecting, storing, and presenting the evidence.
4. **Documentation:** Write down in detail how the evidence was collected, stored, and presented.

Digital evidence encompasses various types of enhanced media used in legal proceedings, such as audio and photo enhancements, forensic video analyses, and digitally enhanced latent fingerprints. Careful handling of this evidence is necessary to maintain its integrity and prevent allegations of tampering or fabrication. The reliability of the judiciary relies on the capability of legal experts to accurately and fairly handle cases involving digital evidence. For this reason, legal professionals must remain informed about advancements in digital forensics and develop effective communication skills to explain complextechnological concepts to judges and juries.[25-28]

Digital Forensics: Tools and Techniques for Legal Admissibility:

In legal cases, allowing digital evidence in court is a significant issue. Specialists in digital forensics use specific techniques and tools to ensure that digital evidence is legally presentedin court.This is donebyfinding, keeping, and analysingdigital information in such a way that it can be used in court.

Legal Aspects and Admissibility

The legality of digital evidence depends on certain technical and legal requirements. In many countries, gathering and analysing digital evidence must follow legal procedures like search warrants or court orders. If these procedures are not followed, accept the evidence maynot in court. Digital forensic experts must protect the privacy and confidentiality of individuals involved in investigations. They must make sure that the collection and examination of digital evidence do not violate anyone's right to privacy.

Technical Aspects:

The accuracy of digital evidencein courtrelies on afewimportant factors. Firstly, the evidence must have a clear history of its handling and storage (chain of custody) to ensure that it wasn't manipulated or changed. Secondly, the tools used to analyze the evidence must beapprovedandup-to-date,andtheinvestigatorswhousethemmusthavethepropertraining and knowledge.

Digital evidence can be used in court cases if it meets the following conditions:

1. Itisrelevanttothecaseandprovidesinformationaboutfactsthatarebeing disputed.
2. Ithasnot beenalteredortamperedwith duringtheprocess ofcollectingandanalysingit.
3. Themethodsusedtoexaminetheevidencearevalidandreliable,andhavebeenpeer- reviewed by other experts in the field.
4. Thefindingsareinterpretedinanunbiasedmanner,andanyerrorsor uncertaintiesinthe findings are disclosed.

Tools and Techniques:

To uncover digital proof, digital forensics employs specific tools and approaches. These tools enable the secure acquisition, preservation, and analysis of digital evidence. In a forensically compliant process, investigators collect digital details, analyse them, and provide interpretations that can be used as evidence in legal circumstances.

Digitalforensicsreliesonspecializedsoftwaretoolsfordataacquisition,analysis,and investigation. Some widely used tools include:

1. **Data Acquisition:** - FTK Imager: It is used to create forensic images of storage devices. - EnCase: This tool allows for the acquisition of digital evidence from various sources.
2. **Data Analysis:** - Autopsy: This open-source tool is designed for comprehensive digital forensics analysis. - X-Ways Forensics: It is a commercial tool used for advanced forensic analysis of digital evidence.
3. **Network Forensics:** - Wireshark: It is a popular network analysis tool used to capture and analyze network traffic.

4. **Memory Forensics:** - Volatility: This tool is used to perform memory forensics analysis of running systems.
5. **Mobile Device Forensics:** - Cellebrite UFED: This tool is widely used for extracting data from mobile devices for forensic analysis. These tools collectively assist forensic examiners in acquiring and examining various forms of digital evidence, including emails, documents, chat logs, images, videos, and metadata, from digital devices and networks.

To ensure the validity of digital evidence in a court setting, a thorough knowledge of the legal and technical considerations of digital forensics is necessary. Following legal procedures, respecting privacy rights, preserving the chain of custody, utilizing reliable forensic tools, and having knowledgeable investigators are key elements in determining the admissibility of digital evidence in court.[29-30]

Digital evidence can take many forms and can be used in various legal cases. Some examples of digital evidence that can be used in legal cases include:

1. **Emails and chat logs:** These can be used to prove communication between parties and can be used to establish intent or motive.
2. **Social media posts:** Social media posts can be used to establish a person's whereabouts, activities, and associations.
3. **Text messages:** Text messages can be used toestablish communication betweenparties and can be used to establish intent or motive.
4. **Computer files:** Computer files such as documents, spreadsheets, and presentations can be used to establish a person's activities, associations, and intent.
5. **Metadata:** Metadata can be used to establish the origin, creation, and modification of digital files.
6. **Videofootage:**Videofootagecanbeusedtoestablisha person'swhereabouts, activities, and associations.
7. **Cellphonerecords:**Cellphonerecordscanbeusedtoe stablishaperson'swhereabouts and activities.
8. **GPSdata:** GPS datacanbeusedtoestablisha person'swhereaboutsandactivities.

In legal cases, various types of digital evidence, such as social media posts, emails, text messages, and GPS data, can be used as supporting evidence. However, the validity of this evidence in court relies on several criteria. These include the significance of the evidence to the case, confirmation of its genuineness, and the credibility of its source. Proper procedures for gathering, examining, and presenting digital evidence should be followed to ensure its acceptance in legal proceedings.[31-35]

Evidentiary Standards for Digital Content in Courtrooms

1. AnvarP.K. vs.P.K. Basheer

This landmark case ruled that electronic evidence must conform to the requirements outlined in Section 65B of the Indian Evidence Act. The Supreme Court overturned previous decisions and clarified that a certificate under Section 65B (4) is mandatory for the admissibility of electronic evidence.[36,37]

2. Arjun Panditrao Khotkar vs. Kailash Kushanrao Gorantyal

Here, the Supreme Court addressed the admissibility of secondary evidence in the form of electronic records when the original records were not available. The court confirmed that the requirements of Section 65B (4) must still be met even when dealing with secondary evidence.[37]

3. State (NCT of Delhi) vs. Navjot Sandhu

Thiscase focused on the interpretationof Section 65B (4)ofthe Indian Evidence Act. The Supreme Court emphasized the importance of strict compliance with the procedural requirements of Section 65B for the admissibility of electronic evidence.[37]

4. Anand Rajendran vs. State

In this case, the Supreme Court of India examined the admissibility of electronic evidence in the form of call records obtained from a cellular service provider. The court held that to admit electronic evidence,

including call records, it is necessary to comply with the provisions of Section 65B.[37]

These cases demonstrate the evolving nature of digital evidence admissibility in Indian courts and the efforts made by the judiciary to adapt to the changing needs of society and technology. They serve as valuable precedents for future litigation involving digital evidence.

Overcoming Obstacles in Digital Evidence Presentation

Challenges in digital evidence presentation include difficulties proving itsauthenticity, maintaining its chain of custody, and translating technical jargon into language laypeople can comprehend.

AuthenticityandIntegrity

Ensure the authenticity and integrity of digital evidence by implementing robust security measures during collection, preservation, and presentation stages. Use tools like encryption, digital signatures, and checksum algorithms to verify the integrity of digital evidence throughout the process.

Chain ofCustody

Establish and maintain an unbroken chain of custody to demonstrate that no changes have occurred to the digital evidence after it was collected. Implement strict procedures for handling digital evidence, and document every action taken with regard to the evidence.

Communication Gap

To bridge the communication gap between technical jargon and legal comprehension, employ plain language summaries and analogies to simplify complex technical concepts for judges,lawyers,andjurors.Avoidoversimplification,but aimtomakethematerialaccessible to those unfamiliar with technology.

Ethical Considerations

Addressprivacyconcerns,dataownershipissues, andthe responsibleuse ofemerging technologies. Ensure that the presentation and scrutiny of digital evidence remain aligned with the principles of justice, the bedrock of our legal systems.

Trainingand Education

Provide regular training and education programs for personnel involved in handling digital evidence to keep their skills current and minimize errors caused by human factors.

DocumentationandRecordKeeping

Document every step of the digital evidence lifecycle, including collection, preservation, and presentation. Create comprehensive reports of each digital evidence file, ensuring evidence integrity at all levels.

Collaborationamong Professionals

Encourage collaboration among legal professionals, technologists, and policy makers to stay abreast of developments in digital evidence and to shape best practices accordingly.

By addressing these challenges, legal professionals can effectively present digital evidence in court, thereby enhancing the administration of justice and promoting public confidence in the legal system.[38,39]

Futureof DigitalEvidence:TrendsandPredictions

The future of digital evidence is influenced by several trends and predictions, including the evolving legal framework, technological advancements, and the increasing volume and diversity of digital evidence.[40,41]

EthicalConsiderationsin Handling DigitalForensicEvidence

Digital forensics plays a crucial role in gathering, securing, and examining electronic evidence for legal proceedings and investigations. Ethical considerations are paramount in digital forensics to guarantee impartial and just investigations while protecting individuals' privacy and rights. The fundamental ethical principles in digital forensics encompass objectivity, confidentiality, integrity, competence, compliance, and respect for human rights. Upholding digital forensics ethics is fundamental in building

trust and credibility between investigators and the client, as well as ensuring unbiased investigations without tampering or alteration of evidence. Maintaining ethical behavior throughout digital forensics investigations can be challenging due to the sensitive nature of the material involved, such as personal data and financial information. Investigators must avoid any bias or conflict of interest that could influence their judgment, and must treat all parties involved in an investigation with respect and professionalism.

In India, the legal framework for electronic evidence is governed by the Indian Evidence Act and the Information Technology Act. To be admissible in Indian courts, electronic evidence must meet certain requirements, including relevance, authenticity, and reliability. The authenticity of digital evidence is ensured through the use of digitalsignatures, hash values, and other technical measures. The Indian courts also require that the chain of custody of digital evidence be maintained to ensure its integrity andadmissibility.[42-44]

Examplesofprivacyconcernsindigitalforensicsinclude:

1. Accessingsensitivedatawithoutproperconsentora warrant,potentiallyviolating individuals' rights to privacy.
2. Biasanddiscriminationinthecollectionandanalysis ofdigitalevidence,leadingto incorrect conclusions and negative impacts on marginalized communities.
3. Potentialforinvasionsofprivacy,whereinvestigator smayaccessprivatecommunications, medical records, or financial information.
4. Misuse of digital forensic tools and methods, resulting in false positives or false negatives, which can lead to miscarriages of justice.
5. Accidental disclosure of personal information, either through careless handling of digital evidence or insufficient safeguarding mechanisms.
6. Deep fake technology, which allows the creation of synthetic videos or images that can deceive viewers, making it difficult to distinguish genuine digital evidence from fabricated ones.
7. Insufficient regulation and oversight of digital forensic activities, leaving room for abuseof power and violation of privacy rights.

The importance of ethical digital forensics practices cannot be overstated in light of the privacy concerns associated with digital investigations. The primary objective of digital forensics is to maintain a balance between the requirement for investigations and the protection of individual privacy rights. Steps need to be taken to address the privacyconcerns. These include creating clear guidelines, fostering collaboration between involved parties, and continually providing training and education for forensic investigators.[45-47]

TheRoleof ExpertWitnessesinDigitalEvidenceCases:

Digital evidence cases rely heavily on expert witnesses who provide professional opinions and testimony during legal case proceedings. Digital forensics experts make complex technical concepts and findings clear and understandable for the jury, majority of whom usually lack technical knowledge. The act of collecting, preserving, and evaluating digital evidenceis known as "Digital Forensics." Theprocess of explainingtheseideas is part oftheirjob. Digitalforensicscoversawiderangeofhighlyspecializedfi eldsthatcanprovide valuable information for achieving justice. In India, the admissibility of digital evidence is governed by the Indian Evidence Act and the Information Technology Act. To be admissible in Indian courts, electronic evidence must meet certain requirements, including relevance, authenticity, and reliability. The authenticity of digital evidence is ensured through the use of digital signatures, hash values, and other technical measures. The Indian courts also require that the chain of custody of digital evidence be maintained to ensure its integrity and admissibility.

To maintain credibility and effectiveness as an expert witness handling digital forensics cases, staying informed of the latest research and literature in the

field is essential. This knowledge aids in demonstrating expertise and handling challenges arising during the consultancyphase.Collaboratingcloselywiththelegalteamiscrucialtoidentifypotential

Daubert challenges, allowing for the development of strategies to address them effectively. Expert witnesses should be prepared to defend their methods and conclusions, including any testing or peer reviews conducted to substantiate their work. Transparency and honesty are vital, with experts clearly communicating any potential limitations or weaknesses in their methods or conclusions.

Expert witnesses play a crucial role in digital evidence cases by providing their professional opinion and testimony during legal proceedings. The admissibility of digital evidence in Indian courts requires that electronic evidence meet certain requirements, including relevance, authenticity, and reliability, and that the chain of custody be maintained to ensure its integrity and admissibility. Expert witnesses should stay up-to-date on digital forensics research and literature, work closely with the legal team, and be prepared to defend their methods and conclusions to ensure a fair judgment.[48-51]

Digital forensics expert witnesses face several challenges when presenting digital evidence in court. Some common challenges include:

1. **Technical complexity:** Digital forensics is a highly technical field, and expert witnesses must be able to explain complex technical concepts in a waythat is understandable to non-technical people, such as judges and juries.
2. **Admissibility:** Digital evidencemust meet certain requirements to beadmissible in court, including relevance, authenticity, and reliability. Expert witnesses must be able to demonstrate that the evidence meets these requirements.
3. **Anti-forensic techniques:** Criminals may use anti-forensic techniques to hide, alter, or remove traces of their crimes, making it difficult for digital forensics experts to collect and analyze evidence.
4. **Privacy concerns:** Digital evidence may contain sensitive personal information, and expert witnesses must ensure that this information is protected and not disclosed without proper consent or a warrant.
5. **Bias and discrimination:** There is a risk of bias and discrimination in the collection and analysis of digital evidence, which can lead to incorrect conclusions and negative impacts on marginalized communities.
6. **Resource challenges:** Digital forensics experts may face challenges related to changes in technology, volume and replication of data, and skill gaps.

Tonavigatethecomplexitiesof digitalforensics,expertwitnessesneedto:

1. Stay informed: Keep up-to-date with the latest research, methods, and techniques in digital forensics. This can involve attending conferences, reading relevant literature, and interacting with experts in the field.
2. Collaborate with the legal team: Establish and maintain open and effective communication with the legal team throughout thecase. This helps in understandingthelegal context, legal arguments, and the specific needs of the case.
3. Be ready to defend: Expect to face challenges and scrutiny of their methods, opinions, and conclusions. Be prepared to defend and explain the basis of their findings, including the methods used and any underlying assumptions or principles.
4. Practice transparency: Be transparent and honest in their work. Disclose any potentiallimitations,uncertainties,orweaknessesin theirmethodsorconclusions.Ifthereare limitations or weaknesses, explain why these do not invalidate the overall validity of the findings.[52-56]

II. CONCLUSION

The main findings of the research paper on the admissibilityand challenges of digital evidence in legal proceedings in the Indian context include:

1. The considerable quantity of evidence analysed by examiners and challenges in obtaining necessary support, in terms of both funding and staffing, as noted by law enforcement attendees.
2. Potential difficulties with prosecutors, judges, juries, and defense attorneys not understanding elements of digital evidence, with defense attorneys being the farthest behind the curve.
3. The importance of reliability as an evidentiary issue with respect to digital evidence, as required by the Indian Evidence Act and the Information Technology Act.
4. Challengesimpactingdigitalforensicjurisprudence andthe admissibilityofevidence, such as inadequate chain of custody, illegal procedures, deficiencies in evidential integrity, and lack of expertise in the field.
5. Theneed to ensurethat digital evidenceis authentic, accurate, complete, and convincing to the jury for its admissibility in court, as required by the Indian Evidence Act and the Information Technology Act.
6. The impact of the rapid evolution of technology on legal standards for the admissibility of digital evidence, and the challenges faced in establishing consistent criteria, as seen in landmark cases such as Anvar P.K. vs. P.K. Basheer.
7. Theneedforcourtstoascertaintherelevance,veracity,andauthenticityofdigitalevidence before acceptingit, along with the limitationsand complexities associatedwith the admission of electronic evidence, as outlined in various judgments by the Supreme Court of India.

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Admissibility of Electronic Record under Section 65B of The Indian Evidence Act Vis-à-vis Judicial Interpretation

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ABSTRACT

In the digital era, persons are interconnected through gadgets, vivid technologies, and internet connections. Digital platforms have been proved as a boon but have given birth to various challenges in the form of vulnerabilities, attacks, and cybercrimes. From time to time, the law has impacted society and society has been impacting law. Until the Information Technology Act, 2000 was introduced; digital data, gadgets, or electronic evidence were not admissible as evidence in India. IT Act made several changes with respect to'Admissibility of Electronic Evidence'in the Indian Evidence Act, 1872. The rampant use of technology has given birth to new laws and regulations and the Apex Court of the land interprets the same from time to time. One such meaningful interpretation is regarding the 'Admissibility of Electronic Records' under Section 65B of the Indian Evidence Act, 1872. Deficiency of simplified interpretations through judiciary on 'Certificate of Authentication' has posed many doubts on the varied electronic evidence viz., Emails, CCTV footages, CDs/DVDs, USBs. This paper focuses on the admissibility of digital evidence in the court of law and presents detailed insight on the relevancy/irrelevancy of the 'Certificate of Authentication.'

Keywords - Certificate of Authentication, Digital Evidence,Cybercrimes,IT Act-2000,Judiciary, Justice.

I. INTRODUCTION

Technological expansions have profited the world by offering many luxuries and comforts of life and also offered newer extents to human abilities and human actions in the modern world. Technology has never been that easy as is today. Its credit goes to convenient digital/electronic inventions. With the arrival of globalisation and the rise in the digitization of everything, a major variation in modes & methods of intercommunication among businesses was observed in last few years. In 1996, The United Nations Commission on International Trade Law (UNCITRAL), by the means of Model Law on Electronic Commerce (MLEC), introduced a set of globally justifiable rules to eliminate lawful difficulties and increase legal expectedness for e-commerce. This enhanced the efficacy in worldwide trade by offering common action to paper-based & electronic data, consequently allowing the usage of paperless statements. MLECaimed at cheering lawmakers to accept a set of universally admissible rules managing electronic commerce. MLEC escorted a guide that gives framework and descriptive guidelines to support the states in making the essential jurisdictive provisions. Digital/electronic evidence, being least appraised, based on significance and credibility are now one of the most important key elements in proving or disproving the case.

The Indian Information Technology Act-2000 (hereinafter referred to as IT Act, 2000) provides lawful admission to digital signatures and electronic records. Prior to this Act, electronic records & digital evidence were not considered vital pieces of evidence. The Indian IT Amendment Act, 2008 provides the admissibility of evidence produced through communication devices. Common digital evidence on the crime scene are CDs, DVDs, Pendrive, Memory Cards, CCTV footages, Mobile handsets, and Cameras. Electronic evidence like social network communications,website information, E-mails, computer-generated documents and even SMS/MMS, pose distinct problems and challenge for appropriate validation and are subjected to a diverse set of interpretations. These days, Pendrive and USBs are some of most common means for transferring digital data. Throughout the courtroom proceeding, Judges are frequently requested to instruct on the admissibility of electronic evidence and it significantly influences the results of civil law cases or conviction/acquittal of an accused. Court of law endures to wrestle with this new electronic edge as the specific behaviour of electronic evidence, whether it's fake or fabricated, creates difficulty to admissibility not confronted with the rest of the evidence.

Digital evidence is used not only in cybercrimes but also useful to solve other crimes. As the applicability of the IT Act and succeeding amendments to the Indian Evidence Act (hereinafter referred to as IEA, 1872), the usage of electronic records in trials/suits in the courts of law has come a long way. Due to the application of Section-92 of IT Act 2000, various laws were amended which includes IEA-1872. This includes: The phrase under section 3 of the IEA, 1872 "*All documents produced for the inspection of the Court*" were substituted by "*All documents including electronic records produced for the inspection of the Court*". Regarding the documentary evidence, in Section 59, for the words "Content of documents" the words "Content of documents or electronic records" have been substituted and Section 65A & 65B were inserted to incorporate the admissibility of electronic evidence. However, in spite of numerous legal precedents emphasising the significance of the 'Certificate of Authentication', the journey of the 'Certificate of Authentication' has developed a confused state for lawmakers.

Admissibility of Electronic Records

Electronic information is very easy to produce, duplicate, modify, terminate, and transfer from one platform to another. In short, by their very nature, electronic records can be easily manipulated. Consequently, their accuracy and trustworthiness are

often suspected. This creates a conflict between the applicability and acceptability of digital evidence, something that has been recognized by authorities across the globe. The rule concerning to the admissibility of evidence in India is dealt under the IEA, 1872. According to Section 65B of the IEA, 1872, 'Any information contained in an electronic record whether printed on a paper, stored, recorded or copied in optical or magnetic media produced by a computer is to be considered as a document, if the conditions mentioned in this section are satisfied then the evidence will be admissible in the court of law.' Section 65B states –"For the purpose of admissibility of evidence, a certification shall have the following matters as necessary: (a) identifying the relevant electronic records relating to the certificate and describing the manner in which it was produced, (b) details of the device producing it, (c) satisfying the conditions of 65B (2) i.e., the computer from which the output was produced was used regularly to store or process information during its regular course of activities and (d) throughout the material part of the said period, the computer was operating properly". Certification of matters is to be stated to the best of the knowledge and belief of the person signing the certificate i.e., the officer in charge of the operation or management of the related activities.

The Supreme court of Nigeria in the case of *Esso West Africa Inc. v.T. Oyegbola* observed that "The law cannot be and is not ignorant of modern business methods and must not shut its eyes to the mysteries of the computer." According to Section 2 (t) of the Indian IT Act, 2000, Electronic Record means "Data, record or data generated, image or sound stored, received or sent in an electronic form or micro film or computer-generated microfiche". It fundamentally means electronic records can be referred to as such data or image or sound or anything which can be either sent or received in the electronic medium. Digital evidence contains thedata that is kept or communicated digitally. Electronic evidence is secondary evidence. Sec. 3 of IT Act, 2000 i.e., the meaning of authentication of the electronic record states that it is compulsory to affix a digital signature so that an electronic record produces the identical hash value every time a particular algorithm is implemented. This is done so that individuals are not able to replicate original evidence and form duplicated or falsified evidence. Electronic records can vary from Pendrive (PDs) to Compact Discs (CDs). It consists of E-Mails, computer generated documents, databases, digital spreadsheets, images, etc. Any data documented by a computer that is produced or received is labelled under an electronic record. There are different interpretations of different countries with respect to the digital evidence and its admissibility in their courts.

In Malaysia, Sections 90A, 90B, and 90C of the Malaysian Evidence Act, 1950 provide the rules which are responsible for admissibility of electronic evidence in the courtof law. Over the dispute of verification of electronic evidence, section 90A (2) of the Malaysian Evidence Act 1950 demands the presentation of a certificate from anindividual accountable for the tasks relatedto the computers. Failure to submit certificate regarding computer generated evidence may result in rejection of the evidence for authenticity failure. While inUnited Kingdom (UK), electronic evidence is accepted at both criminal and civil trials. For civil cases, the Civil Evidence Act, 1995 was approved which provided the admissibility of electronic evidence, the authentication of certain documents, and proof of official actuarial tables in civil proceedings. Computer generated documents are acceptable as an evidenceunder Sec. 3 of the Civil Evidence Act. Similarly, in the United States (US), prior admitting the digital evidence, it shall be certified i.e., the supporter of the evidence must make sufficient findings to supportthe genuineness of an evidence.

Admissibility of Social Media Evidence

The time of two decades is already elapsed after the passing of the IT Act-2000, the laws on digital evidencesare still in its infancy. It is noticed that

social media chats, communications over any of the social media or IM (Instant Messaging) platforms for that case, etc. are still presented in the form of printouts of actual conversations. Such exercises take the social media chats beyond the scope of Sec. 62 of the IEA and thus can't be considered as primary evidence and hence, treated as secondary evidence. Social media platforms like WhatsApp, Instagram, Facebook, Twitter, Telegram, etc.play an important role in present times as the most common ways of online communications. The subsidiary role of social media evidence, considered through judiciary is a hindrance for speedy disposal matters. There requires a strong judicial mindset to prioritise them, which can be considered as effective utilization of technology because the very aim of technology was to reduce the time, human intervention, etc. These evidences have a potential to shift the burden of proof from one party to another.

Admissibility of Electronic Evidences: An Indian Approach

As the exposure to electronic records has increased the evidentiary value of digital evidence, there has been a transition from a time of treating 'Electronic Records' as normal documents to treating them as important pieces of evidence. Unfortunately, it is observed that the legal system does not always keep up with the pace of technological advancements. The court system believes that evidence is acceptable as far as any of the evidence is appropriate. The court must practise judicious options at the time of verdict of the case. Simplyaccepting evidence does not prove any fact in itself.

Over the years, it has been observed that the Indian judiciary shows the 'difference of opinions' in the interpretation of laws. A judgment may vary as per the difference of opinion between/among judges. The differences in the admissibility/ inadmissibility can be observed from the following cases -

In the case of *State (NCT of Delhi) v. Navjot Sandhu,* it was observed that, although the certificate with the details mentioned in Section 65B has not been issued, secondary evidence may be provided if the provisions under Sections 63 and under 65 of the Indian Evidence Act are complied with.

Whereas an overruling judgment was pronounced by the Hon'ble Supreme court through *Anvar P.V v. P.K Basheer*. The court held that the electronic records will not be admissibleasan evidence by way of secondary evidence unless the requirements under Section 65B have been met. As Sec 65A and Sec 65B are special provisions, they have precedence over general law(*Generalia specialibus non derogant*).

The subsequent judgment of *Sanjaysinh Ramrao Chavan v. Dattatray Gulabrao Phalke & Ors* provided that without the source, there is no truth to the translation. Sources and veracity are the two main reasons for accepting electronic evidence in a court of law.

In the case of *Abdul Rahaman Kunji v. State of West Bengal,* the High Court of Calcutta, while determining the eligibility of emails, had said that - an email sent by downloading an email from a person's email account can be proved by Sec. 65B r/w Sec 88A. The court also held that the testimony of witnesses was sufficient to prove the communication in order to carry out such a process for downloading and printing the conversation.

In *Girwar Singh v. CBI* the court directed that the electronic evidence is inadmissible in the court of justice because the electronic record was moved into two new cassettes and the original recording was deleted and was not brought in the eyes of the court for a very long duration. The court stated that the applicability of Section 65B is present only if the original evidence is produced.

In the case of*Vikram v. State of Punjab*the court noted that the tape-recorded conversation in the present case was kept as primary evidence, not secondary evidence which required certification under Sec 65 B of the IEA, 1872. It also gave reference to the *Anvar case* by saying thatElectronic evidence is used as primary evidence to be admissible as evidence, without complying with the terms of Section 65B.

Also, in the case of *Sonu v. State of Haryana*the court held that a call data record (CDR) without any certificate under section 65B was not congenitally unacceptable. The method of such authentication is related to the method of taking evidence and objections and should be raised as soon as possible.The judge in the present case recommended that the appropriate bench consider this in the future. But, despite knowing that their hands were tied, the two judges rejected the appeal by refusing to apply the law in the *Anwar case*.

In the famous *Shafhi Mohammad V. State of U. P.* the court ruled that the need to obtain a certificate of judicial process could be easily met by a court where the interests of justice are preferred. For example, the procedure required under section 65B of the IEA-1872for shop bills, electronic tickets, etc., applies only when electronic evidence is submitted by a person in a position to issue such a certificate,thatis under the control of the device.When the party does not have such a device, the applicability of sections 63, 63 and 65of the IEA-1872cannot be omitted from the application.

In contrast to the above verdict, *State of Karnataka Lokayukta Police Station, Bengaluru v. R. Hiremath*the Supreme Court has ruled that a certificate under Section 65B can be issued after the chargesheet has been filed, which is required to be produced when an electronic record is required to be submitted as evidence at the time of trial.

From the decisions of the above cases, one may go with a 'confused state of mind' concerning the admissibility of electronic evidence and exact need for a certificate of authentication before the court proceeding.

Certificate of Authentication – An Obstacle to Speedy Trial?

Day-by-day, Judiciary is highly burdened due to the rapid increase in the rate of crimes. National Crime Record Bureau (NCRB)report of 2018-19 shows that the crime rate registered per lakh population has increased marginally from 383.5 in 2018 to 385.5 in 2019. Specifically, Crime rate under the cybercrimes category increased from 2.0 in 2018 to 3.3 in 2019 per lakh population. As per the Law Ministry of India records, there is just one judge per 50,000 citizens as against the recommended number of one per 20,000 in the year 2019. This results in the delaying of judgments. In recent times, with the increase of digital devices, it's one of the most difficult tasks to verify each digital evidence present on a crime scene. Preparation of certificate after authentication of each digital device present on the scene is not a good option in every matter. One can lose the crucial/credible information stored on the volatile storage devices due to delay in response to these devices as well as delaying in verification and authentication e.g., data stored on RAM. Digital evidences or gadgets are genuine unless altered or modified. The court should consider that; such evidence may change the order from acquittal to conviction and vice versa. It should also consider the perspective of an expert while admitting evidence.Delay in the presentation of 'Certificate of authentication' is one of the reasons, ultimately leads to a bunch of confusions. The law on electronic evidence under the IT Act 2000 is still in its infancy and is expected to make significant progress in the near future.

II. CONCLUSION

Law must change as per the needs of changing demands of society. Unfortunately, lack of political will from lawmakers, undue execution through executors, and many times, discretion-based interpretation through judiciary is the reason behind many problems. Because discretion may lead to arbitrariness. Recent Indian pronouncements on the admissibility of electronic evidence stress on the requirement of the 'Certificate of Authentication' and non-adherence is violative to section 65B of the Indian Evidence Act. The Hon'ble Supreme Court's verdicts on the admissibility of electronic evidence

evoke mixed feelings. One may say, that it wilfully disregards Section 65B of IEA, by ignoring and wilfully reading with statutory requirements as it sees fit. But, the implementation of time-consuming procedures may lead to injustice. A proper and judicious explanation to the admittance or rejection of electronic evidence is the need of the day and should be reflected through the judgments of the apex court of the land.

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Educational Document Verification through Blockchain: Literature Review

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ABSTRACT

In this survey of the paper, we have described blockchain-based document verification. The blockchain stores the data in the form of blocks, with each block linked to another block to secure the data. Each block contains the hash value of the data. It also contains the hash value of the previous block. Document verification is a time-consuming process. This research paper represents the comparison of hash techniques, namely SHA-3, SHA256, SHA1, and MD5. We are developing a user-friendly and secure document verification system using blockchain technology and QR codes. This work is useful for colleges and universities because a lot of documents are fake and fraudulent, so they can use this system to verify documents.

Keywords: QR Code, HASH Code, Document Verification, Blockchain Technology, SHA-3, SHA256, SHA1 and MD5

I. INTRODUCTION

The degree certificate, mark sheet, and other student-related documents provided by the university or college are of prime importance in the student's life, but the production of fake certificates and document manipulation is very easy because a paper document can easily be forged with the availability of advanced printing and copying technologies. On the other side, when students apply for jobs in any industry, they will have to verify their documents through email, but sometimes institutes, colleges, and universities do not reply in time to document verification emails because they have been effectively document verification processes.[1] Hence, there is a need to adopt document verification through QR codes and publish through blockchain. This process can very easily verify and ensure the authenticity of documents. Data is stored using blockchain technology in an unchangeable format. This is done through cryptography, which involves the encryption of data using a hash function. So the data becomes unalterable. It is practically impossible to hack. In this work, we will design and develop a web-based application in PHP and MySQL using a blockchain system. [2]

A. Blockchain Technology

Blockchain technology stores data in the form of blocks. Each block contains hash value of that block and hash value of previous block. Hence it led to the formation of chronological chain of blocks. Blockchain can be used for document verification because of its immutability that is data recorded on blockchain is extremely difficult to manipulate. The data on blockchain is visible to all participants in the network. [2]

There are three types of block chain that are as follows:

Public Blockchain: public blockchain are open network where anyone can participate, view and validate transaction. [2]

Private Blockchain: Private Blockchain requires permission to access. Private Blockchain is type of blockchain used by organizations. It offers greater privacy. [2]

Consortium Blockchain: Consortium Blockchain is semi decentralized network where group of organizations governs the blockchain. Access is restricted to members. It provides balance between private and public blockchain.

B. Digital Signature

Digital Signature is cryptographic technique. This technique used to verify the authenticity and integrity of digital documents. Data is encrypted using private key and data is decrypted using public key. [3]

C. Hashing

Hashing is used in cryptography to convert data into fixed-size string characters. [3]

D. QR Code

A QR code, or Quick Response Code, is a type of two-dimensional barcode that contains encoded information. QR codes can store various types of data, including text, URLs, contact information, or other types of data. They are widely used for quickly accessing information using a Smartphone or other devices equipped with a camera and QR code scanning software. QR codes consist of black squares arranged on a white square grid, typically with a square shape. When scanned by a QR code reader, the encoded information is extracted and processed. QR codes are commonly used for marketing, advertising, ticketing, product tracking, and various other applications where quick access to digital information is required. [4]

II. LITERATURE REVIEW

Following table shows the some no of research articles based on blockchain technology for the document verification. The problems that exist in blockchain system and the various techniques developed by various research workers to solve these problems have been discussed in the following Table No 1.

TABLE I LITERATURE SURVEY OF BLOCKCHAIN TECHNOLOGY

Authors	Title/Research Article	Methods and Techniques	Conclusion
Iftekher Toufique Imam, Yamin Arafat, Kazi Saeed Alam and Shaikh AkibShahriyar	DOC-BLOCK: A Blockchain Based Authentication System for Digital Documents, IEEE, (2021)	**SHA-256, Ethereum Blockchain, Cryptographic Hash, Peer to Peer Cloud, HTML, JavaScript, Public/Private Key Cryptography, Online Storage Security, Digital**	*This research paper represents the Blockchain Based Authentication System for Digital Documents. They use a web application for peer-to-peer cloud storage and digital document*

		Signatures, Hash, IPFS Hash	*verification that is based on the Ethereum blockchain. They were also used solidity programming language.[1]*
Venkata Marella, Anoop Vijayan.	Document Verification using Blockchain for Trusted CV Information, AMCIS(2020)	**Blockchain, Hash Value, Background Verification process, AES algorithm (Advanced Encryption Standard), Hyperledger fabric.**	*This paper aims to develop a solution for the background verification process of job applicants during the hiring process using by comparing the hash value of the given document with the hash value of the document present on the blockchain.[2]*
Sthembile Mthethwa, Nelisiwe Dlamini, Dr. Graham Barbour.	Proposing a Blockchain-Based Solution to Verify the Integrity of Hardcopy Documents, IEEE(2021)	**SHA – 256, Blockchain, 2D Barcodes, Cryptographic Hashing, Integrity, Optical character recognition (OCR), Secure Hash Algorithm, Tesseract**	*This paper presented a proposed solution for the problem of document forgery. Documents were generated using a font known as Any OCR (which is designed for OCR tools) and Tesseract was used to validate the documents.[3]*
Saqib Rasool, Afshan Saleem, Muddesar Iqbal, TasosDagiuklas, Shahid Mumtaz and Zia ul Qayyum.	DOC-BLOCK: A Blockchain Based Authentication System for Digital Documents, IEEE (2018).	**Docs-Chain, Blockchain, PoE (Proof of Existence), OCR**	*This paper presents a semi-private blockchain based degree verification solution which. It also enables the verification from the photocopies of all the degrees.[4]*
Omar S. Saleh, Osman Ghazali, Muhammad Ehsan Rana.	Blockchain Based Framework for Educational Certificates Verification, Journal of Critical Reviews, 2020	**Hyperledger Fabric Framework, Blockchain**	*This research paper represents blockchain-based framework for Educational certificate verification based on Hyperledger Fabric Framework [5].*
YassynzhanShakan, GalimkairMutanov,	Verification of University Student and Graduate Data	**Blockchain, Smart Contract,**	*This project created the cutting-edge UniverCert*

ZhanlMamykova, YerlanKistaubayev.	using Blockchain Technology,(2021)		*platform, which tracks academic achievement, issues educational certificates, and guards against data forgeries. This system includes a student's registration, verification, and authenticity of educational documents.[6]*
Osman Ghazali and Omar S. Saleh	A Graduation Certificate Verification Model via Utilization of the Blockchain Technology,(JTEC)2019	**blockchain,hash-256, public/private key cryptography, digital signatures, peer to peer network, proof of work**	*This research paper given model for academic certificate issuing and verification using blockchain technology. All the information that is required to validate and authenticate the certificate is hosted on the blockchain itself. In this model one can validate document just by comparing hash value.[7]*
Abdullah Ayub Khan ,Asif Ali Laghari,Aftab Ahmed Shaikh,Sami Bourouis ,Amir Madany Mamloukand Hammam Alshazly	Educational Blockchain: A Secure Degree Attestation and Verification Traceability Architecture for Higher Education Commission, (Appl. Sci.) 2021.	**Blockchain, Hyperledger Fabric, Smart Contracts, HDLU-Ledger Architecture, Digital Signature and Cryptographic Hashing.**	*This paper represents HEDU-ledger architecture document verification. Permission private network architecture created between stakeholders for certificate record traceability. They have used digital signature and hashing.[8]*
Turkanovi, Mrdovi and Marjan Heri	A Preliminary Review of Blockchain-based Solutions in Higher Education,	**Review of Literature Based on Blockchain**	*This research paper represents how blockchain is used in higher education. How blockchain stores student achievement data and makes it available to authorized users. they have made analysis of existed solution.[9]*

Pavitra Haveri , Rashmi U , Narayan D , Nagaratna K , Shivaraj K.	EduBlock: Securing Educational Documents using Blockchain Technology, IEEE (2020).	**Blockchain, Ethereum, Interplanetary File System (IPFS), Smart Contract**	*In this research work they practically checked properties of blockchain i.e. security, traceability, and transparency and data integrity.[10]*

III.METHODOLOGY

The following figure represents the process of document verification using blockchain. In the workflow of that research, first, we create the admin login to upload certificates or details of the certificates for the view of certificate details. [5] Then we create the user login for the authenticated certificate. Then apply the techniques or modules to create QR codes and scanned signatures for scanning documents, document verification, and document validation.[6]

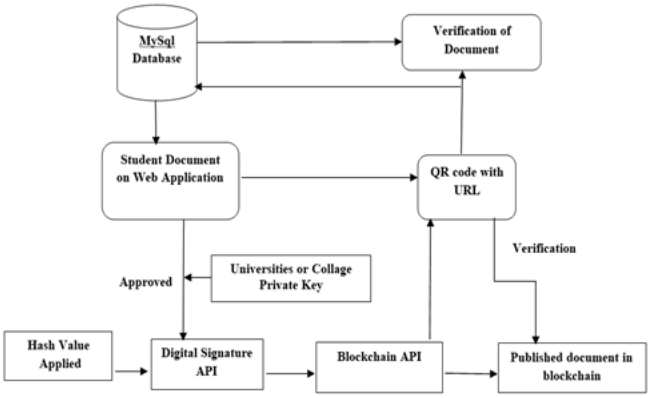

Figure1: Block Diagram of Documents Verification through QR Code

IV. CONCLUSION & FUTURE SCOPE

In this paper, we review a maximum of ten IEEE Scopus Index research papers for the blockchain study. We have observed that SHA256 is the most commonly used algorithm used for document verification using block chain technology. So for this project, we will employ the software engineering life cycle model, which includes database design and web application development, to keep student records. The web application integration and database are being created by us. Using the application's private key, we also generate a digital signature for document authentication. Include digital signatures in the online student document application. Use an API to upload documents to the blockchain. Using a hash value, the blockchain API creates a QR code that contains the document URL. Include, print, and resend the document to the blockchain with the QR code included. Post records to the blockchain. Nobody used SHA1,MD5 hash algorithms for document verification there is future scope for comparison with SHA256 algorithm. In future we will use SHA1 and MD5 algorithms for testing and comparison with SHA256 hash algorithm

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Current Trends in Forensic Analysis of Date Rape Drugs Benzodiazepines

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ABSTRACT

Drugs are typically grouped into categories like analgesics, antipyretics, antibiotics, and others such as antimalarial agents, antihistamines, hypnotics, tranquilizers, and sedatives. Drug-facilitated crimes are on the rise globally, with the prevalence of date rape drugs increasing in both developed and developing nations. The concept of a typical "date rape" scenario, where the victim's drink is secretly "spiked" with a tablet, capsule, or powder containing a sedative-hypnotic, is not supported by the comprehensive forensic database analyzed here. This study emphasizes how important it is to get forensic samples as soon as possible in suspected sexual assault situations. When there are claims of potential drug-mediated sexual assault, law enforcement agencies and medical professionals should set policies and protocols to guarantee that the proper forensic samples (blood and urine) are obtained as soon as feasible. The date-rape drugs includes benzodiazepines, gamma-hydroxy-butyric acid (GHB), ketamine, and alcohol, possess sedative, hypnotic, dissociative, and amnesiac properties. Perpetrators often mix these drugs into food or drinks or administer them covertly in alcohol before committing the crime. This article aims to shed light on these commonly used drugs in sexual assaults, detailing their pharmacology, misuse, and forensic analysis.

Keywords: Date Rape Drugs, Forensic Analysis, Forensic Exhibits

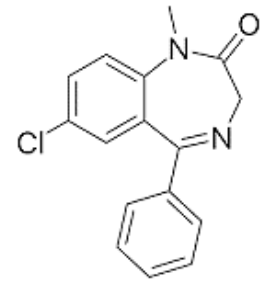
I. INTRODUCTION

Date rape drugs, including benzodiazepines, are a significant concern in forensic investigations due to their potential use in facilitating sexual assaults. Benzodiazepines are a class of psychoactive drugs known for their sedative, hypnotic, anxiolytic, and muscle relaxant properties. They are commonly

prescribed for legitimate medical purposes but can be misused or abused for nefarious activities such as drug-facilitated sexual assaults [1]. The forensic analysis of benzodiazepines in various exhibits plays a crucial role in investigating cases involving alleged drug-facilitated crimes. Different forensic exhibits, including biological samples such as blood, urine, and hair, as well as non-biological samples like beverages or drug residues, are often encountered in forensic casework related to drug-facilitated crimes. Analyzing benzodiazepines in these diverse exhibits requires advanced analytical techniques and methodologies to ensure accurate detection, identification, and quantification [2].

In recent years, there have been notable advancements and trends in the forensic analysis of benzodiazepines, driven by technological innovations and research developments. These trends encompass improvements in sample preparation techniques, instrumental analysis methods, data interpretation approaches, and the expansion of databases for benzodiazepine identification. Additionally, the emergence of novel benzodiazepine analogs and designer drugs poses new challenges for forensic scientists in staying abreast of the latest trends and adapting analytical strategies accordingly [3]. This research paper aims to explore the current trends in the forensic analysis of benzodiazepines, focusing on the advancements in analyzing benzodiazepines in different forensic exhibits. It will delve into the challenges faced, methodologies employed, detection limits achieved, and the significance of accurate benzodiazepine analysis in forensic investigations involving date rape drugs. By examining these trends, this paper seeks to contribute to the ongoing efforts to enhance forensic capabilities in combating drug-facilitated crimes and ensuring justice for victims [4].

A tranquilizer, also known as a depressant, is a type of drug that targets the central nervous system to induce calmness, reduce anxiety, or promote sleep. These medications are utilized to manage mental health disorders as well as alleviate common symptoms of anxiety and insomnia. Examples of tranquilizers include Diazepam (**1**), Flunitrazepam (**2**), Lorazepam (**3**), Alprazolam, and compounds containing Piperidine. They work by suppressing the activity of the central nervous system, resulting in a slowed-down physiological response [5]. It sounds like you're discussing the distinctions between sedatives, hypnotics, and antipsychotic medications in human medicine. Sedatives are primarily aimed at inducing relaxation and reducing anxiety, while hypnotics are used to promote sleep and can produce electroencephalographically similar patterns to natural sleep. Antipsychotic medications, on the other hand, are targeted at treating psychosis, a condition characterized by a loss of contact with reality [6].



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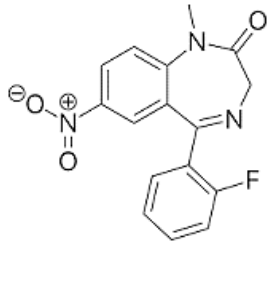

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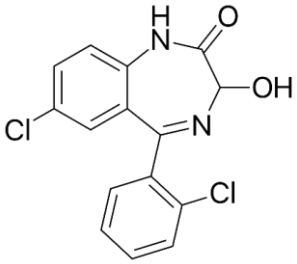

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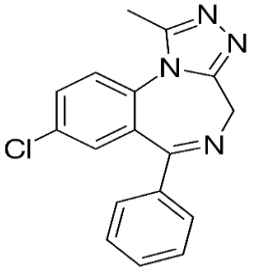

4

II. CURRENT TRENDS IN FORENSIC ANALYSIS OF BENZODIAZEPINES

Walker *et al.* (1997) analyzed beverages by capillary electrophoresis using glycine buffer at pH 9.0. This method is useful for the analysis of caffeine, aspartame and benzoic acid. The detection time of this experiment is 2 minutes. Sample after separation was passed through a UV detector. In this experiment sample was sonicated and mixed with glycine buffer

(1:1). At 260-280nm caffeine peak was obtained. Simple sample preparation has, a shorter run time and it is useful for quantitative analysis [7].

The work of Hung Nguyen and David Rao (2000) was focused on the development of a rapid Solid Phase Extraction for the capture and concentration of flunitrazepam and its metabolite (7-amino flunitrazepam, N-desmethyl flunitrazepam) from human urine and their analysis by GC-MS. GC with an electron capture detector is useful for quantitative detection of drugs. To obtain better extraction results authors gave three washings to the column; first with phosphate-buffered methanol in the first step, second with acetic acid and third with hexane. The developed technique was simple, sensitive, accurate and cost-effective for testing laboratories [8].

In this work, Sandra Bishop *et al.* have developed a new capillary electrophoresis (CE) method in the micellar mode to accomplish this separation in under 16 min using a sodium dodecyl sulfate (SDS)/sodium tetra-borate/boric acid buffer with an acetonitrile organic modifier. A one-step liquid-liquid extraction using ethyl acetate was the only necessary sample pretreatment. The SDS/borate buffer MECC method has proven useful in the analysis of unknown beverage samples. The method shows good separation of all benzodiazepines as well as GHB and provides a rapid screening for many of the common sexual assault drugs and other club drugs. By using the optimized 20 mM SDS in borate buffer with 7.0% acetonitrile we were able to identify GHB in a variety of different beverages. Results have shown that there is no apparent interconversion of GHB into GBL after the drug has been allowed to sit in these beverages for up to 2 days [9].

R. Rao *et al.* was focused on, developing a simple and rapid chromatographic system capable of eluting, resolving and detecting alprazolam, chloral hydrate and diazepam in adulterated toddy. These three substances were subjected to the RP-HPLC on a C-18 column with methanol-water acetic acid as an eluent [10].

Wei Chen *et al.* described a rapid and ultrasensitive detection method using a microfluidic chip for analyzing 7-aminoclonazepam (7-ACZP) residues in human urine. A microfluidic chip-based immunoassay with laser-induced fluorescence (LIF) detection based on the water-soluble denatured bovine serum albumin (dBSA)-coated CdTe quantum dots (QDs)was prepared for the ultrasensitive detection of (7-amino clonazepam)7-ACZP. The whole procedure including the chip and the control software was designed and constructed in the laboratory. The detection of 7-ACZP could be completed within 5 min. The results demonstrated that under the optimal conditions, 7-ACZP residues could be detected with a precision of 5% relative standard deviation (RSD), and the linear range and the limit of detection (LOD) for 7-ACZP were 1.1–60.1 and 0.021 ngmL–1, respectively. This method was compared with ELISA and showed a good correlation. This microfluidic chip with LIF detection was applied to the determination of 7-ACZP residues in positive human urine samples, and the results were confirmed by high-performance liquid chromatography and tandem mass spectrometry [11].

David S.M. Ribeiro *et al.* (2010) developed a Multi-pumping flow system (MPFS) coupled with a photodegradation unit for the determination of the degradation product of benzodizepam. Detection limit: 2mg/L. they used two pumps, one for sample insertion (via stopped-flow technique) and a second for transportation of the sample to the fluorescence detector for measuring the emission intensity at 463 nm. The spiked sample along with the required reagents was exposed to UV rays for 15 minutes, which promotes hydrolysis of diazepam. NaOH was used for baseline establishment. Acid-catalyzed hydrolysis produces a benzophenone compound whereas alkaline media originates different compounds by fission of the amide linkage [12].

In this work, Piotr and Maria Kala (2010) took drug-free urine samples from healthy individuals having no history of drug use. Forensic urine samples were collected from the casework. Spiked urine samples

were subjected to solid phase extraction and analyzed by comparing the mass spectra and retention times using the program with the library for 128 date rape drugs. Despite differences in chemical structure, a sensitive GC-EI-MS method is very useful for screening, identification, and quantification method for 128 date rape drugs in urine samples collected from victims of sexual assault. The proposed method was also useful for toxicologically relevant drugs in other nonbiological materials [13].

Casey T. *et al.* (2011) used NMR spectroscopy for direct analysis of common alcoholic beverages using a new water suppression technique called PURGE (Presaturation Utilizing Relaxation Gradients and Echoes). He also used C13 NMR for the identification of GHB and GBL and to detect GHB in human saliva. PURGE has advised that the only parameters needing adjustment are the pre-saturation power, relaxation delay and transmitter offset. The disadvantage of this method is that labile protons are suppressed in the spectra and it can only suppress one resonance at a time [14].

Anand Lodha and *et al.* (2013) reported a highly sensitive, selective, rapid, reliable and cost-effective method for the detection of trace amounts of clonazepam based on gold nanoparticles (AuNPs) in the presence of melamine. When different concentrations of CN with low concentrations of melamine were simultaneously added into the Au NPs solution, the state of AuNPs changed from dispersed to aggregate and the color of the solution changed from wine red to blue. Coexisting substances including alprazolam, diazepam, nitrazepam and lorazepam did not affect the determination of clonazepam. The sensor developed by this new approach could be used as a spot test and a good alternative means for on-site and real-time screening of clonazepam. The drug-mediated aggregation of Au NPs possesses great potential for detecting the abused drug in post-mortem blood, bone and bone marrow samples in forensic investigations [15].

Hassan Sereshti and Soheila Samadi (2014) made a 1% solution of the above samples. They centrifuged tea and coffee samples and sonicated beverages. After filtration filtrate was used for analysis. Caffeine was extracted and preconcentrated by the simple, fast and green method of dispersive liquid-liquid microextraction (DDLME) and analyzed by gas chromatography-nitrogen phosphorous detection (GC-NPD). The limit of detection and limit of quantification were 0.02 and 0.05 ug/ml. The proposed method was very advantageous because of its short extraction time, low detection limit, wide linear dynamic range, a specific ad-sensitive detection limit (GC-NPD) and the method is eco-friendly because it consumes micro-volume of organic solvent (20ul), thus it is considered as a green method [16].

In the work of Daniel A. Goncalves *et al.* (2015), authors combined standard dilution analysis with microwave-induced plasma optical spectroscopy to determine seven elements (Al, Co, Cu, Zn, Mn, Ni, Cr) in coffee, green tea, energy drink, beer, whisky. For sample preparation, they just did dilution with nitric acid (1% *v/v*). The results were compared with a stock solution of Al, Cu, Cr, and Mn of 1000mg/mL. With the help of this method, 4-5 elements can be detected at the same time [17].

In the study of Giorgio Famiglini (2015), eight BDZs, including diazepam, chlordiazepoxide, clobazam, flunitrazepam, bromazepam, flurazepam, nitrazepam and clonazepam, were extracted from whiskey cream using the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method and analyzed using GC-MS. The kit used for this method includes a 50-mL extraction tube containing 4 g of $MgSO_4$, 1 g of NaCl, 1 g of trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate and a 2-mL purification tube containing 150 mg of $MgSO_4$, 25 mg of PSA (primary and secondary amino sorbents) and 25 mg of C18. The product of the reaction was analyzed by LC-MS and GC-MS. This method is fast, and reliable and allows the reliable determination of targeted compounds at concentrations compatible

with forensic requirements. The proposed method allows the analysis of very small sample volumes (0.5 mL), such as those found in a crime scene. The work can be extended to the study of benzodiazepines from beverages, biological fluids, fruit juices, energy drinks, or other substances added for criminal purposes or voluntary food adulterations [18].

R. Jimenez-Perzerb *et al.* (2017) investigated the electro-oxidation of GHB and ethanol on a glassy carbon/platinum nanoparticles/polyvinyl alcohol-modified electrode (GC/ptnps/PVA) in a weakly acidic medium by cyclic voltammetry and chronoamperometry. Cyclic voltammetry of ethanol-GHB mixtures was also performed observing that the presence of GHB in ethanol solutions significantly suppressed the oxidation peak currents of ethanol. This fact has been discussed in different experimental conditions and suggests that this behavior is due to a competition of both alcohols for the active sites of the platinum surface according to a Langmuir-type expression. Depression of the current density of ethanol in the presence of GHB opens up new prospects for the determination of GHB. The detection limit of GHB, under these experimental conditions, was 0.872 mm (0.110 mg/ml). Analyses of test samples including typical recreational alcoholic beverages (vodka, lemonade + vodka, gin, rum, tequila and whisky) on the surface with the modified electrode have been successfully carried out. Results were analyzed using SEM, TEM, and UV-VIS spectroscopy [19].

Pacioni *et al.* had given the various methods used for the synthesis of silver nanoparticles. They reported the various reduction methods such as reduction by Sodium borohydride, hydroquinone, Gallic acid, etc. They also mentioned the characterization of silver nanoparticles by SEM and TEM. [20]. Dong *et al.* (2009) studied the role of pH in the citrate reduction of silver nanoparticles. Under high pH, due to the high rate of reduction of precursor, they got rod-shaped silver nanoparticles; whereas at low pH, they got triangle or polygon-shaped silver nanoparticles due to the slow reduction rate of precursor. In this way, pH affects on size and shape of silver nanoparticles. As the pH was mediated from 11.5-5.7, the absorption peak of the nanoparticle was shifted from 402 to 466 nm. The mixture of spherical and rod-shaped was obtained at pH 11.5 and 8.3 whereas at pH 6.1 and 5.7 mixture in addition to spherical particles triangular or polygon-shaped silver particles was obtained. [21].

Ivrigh *et al.* (2017) demonstrated the aggregation-based colorimetric sensor for the determination of prothioconazol fungicide using colloidal silver nanoparticles. In the presence of prothioconazole Ag Np shows a colour change from yellow to pink-orange. It was successfully used for the determination of prothioconazole in wheat flour and paddy water samples. They evaluated interference of common ions to determine selectivity such as K^+, Na^+, Cl^-, NO_3^- etc. AgNP color change from yellow to pink-orange in the presence of prothioconazole indicates a highly sensitive naked-eye colorimetric for quantifying prothioconazole in real applications. [22].

III. DISCUSSION

The forensic analysis of benzodiazepines, particularly in the context of date rape drugs, has witnessed significant advancements and trends in recent years. Through this review, we have explored key developments in sample preparation techniques, instrumental analysis methods, data interpretation approaches, and the expansion of databases for benzodiazepine identification. These trends collectively contribute to improving the accuracy, sensitivity, and reliability of forensic analyses involving benzodiazepines in various exhibits. One of the notable trends is the adoption of advanced analytical instrumentation such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) for benzodiazepine analysis. These techniques offer high sensitivity and specificity,

allowing for the detection and quantification of benzodiazepines even at trace levels in complex matrices.

Moreover, the development of novel sample preparation methods, including solid-phase extraction (SPE), liquid-liquid extraction (LLE), and micro-extraction techniques, has enhanced the efficiency of extracting benzodiazepines from biological and non-biological samples. These advancements are particularly valuable in forensic casework where limited sample volumes or challenging matrices are encountered.

In parallel, data interpretation strategies have evolved, leveraging the power of spectral libraries, retention time matching, and isotopic pattern analysis to enhance the reliability of benzodiazepine identification. Furthermore, the continuous expansion of databases containing mass spectral data and retention time information for benzodiazepines and their metabolites aids forensic scientists in comprehensive compound identification and profiling.

Despite these advancements, challenges persist, including the emergence of new benzodiazepine analogs and designer drugs that necessitate ongoing research and adaptation of analytical methodologies. Additionally, the interdisciplinary nature of forensic analysis requires collaboration between forensic scientists, toxicologists, law enforcement agencies, and healthcare professionals to ensure robust and accurate interpretations of benzodiazepine findings in forensic investigations.

IV. CONCLUSION

In conclusion, the current trends in the forensic analysis of benzodiazepines underscore the importance of staying at the forefront of technological innovations, methodological advancements, and collaborative efforts within the forensic science community. By addressing challenges, embracing novel approaches, and prioritizing accuracy and reliability, forensic scientists can continue to strengthen their capabilities in detecting and analyzing benzodiazepines as part of comprehensive forensic investigations involving date rape drug

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Review: Green Synthesis of CuO Nanoparticles and Their Environmental Applications

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ABSTRACT

In this review, we explore environmentally friendlier methods for producing copper oxide (CuO) nanoparticles, with a focus on recent advancements, including those derived from natural sources or extracted from them. The eco-friendly production of metal nanoparticles (such as CuO) has attracted considerable attention because of their promising several properties which enable them to be used in various applications such as in dyes degradation through photocatalysis and detecting chemicals via electrochemical means, detection of latent fingerprints. The green synthesis of CuO offers several advantages over conventional chemical methods. Moreover, green synthesis is a sustainable synthesis method frequently uses botanical, algal, or microbial extracts sourced from nature, which are plentiful, renewable, and economically viable.

Keywords: CuO nanoparticles, Green Synthesis, Plant extract, Forensic applications.

I. INTRODUCTION

Nanoparticles are particles having the size of the order of 10^{-9}m that are suitably developed on an atomic or molecular level. At the Nano level, their properties like physical properties, chemical, and biological properties different than those of bulk structures. Nanomaterials have numerous applications due to the quantum size effect, highsurface to volume ratio, quantum tunneling. Nanomaterials have received a great deal of attention in recent years due to their enhanced properties as compared to the single base nanomaterial. Nanomaterials can combine different properties like electrical, magnetic, photocatalytic, optical, etc. Nanomaterials possess distinctive properties of metals, polymer, or any other material used in their synthesis methods which can be used in different applications. Nanomaterials are new generation smart materials that can besynthesized at the nanoscale by the combination of two or more dissimilar materials which leads to great advancement in their structure and properties. So to develop high-

performance and low-cost multifunctional nanomaterials is an urgent requirement in today's world of renewable energynanomaterials can be synthesized by Sol-gel method, Thermal decomposition, Photochemical decomposition, Sputtering, Hydro-chemical synthesis,Precipitation method, Chemical vapour deposition, Chemical reduction, Electro-assist chemical reduction, emulsion method, Ball milling, Green synthesis method (plant extraction). Chemical synthesis techniques result in the accumulation of potentially harmful chemicals on the surface, which could pose risks in medical applications.

There has been an increasing focus on advancing sustainable and eco-conscious methods for producing nanoparticles. Transition metal oxides like copper oxide (CuO), titanium dioxide (TiO2), iron oxide (Fe3O4), zinc oxide (ZnO), and nickel oxide (NiO) nanoparticles have demonstrated effective utility as advanced nanomaterials in various areas of research such as energy, biomedicine, and environmental studies. Cupric acid originates from two elements in periodic table found in the periodic table: copper and oxygen, belonging to the d-block and p-block elements, respectively. Forensic science appears to be among the rapidly expanding areas in the realm of nano research applications. Cu doped ZnO have been used for enhancing latent fingerprint applications.

Copper oxide (CuO) has drawn interest from nanoparticles due to its robust catalyst properties, effectiveness in electrophoresis, photonics applications, and its potential in textiles, as well as its ability to serve as an antioxidant for bacteria. These properties vary based on factors such as size, shape, and the surrounding environment. Copper oxide (CuO) nanoparticles produced using environmentally friendly methods have demonstrated promise as viable options for applications in electrochemical sensing and safeguarding against corrosion [1]. The present study seeks to investigate the production of CuO nanoparticles through eco-friendly means utilizing a plant extract, as well as to examine their prospective uses. Cu doped ZnO nanoparticles were used as a labelling agent for identifying the latent fingerprints on different surfaces using the powder dusting method [2].

II. METHODS AND MATERIAL

A. Green Synthesis of CuO nanoparticles

The general steps involved in the green synthesis of different nanoscale metals can be summed up as follows: extract plant material, combine it with metal salt solution under particular conditions, and reduce metal particle size, filter, and take additional steps to obtain the desired nanoscale metal. Fig. 1 depicts the schematic of green synthesis of CuO nanoparticles using plant leaf extract.

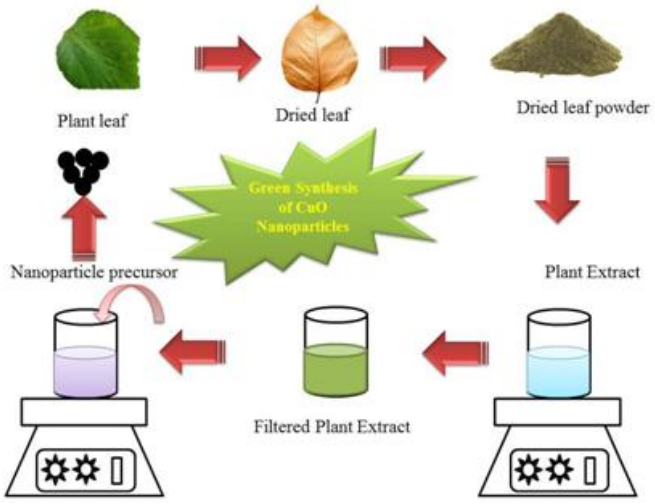

Figure 1 Schematic of green synthesis of CuO nanoparticles using plant leaf extract.

III.RESULTS AND DISCUSSION

Green synthesis of CuO nanoparticles was carried by using Arundinaria Gigantea (Giant cane) leaves extract, the particle size obtained was 36 nm.These nanoparticles are useful in photocatalytic degradation of AR88 dye under ultraviolet light which are prosing for water pollution. These Arundinaria Gigantea (Giant cane) leaves extract synthesis nanoparticles when integrated into an electrochemical sensing device,exhibit enhanced sensitivity and specificity for

the precise and accurate detection of ciprofloxacin in high-concentration aqueous solutions. [3]

Cu nanoparticles were also reported to be synthesized by green synthesis of Betel leaf (piper Betle). The UV, FTIR, XRD, SEM, TEM, and PL analyses of the CuO nanoparticles created through green synthesis confirmed their formation as crystalline assemblies, with particle sizes ranging from 10 to 50 nm when agglomerated. CuO nanoparticles demonstrated significant antibacterial effects against both gram-positive and, notably, gram-negative bacteria[4]. The eco-friendly biosynthesis of nano copper oxide (G-CuO Nps) was successfully achieved using an aqueous extract derived from Cupressocyparis leylandii leaves. G-CuO Nps displayed outstanding electrocatalytic activity for detecting MO dye, as evidenced by cyclic voltammetry when employed to modify a carbon paste electrode (G-CuO-MCPE). G-CuO Nps exhibited notable effectiveness in the photocatalytic elimination of MB dye, serving as an organic model compound. Additionally, G-CuO Nps showed significant antibacterial activity against both gram-negative and gram-positive bacteria [5].

This research outlines the production of copper oxide nanoparticles through a green synthesis approach. The method involved utilizing Gloriosa superba extract as a fuel in the solution combustion process. The PXRD patterns revealed a monoclinic phase, while the UV-visible absorption spectrum indicated a blue shift as the concentration of the plant extract in the reaction mixture increased during the synthesis. The particles seem to exhibit a nearly spherical shape with size of 5-10nm.The copper oxide nanoparticles displayed notable antibacterial effectiveness against all four bacterial strains: Gram-negative K. aerogenes, E. coli, and P. desmolyticum, as well as Gram-positive S. aureus [6].

The green synthesis method effectively produced consistent copper oxide nanoparticles using the leaf extract of the C. gigantea plant in an aqueous solution. These synthesized nanoparticles were then utilized as electrocatalytic materials for crafting the counter electrode in DSSC (dye-sensitized solar cells). The synthesized copper oxide nanoparticles exhibit a crystalline structure that closely aligns with the monoclinic structure of bulk CuO [7].

The research suggested a technique for producing copper oxide nanoparticles via green synthesis employing pomegranate leaf extract, aimed at their utilization in methylene blue adsorption. The process used to synthesize the CuO nanoparticles involved colloidthermal synthesis utilizing gum karaya [8].

The average particle size, ranged from 7.8 ± 2.3 nm to 4.8 ± 1.6 nm depending on the concentration of the metal precursor employed in the synthesis of the nanoparticles. The CuO nanoparticles that have been created exhibit exceptional stability and demonstrate notable antibacterial properties against bacteria from both Gram classifications [9].

Copper oxide nanoparticles were produced by using an extract from Macroptilium lathyroides (L) Copper oxide nanoparticles were produced by using an extract from Macroptilium lathyroides (L) to serve as a reducing, capping, and stabilizing agent. The band gap energy of CuO NPs was reported to be 4.0 eV obtained from UV-visible spectra, indicating that the NPs are good conductors [10].

A sustainable method for the production of CuO NPs using Sal seed DOC, a waste leftover from industrial forests were reported [11]

Jasminum sambac leaf extract was used in the manufacture of copper oxide nanoparticles (CuO NPs), and the concentration of copper sulfate, temperature, reaction time, and solution pH all had a significant impact on the nanoparticles' properties. The resultant CuO NPs had a particle size of 13.4 nm, were monodisperse, crystalline, monoclinic, and showed purity. After 210 minutes of light exposure, these NPs showed encouraging photocatalytic activity, successfully removing 97% of the Methylene Blue (MB) dye. Using Jasminum sambac leaf extract in the green synthesis process shows great efficiency in customizing CuO NPs for photocatalytic applications [12].

Table 1 presents Comparison of Reaction precursors, synthesized by using various leaf extract. particles size and applications of CuO nanoparticles

TABLE 1COMPARISON OF REACTION CONDITIONS, PARTICLES SIZE AND APPLICATIONS OF CUO NANOPARTICLES SYNTHESIZED BY USING VARIOUS LEAF EXTRACT.

Sr.No	Plants used	Chemicals	Size (nm)	Applications	References
1	Arundinaria Gigantea (Giant cane)	$(Cu(NO_3)_2{\cdot}3H_2O$	36	Dye degradation and electrochemical sensor.	[3]
2	Betel leaf (*Piper betle*)	$(CuNO_3.\ 3H_2O)$ $(C_{32}H_{22}N_6Na_2O_6S_2)$	10 to 50	Dye degradationAntibacterial activity and environmental remediation Nanoparticle-modified electrodeapplications	[4]
3	Cupressocyparis leylandii leaf extracts	$(CuSO_4.5H_2O)$	17.09 nm	Antibacterial activityapplications	[5]
5	Gloriosa superba L. extract	$(CuNO_3.\ 3H_2O)$	5-10	Antibacterial activityapplications	[6]
6	Calotropis gigantea	$(CuNO_3.\ 3H_2O)$	20–30	dye-sensitized solar cells applications	[7]
	pomegranate (Punica granatum)	$Cu(CH_3COO)_2$	38.91	Antibacterial activityapplications	[8]
7	gum karaya	$NaOH{\cdot}\ CuCl_2{\cdot}\ 2H_2O$	-	Antibacterial activityapplications	[9]
8	*Macroptilium lathyroides (L)*	$(CuNO_3.\ 3H_2O)>99\ \%)$	7.6	Antibacterial activity Dye degradation, antibacterial, cytotoxicity, anticancer and antifungal applications	[10]
9	Sal seed deoiled cake (Sal DOC) extract a by-product of forest industries	$(CuSO_4.5H_2O)$	-	Antibacterial activity applications.	[11]
10	*Jasminium sambac* leaf extract	$(CuSO_4.5H_2O)$	13.4	Dye degradationapplications.	[12]
11	Achyranthes aspera leaves	$Cu(NO_3)_2.3\ H_2O$ $Zn(NO_3)_2.6\ H_2O$	-	Supercapacitors application	[13]
12	Centratherum punctatum	$(CuSO_4.5H_2O)$	20–30	antibacterial and antibiofilm application	[14]
13	Aegle Marmelos leaves	$(Cu(NO_3)_2{\cdot}3H_2O)$	19	Dye degradation and Antibacterial activity	[15]
14	Allium cepa L.	$(Cu(NO_3)_2{\cdot}3H_2O)$	15.35 nm 13.9	Dye degradationapplications.	[16]

			nm 12.5 nm		
15	neem leaf extract (Azadirachta indica)	($CuSO_4.5H_2O$)	-	Energy storage applications	[17]

IV. CONCLUSION

In this review green synthesis of CuO nanoparticles has been discussed. Research involving different plant materials indicates that it is possible to produce these metal nanoparticles in a sustainable manner. In recent times, numerous studies have emerged focusing on the eco-friendly creation of nanoscale metals through green synthesis methods.Yet, green synthesis encounters several obstacles and deficiencies, including limited output, inconsistent particle sizes, intricate extraction processes, the seasonal and regional availability of raw materials, and various other challenges that must be addressed to enable the practical manufacturing and utilization of green-synthesized nanomaterials. Thus, future research must focus on increasing the production of nanoscale metal particles, utilizing inexpensive raw materials, and implementing straightforward energy-saving technologies.Thus, future research must focus on increasing the production of nanoscale metal particles, utilizing inexpensive raw materials, and implementing straightforward energy-saving technologies.Thus, there is a chance that the green synthesis of nanoscale metals has a lot of promise and room to grow.

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Polyherbal Soap: A Boon for Multipurpose Application

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ABSTRACT

This study focuses on formulating and evaluating a polyherbal soap enriched with botanical extracts from Azardirachta indica, Ocimumtenuiflorum, Curcuma longa, Aloe barbadensis miller and Rosa indica. Various aspects, including phytochemical screening, antioxidant assay, and antimicrobial activity, were investigated. Initially, phytoconstituents were extracted and characterized to establish the soap's chemical foundation, revealing the presence of alkaloids, flavonoids, phenols, sterols, saponins, terpenoids, glycosides, carbohydrates, tannins, and proteins. The antioxidant potential of the extracts was assessed, highlighting their ability to neutralize free radicals. Antimicrobial activity against E. coli, Staphylococcus aureus Pseudomonas aeruginosa various pathogens was also examined, indicating the soap's potential as a hygiene product. Parameters such as pH, moisture content, and physical appearance were scrutinized to ensure quality and efficacy. Results showed a rich phytochemical that are alkaloids, glycosides, proteins, reducing sugar, tannins, resins, sterols, phenolic compounds and saponins, strong antioxidant capacity to neutralize the free radicals, and the antimicrobial activity showed efficacy against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, supporting its potential for skincare and hygiene. This poly herbal soap presents promising attributes for skin health and cleanliness, and the formulation was evaluated for its physical parameters. It is sure that this ingredients on combination behave as an effective bio disinfectant soap. Natural herbal bio disinfectant soap is affordable, effective, and environmentally friendly.

Keywords: Phytochemical Screening, Antimicrobial Activity, Antioxidant Activity, Polyherbal soap, Bio disinfectant.

I. INTRODUCTION

Skin disinfectant cleansers are fatty caustic salts of sodium or potassium that are soluble in water. In contrast to the common usage of the term, soaps are produced by chemically reacting fats and oils with a strong alkaline base. Most conventional soaps contain chemical components with potential depilatory effects on skin pathogens and antibacterial properties. Skin disinfectant cleansers are cleansing agents that can be solid, liquid, semi-solid, or powdered. They are commonly used to remove dirt, grease, microbial stains, unpleasant odors, and other impurities from the skin surface to maintain health, beauty, and odor-free skin. Due to the drawbacks of traditional soaps, people are now gravitating more towards herbal skin disinfectants. Unlike commercial products, herbal skin disinfectants do not contain artificial flavors, colors, or other additives. The advantages of herbal skin disinfectants include smooth skin, rich lather, protection against skin disorders, treatment of skin infections, and gentleness on the skin [1]. Herbal skin disinfectants do not contain artificial colors, flavors, fluorides, etc., unlike conventional soaps. Herbal skin disinfectants help maintain skin pH levels effectively without disrupting the natural balance. Herbs are natural products widely used in the treatment of almost all diseases and skin problems due to their high medicinal value, cost-effectiveness, availability, and compatibility [2].

The origin of the Sanskrit title 'Nimba' is derived from the phrase "Nimbati Swasthyamdadti," signifying the promotion of great well-being. *Azadirachta indica*, commonly known as neem, is rich in natural substances found in its various parts such as leaves, seeds, and bark, which exhibit numerous organic activities against disease-causing organisms due to the presence of around 140 chemical compounds. The active material azadiractrin present in the leaves and seeds of the neem tree has the ability to eliminate disease-causing parasites, infections, and parasites. Neem stands out as a safe and organic resource for the development of modern drugs. [3]Tulsi, also known as Holy Basil, carries significant historical importance in the Indian subcontinent. For more than 3000 years, tulsi has been utilized as an aromatic herb in Ayurvedic medicine. In Ayurveda, Tulsi is commonly referred to as the “Elixir of Life" because of its remarkable healing properties. It has been traditionally used to address various prevalent health conditions. Tulsi has found application in the management of various medical conditions such as epilepsy, asthma, hiccups, cough, skin and blood disorders, parasitic infections, neuralgia, headache, wounds, inflammation, and oral conditions. [4].Tulsi is employed for the treatment of various skin issues such as acne, premature aging, heart diseases, and insect bites. It also aids in combating respiratory problems, aging, kidney stones, headaches, and acne[5]. For centuries, Aloe vera has been employed to address various human ailments. Its applications extend to health, beauty, medicinal, and skincare purposes. The Aloe vera plant consists of fibrous roots, short stems, and greenish leaves. Within the leaf lies a gel, transparent and viscous in nature, primarily composed of water and ploysaccharides. Notably, it possesses a bitter taste [6]. Rose is a member of the family Rosaceae and possesses several important components that contribute significantly to its antibacterial properties. Flavonoids, terpenes, and anthocyanins are among the key components of rose that exhibit antibacterial activity. Flavonoids, which are classified as natural plant compounds, exhibit various properties that aid in the treatment of diseases such as cancer and Alzheimer's. These properties include biochemical and antioxidant effects, antimutagenic impact, and anticarcinogenic effects. [7] *Curcuma longa* is a perennial herb that stands upright, with green leaves, belonging to the Zingiberaceae family. In India, it is commonly known as 'Haldi', and its rhizomes are elongated, ovate, and often with short branches. Recent studies have revealed that curcumin possesses a wide range of potential benefits, including anti-inflammatory and anticancer properties. The

curcumin extracted from the rhizome is used for medicinal purposes [8].

Human beings have been using various parts of plants for medicinal purposes since ancient times. Plants are crucial sources of bioactive compounds, both primary and secondary. It has been observed that secondary metabolites are remarkably diverse in terms of their chemical and taxonomic properties. These metabolites are utilized in various fields such as human healthcare, agriculture, scientific research, veterinary medicine, and many others. Medicinal plants contain numerous organic compounds that exhibit beneficial physiological effects on the human body, including tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids. A wide range of phytochemicals belonging to different chemical classes have been found to have inhibitory effects on various microorganisms in vitro. Plant-derived products have been integral components of traditional medicine for centuries, sourced from barks, leaves, flowers, roots, fruits, and seeds. [9]

Plants contain a variety of naturally occurring compounds that have potential for development as therapeutic agents. Herbal medicines have long been used in developing countries, but recently there has been an increase in the use of herbal remedies in the developed world. Plants offer an alternative approach in the search for new treatments. Hands play a crucial role in the transmission of organisms and diseases, making cleanliness essential to prevent the spread of harmful germs and infections. Hand hygiene is the most important, simplest, and least expensive way to prevent nosocomial infections. Hand contact with ready-to-eat foods is a significant factor through which pathogens can contaminate the food supply[10].Numerous studies conducted so far have confirmed the role of antioxidants, such as Lanthanides, selenium, flavonoids, lycopene, and glutathione, as anti-cancer compounds in bio-coordination chemistry. Recent advancements in medicinal chemistry have been crucial in improving the design of these compounds, reducing their toxic side effects, and understanding their mechanism of action. An antioxidant is a substance that, at low concentration, delays the oxidation of proteins, carbohydrates, lipids, and DNA. [11]A characteristic cleansercan be categorized into three types based on the production method: dissolve-pour cleanser, hot process cleanser, and cold process cleanser. The hot process cleanser, also known as a transparent or translucent cleanser, offers excellent detergency, moisturizing effects, long-lasting scent, and minimal irritation. Herbal cleansers are made by adding various dried herbs, flowers, and stems to the soap base. Herbs are natural ingredients that are commonly used in the treatment of various illnesses and skin problems due to their high medicinal value, cost-effectiveness, availability, and compatibility. The characteristics of a cleanser include gentleness on the skin, rich foam, protection against skin disorders (such as rashes, acne, and scabies), treatment of skin infections (like ringworm), and improvement of skin tone and smoothness [2]

II. METHODS AND MATERIAL

Collection of plant materials:

The leaves of *Azadirachta indica, Ocimum sanctum, Aloe vera barbadensis Miller gel, Rosa indica*, and *Curcuma longa* were collected from different matured plants from the farms. After collection of plant materials, they were dried in shades for about 4-5 days. After drying of plant materials, they were grinded in grinder and the fine powder was made. After that the samples were stored in airtight bottles for study.

Extraction of plant materials:

Extraction *of Azadirachta indica, Ocimum sanctum, Aloe vera barbadensis Miller, Rosa indica,* and *Curcuma longa* was done by traditional method (Maceration) and Soxhlet method.

In Maceration method: fresh leaves of Azardirachta indica, Ocimumtenuiflorum, Aloe barbadensis miller, Rosa indica and rhizoids of Curcuma longa were

washed thoroughly under tap water and then rinse with distilled water. They were dried in shade for 5 to 6 days and then pulverized or fine powdered with the aid of a blender mixed. The solvent used for the preparation of the extract is methanol. The methanol extract was prepare by weighing out 5 gm of milled powder of all medicinal plant for aloe vera gel was measured 5 ml (about 0.17 oz) and soaked in 50 ml of methanol and mixed well for 48 hours at room temperature .The extracts where the filtered using muslin cloth, while same method carried out for aqueous extract .In Soxhlet Method: 5 gm of each plant material were weighed .About 20 gm of powdered plant materials to be extracted separately in 200ml of methanol using soxhlet extractor for 48 hrs., Temperature maintain till boiling point of methanol (64.7^0 C) and aqueous i.e. (100^{0}c)

Qualitative phytochemicals screening:

Subjective screening of phytochemicals was conducted on all extracts to identify the presence of various phytochemicals such as alkaloids, glycosides, proteins, reducing sugars, tannins, gums, sterols, phenolic compounds, and saponins. [12,13,14]

Tests for Detection of Alkaloids:

Wagner's Test:

Several drops of Wagner's reagent were added to a few milliliters of the plant extract along the sides of a test tube. The formation of a reddish-brown precipitate confirms a positive test result.

Test for Detection of Glycoside:

Benedict's reagent Test:

An equal volume of both the extract and Benedict's reagent were mixed and heated to boiling for two minutes. The appearance of a brownish to reddish color indicates the presence of glycosides.

Test for Detection of Proteins:

Millon's Test:

2 ml of the test solution was added to Millon's reagent, resulting in the formation of a white precipitate. Upon heating, the precipitate changes to a reddish color.

Test for Detection of Carbohydrates:

Benedict's reagent Test:

When the crude extract was mixed with 2 ml of Benedict's reagent and boiled, a reddish-brown precipitate formed, indicating the presence of carbohydrates.

Test for Detection of Tannins:

Ferric chloride Test:

A small amount of the filtrate was mixed with a few drops of ferric chloride. The presence of tannins was indicated by the development of a green color.

Test for Detection of Phytosterols / Terpenoids:

Salkowski Test:

The test solution was mixed with 2 ml of chloroform and a few drops of concentrated sulfuric acid. The presence of steroids was indicated by the formation of a reddish-brown color in the lower layer, while the presence of terpenoids was indicated by a yellow color.

Test for Detection of phenols:

A small amount of the extract was treated with 2 ml of ferric chloride solution and shaken for a few minutes. The appearance of a pale brown color in the test indicated the presence of phenolic compounds.

Test for Detection of Saponins:

Foam Test:

A small amount of the extract was treated with 2 ml of sodium bicarbonate and mixed with distilled water. The mixture was shaken vigorously. The presence of saponins was indicated by the formation of foam in the test.

Test for Detection of Flavonoids:

Alkaline reagent Test:

The test solution was treated with sodium hydroxide solution, resulting in a yellow or reddish color.

Test for Detection of Steroids:

Crude extract was mixed with 2 ml of chloroform and gently added concentrated H2SO4. A reddish color was observed in the lower layer, indicating the presence of steroids.

Antimicrobial Activity (Well Diffusion method):

Antimicrobial activity was assessed using the well diffusion method. The test organisms were inoculated

in nutrient broth and incubated overnight at 37°C until turbidity was achieved. Sterile petri plates were used to test the antimicrobial activity of plant extracts against Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. A total of 20ml of sterilized Nutrient Agar was poured into each sterile petri plate. Once solidified, 100ul of fresh culture of human pathogens were swabbed onto the plates. Wells were then punched into the agar plates using a sterile gel puncher, and 10ul of each plant extract was added to the wells. The plates were subsequently placed in an incubator for a duration of 24 hours at a temperature of 37°C. After the incubation period, the diameter of the zone of inhibition (ZOI) was measured in millimeters using a Vernier scale[15].

Antioxidant Assay (Reducing Power Assay):

The Reducing Power Assay, also known as the Antioxidant Assay, is commonly employed to protect cells from damage caused by free radicals (unstable molecules produced during the process of oxidation in normal metabolism) and is used to assess the antioxidant capacity of plant extracts and dietary supplements containing polyphenols. The ferric reducing power test is based on the ability of the antioxidant to reduce Fe3+ to Fe2+ in the presence of potassium ferricyanide and ferric chloride [10].

Protocol:

Different concentrations of the plant extricate in comparing solvents were blended with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This blend was kept at 50°C in water shower for 20 minutes. After cooling, 2.5 ml of 10% trichloro acidiccorrosive was included and centrifuged at 3000 rpm for 10 min at whatever pointfundamental. The topmost layer of the mixture (2.5 ml) was combined with distilled water (2.5 ml) and a naturally occurring ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was arranged in comparativewaybarringtests. Ascorbic corrosive at different concentrations was utilizedas standard. Expanded absorbance of the responseblendshows an increment in diminishingcontrol. Diminishingcontrol was measured by changing the concentration of the extricate and the contact time [16].

Method for preparation of polyherbalSoap:

To create a polyherbal soap, begin by taking the specified amount of soap base in a 500 ml container and gently warm it on a water bath without stirring. As the soap base melts into a liquid form, add all the ingredients to the mixture. Heat the mixture on the water bath until well blended without stirring. Next, pour the mixture into soap molds and allow it to solidify in the molds for 2-3 hours. After 2-3 hours, remove the soap molds from the freezer and let them sit for 5 minutes before unmolding the soap [17].

III.RESULTS

The Phytochemical screening of all the extracts (maceration and soxhlet) were carried out. From the table no 1, phytochemical characteristics of the extracts it could be seen that, Alkaloids: Present in all herbs in both methanolic and aqueous extracts.Glycosides: Present in methanolic extract of rose,tulsi,turmeric and absent in aqueous extract of rose, aleovera and aqueous & methanolic extract of neem. Proteins: Present in methanolic and aqueous extract of tulsi and Turmeric, absent in methanolic and aqueous extract of Rose, Aloe vera, neem.Carbohydrates: Present in all methanolic and aqueous extracts, except aqueous. Extract of neem.Tannins: Present in all herbs in both methanolic and aqueous extracts, except Aloe vera.Terpenoids: Present in methanolic extract of rose, tulsi, neem, Aloe vera,absent in aqueous extract of rose, aloe vera, tulsi and methanolic and aqueous extract of turmeric.Phenols: Present in methanolic and aqueous extract of Rose and tulsi, methanolic extract of neem, absent in methanolic and aqueous extract of Aloe vera, turmeric and aqueous extract of neem.Saponins: Present in all methanolic and aqueous extracts of Rose, Aloe vera, Tulsi, Turmeric, and Neem. Steroids: Present in methanolic and aqueous extract of rose, absent in methanolic and aqueous

extracts of Aloe vera, Tulsi, Turmeric, and Neem. Flavonoids: Present in methanolic and aqueous extracts of rose and tulsi, absent inmethanolic and aqueous extracts of Aloe vera, Turmeric, and Neem.

The results of soxhlet extract were as follows the table no.2, Alkaloids: present in methanolic Soxhlet extract and absent in aqueous Soxhlet extract. Glycoside: present in both the methanolic Soxhlet extract and aqueous Soxhletextract Protein: present in both the methanolic Soxhlet extract and aqueous Soxhlet extract. Carbohydrates: present in both the Soxhlet methanolic extract and aqueous Soxhletextract. Tannins: present in both the methanolic Soxhlet extract and aqueous Soxhlet extracts. Terpenoids: Present in both methanolic Soxhlet extract and aqueous Soxhlet extract. Phenols: Present in both methanolic Soxhlet extract and aqueous Soxhlet extract. Saponins: present in both methanolic Soxhlet extract and aqueous Soxhlet extract. Steroids: Absent in both methanolic Soxhlet extract and aqueous Soxhlet extracts. Flavonoids are present in both methanolic Soxhlet extract and aqueous Soxhlet extract.

Table: 1 qualitative phytochemical screening of Macrenated extracts

Test	Rose (Rosa Indica)		Aloe vera (Aloe BarbeDenisis miller		Tulsi (Ocimum sanctum		Turmeric (Curcuma longa)		Neem (Azardirachta indica)	
	M	A	M	A	M	A	M	A	M	A
Alkaloid	+	+	+	+	+	+	+	+	+	+
Glycoside	+	-	-	-	+	+	+	+	-	-
Protein	-	-	-	-	+	+	+	+	-	-
Carbohydrate	+	+	+	+	+	+	+	+	+	-
Tannins	+	+	-	-	+	+	+	+	+	+
Terpenoids	+	-	+	-	+	-	-	-	+	+
Phenols	+	+	-	-	+	+	-	-	+	-
Saponins	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	-	-	-	-	-	-	-	-
Flavonoids	+	+	-	-	+	+	-	-	-	-

(M- Methanolic, A- Aqueous) (+ positive, - negative)

Table 2: Qualitative Phytochemical Screening of Soxhlet extracts

Test	Methanolic	Aqueous
Alkaloid	+	-
Glycoside	+	+
Protein	+	+
Carbohydrate	+	+
Tannins	+	+
Terpenoids	+	+
Phenols	+	+
Saponins	+	+
Steroids	-	-
Flavonoids	+	+

(+- positive, - negative)

The antimicrobial activity of both the soxhlet extracts against oragnism was performed using well diffusion method. Both the extract produced the widest zone of inhibition against *Escherichia coli, Staphylococcusaureus*, and *Pseudomonas aeruginosa.*

Table 3: Antimicrobial activity by well diffusion method

Sr.no	*Pathogenic Microorganisms*	*Observation*	
		Methanolic	*Aqueous*
1	*Pseudomonas*	*10mm*	*9mm*

	aeruginosa		
2	*Escherichia coli*	*12mm*	*11mm*
3	*Staphylococcus aureus*	*11mm*	*10mm*

The reducing power assay indicated increase in optical density indicating its potential to mitigate oxidative stress

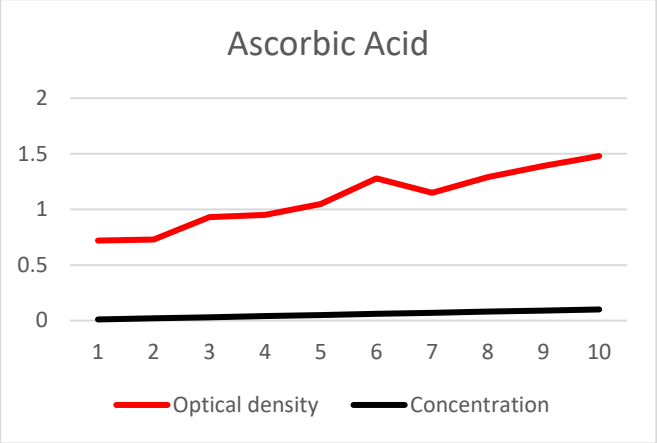

Fig 1: Reducing power assay: Standard Ascorbic acid

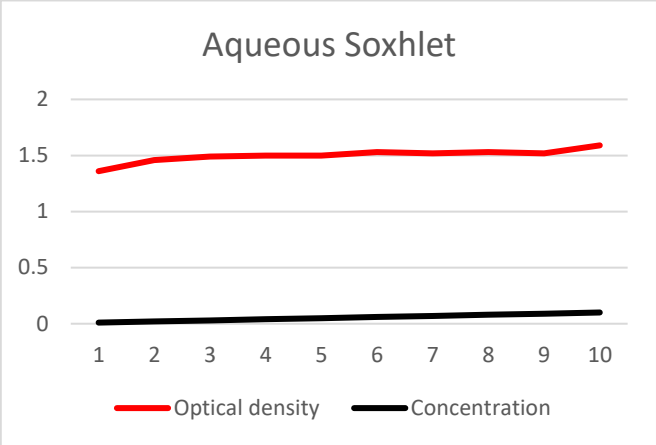

Fig 2: Reducing power assay: Aqueous soxhlet sample

.

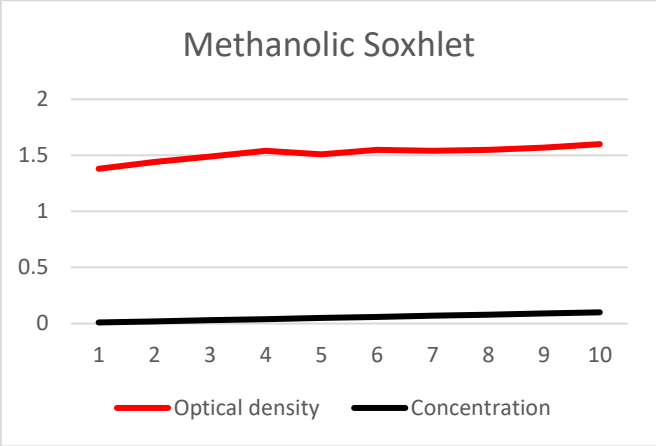

Fig 3: Reducing power assay: Methanolic soxhlet samples

Table 4: Reducing power assay ofAscorbic acid

Sr.no	Different volume of mixture of Methanolic extract (mL)	Phosphate Buffer and Potassium Ferric cyanide	Potassium Ferric cyanide Boiling water bath for 20 min at 50°C	Boiling water bath for 20 min at 50°C	Trichloro acetic acid	Centrifuge at 3000 rpm for 10 min	Upper layer	Distilled water	Ferric chloride	Optical density at 700nm
1	Blank	2.5 mL in each test tubes	2.5 mL in each test tubes		2.5 mL in each test tubes		2.5 mL in each test tubes	2.5 mL in each test tubes	0.5 mL in each test tubes	0
2	1%									0.72
3	2%									0.73
4	3%									0.93
5	4%									0.95
6	5%									1.05
7	6%									1.28

8	7%									1.15
9	8%									1.29
10	9%									1.39
11	10%									1.48

Table 5: Reducing power assay of Aqueous Soxhlet extract

Sr.no	Different volume of mixture of Methanolic extract (mL)	Phosphate Buffer and Potassium Ferric cyanide	Potassium Ferric cyanide Boiling water bath for 20 min at 50°C	Boiling water bath for 20 min at 50°C	Trichloro acetic acid	Centrifuge at 3000 rpm for 10 min	Upper layer	Distilled water	Ferric chloride	Optical density at 700nm
1	Blank	2.5 mL in each test tubes	2.5 mL in each test tubes		2.5 mL in each test tubes		2.5 mL in each test tubes	2.5 mL in each test tubes	0.5 mL in each test tubes	0
2	1%									1.36
3	2%									1.46
4	3%									1.49
5	4%									1.50
6	5%									1.50
7	6%									1.53
8	7%									1.52
9	8%									1.53
10	9%									1.52
11	10%									1.59

Table 6: Reducing power assay of Methanolic Soxhlet extract

Sr.no	Different volume of mixture of Methanolic extract (mL)	Phosphate Buffer and Potassium Ferric cyanide	Potassium Ferric cyanide Boiling water bath for 20 min at 50°C	Boiling water bath for 20 min at 50°C	Trichloro acetic acid	Centrifuge at 3000 rpm for 10 min	Upper layer	Distilled water	Ferric chloride	Optical density at 700nm
1	Blank	2.5 mL in each test tubes	2.5 mL in each test tubes		2.5 mL in each test tubes		2.5 mL in each test tubes	2.5 mL in each test tubes	0.5 mL in each test tubes	0
2	1%									1.38
3	2%									1.44
4	3%									1.49
5	4%									1.54
6	5%									1.51

7	6%									1.55
8	7%									1.54
9	8%									1.55
10	9%									1.57
11	10%									1.60

IV. FORMULATION OF POLYHERBAL SOAP

Table 8: Formulation of polyherbal soap

Sr.no	Ingredients	F1 Neem	F2 Tulsi	F3 Aloe vera	F4 Rose	F5 Turmeric	F6 Poly herbal
1	Soap base	70gm	70gm	70gm	70gm	70gm	70gm
2	Herb in gm	2	2	3ml	2	2	0.400gm of each
3	Vitamin E oil	400mg	400mg	400mg	400mg	400mg	400mg
4	Fragrance	500mg	500mg	500mg	500mg	500mg	500mg

A

B

Fig: A- Formulated Polyherbal soap B- Formulated individual herb soap

The formulated Polyherbal soap and individual herb soaps, physicochemical parameter such as colour, odour, shape, pH, foam height, foam retention, irritation were tested. The fromulated polyherbal soap and individual herb soap of Azardirachta indica, Ocimumtenuiflorum, Curcuma longa, Aloe barbadensis miller and Rosa indica exhibited a good appearance characteristics as well as the ph. was foundto be in the range of 7-9 which is diversed ph. They are safe, beneficial, effective, affordable and overall very useful for humanskin.

Table 9: Physiochemical Parametrs of both polyherbal soap and individual herb soap

Sr.no	parameters	Neem	Tulsi	Turmeric	Aloevera	Rose	Poly-herbal
1	Color	Dark green	Light green	Brickred	White	Brown	Amber
2	Odor	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
3	Shape	Square	Oval	Circle	Circle	Heart	Oval
4	PH	8	7	9	9	8	7
5	Foam Height	2.5cm	2cm	2.4cm	1.2cm	1.5cm	2.5cm

6	Foam retention	9min	8min	8min	9min	10min	8min
7	Irritation	Non-Irritant	Non-Irritant	Non-Irritant	Non-Irritant	Non-Irritant	Non-Irritant

V. DISCUSSION

The formulation of a polyherbal soap using Azadirachta indica (Neem), Ocimumtenuiflorum (Tulsi), Rosa indica (Rose), Curcuma longa (Turmeric), and Aloe barbadensis Miller (Aloe Vera) was successfully achieved. The soap was subjected to various tests to evaluate its phytochemical composition, antioxidant activity, and antimicrobial efficacy.Phytochemical Screening: The soap was found to contain a rich array of bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds. These compounds are known for their antioxidant and antimicrobial properties, which are beneficial for skincare.Antioxidant Assay: The reducing power assay was used to assess the soap's antioxidant activity. The results showed that the soap exhibited significant free radical-scavenging activity, indicating its potential to mitigate oxidative stress-related skin concerns. This is particularly important for maintaining healthy skin and preventing premature aging.Antimicrobial Activity: The soap was tested against pathogenic microorganisms, including *Escherichia coli* (E. coli), *Staphylococcus aureus* (S. aureus), and *Pseudomonas aeruginosa* (Pseudomonas). The results demonstrated the soap's notable efficacy against these strains, highlighting its utility as a hygiene product for preventing infections and promoting skin health.Soap Parameters: After formulation, the soap was evaluated for parameters such as pH, moisture content, and physical attributes. The soap was found to have a pH within the acceptable range for skincare products, ensuring it is gentle on the skin. The moisture content was also optimal, providing hydration without leaving a greasy residue. Additionally, the soap had a pleasant fragrance and a smooth texture, enhancing the overall user experience.

VI. CONCLUSION

The result of this ponder leads to the definition of polyherbal extricate cleanser which had powerful antibacterial properties against different microbes. The polyherbal cleanser was defined utilizing cold handle method. The planned detailing comprising of 75 gm of cleanser base and different concentration of herbs was found to be promising polyherbal cleanser with antibacterial and antioxidant properties. Too this definition can be utilized in every day utilize without causing any skin bothering. Oils and extricates were included to treat different skin disease and are idealize for every day usage. The physico-chemical and organic parameters of the arranged cleanser were examined. Amid this work we accomplished the required comes about and definition to form the polyherbal cleanser which does not contain any hurtful chemicals. Based on the think about comes about, it can be concluded that home grown cleanser can be defined utilizing cold prepare strategy taking distinctive parameters in thought as that of skin condition and as that of home grown possibilities and its action. This sought of home grown detailing can bring a enormous distinction within the field of home grown beauty care products as there are numerous arrangement and related imperfections completely different polyherbal Or chemical - based definitions which can be removed.

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Discover the Beauty of Natural Art Supplies: Watercolours, Crayons, and Sketch Pens

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ABSTRACT

Choose natural watercolor paints, crayons, and sketch pens for a confident, sustainable, and artistic experience that celebrates the beauty of nature. Made with eco-friendly materials like plant-based pigments and natural edible waxes which are vegan, these art supplies allow you to unleash your creativity while promoting environmental awareness, making a positive impact on the planet with natural art materials. plant materials like Bougainvillea, ClitoriaTernetea, Cestrum Nocturnum, Piper Betle, and Red Malabar Spinach seeds have been used to dye the clothes.

Keywords: Natural, watercolor paints, crayons, sketch pens, vegan wax.

I. INTRODUCTION

The earliest known form of painting is cave painting started 50,000- 60,000 years ago in the paleolithic age for ex: Bhimbetka rock shelters (Madhya Pradesh). From 15,000 to 40,000 years old, more than 500 caves with ancient artworks were found. Barkheda, a significant location during the Neolithic period, was a major source of two raw materials - "chert" and "chalcedony". Chert, a silica-rich sedimentary rock, comes in various colors and is known for its durability. Chert is found in Greenland, chalk, and limestone. Chalcedony is a unique microcrystalline form of Quartz. Ground pieces of ochre and hematite are used during the Mesolithic period. Extraterrestrial life is a reality we cannot ignore. Reported UFO sightings, ancient epics, and a 10,000-year-old cave painting all point to its existence. It's time we acknowledge the possibility of life beyond our planet. (Bipin Shah, August 2015, Prehistoric Cave Paintings of India some depictions puzzling archaeologists as representation of extra-terrestrial beings) [1-2] At Ajanta caves, Mud mortar is used. One of the recipes used according to the - Vishnudharmottara Purana – In guggula (gum) or beeswax, 3 types of brick dust and 3 parts of mud mortar are added and mixed with one-third of powdered burnt lime and the necessary amount of salt. For the binder, Vajralepa glue is used. [It contains buffalo horns, buffalo or goat or cow skin with juice of bimbo (Momordica monadelpha) and kapittha (Feronia elephantum)] [3]Ready-made paints were introduced to the market in 1766 when William

Reeves (UK) sold the first water-soluble dry cake watercolors. In 1780, honey was added to the paint to make it more pliable and easier to manufacture. Honey, being a natural humectant, attracts and retains moisture.Winsor Newton made a bold move by introducing their glycerine-softened formula moist cakes. These moist cakes set a new standard in the industry.John Goffe Rand (1801-1873) made a “metal paint tube”.[4]

A. Watercolor

How watercolor paint is made today-

Paint is made with the finest pigments like aniline and arizarine and brighteners.The medium or vehicle used comprises carefully selected elements to ensure the best possible results.The binder, typically made of gum arabic or synthetic glycol, holds the paint together perfectly.

Adding a plasticizer keeps the dried gum arabic soft, while a humectant ensures our paint retains moisture. An extender or filler to thicken the paint without affecting the colour.Manufacturing additives prevent clumping and speed up the milling of ingredients.Also added a fungicide or preservative to prevent the growth of mold or bacteria. Finally, use only pure water to dissolve or suspend all the ingredients, carrying them onto the paper.[5]

B. Crayons:

Crayons are often a child's first tool for coloring and exploring the world around them. Edwin Binney and C. Harold Smith's expertise in the coloring industry was well-known through Binney's Peekskill, New York, chemical works. Their products, including lampblack, carbon black, and chalk, were highly regarded. In 1902, they launched the revolutionary Staonal marking crayon, quickly becoming a customer favourite. With their years of experience and innovative approach, Edwin Binney and C. Harold Smith remain reliable and trustworthy in the market. [6].

The manufacturing of crayons involves several steps. The first step is the storage of hot paraffin (wax). Next, the color pigment is prepared by either using individual colors or mixing the pigments to produce the various colors of Crayola Crayons. The liquid beeswax is then mixed correctly with the powder form of colour pigment, along with some white additive to enhance the quality of the crayons. The mixture of wax and pigment is then injected into the crayon mould and solidification takes place. [7] [8]

Benefits of Soy Wax used to make crayons: Soy wax is derived from soybeans, whereas paraffin wax is derived from refined gasoline products. It is a natural, renewable resource.[9]Lee Newman patented sketch pens in 1910, although Benjamin Paskach patented a sponge tip pen in 1926. Sketch pens were popular in the 1950s for creating labels, letters, and posters.A sketch pen has its ink source and usually comprises a tip made of a porous fibre such as felt. You may still remember the smell of sketch pens from your childhood; until the 1990s, these sketch pens had ink made from solvents such as xylene and toluene.

Crayons are often a child's first tool for colouring and exploring the world around them. Edwin Binney and C. Harold Smith's expertise in the colouring industry was well-known through Binney's Peekskill, New York, chemical works. Their products, including lampblack, carbon black, and chalk, were highly regarded. In 1902, they launched the revolutionary Staonal marking crayon, quickly becoming a customer favourite. With their years of experience and innovative approach, Edwin Binney and C. Harold Smith remain reliable and trustworthy in the market. [6]. The manufacturing of crayons involves several steps. The first step is the storage of hot paraffin (wax). Next, the colour pigment is prepared by either using individual colours or mixing the pigments to produce the various Crayola Crayons colors. The liquid beeswax is then mixed correctly with the powder form of colour pigment, along with some white additive to enhance the quality of the crayons. The

mixture of wax and pigment is then injected into the crayon mould and solidification takes place. [7] [8]

C. Sketch Pens:

Lee Newman patented sketch pens in 1910, although Benjamin Paskach patented a sponge tip pen in 1926. Sketch pens were popular in the 1950s for creating labels, letters, and posters.A sketch pen has its ink source and usually comprises a tip made of a porous fibre such as felt. You may still remember the smell of sketch pens from your childhood; until the 1990s, these sketch pens had ink made from solvents such as xylene and toluene.

II. METHODS AND MATERIAL

Materials:

Firstly, various flowers were collected from different locations such as the *Clitoria Ternatea* flowers, *Piper Betle* leaves and *Colocasia* leaves were collected from Ramakrishna Mission Ashram, Satara Parisar, *Red Malabar Spinach* seeds was collected from theShivsena Pramukh Sriman Balasaheb Thakre Botanical Buddi Garden, sector N4, Cidco, *Bougainvillea* flower was collected from Prayas Biodiversity HotSpot, Nyay Nagar, *Curcuma Longa* (Turmeric) was collected from K.K Dawasaz, shahganj, *Cestrum nocturnum* (Night Jasmine) flower was collected from the Parijat Botanical Garden, sector N4 Cidco, All samples collected near city of Chhatrapati Sambhajinagarand *Physiculus nematopus* (coal) was prepared by burning the wood pieces. Whole Wheat flour or corn starch was collected from the local retail store of Chhatrapati Sambhajinagar, glycerine was bought from the local medical store, and for crayons, soya wax is used. For sketch pens, cotton wig, plastic, and sketch pen's structure is collected from local general store of Chh. Sambhajinagar.

Fig. 1: *Clitoria Ternatea* (Blue) Soaked in water.

Fig. 2: *Colocasia* leaves (light green) soaked in water.

Fig. 3: *Bougainvillea* (Red) soaked in water.

Fig. 4: *Piper Betle* (Grey) ground in water.

Fig. 5: *Cestrum Nocturnum* (yellow) soaked in water.

a) Method of Watercolor paint production:

There is a common watercolor method for flowers such as *Clitoria Ternata*, *Cestrumnocturnum*, *Colocasia* leaves, *Bougainvillea*, *Piper Betle,* and *Physiculusnematopus* (charcoal powder) as follows

Above flowers and *Physiculusnematopus* (charcoal powder) were soaked and *PiperBetle* was ground in water till pigments were extracted in the beakers.

1/2 or 1 tablespoon of Wheat flour or wheat starch was added to the extracted pigments.

Then the beaker was placed on the burner and stirred well till the Wheat flour was dissolved completely into the pigments.

½ teaspoon of glycerine was added to the pigment to saturate the mixer whilestirring on the burner resulting in the formation of watercolor paint.

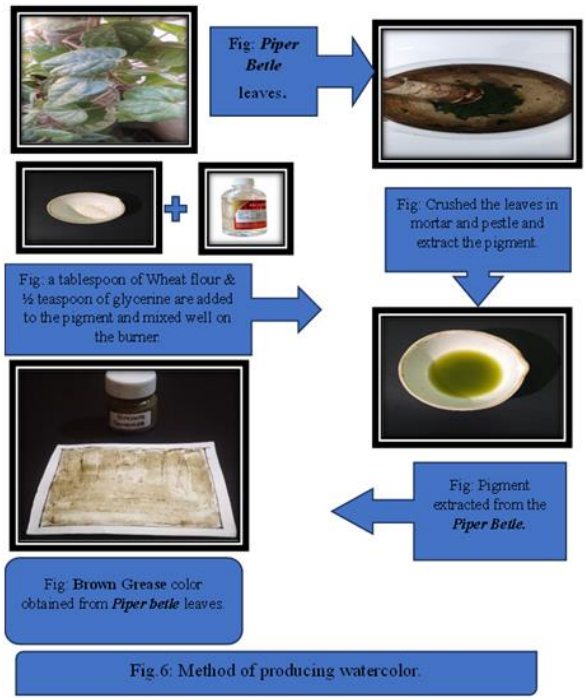

Fig.6: Method of producing watercolor.

Every type of watercolor from flowers and seeds of red Malabar spinach was prepared by this method.

b) Method used to make the Crayons:

Plant pigments were extracted by dissolving the plant material in water, as shown in the diagrams above. Approx 4 g of Soy wax was taken in the beaker and dissolved on the burner.The extracted pigment was added to the dissolved soy wax. The mixture was poured into the mould to get the desired shape of the crayons.Crayons can be made using the same method with other pigments as well.

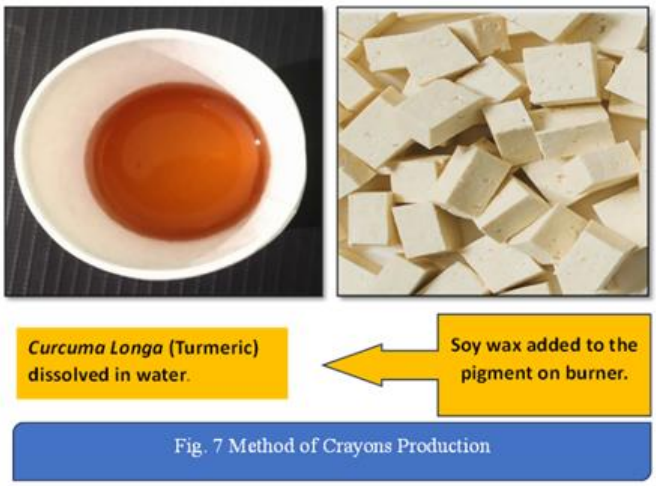

Fig. 7 Method of Crayons Production

c) Method used to make Sketchpens

To extract the pigments from the plant, leaves and flowers were first crushed and ground intoa fine powder. This powder was then mixed with a small amount of water and left to soak for several hours. The mixture was then filtered to remove any solids, leaving behind a solution of the plant pigment dissolved in water. To prepare the refill for the sketch pen, cotton wick was tightly wrapped with a thin layer of plastic to create a seal. The cotton wick refill was then immersed in the solution of pigment and water, allowing it to absorb the liquid. Once fully soaked, then cotton wick was carful inserted into the sketch pen structure, providing a steady and consistent flow of ink during use.

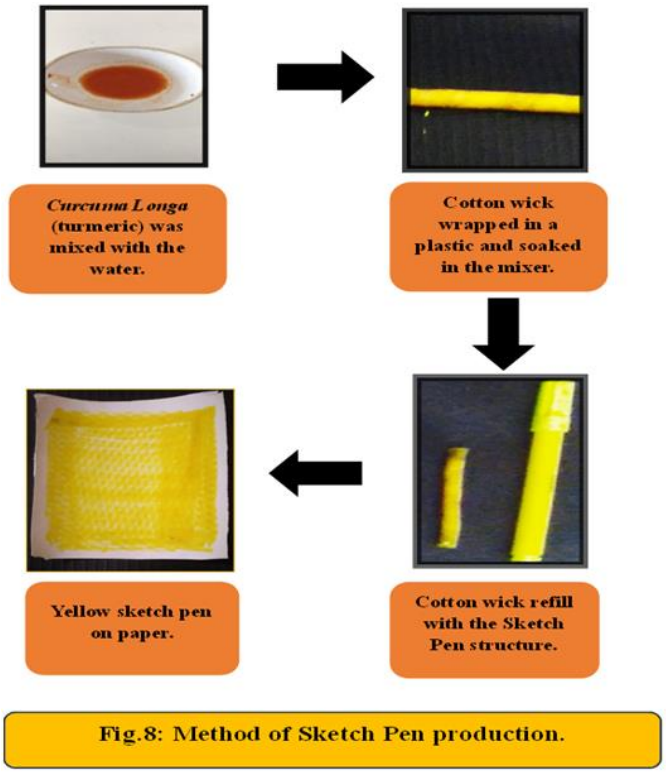

Fig.8: Method of Sketch Pen production.

III.RESULTS AND DISCUSSION

A) Watercolors:

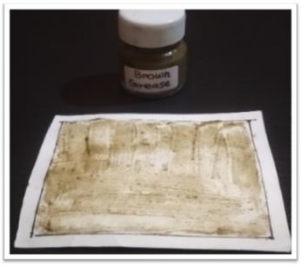

Fig.9: Brown Grease

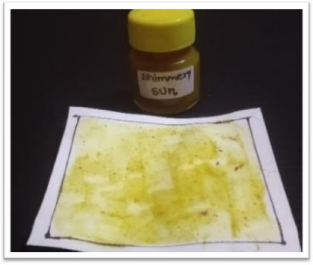

Fig. 10: Shimmery Sun

Fig.11: Blackberry

Fig.12: Mint Green

Fig.12: Rosy

Fig.9: shows Brown Grease, a brown-grey color obtained from the leaves of *Piper Betle*. Fig.10: shows a Shimmery Sun, a dark yellow color obtained from *Curcuma Longa* extract. Fig.11: shows Mint Green, a green color obtained from *Colocasia* leaves. Fig. 12: shows Rosy, a Magenta color obtained from *Red Malabar Spinach* seeds. Finally, Fig. 13: shows blackberry, a black color obtained *from Physiculus Nematopus* (charcoal) extract. All the pigments provide their respective results as shown above. These watercolor paints are safe for children under five, as they are edible and non-toxic in nature. They are temporary and not contains any preservatives.

B) Crayons:

Fig. 14: Mango

Fig.14: Mangonamed yellow colored crayon is made of Curcuma Longa extract. Crayons are now can be eaten as they are vegan, biodegradable, and healthy.

C) Sketch pen:

Fig. 15: Sketch pen

Fig. 15:By soaking the refill of cotton in *CurcumaLonga* extract, a yellow-colored sketch pen is made that does not cause any skin allergies.

Tests:

Thin Layer Chromatography (TLC), Paper chromatography, and spectrophotometric analysis are done to check the absorption rate of pink, pale yellow, blue, and dark yellow color pigment obtained from *Bougainvillea*, *CestrumNocturnum*, *ClitoriaTernetea*, and *CurcumaLonga*. [10]

Fig.16: TLC and Paper Chromatography

Table No 1: Plant pigments separated using Paper chromatography

S.R. No.	Descriptionof color	Distance Solvent Front Travelled	Distance color travelled	Rf value	Name of Pigment
1	Blue	4	3.5	0.87	Anthocyanins
2	Dark pink	4	3.8	0.95	Astaxanthin
4	Brown	4	2.7	0.65	Umber
5	Dark yellow	4	1.6	0.4	Havana ochre

The Astaxanthin pigment, which has a dark pink color, was obtained from *Bougainvillea* and had the highest RF value of 0.95 when calculated for paper chromatography.

Table No2: Optical density observed at 360 nm in spectroscopy

Sr. no	Flower name	Water OD (360 nm)	Isopropanol OD (360 nm)
1.	*Bougainvillea* dark	2.10	3.78
2.	Cestrum *Nocturnum* (Night jasmine)	3.37	0.76
3.	*CurcumaLonga*	3.10	0.98

.Spectrophotometry was done to measure the absorbance of pigments. *CestrumNocturnum* had the highest OD of 3.37 in water at 360 nm, while *CurcumaLonga* had the highest OD of 0.98 in isopropanol at 360 nm.

IV. DISCUSSION

A survey is conducted to determine people's awareness of the harmful effects of watercolor paints, crayons, and sketch pens, and whether they prefer to

use natural alternatives. Whether they want to use the colors which are natural and edible.

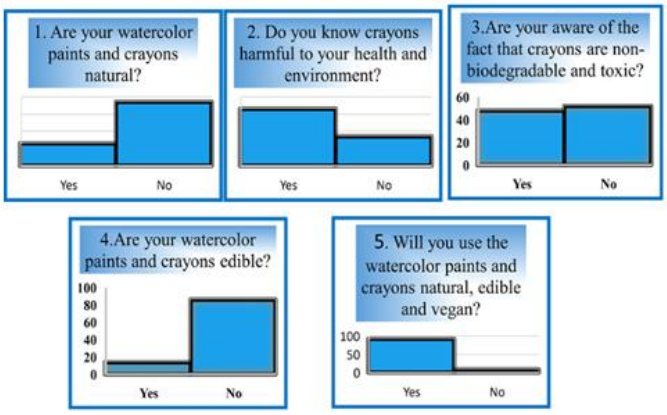

Fig. 17: Histogram Representation of the survey[11]

V. CONCLUSION

Generally, watercolor paints, crayons, and sketch pens we use contain pigments like alizarin crimson, iron blue and the phthalocyanines, red quinacridones, dioxazine violet, Prussian blue, carbon black, zinc or titanium white, yellow quinacridones, benzimidazoles and most other synthetic organic pigments, viridian, ultramarine blue, ultramarine violet and the finer-grained cobalt pigments (blue, cerulean, turquoise, green); cadmium yellows, cobalt violet, red and yellow iron oxides, cadmium orange, the cadmium reds, manganese violet and manganese blue. But we used natural pigment-containing colors from Curcuma *Longa* (turmeric), *Colocasia* leaves, *PhysiculusNematopus*, *PiperBetle*, *Red MalabarSpinach* seeds, *Bougainvillea* dark, and *Cestrumnocturnum*. In watercolor paints, instead of synthetic glycol, we used wheat flour or wheat starch which we consume in our diet that is rich in fibre, protein, and vitamin B. Also, we used glycerine to soften the watercolor paints. [5] In crayons, we used soy wax instead of animal wax (Bees wax) which is indigestible for the human body. Soy wax is made of soyabean oil. It is non-carcinogenic, edible (only if 100% pure), and vegan. Even if children below 5 years old ate our crayons it will not harm them.

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Honor Killing: Case Story Revealed By Forensic Crime Scene Investigation

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ABSTRACT

Honor killing is seen as dirty behaviour by the family members thinking about bringing purity to the family. It is an act of murder by the family on female family members to bring honor to the family who has violated the wishes and honor of the family. Here we are reporting a case study of investigation of an honour killing in Maharashtra state. A girl had chosen a life partner herself. The family refused a matrimonial choice and a pre-marital relationship of a daughter. Parents pre-planned the murder of their daughter and claim she committed suicide by hanging. Later, they did an immediately doubtful funeral without informing police and relatives. Police didn't find any clues or statements from family members or relatives. Police reported an accidental death case as they didn't have any evidence to file a criminal case. After three days, the investigating officer informed the forensic team. The place of the funeral was identified as near the suspect's house. Forensic investigation of the scene of the crime was done by a forensic team, as it was wiped fully by the suspect after the killing. Evidence such as bone samples, earth mixed with blood, wall scrapping and prepared stain of floor from the scene of crime was collected, tested and concluded as a murder. After a forensic investigation, police filed a criminal case against the parents and statements were recorded. Mother confessed to the act of killing and statements matched the scene of the crime, related evidence and place of funeral.

I. INTRODUCTION

Crime scene investigation is a crucial part of any investigation. It's the tri-junction of Science, Law and logic. Investigation begins at the scene of crime with the identification and recovery of physical evidence. It then moves on to provide all findings to the court of law following the study and evaluation of the findings of the retrieved physical evidence and documents, as well as the statements of witnesses. From the information of the first responding officer to the last visiting officer, all personnel should have

adequate scientific knowledge of the forensic process, like identification, recovery, collection, preservation, transportation, and proper documentation to maintain the chain of custody. [1] A crime scene investigation is a process that aims to document the scene of crime to the point of conviction by collecting potential physical evidence necessary to solve the case. The exact spot or location where the incident occurred is known as the scene of the crime or place of occurrence. According to the locard Mutual exchange principle, every contact leaves a trace of evidence. This principle holds that the perpetrator of a crime will bring something into the crime scene and leave with something from it and that both can be used as forensic evidence. Suspect, Victim, Evidence and crime scene are interlinked in one circle.

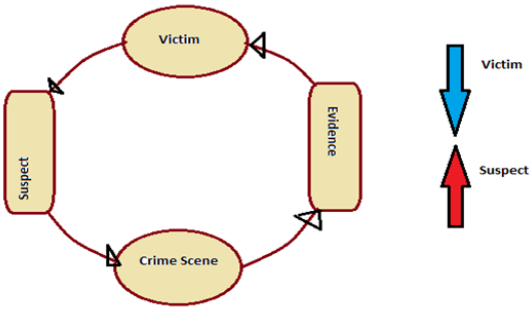

Fig: Locard Exchange Principle

Mostly, police officers reach the scene of a crime first and start the investigation process by securing the scene of the crime. The first responding officers should have the ability to correctly locate, segregate, and safeguard the evidence precisely; this is crucial to the success of the overall investigation. The case story involves a mystery of honor killing revealed by forensic investigation. Honor killing is the “unlawful killing of a woman for her actual or perceived morally or mentally unclean and impure behavior” (Hassan 1995). [2] Honor is most frequently referred to as a family's reputation or social position as determined by the conduct and morals of its female members. In general, honor violence is used to protect a family's reputation by punishing or eliminating young women whose behavior sparks rumours of sexual impropriety or disobedience. Honor killing is a flagitious crime. In patriarchal societies, women are constantly treated as they are considered weak compared to males. The males in the woman's family, first her father and brothers, then her husband, are thought to be responsible for keeping her virginity and "sexual purity". In case of Victims of honor killings are generally supposed to have engaged in matrimonial choices and “sexually immoral” actions by causing damage to their family name. [3] A scientific approach to help in case solving is necessary in distinguishing homicide and suicide cases. This approach also works here in suicide and honor killing cases to differentiate the motive of the death. Many of these honor killing cases are unreported and filed as accidental death and suicidal death. On examining the physical evidence, the investigating officer may get any clue that can reveal the mystery of the case. Such heinous actions on family members are a violation of human rights. The most disturbing and inhumane scenarios are those that result in honor killings. The decision of the family or society to kill a particular family member is irrational. This is the homicide of a female member of a family by the family members of the woman, due to the belief of the accused that the deceased had broughtdishonour upon the family or relatives. Honor killings are not only against women and girls but it has also been extended to men. [4] Men can also be the victims of honor killings by the family members of the woman with whom they are in inappropriate relationships. The major causes for honor killing include falling in love with a person of another caste, similar gotra or different religion as well as Matrimonial choices against family, pre-marital relation, and heterosexual or homosexual acts. Love is considered to be one of the most sacred feelings which are propagated by all religions and when this same feeling becomes a reason for killing someone, it becomes heinous. In most of the cases, even a suspicion, and not an evident confirmation of the woman’s involvement in any of this contravention can be enough to murder of victim. After more than 75 years of independence to India, some people think

caste, gotra, and social status are superior to the life of a child. Every individual in the democratic nation of India is entitled to both equality and life. But as we can see, this horrific crime of honor killing was motivated by caste, society, and the people's narrow-minded thinking. The lack of scientific knowledge of the investigator regarding proper collection, preservation, storage and transportation of evidencewhich are collected from the scene of crime destroys its potential before the court of law. [5]

Crime scene investigation is done by a forensic expert with the help of their team members and the tools. A mobile forensic van equipped with different light sources, as well kit containing scrapping tools, a scale, cutter, forceps, scissors, tape, packaging envelops, test tubes, vials, gauze cloth, cotton, sample collection tubes, cavity tile, plain slides as well Blood and semen detection kit, narcotics detection kit and explosive detection kit.

The method used to investigate the crime scene is the Grid method followed by the spiral searching. The suspected stain was lifted by Cotton Gauze and tested with Castel-Mayer Reagent. The castle-Mayer test is a presumptive blood test, in which indicator phenolphthalein is used to detect the possible presence of hemoglobin. It relies on the peroxidase-like activity of hemoglobin in blood to catalyze the oxidation of phenolphthalein by hydrogen peroxide which is visible as a bright pink colour. [6]

II. REPORTED FACT ACCORDING TO THE STATEMENT OF THE PARENT

Case study of suicide mystery of a 16-year girl who is residing in a small village with her parents. According to their parents' statement, she was tense during study and exams. She didn't talk about that but the facial expression shows it clearly. The family had dinner at night and went to sleep. In about early morning when they are in deep sleep, their daughter commits suicide by hanging. After the suicide, they decide on a funeral. Each member of the family was firm on statements.

III. INFORMATION AND EVIDENCE COLLECTED BY POLICE

Police visited the scene of crime after the funeral of the victims. Police recorded statements from family members, relatives and neighbour's. The victim's parents and family made a dramatic illustration of a suicidal hanging committed by their daughter and the family agreed that they had a funeral near to their house. The first responding officer investigated the place of the funeral and reported a suicide case by hanging. The police investigating officer couldn't link a doubtful immediate funeral without informing the relatives and police. They didn't find any clue or evidence to file a criminal case against the family. Later, the first responding officer secured and preserved the scene of the crime and called to forensic team.

IV. FORENSIC CRIME SCENE INVESTIGATION AND DISCUSSION

The investigation begins at the crime scene with the identification and recovery of physical evidence followed by reconstruction of the crime scene.

a) Observation and examinations

After securing the scene of crime forensic team observed that the family members were feeling unwell when the team entered room no (2). Facial expressions show something is here and the family members are trying to hide from the forensic team. Room no (3) is open from the front and has no concrete flooring as it was wet due to rain. The surface of the floor in room no (2) was cleaned properly by detergent or maybe a toilet cleaner which is generally used in house cleaning. The cleaning marks were wiped on the surface and made doubtful. The forensic team tried to collect any of the blood

stains or other trace evidence from the surface and wall.

During the examination, Castle Mayer reagent, Alternate light sources with filters, Cotton Gauze pieces, and different tools were used. The team searched the house by grid and spiral method to find evidence in the context of a suicide or murder case.

b) Description and Reconstruction of the scene of crime

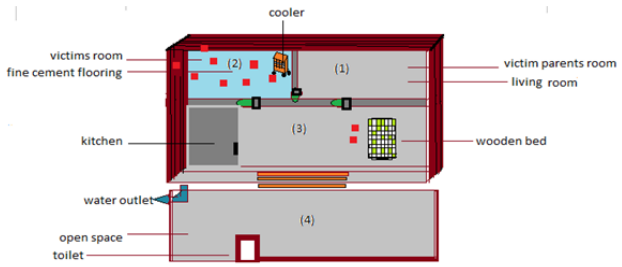

Fig: Reconstruction of scene of crime (Not to Scale)
Blood observed area

The said house consisted of two closed rooms (1) and (2) and one open room (3) covered with iron sheet roofing. When the forensic team reached the scene of the crime they neither found any clue in the relevance of suicidal hanging nor any rope used for this action as the story stated by the parents. Later forensic expert takes a different investigation perspective. The possibility of murder can't be ignored by the forensic team and the whole area of the house and open space is secured. Open place (4) was cleaned and family members used this place for cattle. Room no. (1) And (2) are the back portion of the house and room no. (2) Used by victims for sleeping.Reconstruction of crime scenes shows ideas in which direction forensic experts should go. The forensic team reconstructs the scene of the crime and the expert starts the investigation from a murder perspective. In the figure objects are shown in their original position and not to scale.

c) Revealed from the crime scene

All members of the family are stuck on the hypothetical statement of suicidal hanging. The police registered a suicidal hanging, and again, they started an investigation. Police tried to get clues or witnesses or any evidence from a victim's home. They recorded the statements of relatives, family members and neighbour's, but they didn't find any clues. The parent claims the place of the funeral and shows it to the police. Still, police authorities cannot register a criminal case because of a lack of evidence. After three days, they invited a forensic team, and crime scene management started. Firstly, the forensic team secured the scene of the crime and identified the place of the funeral as the parents stated. The forensic team collects the bone sample residue at the funeral place. Later, a forensic investigation starts at the victim's house, 200 meters away from the funeral site. After the forensic team reached to crime spot they tried to identify a blood or any trace evidence. At last they found blood traces on the wheels of the cooler stand which is present room in no. (2) as shown in the reconstructed diagram.

V. THE REAL FACT COMES OUT AFTER FORENSIC EXAMINATION

The place where the family used to kill the victim is her bedroom no. (2) as shown in the figure reconstruction of the crime scene. Forensic investigation was carried out by experts and found blood stains on the surface but it was not visible as it was wiped multiple times. But the wheel of the cooler which is present in room no (2) was stained with blood. Suspect cleaned room no. (2) Very well but a trace amount of blood was found on one of the wheels of the cooler stand. The scene of the crime is confirmed by the presence of blood on the wheel of the cooler stand in room no. (2) and concludes that the heinous crime committed by family members on a victim. After a forensic investigation police filed a criminal case against the family member under section 302 IPC. again statements were recorded and they agreed and confirmed the scene of the crime and handed over the weapon to police which was used for murder. The mystery of suicide cases reveals a

punishable criminal offense under section 302 IPC by crime scene investigation.

This case study is an excellent example of crime scene investigation for forensic and law enforcement professionals in murder cases in honor of the family. The fact of this case is that one 16-year-old girl was in love with a sugarcane worker residing in the nearest village, and she decided to marry him. But these things, like matrimonial choices or love marriages, are refused by family members. The day she asked her parents and other family members about the matrimonial choice, her parents listened and tried to convince her that she should decide according to family. The victim stuck to her choice. To maintain the so-called honour of family they decided to eliminate her. After the crime scene examination, they can't deny the facts and the Mother confessed to the act of killing. Their statements matched the scene of the crime, related evidence, the place of the funeral and the weapon used.

VI. CONCLUSION

The so-called "honor" crime has its roots in a widespread tradition of discrimination against women, where women are viewed and treated as objects and commodities rather than as human beings deserving of respect and rights on the scale of men. Additionally, a sizable portion of society supports "honor" killings to uphold family honor and shares traditional notions of what that honor should be. Women's right to life shouldn't be dependent on adhering to cultural and social standards that are frequently biased and discriminatory against women. [7]

After considering the above observation it could be concluded that a girl had matrimonial choices and she always told her parents about that. But the family belongs to the male partner whom she wants to marry is from a lower economic class as well illiterate and works as a sugarcane worker. Parents and family didn't agree because family belong to boy didn't have same social and economic status as comparedthem. Parents tried much more to convince their daughter that she should get a better life partner have high social and economic status and be well employed. But the victim stuck with her choice. The parent and family decide to eliminate the future threat to the so-called honor of the family from their daughter. A family pre-planned a murder and committed a heinous crime proved by a crime scene investigation.

Acknowledgment

We are thankful to the Director and Director-General, Legal and Technical, Home Department, Mantralaya, Mumbai, Government of Maharashtra, India for his guidance.

Ethical standards

All data used for this case study were collected by maintaining all legal formalities and the crime scene investigation method has been used in the case study according to the guidelines mentioned in the Directorate of Forensic Science Laboratory, Maharashtra State and Directorate of Forensic Science Services India. This study did not disclose the identity of anybody byany means.

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Agriculture Pesticides Advantages In Society and a Forensic View

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ABSTRACT

Pesticide application plays an important role in pest management. Proper technique of application of pesticide and the equipment used for applying the pesticide are vital to the success of pest control operations. The main purpose of the pesticide application technique is to cover the target with maximum efficiency and minimum efforts to keep the pest under control as well as minimum contamination of non-targets. All pesticides are poisonous substances and they can cause harm to all living things. In the toxicology department we have seen thousands of pesticide poisoning cases.

Keywords : Pesticides, Forensic Toxicology, pest control.

I. INTRODUCTION

Toxicology is the branch of science that deals with the study of harmful toxic effects of agents on people, animals, and other living organisms. One aspect of toxicology is to evaluate the likelihood that adverse effects will occur under specific chemical exposure scenarios; referred to as risk assessment.The word 'Toxicology' is derived from the Greek word 'Toxicon' which was used as a poisonous substance in arrowheads. Traditionally, toxicology is defined as the science embodying the knowledge, source, character, fatal effects, lethal dose, analysis of poisons, and the remedial measures.

The professional activities of toxicologists fall into four main categories i.e., forensic, industrial, clinical,

and environmental toxicology. Forensic toxicology emerged as the hybrid of analytical chemistry and toxic principal effects. Forensic toxicologists are also primarily concerned with the medico-legal aspects of the harmful effects of chemicals on humans and animals. The expertise of forensic toxicologists is primarily utilized in establishing the cause of death and elucidating its circumstances in postmortem investigation. The work of a forensic toxicologist is therefore considered as highly complicated as small quantities of poisons and their metabolites are to be isolated, purified, and quantified from highly complex matrices.[1]

II. NEED OF THE STUDY

The exact scale of this problem in India remains uncertain, but it is reported that 1 to 1.5 million cases of poisoning occur every year, from which nearly 50,000 die [2] every year. India Stands in 13th position in the country for uses of pesticides worldwide [3].

Pesticide Use by Country worldwide is shown in Table 1.

Sr No.	Country Name	Pesticide Use (tons)	Kg of Pesticide per Hectare of Cropland
1	China	1,763,000	13.1
2	United States	407,779	2.5
3	Brazil	377,176	6.0
4	Argentina	196,009	4.9
5	Canada	90,839	2.4
6	Ukraine	78,201	2.3
7	France	70,589	3.6
8	Malaysia	67,288	8.1
9	Australia	63,416	2.0
10	Spain	60,896	3.6
11	Italy	56,641	6.1
12	Turkey	54,098	2.3
13	**India**	**52,750**	**0.3**
14	Japan	52,249	11.8
15	Germany	48,193	4.0
16	Mexico	47,128	1.8
17	Colombia	37,698	9.9
18	Thailand	35,287	1.7
19	Ecuador	34,253	13.9
20	South Africa	26,857	2.2

Table 1: Pesticide Use by Country Worldwide

Data is taken from the website of the Food and Agriculture Organization of the United Nations) the pie chart is shown below.

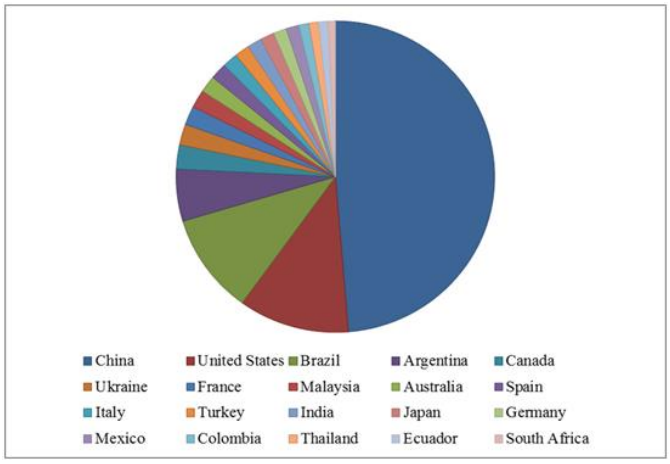

Figure 1: Pie chart explaining worldwide consumption of pesticides

A pie chart explaining the worldwide consumption of pesticides is shown in figure-1. The present-day poisoning scenario is altogether different from that of the past and poisoning in the present context needs to be seen in the light of the profile of poisoning, commonly used/abused poisons, the quantum of pesticide consumption, legal expectations, diagnostic difficulties, treatment, and analytical facilities in developing countries. Knowledge of the general pattern of poisoning in a particular region would help in early diagnosis and treatment, which in turn should result in a reduction of morbidity and mortality [3].

A large number of different pesticides are used in India and they are easily available in every home in rural areas the poisoning cases ratio is increased day by day in forensic toxicology laboratories.

Figure 2: Farmer spraying pesticide on crops

Pesticides that are approved for use in India by the government as per Insecticides/Pesticides Registered under section 9(3) of the Insecticides Act, 1968 (As of 1/07/2021) [source: ppqs.gov.in, website of directorate of plant protection, quarantine, and storage, Government of India].pesticide 299 are seen in the list is dynamic. It is revised from time-to-time based on government decisions. Pesticides may be added or removed based on the decision of the government. According to the law, commercial formulations must be prepared with only approved pesticides at any specific time. The entire pesticide manufacturer needs to follow the given guidelines for production and distribution of these formulations.

III.RESEARCH METHODOLOGY

Forensic science laboratories provide scientific aid to the police investigating officers in crime investigation, about crimes registered under various acts of the Indian penal code and criminal procedure code, etc. Forensic science laboratories are multidisciplinary institutions doing highly specialized and sophisticated analytical work.

The Toxicology department of Forensic science laboratories mainly analyzes cases of poisoning in which pesticides, drugs, and household poison products are used to kill cockroaches and bugs, etc. Poisoning may be suicidal, accidental, or homicidal. Suicidal poisoning is one in which a person takes poison at his own will. Accidental poisoning happens to self or others without any intentions. Homicidal poisoning, which is mostly encountered in criminal cases is one in which poison is intentionally given to someone else.

The indispensable analytical reports issued by the forensic science laboratories help the police and the judiciary in the detection of crime and administration of criminal justice by providing objective scientific evidence against the guilty or at times clearing the innocent.

The main laboratory examination is directed towards material identification. The main and biggest division of the forensic science laboratory is forensic toxicology which is pressed into service whenever a chemical or toxicological examination such as identification of drugs or pesticides or any synthetic insecticides, like poisonous materials is required in a suspected article or in a biological materials like viscera, postmortem blood, stomach wash and vomit etc. of a victim of poisoning.[4] There is no universal method of analysis for all substances, particularly where different analytical schemes are required to exclude even the most commonly encountered poisons (Figure 3).

Forensic toxicology demands an overall analytical system designed to exclude or indicate the presence of any poison in each of the chemical groups shown in Figure 3. Most of the numerous screening procedures reported in the literature are too limited to permit a confident negative report.[5]

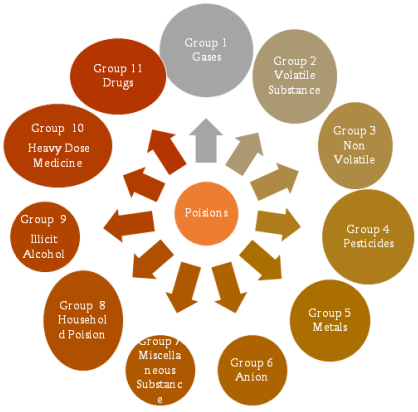

Figure 3: The eleven major groups of poisons

The eleven major groups of poisons are shown in

Figure 1. Group 4 consists of pesticides which are mainly divided into the following groups viz.

- Organophosphorus Insecticides
- Organochlorine Insecticides
- Carbamate Insecticides

ORGANOPHOSPHORUS INSECTICIDES (OP)

The Organic chemistry of phosphorus goes back to 1820, when Lassaigne first studied the reaction of alcohols with phosphoric acid[6], serious investigation into the synthesis of toxic organophosphorus compounds as potential nerve gases began during the Second World War. At Cambridge, Saunders and his colleagues studied alkyl fluorophosphates such as tetraethyl phosphoramidic fluoride or dimefox, while in Germany, Schrader made the highly active nerve gases tabun and sarin.

As such today, organophosphorus compounds form an important class of pesticides. More than 100,000 different OP compounds have been synthesized and evaluated as pesticides; out of these more than 70 to 80 are widely used in agriculture.

There is enormous structural diversity of OP insecticides. There are few studies available about their chemistry. [7-8] Some typical oprganophosphorus and organothiophosphorous pesticides structure are given in Figure-4.

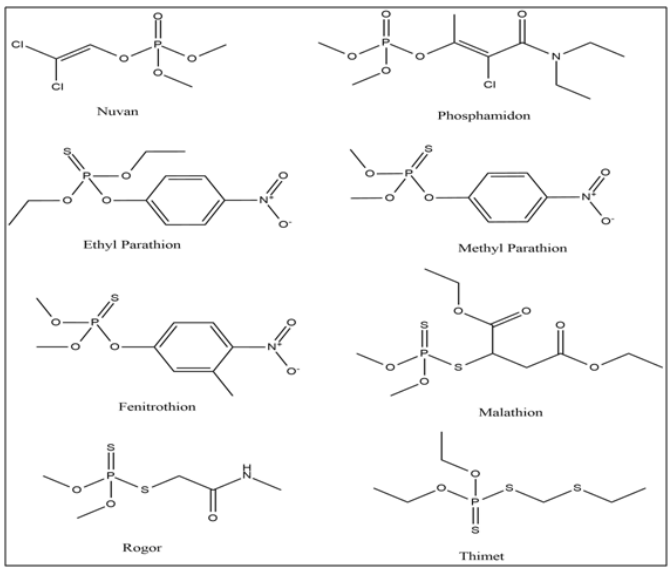

Figure 4: Chemical structure of organophosphorus and thiophosphoric insecticides

Various methods have been reported in the literature for the detection and determination and identification of OP insecticides those includes, Gas Chromatography mass spectrometry , Spectrophotometry, High Performance Liquid Chromatography, Fluorometryetc. But all these methods are reported for pure compounds or for formulated products or for extract from water samples, grains, soil sample etc., and are highly susceptible to the impurities co-extracted with insecticides from biological materials (viscera, blood, urine and stomach wash). In poisoning cases, however an elaborate clean up procedure is essential for their detection and determination in biological materials by above reported methods.[9]

TLC and HPTLC have many advantages as an analytical and/or screening tool. Visualization of many of the insecticides may be achieved by a variety of methods however, spraying with universal most famous spray reagents like mercurous nitrate and potassium iodate-starch reagent is suitable for screening many organophosphorus insecticides.[11]

ORGANOCHLORINE INSECTICIDES (OC)

Organochlorine insecticides are widely used in agriculture for the protection of crops. The extreme toxicity of these insecticides towards insects particularly those affecting humans and its low toxicity against mammals helped its rapid spread. Dichlorodiphenyltrichloroethane (DDT) is the first synthetic insecticide prepared by Zeidler (1874)[12].DDT was introduced as an insecticide in 1942 and was manufactured on a large scale during the war. Alsobenzene hexachloride were first synthesized by Michael Faraday in 1825. In 1842, the imperial chemical industrial laboratories in England found this compound to have considerable insecticidal properties but did not give consistent results because it contains isomers of different toxicity. Endrin [14] is a stereoisomer of dieldrin.

It is produced by the oxidation of isodrin with hydrogen peroxide in acetic acid at the lowest

possible temperature. Endrin is a white crystalline solid melting at 200 ^{0}C. It is insoluble in water but is soluble in most organic solvents. Endrin exceeds in toxicity for man, domestic animal and insects than many other compounds of this series and hence recently banned by the government of India. Endosulfan[15] (Thiodan).

Detection and determination of organochlorine insecticides in food materials, milk, water samples, urine, meat and meat products and in formulated products. However, a tedious and time consuming clean up procedure is essential before GC analysis. Hence, TLC and HPTLC is the method of choice for the detection and determination of chlorinated insecticides in biological and non-biological materials of forensic interest.

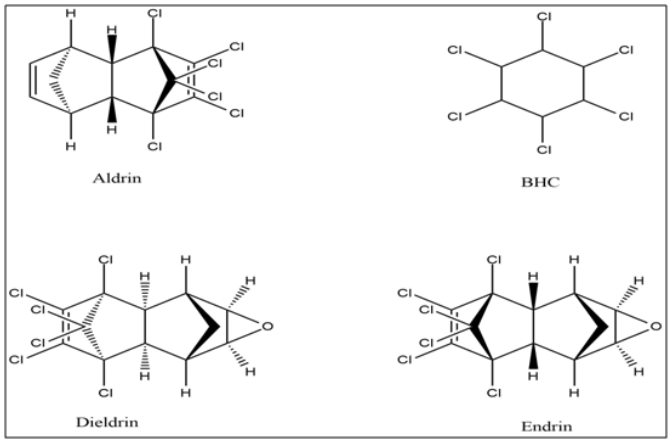

Figure-5: Chemical structure of organochlorine insecticide

The chemical structure of some of the organochlorine insecticides is given in Figure-5.

CARBAMATE INSECTICIDES

In terms of toxicity, carbamate insecticides [16] have a similar action to that of the OP compounds in causing a decrease in cholinesterase activity but the binding to the active site of the cholinesterase enzyme is reversible. Carbamates can be divided into various subclasses, characterized by their different thermal stabilities. *N*-methyl carbamates give thermal decomposition products mainly substituted phenols.*N*-methyl and *N*, *N* dimethyl carbamic esters of phenols and heterocyclic enols possess useful insecticidal properties. Carbamate insecticides are generally crystalline materials of low water solubility but soluble in organic solvents. Among the derivatives of carbamic acid, the aryl esters of *N*-methyl carbamic acid are used for control of insect or pests. Maximum insecticidal effect is shown by the aryl esters of *N*-methyl carbamicacid.

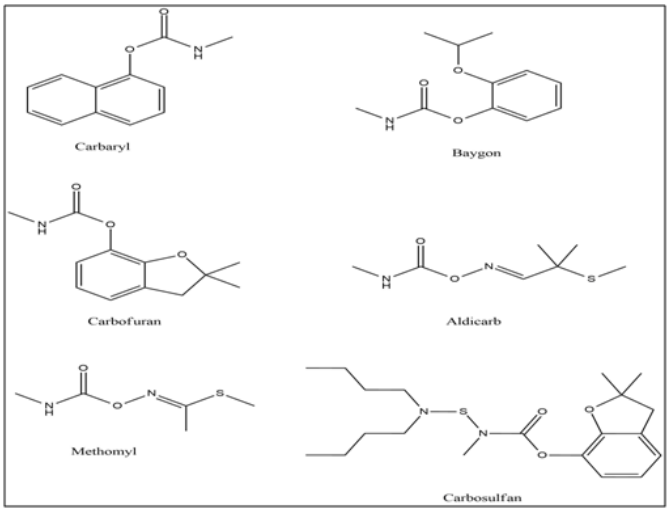

Figure-6: Structure of carbamate insecticide

The widely used carbamates include propoxur, carbaryl, carbofuran, carbosulfan and zineb.Figure-6 shows the structural formula of some of the carbamate insecticide. Among the various analytical methods used for the detection and determination of

Carbamate insecticides, TLC and HPTLC are the most useful and applicable methods for the detection of these insecticides in biological material in forensic toxicology.

Due to easy availability, they are frequently misused for homicidal and suicidal purposes in India. Among the various analytical methods used for the detection and determination of glyphosate and atrazine herbicides, TLC and HPTLC is the most useful and most applicable method for the detection of these insecticides in biological material in forensic Toxicology.

IV. RESULT AND DISCUSSION

The requirement is to be able to detect, identify and

quantify the substance, typically at low concentrations and in complex matrices such as organ, tissues and blood. The present day requirement is not merely identifying the toxic material qualitatively by general and non-specific tests of years but also to identify it specifically using efficient extraction procedures and modern techniques like Thin-Layer Chromatography (TLC), High Performance Thin-layer chromatography (HPTLC), High Performance Liquid chromatography (HPLC), Gas Chromatography (GC), Spectrophotometry and hyphenated technologies like GC-MS, FT-IR etc.In recent years in India, the use of different types of insecticides, fungicides, rodenticides, and herbicides is increased in agriculture to protect the crops and commercial plants from insects to get good yields. Also, they are often used in houses to kill the mosquitoes, cockroaches, bed bugs and rats. Easy availability of these insecticides is frequently misused in suicidal or homicidal poisoning cases occurred every year drastically.

Significance of the work

Pesticide's in which household poison(Rat kill.Mosquito repellents etc.) mainly Organophosphorus,Organochloro,Herbicides,Plant growth regulator etc.many cases found in large amount in poisoning cases.

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Application of Actinomycetes for Ecological Sustainability

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ABSTRACT

Various actinomycetes species can degrade textile dyes; however, very less work has been done to elucidate the role of these microbes in plant growth promotion (PGP). This study was designed to isolate and evaluate actinomycetes strains carrying dual traits for textile dye degradation and PGP. The isolates were studied for dye removal activity using a bandhani dye (red), in minimal media with 1mg/ml concentration. The isolates were also screened for their PGP activities such as IAA ammonia, HCN production, phosphate solubilisation, and enzyme production including amylase and cellulose. Seven isolates (isolate no-A6, A7, A9, A13, A18, A19) depicted promising potential to treat the textile dye. Other thirteen isolates(A1,A5,A6,A7,A8,A9,A13,A14,A15,A16,A17,A19,A20) showed prominent activity in IAA, HCN & Ammonia production, phosphate solubilization, & various enzyme production. Three isolates (A6,A9, and A20) among those thirteen were used in pot assay with chickpea and wheat seeds. Our findings show that one isolate is common (isolate no A6) in both studies having dual traits i.e. dye removal and PGP activity. That common strain of actinomycetes could be used simultaneously for textile effluent treatment and PGP.

Keywords: Actinomycetes, IAA production, dye degradation, plant growth promotion (PGP).

I. INTRODUCTION

The textile dyeing industry is one of the main industrial sectors responsible for the production of a large quantity of wastewater. After the dyeing process, a significant fraction of the applied dyes is released into wastewater streams. (Shafkat M *et al.*, 2017) The released dye residue in the water bodies has a diverse negative effect on the ecosystem. (Sinha *et al.*, 2019) The effluent consists of an array of compounds such as heavy metals, aryl phenols, chlorinated compounds, aromatic amines, volatile organic compounds (VOC), etc., which are toxic to the environment when exposed for a prolonged period. In addition, the 'azo dyes' toxicity is a major concern (Khandare and Govindwar, 2015). Therefore restoration through a cost-effective biological strategy is the need of the hour. Bioremediation of wastewater using microorganisms has emerged as an eco-friendly option. It is a cost-effective and attractive strategy for the removal of recalcitrant synthetic dyes (Sinha *et al.*, 2019). The reuse of treated wastewater in agriculture is gaining popularity as a low-cost and useful alternative for irrigation (Thapliyal *et al.*, 2013)

Plant growth-promoting rhizobacterium (PGPR) are free-living bacteria that actively colonize the rhizosphere region and are considered a potential tool capable of promoting plant growth. (De-Bashan *et al.*, 2012). They possess various mechanisms including the production of ACC deaminase, growth hormones like IAA and GA, phosphate solubilization, and the production of various valuable enzymes.(Penrose *et al.*, 2011; Pattern and Glick, 2002; Gulati *et al* ., 2002).

Actinomycetes, found mostly in soils, are widely known for their antibiotic and bioactive secondary metabolites production as well as their outstanding ability to survive in unfavourable environments (Passari *et al.*, 2015). They are also known for their ability to produce potential bioactive compounds that can be applied in medicine, agriculture, and other industries (Poppeliers, Sánchez-gil and Jonge, 2023). Many microorganisms' PGPR characteristics have been extensively studied, but it has only been in the last two decades that actinobacterial germ's capacity to stimulate plant growth has been increasingly recognized. They may have a direct, indirect, or combined effect on the health and productivity of plants (Boukhatem, Merabet and Tsaki, 2022). Some of the rhizosphere actinomycetes have been widely developed for increasing agricultural crop yields, such as *Actinoplanes*, *Streptomyces*, and *Micromonospora*. Among them, *Streptomyces* is the most explored genera concerning plant growth-promoting activity (Wahyudi *et al.*, 2019). Actinomycetes have also been studied for dye-degrading properties. Few reports have been presented on the degradation studies of azo dyes. *Salinispora arenicola*, *Streptomyces spp*. and *Micromonospora fulviviridis* were able to degrade erichrome black T and congo red from effluent sample (Shanmuga *et al.*, 2016). *Streptomyces cacao subspp. cacaoi* was able to degrade red azo dye (Janaki T, 2016). Isolation of such multifunctional bacteria expands the range of potential bioresources which might be exploited for concurrent agricultural production and remediation of pollutants in the soils under stress due to industrial effluents (Shahid *et al.*, 2018).

The present study deals with the study of dual-functioning microbes that can be exploited in a way that enhances plant growth by showing PGP activity and reducing the toxicity of dyes from wastewater.

II. METHODS AND MATERIAL

Reagents: All the chemicals and reagents used were of analytical grade. The red bandhani dye was obtained from a local market. Actinomycetes isolation agar, calcium carbonate (cat. No. GRM397) were procured from Himedia Laboratories Pvt. Ltd. Mumbai.

Phenol, K2HPO4, FeSO4, MgSO4, NH4Cl, NaCl, and dextrose were obtained from SDFCL, S D Fine Chem Ltd. Mumbai.

Culture media used in study:

Actinomycetes isolation agar: Agar 15 g/L , L-asparagine 0.1 g/L , dipotassium phosphate 0.5 g/L , ferrous sulfate 0.001 g/L , magnesium sulfate 0.1 g/L , sodium caseinate 2 g/L , sodium propionate 4 g/L, final pH 8.1±0.2

ISP-2 medium: Yeast extract 4 g/L, Malt extract 10g/L, Dextrose 4 g/L, agar 20g/L, pH 7.2

Minimal media: 0.18% dipotassium hydrogen phosphate, 0.001% iron (II) sulfate heptahydrate, 0.02% magnesium sulphate heptahydrate, 0.4% ammonium chloride, 0.01% sodium chloride

Peptone water: Peptone 10.0 g/L, Sodium Chloride 5.0 g/L, pH 7.2

Pikovaskaya's medium: Yeast extract 0.5 g/L, Dextrose 10.0 g/L, Calcium Phosphate 5.0 g/L, Ammonium Sulphate 0.5 g/L, Potassium Chloride 0.2 g/L, Magnesium Sulphate 0.1 g/L, Manganese Sulphate 0.0001 g/L, Ferrous Sulphate 0.0001 g/L, Agar 15.0 g/L, pH 7

Starch agar: Peptone 5g/L, meat extract 3g/L, agar 15g/L, starch 1%, pH 7

Cellulose agar: Potassium dihydrogen phosphate 0.5 g/L, Magnesium sulphate 0.25 g/L, cellulose 2.0 g/L, agar 15 g/L, gelatin 2 g/L, pH 6.8–7.2.

Collection and enrichment of soil samples: Soil samples were collected from various locations around Aurangabad such as gardens, farms, lake mud, etc. The samples were collected by using the method previously used by A. Sapkota and colleagues (Sapkota *et al.*, 2020) and stored in sterile zip-lock polythene bags and stored at a lower temperature (4°C) until used.

The samples were treated in four different ways for the enrichment of actinomycetes. The first three treatments in table no. 1 were done according to (Shivabai and Gutte, 2019)

Table no.1 Enrichment methods for actinomycetes.

Enrichment method	Procedure	Reference
1. $CaCO_3$ treatment	10gm soil sample, air dry, add 1% $CaCO_3$, and incubate at 30°C for three days.	Shivabai and Gutte, 2019
2. Hot air oven treatment	1gm of soil sample incubated in a hot air oven at 60°C for 3 days.	
3. Phenol treatment	1gm soil sample, air dry, add 1.5% (w/v) phenol solution, incubated at 30°C for 30 min.	
4. Sun drying treatment	10gm soil sun-dried dried for 7 days. Suspend in sterile distilled water at 50oC for 30 min.	

Soil samples after pre-treatment were serially diluted up-to 10-4 & 10-5 and inoculated in actinomycetes isolation agar. Plates were incubated for 7 to 10 days. Further isolated strains were preserved on AIA slants. Morphological properties were studied according to Mohan et. al.(2014).

Screening for dye removal activity:

Dye removal activity was studied using minimal salt media (both agar and broth) supplemented with red tie dye (bandhani red-dye) in 1mg/ml concentration (Wai, Yusop, and Pahirulzaman, 2020). Both the agar and broth medium were inoculated with the isolates and incubated at room temperature. The decolourization activity was checked every 48 hrs.

Aliquots of 500ul were centrifuged at 5000 rpm. From each broth sample and the supernatant was collected. The absorbance of each supernatant was checked at

500nm. The plates were observed for a zone of clearance around the colonies.

Screening for PGPR activity:

All the isolates were screened for PGP activity. Screening for IAA production was done by using a method explained by (Chandwad and Gutte, 2019).

IAA test: A loopful culture of all strains were inoculated in ISP2 broth containing 0.2% tryptophan and kept for incubation on a shaker incubator at 120 rpm at 280 C for 7 days. After incubation, the broth was centrifuged and the supernatant was collected. 2ml of Salkowski's reagent was added to 1ml of supernatant. The formation of the pink-colored complex shows the production of indole acetic acid.

HCN and ammonia production were screened by the method from (Malviya *et al* ., 2014).

Ammonia test: For ammonia production peptone water tubes were inoculated with loopful culture of all strains and incubated at 280C for 7 days. After incubation few drops of Nessler's reagent were added to the medium. The formation of red colour indicates a positive result.

HCN Test: HCN production was screened with the help of ISP2 broth amended with glycine was inoculated with loopful culture and strips of Whatman filter paper no.1 dipped in 2% sodium carbonate in 0.5% picric acid solution were hanged in the inoculated tubes and kept for incubation at 280C for 7-12 days. The change in colour of the filter paper from orange to red indicates a positive result.

Phosphate solubilisation test: For phosphate solubilization, all isolates were spot inoculated on Pikovaskaya's medium and incubated at 280 C for 7 days. After incubation plates were flooded with 0.1% methyl red indicator. The formation of red color shows positive results.

The isolates were also screened for enzyme production such as amylase and cellulase as explained by (Vishvanathan and Jeyanthi, 2019).

Amylase test: Starch agar plates were spot inoculated with all strains and incubated at 280C for 7 days. After incubation plates were flooded with 1% iodine solution, and colonies showing a clear zone against the blue background indicate positive results.

Cellulase test: All strains were spot inoculated on cellulose agar plates and incubated at 280C for 7 days. Colonies showing clear zone around themselves indicate positive results.

III.RESULTS AND DISCUSSION

Using diverse procedures, a total of 20 isolat*es were* isolated from various soil samples. The use of phenol and calcium carbonate as treatments for actinomycetes isolation was shown to be successful and suitable. Table no.2 indicates the percentage of isolates detected in different soil samples with different treatments.

Table no. 2. Percentage of actinomycetes isolated from soil samples using various treatment.

Pre-treatment	No. of colonies obtained	% of total isolates obtained
1.Calcium carbonate	8	40
2.Phenol	5	25
3.Hot air oven	3	15
4.Sun drying	4	20

Morphological properties:

The morphological characters of the actinomycetes isolates along with the pigment colour are presented in table no. 3 and figure no. 1. As from the obtained data some of the isolates were able to produce melanin-like pigmented colonies. Others were able to produce dark pigment on the reverse side of agar.

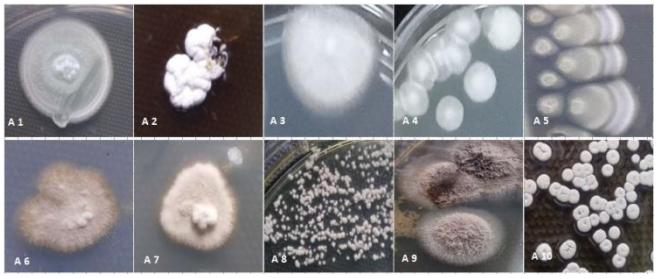

Figure no. 1 *Actinomycetes* Isolates with their images

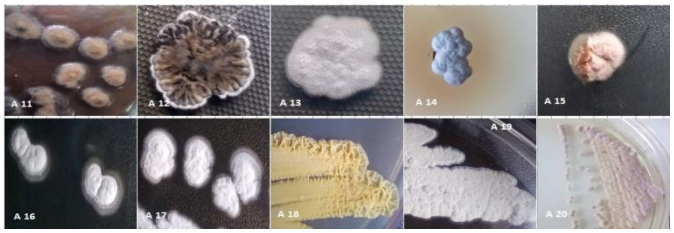

Figure no.2 *Actinomycetes* Isolates with their images

Table no. 3 Morphological properties of isolates.

Sr. no	Culture	Colour	Reverse side pigments	Diffused pigmentation
1.	A1	White	White	-
2.	A2	White	Pale white	-
3.	A3	White with pink tinge	Pale white	-
4.	A4	White	Pale white	-
5.	A5	White	Pale white	-
6.	A6	Pale white (later grey)	Pale white (later black)	-
7.	A7	Pale white (later grey)	Pale white (later black)	-
8.	A8	White with pink at the centre	White	-
9.	A9	Grey-brown	Dark brown	-
10.	A10	White	Brown	Brown
11.	A11	Brown	Dark brown	Light brown
12.	A12	Brown with white margin	Brown	-
13.	A13	White	Pale white	-
14.	A14	White	Pale white	Light brown
15.	A15	Pink	Pale white	-
16.	A16	White	White	-
17.	A17	White	White	-
18.	A18	Yellow	Yellow	-
19.	A19	White	Pale white	Light brown
20.	A20	White to light violet/pink	White	-

Table no.4 PGP activities of Actinomycetes

Culture number	Indole acetic acid	Phosphate solubilization	Ammonia production	HCN	Amylase production	Cellulase production
1	-	+	+	-	-	-
5	-	-	+	-	-	-
6	+	+	+	-	-	+
7	+	+	+	-	+	-
8	+	+	+	+	-	+
9	+	+	+	-	+	+
13	+	+	+	-	+	+

14	+	-	-	-	+	+
15	-	+	+	-	+	+
16	-	-	-	-	+	+
17	-	+	+	-	+	+
19	-	-	+	-	+	+
20	+	-	+	+	+	+

Primary screening of dye decolorizing isolates:

Most of the isolates were able to degrade the dye, both on plate and broth. It was noticed that among the 20 isolates, isolates no. 6, 7, 9, 13, 18, and 19 had shown the maximum ability to degrade the red bandhani dye. A zone of clearance around the colonies on plates can be seen. That shows the dye degrading capacity of the isolates. In broth the samples in test tube can be see getting colour-less indicating the degradation of dye with particular isolates. Isolate no. 6,7,19 were found to be highly efficient strains and were selected for further studies. The details can be observed in fig. no. 3 & 4

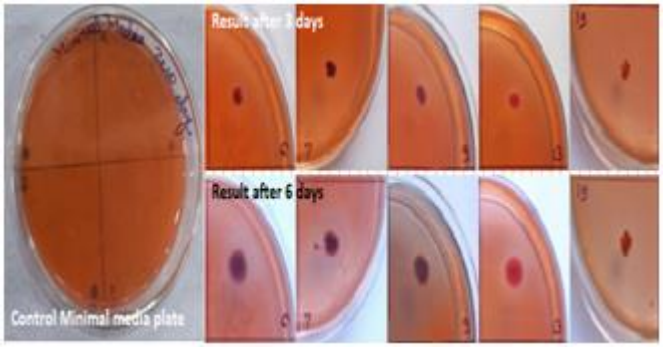

Figure no. 3 The dye degradation (Before and after)

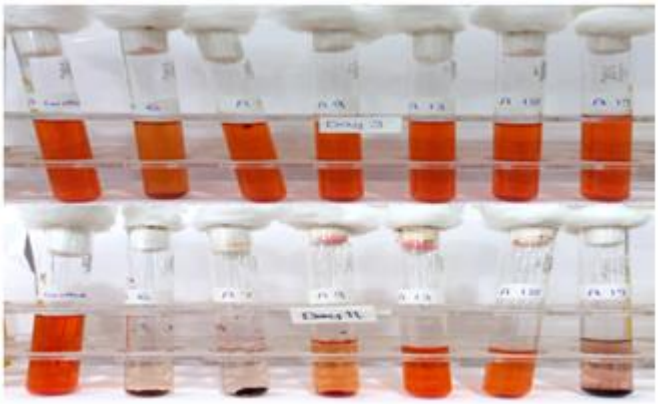

Figure no. 4 The dye degradation (showing the results from day 3 and 11)

Screening of Actinomycetes for PGPR activity:

The findings of the PGPR activity revealed that some of the isolates had almost all of the PGP activities (table no. 4).Out of 13 isolates, 4 isolates were showing IAA production, 8 isolates showed phosphate solubilization, 6 isolates were showing ammonia production, 2 isolates were showing HCN production, 5 and 11 isolates were showing amylase and cellulase production activity respectively.

Discussion: Several studies have shown that various microorganisms can degrade synthetic textile dyes in liquid medium and can be used to treat textile effluent to prevent soil and water contamination (Shafkat *et al* ., 2017). Bacterial strains have been studied mostly for the same approach but less work has been done with actinomycetes. However, no work has been done to develop a strategy for removing dye-pollutants using plant growth promoting bacteria, even though that different studies have shown that bacterial species belonging to the same genus can degrade dyes and promote plant growth (Hsueh *et al* ., 2009; Kang *et al* ., 2009).

The Plant growth promoting potential of bacterial strains is primarily attributed to their ability to solubilize inorganic phosphates, which facilitates phosphates acquisition from soil due to acidosis caused primarily by microbial synthesis of organic acids. The organic acid produced in the rhizospheric environment lower the microenvironment's pH. This enables the cation-free phosphates to be utilized by plants in its primary (HPO4) and secondary (H2PO4) ortho-phosphate. (Trivedi, P.and Sa, T., 2007). Results revealed that the isolated strains possess the said activity. Nearly 8 different strains studied for

phosphate solubilisation activity showed positive results.

Majority of rhizospheric actinomycetes are able to produce IAA. It is responsible for enhancing the growth of adventitious root system that helps in uptake of large volume of nutrients and water from soil. The tryptophan in the media serves as a precursor for IAA synthesis. Actinomycetes isolated from rhizosphere mostly consist of IAA production capacity (Wahyudi *et al.*, 2019). 7 strains were able to produce IAA from the total selected isolates.

Ammonia and HCN production is also one of the characteristic features of rhizospheric bacteria. They can synthesize ammonia and provide nitrogen to the plants in vicinity. It has important role in HCN production. HCN provides protection from plant pathogenic disease (Marques *et al.* 2010). 11 of the isolates revealed the ammonia production capacity and 2 of them were able to produce HCN.

Some of the actinomycetes that live in extreme habitat also show significant effects on plant growth in adverse conditions. Species from thermophilic conditions reaching 60°C can be used as an inoculum and hence are known as the winners of arid conditions (Marasco *et al.*, 2021). According to Marasco the change in arid conditions changes the interaction between the plant and microbial community. In addition to shielding plants from the damaging effects of drought, actinobacterial inoculation has been shown to significantly improve physiological indices in plants (Chukwuneme *et al.*, 2020). Numerous halotolerant Actinobacteria have been identified from saline environments, and they have demonstrated their value as protective agents for plants under stress (Boukhatem, Merabet and Tsaki, 2022).

The present study demonstrates the dual ability of the isolates that is, the degradation of synthetic dyes and also plant growth promotion activity. This distinguishing feature makes it possible to recycle the industrial wastewater in a manner that is used as an irrigation source for cropping. Treated water when applied for seed germination, had positive effects on plant growth parameters such as root and shoot length, compared with those of non-inoculated plants. Among all the isolates studied for dye degradation and PGP activity, isolate no. 6 showed the dual nature that can be applied for both the purpose simultaneously. Hence, shows applicability for sustainable environment formation.

IV. CONCLUSION

Due to water scarcity in drought regions it is absolute necessity to make waste water reusable for cropping purpose. The direct use of waste water from industries such as textile industry is unacceptable for crop development because of harmful effects. These effects can be reversed by the use of actinomycetes capable of removing the pollutants from environment. The isolates with dye removal capacity along with additional traits such as P-solubilisation, IAA production, ammonia and HCN production capacity are a very effective in treating waste water, making it available for cropping and increasing plant biomass.

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The Vital Role of Organic Chemistry in the Process of Drug Development

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ABSTRACT

The foundation of contemporary drug development is organic chemistry, which is essential to the creation, synthesis, and refinement of medicinal compounds. The critical roles that organic chemistry plays in each phase of the drug development process—from target identification to clinical trials are examined in this article. It explores medicinal chemistry, synthetic techniques, and Structure-Activity Relationship (SAR) research, emphasising their importance in the development of new therapeutic drugs. It also covers the development of computer tools and methods for organic synthesis, which have transformed the effectiveness and accuracy of drug design. This article explains how the study of organic chemistry enables scientists to tackle challenging biological problems, find effective and targeted medications more quickly, and ultimately enhance patient outcomes.

Keywords: Drug Development, Organic Chemistry, Medicinal Chemistry, Structure-Activity Relationship (SAR)

I. INTRODUCTION

The development of novel pharmaceuticals relies heavily on organic chemistry. By using their understanding of organic chemistry principles, medicinal chemists synthesize and manufacture molecules with specific biological effects. This entails making changes to molecular structures to enhance characteristics including safety, selectivity, and potency. A large number of pharmaceutical medications are organic molecules that are created using intricate chemical processes (Fig. 1). The basis for the regulated and repeatable synthesis of these active compounds is provided by organic chemistry. Microorganisms, fungi, and plants are among the natural sources from which many medications are produced. These natural products are valuable sources of lead compounds for drug development, and organic

chemistry is essential in their isolation, characterization, and synthesis. The whole pharmaceutical development process relies on organic chemistry, from the identification of potential novel drugs to their synthesis, optimization, and pharmacological property analysis[1], [2], [3].

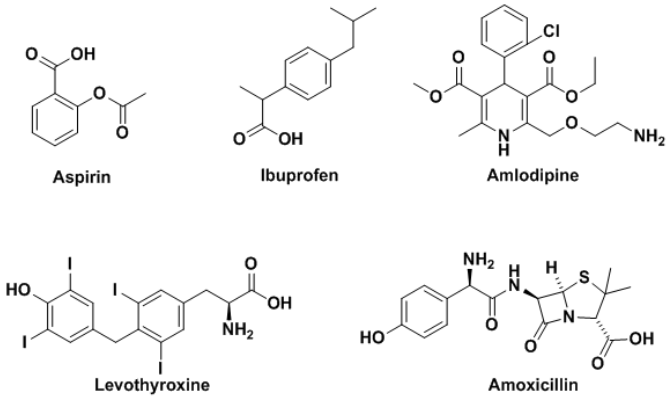

Figure 1 Highly Prescribed Drugs

II. ORGANIC CHEMISTRY PLAYS A CRUCIAL ROLE IN DRUG DISCOVERY

Organic chemistry serves as the backbone of modern drug discovery, bridging the gap between scientific theory and practical application. It is the art and science of designing, synthesizing, and optimizing chemical compounds that can effectively combat diseases and improve human health. Let's delve into how organic chemistry contributes at various stages of drug development[3], [4].

The first step in this process is target identification where organic chemistry principles help in understanding the target's structure and function and investigators identify specific biological targets (such as proteins or molecules) associated with diseases. Later, in the Lead Discovery process, organic chemists synthesize and modify small molecules (leads) that can interact with the target. It involves the hunt for potential compounds or molecules that have the desired biological activity to become a drug candidate. After the successful synthesis of a small molecule, it is subjected to the Structure-Activity Relationship (SAR) where organic chemists study the relationship between the chemical structure of a compound and its biological activity. SAR guides lead optimisation by modifying functional groups, stereochemistry, and framework. Structure-Activity Relationship (SAR) is a fundamental concept in medicinal chemistry and pharmacology. It refers to the relationship between the chemical structure of a molecule (such as a drug or a lead compound) and its biological activity or pharmacological effect. SAR studies aim to understand how specific structural features of a molecule influence its interaction with a biological target, such as a receptor, enzyme, or ion channel, and ultimately determine its pharmacological properties.

Figure 2Steps Involved in Drug Discovery

After successful lead optimisation, the organic chemist provides adequate quantities of the synthesized lead compound for preclinical testing. A preclinical trial of a lead compound involves testing the compound in laboratory and animal studies before it's tested in humans. This stage helps determine the compound's safety profile, efficiency, and potential toxic effects, providing vital data for designing human clinical trials. After preclinical trials, a lead compound moves into clinical trials, which involve testing the compound in humans. Clinical trials are conducted in several phases (Phase 1, 2, 3, and sometimes Phase 4), each aimed at gathering specific data regarding safety, dosage, effectiveness, and side effects in human subjects. These trials are essential steps in the drug development process before regulatory approval and eventual market release[5], [6].

Process chemistry focuses on refining and perfecting chemical reactions and procedures to be used in production on a large scale. It aims to convert small-

scale laboratory synthetic methods into efficient and scalable procedures for the mass production of chemicals, medicines, and other goods. This discipline combines ideas from chemistry, engineering, and economics to guarantee a safe, efficient, and ecologically sustainable manufacturing process. Organic chemistry contributes to the NDA submission by providing data on synthesis, purity, and stability[7].

III.CONCLUSION

In summary, organic chemistry continues to be the most useful science, and it is also the one that plays the most important part in the process of developing new drugs. It is the foundation upon which the framework for drug development is built, and it is the driving force behind the expansion of the pharmaceutical sector.

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The Role of Co^{+3}, Ni^{+2}, Cu^{+2} and its Hydrazone Metal Ion Chelates in Alcohol Fermentation

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ABSTRACT

The utilization of metal complexes as catalysts in alcohol fermentation processes represents a burgeoning area of research with promising implications for sustainable biofuel production. This abstract outlines the current state of knowledge regarding the application of metal complexes in enhancing alcohol fermentation reactions. Transition metal complexes, owing to their diverse coordination geometries and redox properties, exhibit catalytic activity in various steps of the fermentation pathway. This includes facilitating the conversion of sugars into alcohol, improving the efficiency of microbial fermentation, and mitigating inhibitory effects on microorganisms. Notably, cobalt, nickel, and iron complexes have emerged as prominent candidates for catalyzing alcohol fermentation reactions due to their versatile reactivity and low toxicity. The synergistic interplay between metal complexes and biological catalysts presents opportunities for optimizing fermentation conditions and enhancing overall process efficiency. However, challenges such as catalyst stability, selectivity, and economic viability warrant further investigation. This abstract provides a comprehensive overview of recent advancements, challenges, and future prospects in the utilization of metal complexes as catalysts in alcohol fermentation, thereby contributing to the advancement of sustainable biofuel production.

Keywords: Ligands, metal ion chelates, Biomass, Alcohol.

I. INTRODUCTION

Alcohol fermentation, a biochemical process converting sugars into alcohol and carbon dioxide, has been utilized for centuries in various applications, from brewing beverages to producing biofuels. In recent years, the exploration of novel catalysts to enhance the efficiency and sustainability of alcohol fermentation has gained significant attention. Metal complexes, with their diverse coordination geometries

and redox properties, offer promising avenues for catalyzing key steps in the fermentation pathway, thereby improving overall process performance.Transition metal complexes, in particular, have emerged as notable candidates for catalytic applications in alcohol fermentation. These complexes exhibit a wide range of catalytic activities, including facilitating sugar conversion, enhancing microbial fermentation rates, and mitigating inhibitory effects on fermentation microorganisms. Among the transition metals, cobalt, nickel, and iron complexes have garnered significant interest due to their abundance, low toxicity, and versatile reactivity.

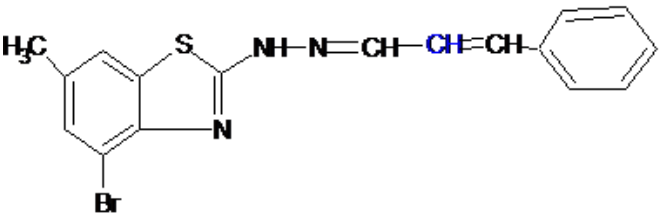
The synergistic interaction between metal complexes and biological catalysts presents opportunities for optimizing fermentation conditions and increasing product yields. Metal complexes can act as cofactors, modulating enzymatic activity, or as independent catalysts accelerating specific biochemical reactions. Additionally, the design and synthesis of tailor-made metal complexes allow for fine-tuning their catalytic properties, such as selectivity, stability, and substrate specificity.

Despite the considerable advancements in the field, challenges remain in the utilization of metal complexes as catalysts in alcohol fermentation. These include ensuring catalyst stability under fermentation conditions, minimizing side reactions, and addressing the economic feasibility of catalyst production and recovery. Furthermore, understanding the intricate interplay between metal complexes and fermentation microorganisms is crucial for optimizing catalyst performance and process efficiency.

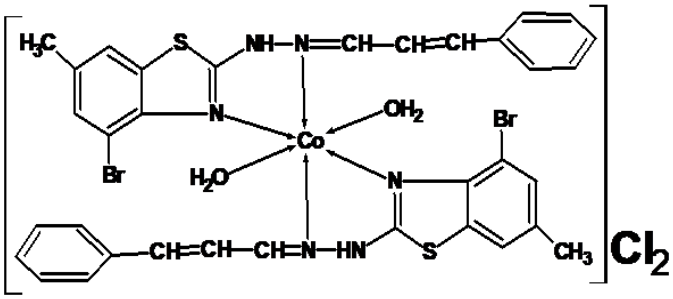
This paper aims to provide a comprehensive overview of the current state of research on metal complexes used as catalysts in alcohol fermentation. It will explore recent advancements, challenges, and future prospects in this rapidly evolving field, with a focus on the catalytic mechanisms, applications, and potential implications for sustainable biofuel production.

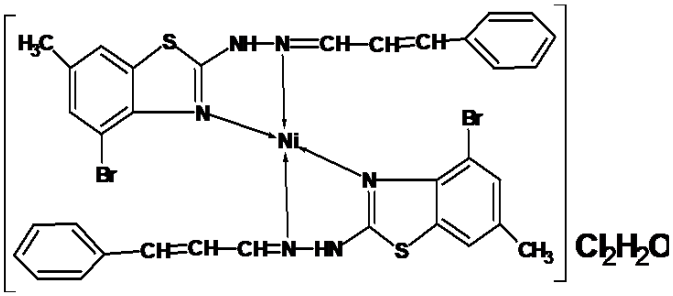
II. MATERIAL AND METHODS

Ligamd-2-(Cinnamyl)-4-bromo-6-methyl benzothiazolyl hydrazones (It consider CBMBTH) and its metal chelates of Co^{+3}, Ni^{+2}, Cu^{+3} are used which are previously Reported. Structure of ligand and its metal chelates of Co^{+3}, Ni^{+2}, $Cu^{+3}$are as.

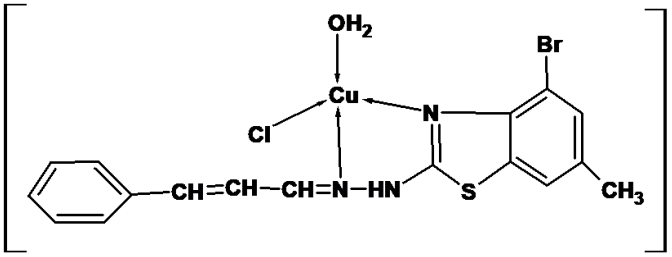


2-(Cinnamyl)-4-bromo-6-methyl benzothiazolyl hydrazones (It consider CBMBTH)





$[Co(HMPBMBTH)_2]\ Cl_2H_2O[Ni(CBMBTH)_2]\ Cl_2\ H_2O$



[Cu(CBMBTH) H_2O Cl] Cl

Microorganism- yeast used in the study was Saccharomyces Cerevisieae collected from local market. The culture was mainted on solid yeast medium.

Molasses.- The molasses were obtained from Bhaurao Co.op. sugar industries, Laxmi Nager, Nanded.

Batch process is adopted in fermentation.

The % of reducing sugar is about 40% the molasses were diluted to prepare different concentration of sugars. The production medium is supplemented with nitrogen and Phosphate . The pH of medium was adjusted to 5.

Media composition.

KH_2PO_4- 0.1%

$(NH_4)_2SO_4$ 0.5%

$MgSO_4$ $7H_2O$ 0.05%

Yeast extract 0.1%

pH 5

The pH of medium was adjusted by putting drop by drop dil. sulphuric acid .2-(Cinnamyl)-4-bromo-6-methyl benzothiazolyl hydrazones(It consider CBMBTH) metal chelates and metal salts were added in different experimental fermentation containers.

The fermentation flask were arranged and they were labeled along with yeast catalyst. Metal salt and metal chelates were added. pH were adjusted.

Experimental:-To study the effect of the metal ions and metal chelates different experiment were carried out. The entire fermentation flask were sterilized. All solution which are used for the experiment were also sterilized. 6 fermentation flask (sterilized) were arranged and they were labeled from 1 to 6.Fermentation flask no.1 is used as control, Fermentation flask no. 2 is used to study the effect of 2-(Cinnamyl)-4-bromo-6-methyl benzothiazolyl hydrazones and flask no.3were used to study the effect of $[Co(HMPBMBTH)_2]$ Cl_2. H_2O on fermentation process In conical flask no. 4 $[Ni(CBMBTH)_2]$ Cl_2 H_2O and Conical no. 5 [Cu(CBMBTH) H_2O Cl] Cl and Conical No. 6$CrCl_3$ salt 0.05 gm were added.Solution like 0.05% $KHSO_4$, 0.5% $(NH_4)_2SO_4$, 0.05 % $MgSO_4$ $7H_2O$ and as mentioned above different metal ion were added . The pH of the experiment is adjusted by putting drop by drop dil. H_2SO_4.The fermentation reaction were carried out for 24 hours. In each fermentation flask 10 ml 1% molasses solution and media

Estimation of biomass.-

The quantity of biomass depends upon yeast growth takes place. The dry biomass was measured by transferring the content of conical flask through the filter paper. The residue of biomass which is collected on the filter paper is dried by keeping it in oven at 100°C. The mass of biomass were recorded and it is given in the table.

Estimation of ethyl alcohol.-

Spectroscopic method is used to determine the alcohol generated in the fermentation process.Fermented wash were taken in distillation flask. 15 ml distillate were collected in the conical flask. 5 ml $K_2Cr_2O_7$ (0.1N) in 0.1N H_2SO_4 solution were added it is warmed at 60°C . The color obtained. The optical density of solution were measured from standard graph. The ethanolgenerated during fermentation were determined.

Table of Observations

Observation table.

Sr. No.	Flask No.	Compounds	%wt. of biomass	% Alcohol
1	1	Control	1.09	2.05
2	2	(CBMBTH)	1.15	2.16
3	3	$[Co(HMPBMBTH)_2]$ Cl_2. H_2O	1.48	2.38
4	4	$[Ni(CBMBTH)_2]$ Cl_2 H_2O	1.33	2.30
5	5	[Cu(CBMBTH) H_2O Cl] Cl	1.75	2.58
6	6	$CoCl_2$	1.13	2.19
7	7	$NiCl_2$	1.18	2.20
8	8	$CuCl_2$	1.15	2.13

III.RESULT AND DISCUSSION

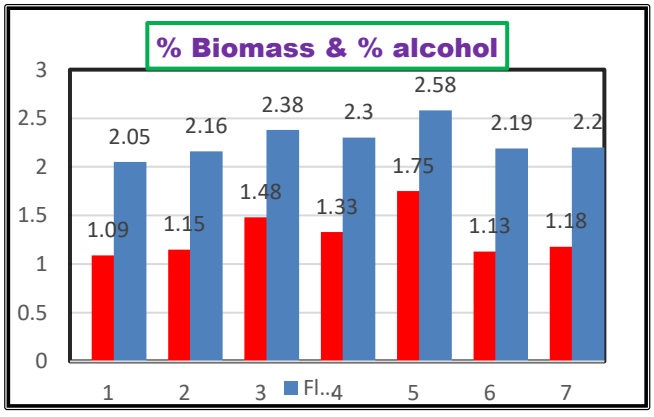
The experiment involved investigating various compounds for their catalytic activity in the conversion of biomass to alcohol. Each compound was tested for its effectiveness in promoting this conversion, with the percentage weight of biomass and the resulting alcohol yield recorded.

The control group, without any added catalyst (Flask No. 1), exhibited a biomass weight percentage of 1.09% and produced alcohol at a rate of 2.05%. Comparatively, the introduction of CBMBTH (Flask No. 2) resulted in a slightly higher biomass weight percentage of 1.15% and a marginally increased alcohol yield of 2.16%.The compounds $[Co(HMPBMBTH)_2]$ $Cl_2 \cdot H_2O$ (Flask No. 3), $[Ni(CBMBTH)_2]$ $Cl_2 \cdot H_2O$ (Flask No. 4), and $[Cu(CBMBTH)H_2OCl]Cl$ (Flask No. 5) displayed progressively higher biomass weight percentages of 1.48%, 1.33%, and 1.75%, respectively. Consequently, these compounds also exhibited higher alcohol yields of 2.38%, 2.30%, and 2.58%, indicating their potential as effective catalysts in the conversion process.

Flasks containing individual metal chlorides—$CoCl_2$ (Flask No. 6), $CuCl_2$ (Flask No. 7), and $NiCl_2$ (Flask No. 8)—were also tested. These exhibited biomass weight percentages ranging from 1.13% to 1.18% and alcohol yields ranging from 2.13% to 2.20%. While these results show some catalytic activity, they generally fell short in comparison to the performance of the organic-metal compounds tested.

Overall, the experiment highlights the varying catalytic efficiencies of different compounds in biomass conversion to alcohol. Compounds featuring organic ligands, such as CBMBTH, along with specific metal ions, demonstrated more pronounced catalytic effects compared to metal chlorides alone. This suggests the potential for further exploration and optimization of organic-metal catalysts in biomass conversion processes and generation of alcohol.

Graphical representation of Results.



IV. CONCLUSION

In conclusion, the experiment investigated the catalytic activity of various compounds in converting biomass to alcohol. Results showed that compounds incorporating organic ligands, particularly CBMBTH, in combination with specific metal ions, displayed superior catalytic efficiency compared to metal chlorides alone. The control group and metal chloride-containing flasks exhibited moderate catalytic activity, but organic-metal compounds consistently outperformed them in both biomass weight percentage and alcohol yield. These findings underscore the potential for further exploration and optimization of organic-metal catalysts in biomass conversion processes. This study suggests promising avenues for enhancing alcohol production through the utilization of tailored catalysts, which could contribute to the advancement of sustainable energy generation and biofuel production.

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Review on Anti-Inflammatory, Anti-Bacterial and Anti-Cancer Properties of Thiazole Derivatives

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ABSTRACT

A review study contains synthesis, characterization and biological significance of substituted thiazole derivatives. Thiazole derivatives have great importance in the field of medical science due to their anti-inflammatory and anti-bacterial and anti-cancer properties.

Keywords: Thiazole derivatives, anti-cancer, anti-bacterial and anti-inflammatory properties.

I. INTRODUCTION

One of the leading causes of death worldwide is still cancer. Due to resistance, toxicity, and a lack of target specificity and selectivity, the anticancer drug therapy now in use is inadequate[1]. Antimicrobial drug researchers are actively focusing on finding novel targets and chemical entities with antibacterial activity in order to counteract the rapid development of medicine resistance. In light of the aforementioned, continued progress in the creation and development of antibacterial and anticancer medications is desperately needed. Because thiazoles contain a wide range of biological activities, including antibacterial[2-5], cytotoxic[4-14], antiviral[15], anti-HIV[16], antimicrobial[17] and analgesic properties[18], many research have been

conducted using them to treat various illnesses. Furthermore, one of the most popular scaffolds for creating and finding novel lead compounds particularly anticancer drugs is the thiazole derivative[19-23]. Furthermore, thiazole-containing substances have been identified in a number of clinically accessible anticancer medications, including dabrafenib[24], ixabepilone[25] and dasatinib[26].

II. RESULTS AND DISCUSSION

All paragraphs Aejaz Ahemad and etal[27] demonstrated a novel collection of trisubstituted thiazole compounds was synthesized and described, and their anti-inflammatory properties were assessed both in vitro and in vivo. 2,3,5-trisustituted thiazole observed maximum HBRC membrane stabilization. In rats, this combination of trisubstituted thiazole compounds offered the same level of protection against inflammation of the paw. The potential antibacterial efficacy of synthesized trisubstituted thiazole compounds against harmful bacteria, such as S. aureus, E. faecalis, E. coli, and P. aeruginosa, was also evaluated. These compounds have promise antibacterial action, according to the preliminary screening results.

W. X. Cai and etal[28] revealed the synthesis of novel 2-phenyl-4-trifluoromethyl thiazole-5-carboxamide derivatives and evaluate the and evaluated for their anticancer activity against A-549, Bel7402, and HCT-8 cell lines.

L. A. Al-Mutabagani[29] elaborated the synthesis of thiazolylhydrazonothiazole derivatives. Most of the thiazole compounds screened were effective against Gram-positive and Gram-negative bacteria. Most of prepared compounds showed antibacterial properties through minimum inhibitory concentration method. HepG-2 (liver carcinoma), HCT-116 (colorectal carcinoma), and MDA-MB231 (breast carcinoma) cell lines were used to investigate the cytotoxic activities of the drug in comparison to the reference drug cisplatin and using the colorimetric MTT assay.

K. N. Weib[30] developed A simple two-step, one-pot method has been established for the synthesis of 1,3-thiazole heterocycles. It involves the epoxidation of nitro-olefins using the t-BuOOH/DBU system, followed by a moderate reaction between a-nitro-epoxides and thioamides.

J. Ramirez and co-workers[31] were obtained a new series of novel thiazole-based 8,9-dihydro-7H-pyrimido[4,5-b][1,4]diazepines with high regioselectivity from the reaction of triamino- or tetraaminopyrimidines with α, β-unsaturated carbonyl compounds based on 2,4-dichlorothiazol-5-carbaldehyde. The US National Cancer Institute (NCI) evaluated twelve of the produced compounds for their anticancer efficacy against 60 distinct human tumor cell lines.

A. R Sayed and etal[32] studied the efficient synthesis of novel 1-(2-(2-benzylidenehydrazinyl)-4-methylthiazol5-yl)ethenone. Also demonstrated the in vitro growth inhibitory activity of the synthesized compounds against three tumor cells (HCT116, HT-29 and HepG2) was investigated in comparison with harmine and cisplatin reference drugs using an MTT assay and the results revealed promising activities of three compounds.

Shivkant Sharma and co-workers[33] studied retrospective study on synthesis, structure-activity Relationship and therapeutic Significance of thiazoles.

Sobhi M. Gomha and etal[34] revealed the preparation of thiazoles contain 1,3,4-thiadiazole which were synthesized via the reaction of the 2-(4-methyl-2-phenylthiazole-5-carbonyl)-N-phenylhydrazinecarbo-thioamide with the appropriate hydrazonoyl chlorides. The cytotoxic effect of thiadiazoles were due to ability to suppress the growth of the liver HepG2 cancer cell line.

III.CONCLUSION

The literature study reported the synthesis and characterization of thiazole derivatives. Thiazole derivatives have remarkable attention due their anti-inflammatory and anti-bacterial and anti-cancer

properties. The anti-cancer study indicated that thiazole derivatives shown anti-cancer activity againstHepG-2 (liver carcinoma), HCT-116 (colorectal carcinoma), MDA-MB231 (breast carcinoma),A-549, Bel7402, and HCT-8 cell lines.

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Micro Expression to Detect Criminal Behaviour Using AI

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ABSTRACT

This project represents a significant advancement in the realm of human-computer interaction, focusing on real-time emotion detection using deep learning techniques. Emotion recognition, a critical face to fhuman communication, has garnered substantial interest for its applicability in diverse fields, from user experience enhancement to mental health assessment. Leveraging the power of Convolutional Neural Networks (CNNs), this system has been meticulously engineered to instant aneously discern human emotions from live webcam feeds. This project's advantages encompass its real-time capability, non-intrusiveness, automation, and educational potential. It can be employed for diverse applications, including human-computer interaction, affective computing, and entertainment. However, its limitations encompass a limited emotion repertoire, reliance on facial expressions alone, potential cultural variations, and susceptibility to environmental factors. This is useful to find out the emotion of criminal during investigation.

Keywords: FER, HCI, DL

I. INTRODUCTION

Facial emotions are important factors in human communication that help to understand theintentions of Criminal. In general, people identify the emotional state of other people, such as joy,sadness and anger, using vocal tones and facial Expression. Facial expressions are one of medium channels in interpersonal communication. Therefore, it is natural that facial emotion research used to gained a lot of attention over the past decade with applications in perceptual and cognitive sciences. Interest in automatic Facial Emotion Recognition (FER) has also been popular recently with the rapid development of Artificial Intelligent (AI) techniques. They are now used in many applications and their exposure to humans is increasing. To improve Human Computer Interaction (HCI) and make it more real, machines must be provided with the capability to understand the surrounding environment, especially the

intentions of humans. Machines can gather their environment state through cameras andsensors. In recent years, Deep Learning (DL) algorithms have proven to be successful incapturing environment states. Emotion detection is necessary for machines to provide information about the inner state of humans. A machine canuse an umber offacial images with DL techniques to determine humane motions .

II. METHODS AND MATERIAL

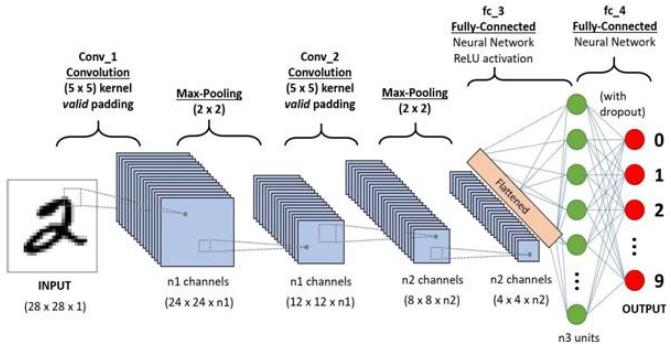


Fig 1 Block diagram of CNN model

1. Convolutional layers:

Filters are applied to the original image, or to other feature maps in a deep CNN.Here most of the user-specified parameters are in the network.The most important parameters are the number of kernels and the size of the kernels.

2. Pooling layers:

Similar to convolutional layers, but it performs max pooling oraverage pooling.It is used to reduce the dimensionality of the network

3. Fully connected layers:

Placed before the classification output of a CNN.Used to flatten the results before classification.This is similar to the output layer of an MLP.

2.1 Working of Project

1. Importing Libraries:

The code starts by importing various Python libraries such as Keras for building the CNN, OpenCV for computer vision tasks, and pandas for data manipulation.

2. Data Loading:

It reads a CSV file named 'fer2013.csv' containing facial expression data. This dataset is divided into training and testing sets.

3. Data Preprocessing:

The pixel data for each image is extracted and reshaped into a 48x48 grayscale image.
Labels (emotions) are also extracted from the dataset.
The data is split into training and testing sets for model training and evaluation.

4. CNN Model Definition:

A convolutional neural network (CNN) model is defined using Keras. This model consists of multiple convolutional layers, max-pooling layers, and fully connected layers.
It's designed to learn features from the facial expression images.

5. Model Compilation:

The model is compiled with a categorical cross-entropy loss function and the Adam optimizer.

6. Data Preparation:

The labels are one-hot encoded for training and testing data.

7. Model Training:

The model is trained using the training data and labels. The training process is visualized with accuracy and loss plots for both training and validation data.

8. Model Saving and Loading:

The trained model weights are saved to a file named 'model.h5'.
Later, the model is loaded from the saved file.

9. Emotion Detection:

The code captures video from the webcam in real-time.

It uses a Haar Cascade Classifier to detect faces in each frame.

For each detected face, it extracts the region of interest (ROI) and resizes it to 48x48 pixels.

The CNN model predicts the emotion label for each ROI.

The predicted emotion is overlaid onto the frame.

10. Displaying the Webcam Feed:

The webcam feed is displayed in a window.

Emotion predictions are shown in real-time above each detected face.

Pressing 'q' quits the webcam feed, ending the program.

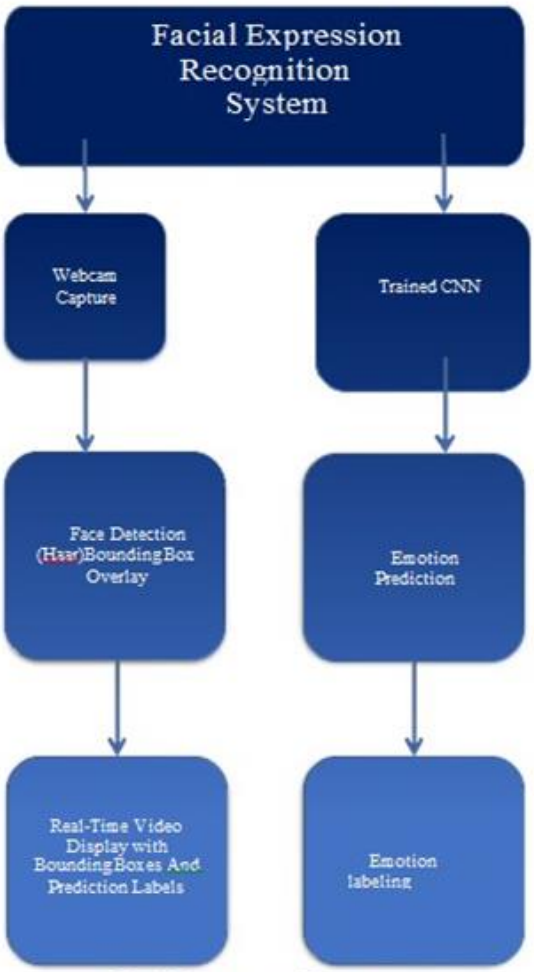


Fig 4.1.Structure of System

2.2 Literature Survey

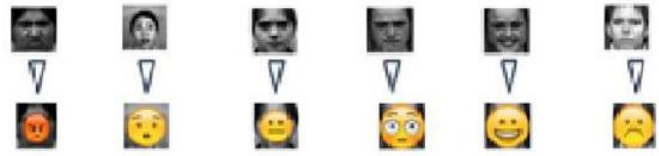
Paper	Techniques used	Accuracy	Research gap
Xiangwei Mou et al.IEEENov2023	CNN+FACS	80.55%	Small sample size and the constraints related to the age, gender, and subject background of the participants,
Irfanali.Hindawi. et.al Nov2023	CNN	97%	Match with selected images available in datasets
Gulnaz perveen.et.al.IEEE oct23	GDCNN+FER	95.33%	Limited dataset with difficultly for low level image
Sakshi Indolia.et.al.IEEE sept23	CASME-I+ CASME-II+ CASME-III	95.97% 98.59% 100%	Dataset are highly imbalance
Aamir Anwar.et.al.IEEE sept23	CNN+SVM	93.5%	challenges and limitations associated with

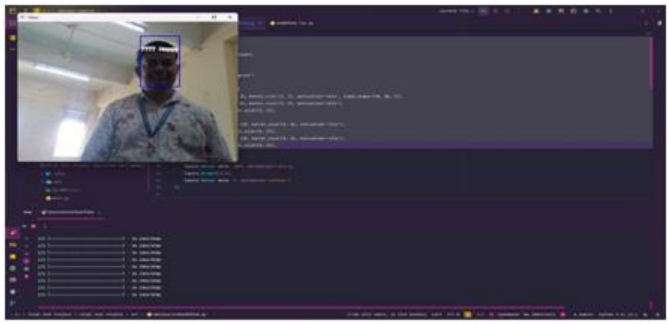
			capturing learners' emotions using FSA
Fei Li, Ping Nie et al.IEEE 22	MER	NIL	Difficult to recognize 3D facial Expressio n
Yante Li.et.al IEEE Dec22	MER	35%	MER poses significan t challenge s due to the limitation s of MEs
Chaehyeon et.al.IEEE Sept22	CASME-II	NIL	transferre d the model multiple times on various datasets in a specific order
GangWang et.al.IEEE Sept22	CASME-II+CNN	47%	Difficult to recognize multiple facial expressio n
Jinming Liu et.al.IEEE 21	CASME-II+CNN+M E	NIL	Difficult to analysis datasets

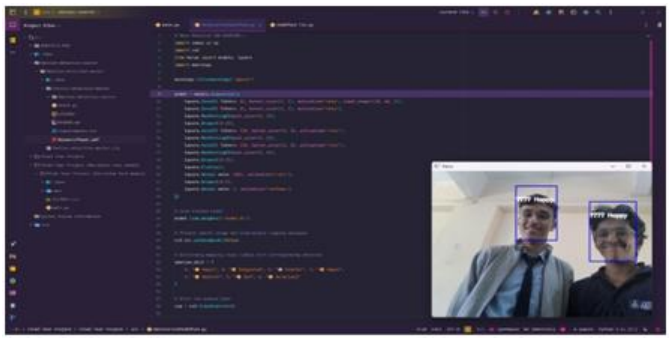
In above literature survey all reasearcher worked on single face emotion detection but my research work on multiple face emotion detection result on individual face emotion.This is the common research gap found in literature survey

III.RESULTS AND DISCUSSION

In this deep learning project, we have built a convolution neural network to recognize facial emotions. We have trained our model on the FER2013 dataset. Then we are mapping those emotions with the corresponding emojis or avatars. We are using OpenCV's haar cascade xml we are getting the bounding box of the faces in the webcam. Then we feed these boxes to the trained model for classification.







IV. CONCLUSION

A Conclusion of this research is that you identify the criminal behaviour by there facial expression.whether the person is anger,neutral,calm during investigation

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Exploring the Synergy: Analytical Chemistry Techniques in Forensic Science - Progress and Practicalities

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ABSTRACT

This paper explores the symbiotic relationship between analytical chemistry techniques and forensic science, focusing on the advancements made and the practical considerations involved. Analytical chemistry methods play a pivotal role in forensic investigations, enabling the precise analysis of evidence crucial for criminal proceedings. Through the integration of chromatography, mass spectrometry, spectroscopy, and microscopy, forensic scientists can identify substances, elucidate complex mixtures, and provide valuable insights into crime scenes. This review delves into the progress achieved in analytical techniques and their applications in forensic science, highlighting their role in enhancing investigative methodologies and ensuring justice. Additionally, practical challenges such as sample handling, instrumental limitations, and data interpretation are discussed, emphasizing the importance of robust methodologies and quality assurance measures. By exploring the synergy between analytical chemistry and forensic science, Overall, this paper underscores the indispensable role of analytical chemistry in forensic science and its contribution to the pursuit of justice. this paper aims to provide a comprehensive overview of the progress made and the practical considerations involved in utilizing these techniques for forensic analysis.

Keywords: Exploring Synergy, Analytical Chemistry, Techniques, Forensic Science, Progress, Practicalities.

I. INTRODUCTION

Analytical chemistry techniques serve as indispensable tools in the realm of forensic science, fostering a symbiotic relationship that drives advancements in investigative methodologies and ensures the pursuit of justice. This paper aims to

explore the intricate interplay between analytical chemistry techniques and forensic science, shedding light on the remarkable progress achieved and the practical considerations that underpin their application. Forensic science plays a pivotal role in the legal system by providing scientific evidence to support criminal investigations and court proceedings. Analytical chemistry is a key component of forensic science, enabling the identification and quantification of substances crucial for establishing facts in legal cases. This section provides an overview of the importance of analytical chemistry in forensic science and the specific challenges addressed by these techniques. By exploring the synergy between analytical chemistry and forensic science, this paper provides a comprehensive overview of the progress made and the practical considerations involved in harnessing these techniques for forensic analysis. Through a deeper understanding of this symbiotic relationship, we can further propel advancements in forensic science and contribute to the continual refinement of investigative practices.

II. INSTRUMENTATION IN FORENSIC ANALYTICAL CHEMISTRY

2.1 Mass Spectrometry in Forensic Toxicology:

Advances in mass spectrometry have significantly enhanced forensic toxicology, allowing for the detection and quantification of drugs, poisons, and metabolites in various biological samples. This section explores how techniques like liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) contribute to the accurate analysis of forensic samples.Mass spectrometry (MS) is a crucial analytical technique widely used in forensic toxicology for identifying and quantifying drugs, poisons, and other substances in biological samples. Forensic toxicologists employ MS alongside other techniques like liquid chromatography (LC) or gas chromatography (GC) to separate complex mixtures of compounds before subjecting them to MS analysis.

Here's how mass spectrometry is typically applied in forensic toxicology:

Sample Preparation: Biological samples such as blood, urine, saliva, or hair are collected from individuals suspected of drug use or poisoning. These samples are then processed to extract the target compounds of interest. Sample preparation methods may involve protein precipitation, liquid-liquid extraction, solid-phase extraction, or derivatization to improve the detection sensitivity and selectivity of the technique.

Chromatographic Separation: Before entering the mass spectrometer, the extracted compounds are often separated using techniques like liquid chromatography (LC) or gas chromatography (GC). LC-MS and GC-MS are two common configurations used in forensic toxicology. LC-MS is preferred for analyzing polar and thermally labile compounds, while GC-MS is suitable for volatile and thermally stable compounds.

Ionization: In mass spectrometry, ionization is a critical step where the analyte molecules are converted into ions. Common ionization techniques used in forensic toxicology include electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) for LC-MS, and electron ionization (EI) or chemical ionization (CI) for GC-MS. The choice of ionization technique depends on the nature of the analyte and the chromatographic method employed.

Mass Analysis: Once ionized, the ions are accelerated into the mass analyzer, which separates them based on their mass-to-charge ratio (m/z). Quadrupole, time-of-flight (TOF), ion trap, and magnetic sector analyzers are among the commonly used mass analyzers in forensic toxicology. The mass analyzer provides information about the mass and abundance of each ion present in the sample.

Detection and Data Analysis: The detector records the ions that pass through the mass analyzer, generating mass spectra that represent the distribution of ions in

the sample. Forensic toxicologists analyze these spectra to identify the presence of target compounds based on their unique mass-to-charge ratios and fragmentation patterns. Quantification is often achieved by comparing the signal intensity of the analyte ions to that of internal standards or calibration curves.

Interpretation and Reporting: Once the analysis is complete, forensic toxicologists interpret the results in the context of the case and prepare reports summarizing their findings. This information may be used as evidence in legal proceedings to determine issues such as drug intoxication, poisoning, or compliance with drug treatment programs.

Overall, mass spectrometry plays a crucial role in forensic toxicology by providing accurate and reliable analysis of a wide range of compounds, helping to support criminal investigations and legal proceedings.

2.2 Chromatographic Techniques in Trace Evidence Analysis:

Analytical chemistry methods such as gas chromatography (GC) and liquid chromatography (LC) are crucial for analyzing trace evidence found at crime scenes. The paper discusses how these techniques aid in the separation and identification of complex mixtures, including fibers, paints, and gunshot residue.

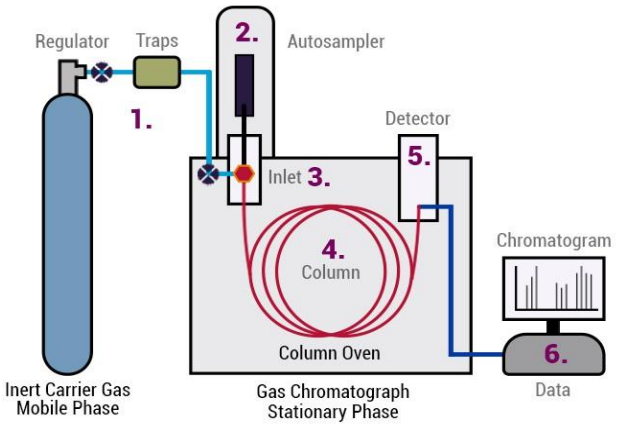
2.3 Spectroscopic Techniques for Substance Identification:

Spectroscopic methods, including infrared spectroscopy and Raman spectroscopy play a vital role in the rapid and non-destructive identification of substances in forensic analysis. This section explores their applications in identifying drugs, explosives, and other materials encountered in criminal investigations.Chromatographic techniques are essential in trace evidence analysis, allowing forensic scientists to separate complex mixtures of compounds into individual components for identification and quantification. These techniques are widely used in various forensic disciplines, including arson investigation, drug analysis, environmental forensics, and materials analysis. Here are some of the chromatographic techniques commonly employed in trace evidence analysis:

Gas Chromatography (GC):

Principle: GC separates volatile compounds based on their partitioning between a stationary phase (typically a liquid coating inside a capillary column) and a mobile phase (inert gas such as helium or nitrogen).

Applications: GC is particularly useful for analyzing volatile organic compounds (VOCs) such as accelerants in arson investigations, drugs of abuse, explosives residues, and environmental pollutants.



Instrumentation

Liquid Chromatography (LC):

Principle: LC separates compounds based on their differential interactions with a stationary phase (solid or liquid) and a mobile phase (liquid solvent).

Applications: LC is versatile and can be used for analyzing a wide range of compounds, including drugs, pesticides, dyes, and biomolecules like proteins and peptides.

High-Performance Liquid Chromatography (HPLC):

Principle: HPLC is a form of liquid chromatography that utilizes high pressure to improve separation efficiency and speed.

Applications: HPLC is commonly employed in forensic laboratories for analyzing drugs, toxins, and other trace substances in biological samples such as blood, urine, and tissue.

Thin-Layer Chromatography (TLC):

Principle: TLC separates compounds based on their differential migration rates through a thin layer of stationary phase (usually silica gel or alumina) coated on a glass or plastic plate.

Applications: TLC is often used for the preliminary screening of substances in forensic samples due to its simplicity, rapidity, and low cost. It can be applied to analyze drugs, explosives, ink, and dyes.

Ion Chromatography (IC):

Principle: IC separates ions based on their interactions with ion exchange or ion exclusion stationary phases.

Applications: IC is particularly useful for analyzing inorganic ions, such as those found in explosives residues, drugs, and environmental samples.

Supercritical Fluid Chromatography (SFC):

Principle: SFC uses supercritical fluids (typically carbon dioxide) as the mobile phase, offering advantages such as rapid analysis and compatibility with both polar and non-polar compounds.

Applications: SFC can be applied to analyze drugs, natural products, and other trace substances in forensic samples.

These chromatographic techniques are often coupled with detection methods such as mass spectrometry (GC-MS, LC-MS) or ultraviolet-visible spectroscopy (UV-Vis) to enhance sensitivity, selectivity, and identification capabilities in trace evidence analysis. Their integration enables forensic scientists to accurately identify and quantify trace substances, contributing valuable information to criminal investigations and legal proceedings.

III.METHODOLOGIES IN FORENSIC ANALYTICAL CHEMISTRY

3.1 DNA Analysis:

While not a traditional analytical chemistry technique, DNA analysis is an essential forensic tool. This section discusses the integration of analytical methods in DNA profiling, including polymerase chain reaction (PCR) and capillary electrophoresis, highlighting their role in establishing identity and relationships in criminal cases.

3.2 Forensic Imaging Techniques:

Analytical chemistry contributes to forensic imaging through technologies like mass spectrometry imaging (MSI) and nuclear magnetic resonance imaging (MRI). These methods aid in visualizing the distribution of substances within forensic samples, providing valuable spatial information.

IV. APPLICATIONS IN FORENSIC SCIENCE

4.1 Forensic Toxicology: Analytical chemistry techniques are integral in identifying and quantifying drugs, poisons, and alcohol in biological samples, contributing to determining the cause of death and evaluating impairment levels.

4.2 Trace Evidence Analysis: The paper explores how analytical chemistry assists in the analysis of microscopic traces found at crime scenes, including fibers, hair, and gunshot residue, aiding in establishing connections between individuals and locations.

4.3 Detection of Illicit Substances: Analytical chemistry methods are crucial in the identification and analysis of illicit substances such as drugs, explosives, and chemical warfare agents, providing essential evidence in criminal investigations.

Chromatographic techniques find extensive applications across various disciplines within forensic science, aiding in the analysis and interpretation of

evidence encountered in criminal investigations. Here are some key applications:

Drug Analysis:

Chromatography is used to identify and quantify drugs of abuse in biological fluids (blood, urine, saliva) and solid samples (powders, tablets).

It helps in detecting controlled substances, pharmaceutical drugs, and their metabolites, providing crucial evidence in cases related to drug trafficking, overdose, and impaired driving.

Arson Investigation:

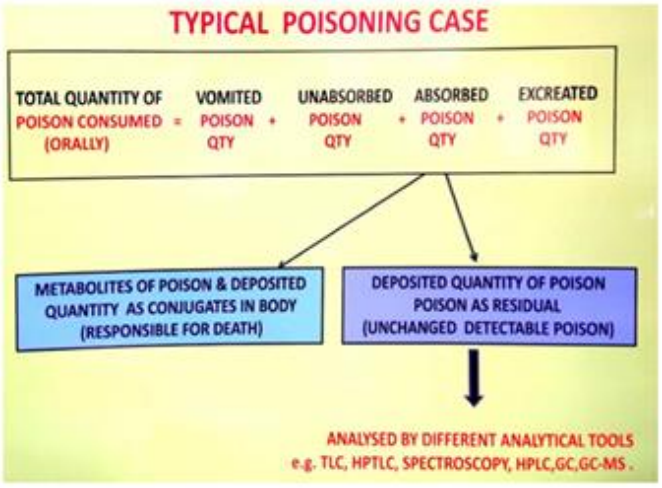
Chromatography assists in the analysis of accelerants (such as gasoline, kerosene, and lighter fluid) found at fire scenes.

Gas chromatography coupled with mass spectrometry (GC-MS) is commonly used to identify and characterize volatile residues left behind by accelerants, aiding in determining the cause and origin of fires.

Toxicology:

Chromatographic techniques are vital in identifying and quantifying toxic substances, poisons, and metabolites in biological samples.

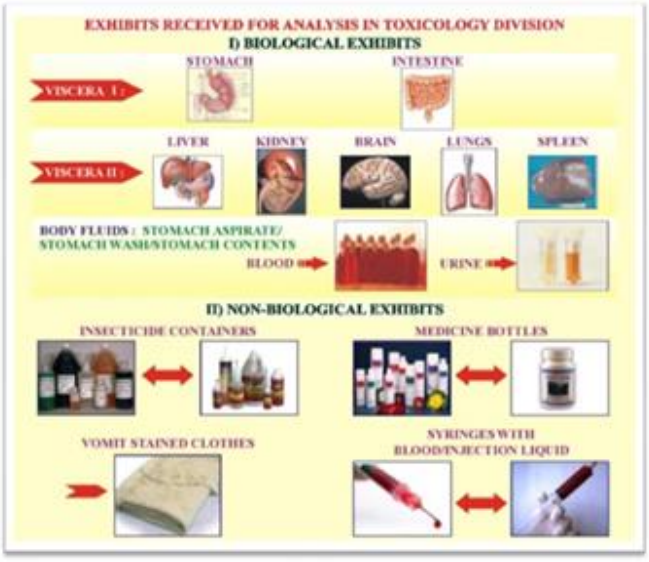
They provide essential evidence in cases involving poisoning, homicide, accidental deaths, and workplace exposures to hazardous chemicals.



Trace Evidence Analysis:

Chromatography helps analyze trace substances such as fibers, paints, ink, explosives residues, and gunshot residues.

It enables forensic scientists to identify and compare materials found at crime scenes with those from suspects or known sources, aiding in linking suspects to crime scenes or determining the source of evidentiary materials.



Forensic Anthropology:

Chromatographic techniques are used in analyzing organic residues on bone samples, such as blood, urine, or environmental contaminants.

They assist in determining postmortem interval, cause of death, and identifying drugs or toxins present in skeletal remains.

Environmental Forensics:

Chromatography helps analyze environmental samples such as soil, water, air, and vegetation for pollutants, toxins, and contaminants.

It aids in investigating environmental crimes, illegal dumping, and chemical spills, and assessing the impact of pollution on human health and ecosystems.

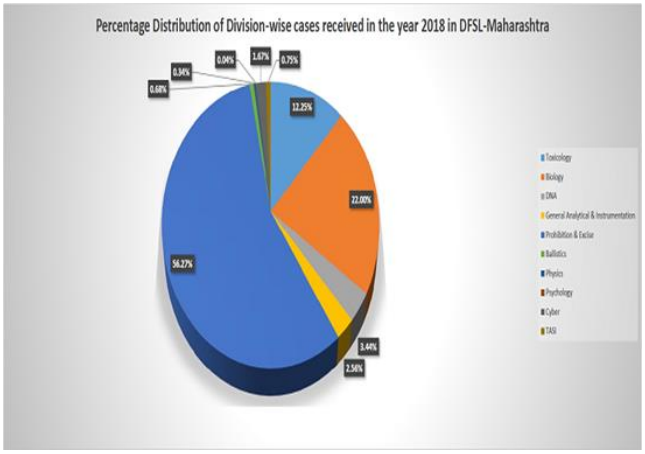
Forensic Chemistry:

Chromatography is widely utilized in analyzing non-biological evidence such as fibers, paints, glass fragments, and explosive residues.

It assists in identifying and characterizing chemical components of evidence materials, providing crucial information for reconstructing crime scenes and

establishing associations between suspects, victims, and crime scenes.

Overall, chromatographic techniques are indispensable tools in forensic science, providing valuable information and evidence critical for criminal investigations, legal proceedings, and the pursuit of justice.



V. CHALLENGES AND FUTURE DIRECTIONS

The paper concludes with a discussion of the challenges faced by forensic analytical chemistry, including sample complexity, legal admissibility, and the need for standardization. Future directions highlight potential advancements in technology and methodologies, emphasizing the continuous improvement of forensic analytical techniques.

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Biotransformation of 1, 3-Diaryl-2-Propene-1-Ones To Benzopyran 4-One By Using Enterobacter Hormaechei SSwC2 As A Biocatalyst

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ABSTRACT

Microorganisms showed their potential ability to degrade bioactive natural and synthetic compounds. Enterobacter hormaecheiSSwC2 isolated from sweet curd obtained from Seema milk dairy Aurangabad and used for biotransformation of heteroaldehyde under laboratory conditions on MSM (minimal salt medium) containing malt extract-0.1%, yeast extract-0.2%, glucose-0.5%, peptone-0.2%medium at 30oC for 10 days. After incubation sample was harvested and analysed by TLC, 1H NMR, LR MS. The product which formed in the reaction using Enterobacter hormaechei SSwC2 was confirmed from spectral analysis was flavone derivitive.The biotransformation of heteroaldehyde by Enterobacter hormaechei SSwC2reaction was regioselective.Benzopyranwas described here by the condensation of heteroaldehyde.

Keywords: Biotransformation, Enterobacter hormaechei SSwC2, benzopyran.

I. INTRODUCTION

Microorganisms and their enzymes have proved to be versatile biocatalyst and are involved in the conversion of complex organic materials[1]. Microorganisms performed several chemical reactions as an alternative to obtain products of chemical and biological interest[2]. Biotransformation is a conversion of natural or synthetic precursors into products of increased value. Whole cell or catalytic enzyme used many different conditions such as free, immobilized. Biotransformation is a combinational work of chemistry and microbiology [3]. Such reactions certain advantages over the conventional reactions as performed in aqueous systems and at neutral pH, preventing the hazards of solvents in conventional synthesis [4-7].

Chalcones are α,β unsaturated ketones and precursors of flavonoids in which olefinic and carbonyl fragments are linked to an aromatic ring[6].

Chalcones can be obtained from natural sources or by synthesis. The functional group present on chalcone shows biological activity like antiviral activity, inhibition of NS3 protease of dengue virus [9], activity against herpes simplex virus, HIV-1 replication inhibition in lymphocytes [10]. The main reaction during biotransformation are hydrogenation, dehydrogenation, O-methylation, glycosylation, hydroxylations, dehydroxylations, C-ring cleavage, cyclization and carbonyl reduction. Chalcones were regioselectively cyclized to flavones. Hydrogenation of flavonoids was reported on transformation of chlacones to dihydrocalcones [6].

The *Enterobacter hormaechei* SSwC2 are psychrotrophic bacteriaand optimally grow at low temperature.

Bystudyingthecharacteristicsofenzymesfromcold-adaptedmicroorganismsmay providenovelwaysofunderstandingthermobiochemistryaswellasleadtopossibleapplicationsofsuchenzymesintheprocessingoffoodandthebiotransformationofchemicalsatlowtemperature.

II. MATERIALS AND METHODS

All the chemicals used in present research work were of AR grade. Melting points of samples were corrected and determined in an open capillary tube. IR spectra were recorded on FTIR Shimadzu spectrometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on Advance 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer. Heteroaldehyde were synthesized in chemistry laboratory and used for biotransformation.

Synthesis of Heteroaldehyde

The starting Heteroaldehyde 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxyaldehyde was prepared by chloro formylation of pyrazoloneunder Vilsmeir-Haack reaction condition Initially, the condensation of simple aromatic acetophenone with 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxyaldehyde in ethanol to give theCorresponding product benzopyran in good yield reference.

Biotransformation of Heteroaldehyde

Psychrotrophicbacteriawasisolatedbyserialdilutionmethod.1mLofsweetcurdobtainedfromSeemaMilkDairy,Aurangabad,(MH),Indiawasseriallydilutedinsixtubescontaining9mLof0.1%sterilepeptonewaterand0.1mLofeachdilutionwasplatedontoPlateCountAgar(M1025)andincubatedat7°Cforupto10days.Afterincubationisolatedcoloniesweretransferredonagarslantsandtheircolonycharactersand biochemicalcharacterswerestudied.*Enterobacterhormaechei*wasconfirmedbyusingBergey'sManualofDeterminativebacteriology(9thedition).

Furtheridentificationwascarriedoutby16srRNAsequencinginwhichisolationofgenomicDNAwascarriedoutusingprepmanultrasamplepreparationreagent(Applied biosystem,Applera,USA).Thefacilitywasavailedfrommoleculardiagnosis,ZoologyDepartment,Dr.BAMU,Aurangabad,(MH),India.Generatedsequencessearchedforthehomologoussequencesin NCBIdatabasebyusingBLASTn.GeneBankaccessionnumberswereobtainedfortheisolates.Phylogenetictreeof10closelyrelatedtaxawascarriedoutbyusingMEGAXsoftware.

After enrichment the 2 ml of inoculum is transferred to 100 ml of MSM (minimal salt medium) containing malt extract-0.1%, yeast extract-0.2%, glucose-0.5%, peptone-0.2%. chemically synthesized chalcone 0.05 gm. dissolved in 0.5 ml DMSO was added under sterile conditions in 250 ml of Erlenmayer flask and shaken at 160 rpm at 30°C. After 10 days the sample was harvested by sterile centrifugation technique (8000 rpm for 20 min.) and extracted by the same value of ethyl acetate three times.

The organic phase were grouped , dried using sodium sulphate (Na_2So_4) filtered and evaporated at reduced pressure. Experiments were carried out in triplicates and analysed by TLC. From one of the experiment purification was carried out. The crude residue 90 mg obtained from biotransformation of chalcone was

chromatographed on silica gel with ethyl acetate 200 ml and methanol 100 ml. The ethyl acetate extract of 45 mg by TLC analysis indicate interested substance. This was further chromatographed sequentially on silica gel eluting with hexanes and increasing polarity of ethyl acetate and 11mg of fraction were obtained.

III.RESULT AND DISCUSSIONS

Heteroaldehyde (61% yield) was synthesized by the reported method[15] and structure was determined by the analysis of 1D- and 2D-NMR, mass spectra and comparison with reported physical spectroscopic data. *Enterobacter hormaechei* SSwC2 was used to bio transform the heteroaldehyde. Reduced benzopyrans was identified as 32% yield.Biotransfomation of heteroaldehyde showing condensation reaction by *Enterobacter hormaechei*SSwC2as metabolic activity. Out of the different reactions reported none of the reaction showed regioselectivity for double bond C-2 and C-3. Shindo, Kagiyama, et al reported the unsubstituted chalcone was converted to 2"-hydroxychalcone and 2", 3" – dihydroxychalcone in 25% and 59 % yield by *E. coli*[13]. Similarly Herath W. et al reported biotransformation of chalcone xanthohumol using the culture broth of *Pichia membranifaciens* synthesized three metabolites, one isomeric prenylflavonone 3.3% yield and to modified chalcones in 0.55 and 0.58% yields[14].

Spectra of substituted benzopyran derivatives

2-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-methyl-4*H*-1-benzopyran-4-one (2a)

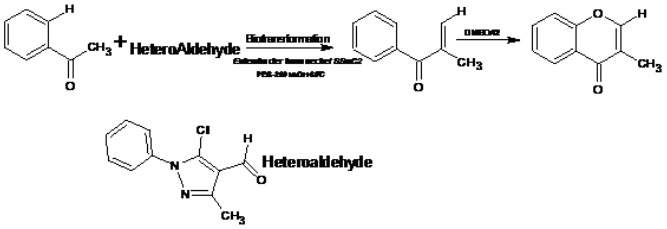
IR (KBr): 1602, 3162 ^{1}H NMR (DMSO-d_6): δ 0.9 (s, 3H, CH_3), δ3.1 (d, 1H, CH), δ3.4(d, 1H, CH), δ 7.1-8.0 (m, 7H, Ar-H), ppm; M.S. (m/z): 502(M^+), Anal. Calcd for$C_{19}H_{12}N_2O_2Cl$: C, 45.9; H, 2.25; N, 5.51%. Found: C, 45.80; H, 2.20; N, 5.31%

7-chloro-2-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-3-methyl-4*H*-1-benzopyran-4-one

(2b).

IR (KBr): 1602, 3162 ^{1}H NMR (DMSO-d_6): δ1.2 (s, 3H, CH_3), δ3.30 (d, 1H, CH), δ3.5(d, 1H, CH), δ 7.0-8.2 (m, 7H, Ar-H), ppm; M.S. (m/z): 456(M^+), Anal. Calcd for $C_{19}H_{11}N_2O_2Cl_2$: C, 50.07; H, 2.53; N, 6.33%. Found: C, 50.10; H, 2.45; N, 6..22%.

Scheme



The product which formed in the bio reduction reaction using *Enterobacter hormaechei*SSwC2was confirmed from spectral analysis is benzopyran.

IV.CONCLUSION

Biotransformation is a effective tool for structural modification of biologically active natural and synthetic compounds such as chalcones. In this present study the heteroaldehyde converted into reduced benzopyran with better yield. The biotransformation reaction was regioselective, showing condensation reaction which is a part of Psychrotrophic bacterial metabolic activity.

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Experimental Analysis Considering Dimensional Variations of Bite Marks: A Pilot Study

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ABSTRACT

The aim of this study is to examine the variations (if any) in bite marks on food items in co- relation with the Law of Progressive Change. Forensic odontology plays an essential role in many cases, such as sexual offenses, homicides, child abuse cases, etc. The elasticity of human skin influences bite-mark distortion. The tooth wounds of every individual are different, and bite pressure varies from individual to individual based on analysis. Every person has a unique set of teeth, and an examination shows that bite pressure differs among individuals. In this research, fruits and chocolate were used to analyze it. Bite marks on food items found at crime scenes start changing in dimension after some time. Bite marks on food at the crime scene provide a three-dimensional representation of the suspect's dental structure, and its analysis might provide helpful hints about the perpetrator, implying or finding out the person under investigation. As bite marks are considered as an individual characteristics; as a result we are able to correlate them with the culprit or suspect in a harmonious way.

Keywords: Bite marks, forensic odontology, individual characteristics

I. INTRODUCTION

Forensic odontology is a branch of forensic science. It is defined as “a branch of dentistry that deals with the proper handling and examination of dental evidence and, with the proper evaluation, the presentation of dental findings in the interest of justice (Keiser-Nielson, 1970).” In order to ensure justice, evaluate dental evidence, and present dental findings correctly, forensic odontology is a branch of forensic science that deals with the appropriate collection, handling, careful examination, appropriate interpretation, and preservation of dental evidence for future reference[1]. Bite marks are a pattern made by the teeth on a substrate. In forensic casework, bite mark analysis is crucial to personal identification. Bite marks may be documented in cases of child abuse, homicides, sexual assaults and athletic events. Each person's teeth are unique in terms of size, alignment, and arrangement. Using transparent overlays or machine learning

programs, the most popular techniques for identifying bite marks involve comparing the morphology of the dentition—that is, the size, shape, and position of the teeth as well as the shape of the dental arches—with similar features and characteristics found in life-sized photographs of the injury[2,3].It has been reported that bite marks have a similar evidentiary value to fingerprints. Bite mark distortion, in our opinion, would affect the precision and dependability of bite mark interpretation. In addition, teeth defy decay and are a valuable source of evidence when the majority of other body components are rendered useless by putrefactive changes in the body[4].When King Canouj, JayachandraRathore, died in battle in 1191, his body was identified by his artificial anterior teeth, making it the earliest identification instance in Indian history[5].Forensic odontology not only assists with man-made disasters, but also during natural disasters such as earthquakes and tsunamis, or the identification of burned, decomposing, or drowning fatalities, as well as victims of auto accidents[6]. Evidence of bite marks can be discovered on objects, the corpse, or the body of a living person. Although the victim's entire body may be affected, the face, neck, arm, breast, legs, buttocks, waist, and female genitalia are theareas most frequently affected. Other items, such as food items like cheese, chocolate, apples, or chewing gum, may also have bite marks on them; the evaluation of these items may play a significant role in the accused's conviction. The anterior teeth of the upper and lower jaw are clearly visible in bite marks, which have a characteristic double horseshoe form.

Principles of Forensic Science:

- Law of individuality
- Law of progressive change
- Principle of comparison
- Locard’s principle of exchange
- Law of probability
- Law of circumstantial facts [7]

Future aspects of forensic odontology

Person identification, particularly in criminal cases and large-scale catastrophes[8]

The process of personal identification is greatly aided by the ability to estimate an individual's age. Three factors contribute to this process: the appearance of tooth germs, the presence of mineralization remnants, the growth status of unerupted teeth, the rate at which hard structures such as enamel, dentin, and neonatal line are formed, and the transparency of root dentin [9].

Legal requirements and professional obligations require dentists to create and keep up-to-date patient records. Accurate and consistent antemortem and postmortem data are essential for the proper identification of a victim [10].

A few characteristics that can help with identification and provide some insight into the deceased's ethnicity are taurodontism, hypocones, peg-shaped teeth, and the cusp of Carabelli [11].

II. METHODS AND MATERIAL

The present seventeen-day pilot study was carried out in the Department of Basic and Applied Science, MGM University of Chhatrapati Sambhajinagar, Maharashtra, India in the month of February, 2024. For this research, we used bananas, apples, Perk chocolate, and Five Star chocolate as samples. We asked two individuals to provide their bite marks, which were used in this research for tooth alignment differentiation. Both individuals left their bite marks on the samples. Then we measured samples every day, both of maxillary and mandibular arch. Photography was done on each day and the dimensional variations were studied. The ABFO no. 2 scale is used for bitemark measurement and photography.

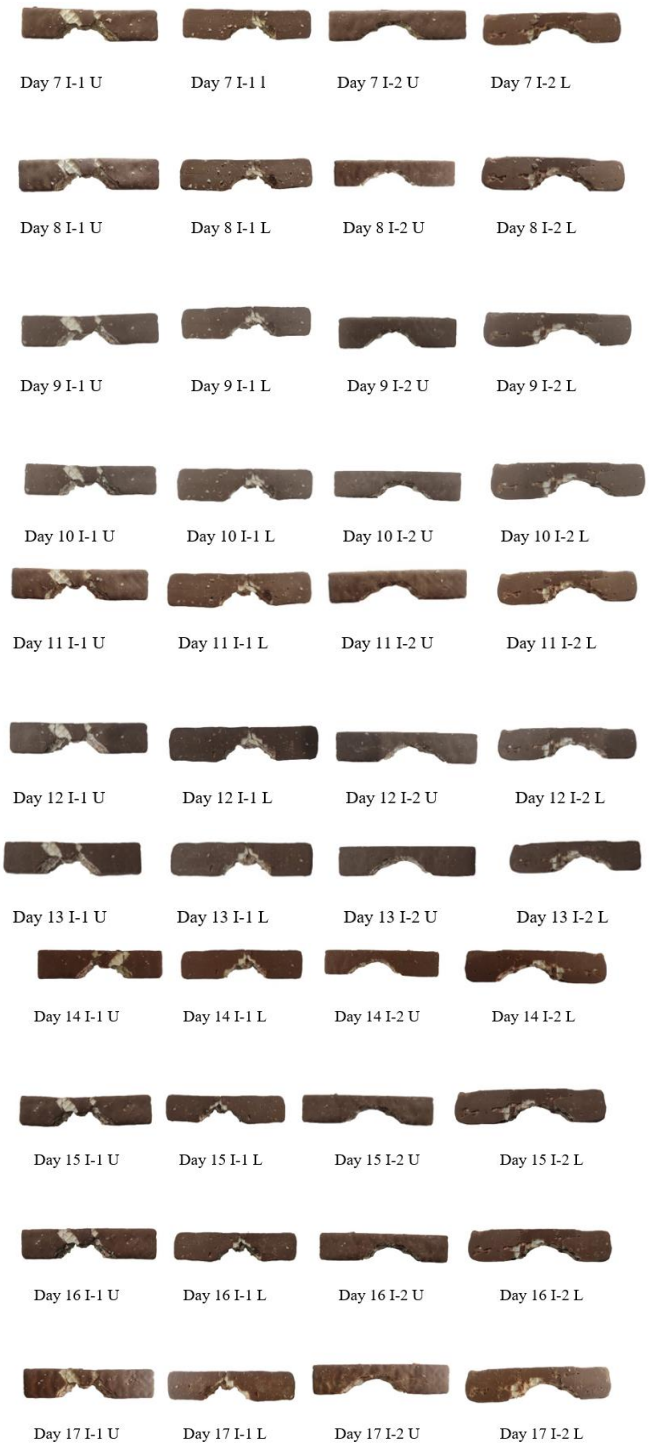
Following abbreviationsare used:

- Individual 1 (I-1)
- Individual 2 (I-2)
- Upper (Maxillary)(U)
- Lower (Mandibular) (L)

- Indicates that the particular samples were analysed for 4 and 8 days respectively (Banana and Apple).

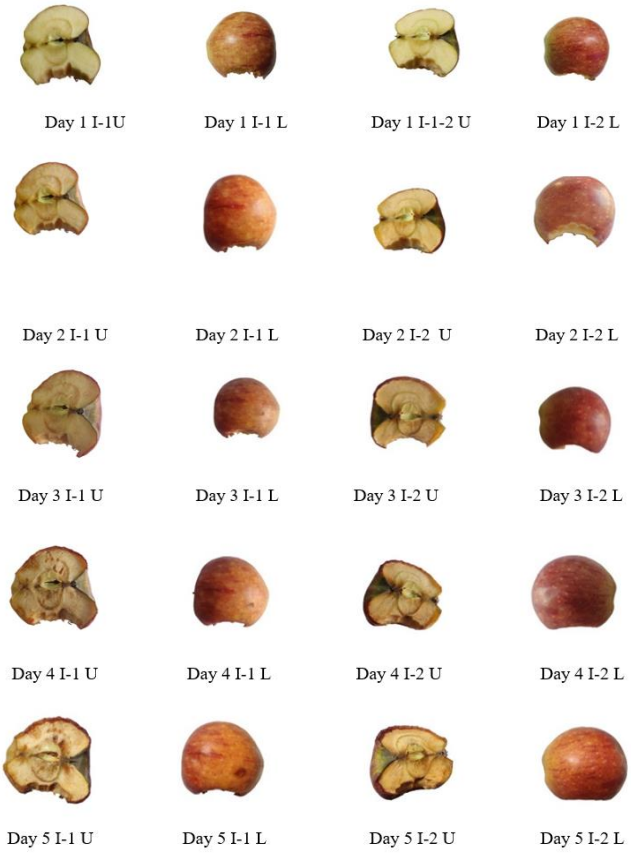
FIVE STAR

PERK



BANANA

APPLE




Day 6 I-1 U


Day 6 I-1 L


Day 6 I-2 U


Day 6 I-2 L


Day 7 I-1 U


Day 7 I-1 L


Day 7 I-2 U


Day 7 I-2 L


Day 8 I-1 U


Day 8 I-1 L


Day 8 I- 2 L


Day 8 I-2 L

III.RESULTS AND DISCUSSION

Temperature (°C)		+30	+30	+30	+31	+31	+32	+32	+31	+31	+31	+32	+33	+33	+33	+33	+34	+33
Sample/ Date	U/ L	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Fivestar	I-1 U	35	35	35	35	34	34	34	34	34	34	34	34	34	35	34	32	32
	I-1 L	34	34	36	35	36	36	36	36	36	36	36	36	36	36	36	35	35
	I-2 U	30	30	30	31	30	30	30	30	30	30	31	31	31	31	30	28	28
	I-2 L	33	33	33	32	32	32	32	32	32	32	32	32	32	32	31	31	31
Perk	I-1 U	40	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39
	I-1 L	34	33	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
	I-2 U	39	40	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
	I-2 L	40	40	40	39	39	39	39	39	39	39	39	40	40	40	40	40	40
Banana	I-1 U	30	28	27	26	-	-	-	-	-	-	-	-	-	-	-	-	-
	I-1 L	28	27	26	25	-	-	-	-	-	-	-	-	-	-	-	-	-
	I-2 U	30	29	28	25	-	-	-	-	-	-	-	-	-	-	-	-	-
	I-2 L	28	27	26	25	-	-	-	-	-	-	-	-	-	-	-	-	-
Apple	I-1 U	36	33	31	30	29	27	27	26	-	-	-	-	-	-	-	-	-

	I-1 L	26	25	24	23	22	22	21	21	-	-	-	-	-	-	-	-	-
	I-2 U	38	35	34	32	30	28	27	26	-	-	-	-	-	-	-	-	-
	I-2 L	28	27	27	26	25	23	22	22	-	-	-	-	-	-	-	-	-

Since these were organic fruits (bananas and apples), they shrank quickly, and due to the humid climate, the fungal growth was seen.Whereas in the other two samples (Perk chocolate and Five Star chocolate), the changes including fungal growth was not noticed.

IV. CONCLUSION

The principle "Law of Progressive change" was applied successfully.Bite marks might be harmoniously associated with the criminal or suspect because they are regarded to be individual characteristics. From the pilot study we can understand that there are dimensional changes with respect to climatic change as well as the food product. As a result, and application of law of progressive changefollowing dimensional changes were noticed as mentioned

1. Fivestar: Individual 1 U/L: Day 1(35/34mm) to last day (32/35mm),
2. Fivestar: Individual 2 U/L: Day 1 (30/33mm) to last day (28/31mm),
3. Perk: Individual 1 U/L: Day 1 (40/34mm) to last day (39/32mm),
4. Perk: Individual 2 U/L: Day 1 (39/40mm) to last day (41/40mm),
5. Banana: Individual 1 U/L: Day 1 (30/28mm) to last day (26/25mm),
6. Banana: Individual 2 U/L: Day 1 (30/28mm) to last day (25/25mm),
7. Apple: Individual 1 U/L: Day 1 (36/26mm) to last day (26/21mm),
8. Apple: Individual 2 U/L: Day 1 (38/28mm) to last day (26/22mm).

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Mechanobiology: Biophysical Regulation of Cell Behavior and Tissue Morphogenesis

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ABSTRACT

Dopamine, a neurotransmitter associated with pleasure, motivation, and reward, plays a crucial role in shaping human behavior. In the present era, characterized by rapid technological advancements, social media, and changing lifestyles, understanding the impact of dopamine on human behavior is essential. This paper explores the multifaceted influence of dopamine on various aspects of human behavior, including addiction, decision-making, motivation, and social interactions. Through an examination of recent research findings and theoretical frameworks, this paper elucidates the intricate interplay between dopamine and human behavior in contemporary society. Additionally, implications for mental health, well-being, and societal dynamics are discussed, highlighting the need for further interdisciplinary research and interventions to navigate the complexities of dopamine-driven behaviors in the modern world.

Keywords: Dopamine, Human Behavior, Addiction, Decision-Making, Motivation, etc.

I. INTRODUCTION

Dopamine, a neurotransmitter renowned for its role in modulating human behavior, stands as a central protagonist in the intricate workings of the brain. Its influence permeates various facets of cognition, emotion, motivation, and reward processing, rendering it indispensable to our understanding of human behavior in the present era. As society traverses through rapid technological advancements, shifting cultural norms, and evolving lifestyles, the significance of dopamine in shaping human behavior becomes increasingly pronounced.

At its core, dopamine is a catecholamine neurotransmitter synthesized in multiple regions of the brain, notably the substantia nigra and ventral tegmental area (VTA). This neurotransmitter operates within intricate neural circuits, projecting to diverse brain regions such as the prefrontal cortex, nucleus accumbens, and amygdala, where it orchestrates a symphony of physiological processes critical to human functioning.

Dopamine's involvement in the brain's reward system has garnered significant attention from researchers and clinicians alike. Through its modulation of mesolimbic and neocortical pathways, dopamine mediates responses to pleasurable stimuli, reinforces reward-seeking behaviors, and shapes our subjective experiences of pleasure and motivation. Furthermore, dopamine plays a pivotal role in reinforcement learning, facilitating the encoding of reward-related information and guiding adaptive decision-making processes.

However, dopamine's influence extends far beyond the realms of pleasure and reward. Emerging research has highlighted its involvement in various cognitive functions, including attention, memory, and executive control. Dopaminergic dysfunction has been implicated in a myriad of neuropsychiatric disorders, ranging from addiction and depression to schizophrenia and attention-deficit/hyperactivity disorder (ADHD). Understanding the nuanced interplay between dopamine and human behavior is thus imperative for elucidating the underlying mechanisms of these disorders and informing targeted interventions.

In the contemporary era, characterized by an unprecedented influx of information, ubiquitous digital technologies, and shifting social dynamics, the impact of dopamine on human behavior assumes heightened significance. The omnipresence of smartphones, social media platforms, and online entertainment avenues has revolutionized the way individuals seek and experience rewards, presenting both opportunities and challenges for dopamine regulation and mental well-being.

Against this backdrop, this paper endeavours to delve into the multifaceted influence of dopamine on human behavior in the present era. By synthesizing insights from neuroscience, psychology, and sociology, we aim to unravel the intricate web of dopamine-driven behaviors, shedding light on their implications for mental health, societal dynamics, and human flourishing. Through an exploration of recent research findings and theoretical frameworks, we seek to navigate the complexities of dopamine's role in shaping human behavior, offering a nuanced understanding of its relevance in contemporary society.

As we embark on this journey of inquiry, it becomes evident that unraveling the mysteries of dopamine holds profound implications for our understanding of human nature and the challenges we face in the modern world. By illuminating the neurobiological underpinnings of dopamine-driven behaviors, we aspire to foster interdisciplinary dialogue, inspire innovative research endeavours, and pave the way for interventions aimed at promoting healthy dopamine regulation and enhancing human well-being in the present era.

This paper aims to explore the multifaceted influence of dopamine on human behavior in the context of the contemporary environment.

II. DOPAMINE: NEUROBIOLOGY AND FUNCTION

Dopamine, a neurotransmitter belonging to the catecholamine family, serves as a fundamental mediator of neural communication within the central nervous system. Synthesized primarily in dopaminergic neurons located in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), dopamine exerts its influence across diverse brain regions, orchestrating a myriad of physiological and behavioral processes.

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A. Dopaminergic Pathways:

1. Mesolimbic Pathway: Originating in the VTA, the mesolimbic pathway projects to the nucleus accumbens, amygdala, and prefrontal cortex. This pathway is critically involved in reward processing, reinforcement learning, and motivational behaviors.
2. Mesocortical Pathway: Arising from the VTA, the mesocortical pathway innervates the prefrontal cortex, where it modulates executive functions, working memory, and cognitive control.
3. Nigrostriatal Pathway: Emerging from the SNc, the nigrostriatal pathway extends to the dorsal striatum (caudate nucleus and putamen), regulating motor coordination, voluntary movement, and procedural learning.

B. Dopamine Receptors:

1. Dopamine exerts its effects through interaction with five distinct receptor subtypes: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4). These receptors are distributed heterogeneously throughout the brain and exhibit divergent signaling cascades, contributing to the complexity of dopamine-mediated neurotransmission.
2. D1-like receptors primarily couple to stimulatory G-proteins (Gs), activating adenylate cyclase and increasing cyclic adenosine monophosphate (cAMP) levels, thus promoting excitatory signaling cascades.
3. In contrast, D2-like receptors predominantly couple to inhibitory G-proteins (Gi/o), exerting inhibitory effects on adenylate cyclase activity and modulating neuronal excitability.

C. Functions of Dopamine:

1. Reward and Reinforcement: Dopamine plays a central role in the brain's reward system, mediating responses to pleasurable stimuli and reinforcing reward-seeking behaviors. Phasic dopamine release in response to unexpected rewards facilitates reinforcement learning and the encoding of reward-related information.
2. Motivation and Arousal: Dopamine is intricately involved in motivational processes, regulating incentive salience and energizing goal-directed behaviors. Dysregulation of dopaminergic neurotransmission can lead to motivational deficits, apathy, or anhedonia, characteristic symptoms of neuropsychiatric disorders.
3. Motor Control: In the nigrostriatal pathway, dopamine modulates motor function by exerting tonic inhibition on the indirect pathway and disinhibiting the direct pathway, thus facilitating smooth and coordinated movements.
4. Cognitive Functions: Dopamine modulates various cognitive processes, including attention, working memory, and executive control. Optimal dopamine levels are essential for maintaining cognitive flexibility, response inhibition, and goal-directed behavior.
5. Emotional Regulation: Dopamine contributes to the regulation of emotional responses and affective states, influencing mood, arousal, and stress responses. Dysregulation of dopaminergic neurotransmission has been implicated in mood disorders, anxiety disorders, and post-traumatic stress disorder (PTSD).

In summary, dopamine serves as a versatile neurotransmitter with diverse neurobiological functions, ranging from reward processing and motivation to motor control and cognitive regulation. Its intricate interplay with dopaminergic pathways and receptor subtypes underlies the complexity of dopamine-mediated behaviors and underscores its significance in shaping human cognition, emotion, and behavior. Understanding the neurobiology of dopamine is essential for elucidating the pathophysiology of neuropsychiatric disorders and informing targeted therapeutic interventions aimed at

restoring dopaminergic homeostasis and promoting optimal brain function..

III.MECHANOTRANSDUCTION PATHWAYS

Mechanotransduction pathways represent the intricate molecular machinery through which cells sense and respond to mechanical cues from their microenvironment. This process is pivotal for numerous cellular functions, including adhesion, migration, differentiation, and proliferation. At the forefront of mechanotransduction are integrins, transmembrane receptors that bridge the extracellular matrix (ECM) with the intracellular cytoskeleton. Integrins play a central role in mechanosensing, transmitting mechanical signals across the cell membrane and initiating downstream signaling cascades. Focal adhesions, dynamic protein complexes localized at the cell-ECM interface, serve as molecular hubs for mechanotransduction. They anchor the actin cytoskeleton to the ECM and enable the transmission of mechanical forces from the external environment to the cell interior. Talin, vinculin, and focal adhesion kinase (FAK) are key components of focal adhesions, orchestrating signaling events that regulate cell adhesion, migration, and mechanosensitivity. [8-10]

Moreover, the nucleus emerges as a mechanosensitive organelle that responds to changes in mechanical forces within the cellular microenvironment. The LINC complex, consisting of linker proteins connecting the cytoskeleton with the nuclear lamina, facilitates force transmission between the cytoskeleton and the nuclear envelope. Mechanical signals exerted on the nucleus regulate gene expression, chromatin organization, and nuclear shape, influencing cellular functions and fate decisions. [11-14]

In addition to direct mechanical cues, biochemical signaling pathways intersect with mechanotransduction networks to regulate cellular responses to mechanical stimuli. For instance, growth factors, cytokines, and ECM-derived proteins modulate mechanotransduction pathways through crosstalk with receptor tyrosine kinases and intracellular signaling cascades. Integrin-mediated activation of focal adhesion kinase (FAK) and Src family kinases links mechanical cues with growth factor signaling, coordinating cellular responses to extracellular cues.Overall, mechanotransduction pathways represent a complex network of molecular interactions that translate mechanical signals into biochemical responses, regulating diverse aspects of cellular physiology and tissue homeostasis. Elucidating the mechanisms underlying mechanotransduction is essential for understanding the biophysical regulation of cell behavior and tissue morphogenesis. Moreover, dysregulation of mechanotransduction pathways has been implicated in various pathological conditions, highlighting the therapeutic potential of targeting mechanobiological mechanisms for disease intervention and tissue regeneration. [15-19]

IV. REGULATION OF CELL BEHAVIOR

The regulation of cell behavior by biophysical cues is a fundamental aspect of mechanobiology, encompassing processes such as adhesion, migration, proliferation, and differentiation. Cells possess exquisite sensitivity to their mechanical microenvironment, integrating physical signals to modulate their behavior and function. Cell adhesion, the process by which cells attach to the extracellular matrix (ECM) or neighboring cells, is a critical determinant of cell behavior and tissue organization. Integrins, transmembrane receptors that bind to specific ECM proteins, mediate cell adhesion and transmit mechanical forces between the ECM and the cytoskeleton. Engagement of integrins with the ECM initiates signaling cascades that regulate focal adhesion dynamics, cytoskeletal organization, and cellular responses to mechanical cues. Substrate stiffness, a key biophysical property of the ECM, profoundly influences cell behavior and fate

determination. Cells exhibit differential responses to substrate stiffness, with increased stiffness promoting cell spreading, proliferation, and differentiation. Mechanistically, changes in substrate stiffness modulate the formation and maturation of focal adhesions, as well as the activation of signaling molecules such as Rho GTPases and YAP/TAZ transcriptional coactivators, which regulate cytoskeletal dynamics and gene expression. [20-22]

Topographical cues, including surface patterning and micro/nanostructures, also exert significant effects on cell behavior and function. Cells sense and respond to topographical cues through mechanotransduction pathways, altering their morphology, migration, and gene expression profiles in response to surface features. Nanostructured substrates, such as nano topographic patterns and nanopillar arrays, can modulate cell adhesion and alignment, offering precise control over cellular responses for applications in tissue engineering and regenerative medicine.[23]

Cell migration, the directed movement of cells in response to extracellular cues, is intricately regulated by mechanical signals from the microenvironment. Cells exhibit distinct modes of migration, including mesenchymal migration, characterized by protrusion of lamellipodia and filopodia, and amoeboid migration, driven by actomyosin contractility and cytoskeletal reorganization. Mechanical cues, such as substrate stiffness gradients and spatial confinement, guide cell migration by modulating cytoskeletal dynamics and adhesion turnover, enabling cells to navigate complex tissue environments during development, wound healing, and cancer metastasis.[24]

Cell proliferation, the process of cell division and expansion, is tightly regulated by mechanical cues and biochemical signals from the microenvironment. Substrate stiffness, cell-cell interactions, and soluble factors such as growth factors and cytokines influence cell proliferation rates and cell cycle progression. Mechanotransduction pathways, including the Hippo-YAP/TAZ pathway and the mechanosensitive mTOR signaling pathway, integrate mechanical signals with cell proliferation pathways, coordinating cell growth and tissue morphogenesis in response to changing environmental cues.

Cell differentiation, the process by which cells acquire specialized phenotypes and functions, is governed by a combination of mechanical and biochemical cues. Substrate stiffness, ECM composition, and cell-cell interactions play crucial roles in directing cell fate determination and lineage commitment. Mechanical cues regulate stem cell differentiation by modulating transcriptional programs, chromatin organization, and epigenetic modifications, providing insights into the mechanisms underlying tissue development and regeneration.[25]

In summary, the regulation of cell behavior by biophysical cues is a multifaceted process that integrates mechanical signals with biochemical pathways to orchestrate cellular responses and tissue morphogenesis. Elucidating the mechanisms underlying cell-matrix interactions, cytoskeletal dynamics, and mechanotransduction pathways is essential for understanding tissue development, homeostasis, and disease pathogenesis. Harnessing the biophysical regulation of cell behavior holds promise for engineering functional tissues, developing novel therapeutic strategies, and advancing regenerative medicine in the future.[26]

V. TISSUE MORPHOGENESIS

Tissue morphogenesis embodies the dynamic process through which cells organize and shape into complex tissue structures during embryonic development and homeostasis. It is governed by intricate interactions between mechanical forces, cell-cell adhesion, and extracellular matrix (ECM) remodeling, orchestrating the emergence of functional tissue architectures. During embryogenesis, tissue morphogenesis unfolds through a series of coordinated events, including epithelial folding, tissue elongation, and organogenesis. Mechanical forces generated by cell contractility, cell-cell adhesion, and tissue tension

play pivotal roles in sculpting tissue morphology and guiding organ formation. Epithelial-mesenchymal interactions drive tissue patterning and differentiation, as cells undergo dynamic changes in shape, polarity, and motility in response to mechanical cues from the microenvironment.[27]

The extracellular matrix (ECM), a complex network of proteins and polysaccharides surrounding cells, provides structural support and biochemical cues that influence tissue morphogenesis. ECM composition, stiffness, and topography regulate cell behavior and tissue organization, guiding cell migration, proliferation, and differentiation during development. Changes in ECM architecture and mechanical properties drive morphogenetic processes such as branching morphogenesis in the lung, tubulogenesis in the kidney, and neurulation in the nervous system.[28]

Moreover, cytoskeletal dynamics and contractile forces generated by actomyosin networks drive tissue morphogenesis by regulating cell shape changes and tissue remodeling. Actin polymerization, myosin contractility, and cytoskeletal rearrangements coordinate cell movements and tissue morphogenesis, facilitating processes such as gastrulation, neurulation, and organogenesis during embryonic development.

Disruption of mechanotransduction pathways and ECM remodeling can lead to developmental defects and congenital abnormalities, highlighting the importance of understanding the biophysical regulation of tissue morphogenesis. Advances in imaging techniques, biophysical tools, and computational modeling have enabled researchers to elucidate the mechanisms underlying tissue morphogenesis and unravel the dynamic interplay between mechanical forces and cellular behavior.[29]

In summary, tissue morphogenesis represents a dynamic process driven by mechanical forces, cell-cell interactions, and ECM remodeling, shaping the architecture and function of tissues and organs during development and homeostasis. Deciphering the mechanisms underlying tissue morphogenesis holds promise for understanding embryonic development, tissue regeneration, and disease pathogenesis, paving the way for innovative therapeutic strategies and tissue engineering approaches in regenerative medicine.Finally, mechanobiology holds immense therapeutic potential across a wide range of medical disciplines, from regenerative medicine and tissue engineering to cancer therapy and cardiovascular health. By elucidating the biophysical regulation of cellular processes and tissue morphogenesis, researchers can develop innovative therapies that harness the power of mechanical forces to promote tissue repair, inhibit disease progression, and improve patient outcomes in diverse pathological conditions.[28-29]

VI. THERAPEUTIC IMPLICATIONS

Insights gained from mechanobiology have profound implications for regenerative medicine, tissue engineering, and disease therapeutics. By manipulating mechanical cues in the cellular microenvironment, researchers can guide stem cell fate determination, enhance tissue regeneration, and engineer biomimetic scaffolds for tissue repair. Moreover, targeting aberrant mechanotransduction pathways holds promise for treating various diseases, including cancer, fibrosis, and cardiovascular disorders. Developing innovative therapeutic strategies that leverage our understanding of mechanobiology represents a promising avenue for addressing unmet clinical needs and improving patient outcomes. [27-29]

A. **Regenerative Medicine**: Mechanobiology offers promising avenues for regenerative medicine by leveraging the biophysical cues that regulate cell behavior and tissue morphogenesis. Understanding the mechanotransduction pathways involved in tissue regeneration can guide the development of biomimetic scaffolds, growth factors, and stem cell therapies aimed at

promoting tissue repair and regeneration in various pathological conditions.

B. **Tissue Engineering:** Biophysical regulation of cell behavior provides essential insights for tissue engineering strategies aimed at creating functional tissue substitutes. By mimicking the mechanical properties and microenvironmental cues of native tissues, engineered scaffolds can guide cell adhesion, migration, and differentiation, facilitating the formation of organized tissue structures for transplantation and organ replacement therapies.

C. **Cancer Therapy:** Dysregulation of mechanotransduction pathways is implicated in cancer progression, metastasis, and treatment resistance. Targeting mechanosensitive molecules and signaling pathways in tumor cells holds promise for developing novel cancer therapies aimed at disrupting tumor-stroma interactions, inhibiting metastatic spread, and sensitizing cancer cells to conventional treatments such as chemotherapy and radiotherapy.

D. **Fibrosis and Scar Formation:** Aberrant mechanical signaling contributes to the development of fibrosis and scar formation in various organs, including the liver, lung, and heart. Therapeutic interventions targeting mechanotransduction pathways can mitigate fibrotic responses, inhibit excessive extracellular matrix deposition, and promote tissue remodeling, offering new strategies for treating fibrotic diseases and promoting tissue regeneration.

E. **Cardiovascular Health:** Mechanobiology plays a crucial role in cardiovascular health by regulating vascular function, cardiac remodeling, and blood vessel formation. Understanding the biomechanical forces governing vascular homeostasis can inform the development of therapies for cardiovascular diseases such as atherosclerosis, hypertension, and heart failure. Strategies targeting mechanotransduction pathways in endothelial cells and vascular smooth muscle cells may enhance vascular repair and improve cardiovascular outcomes.

F. **Musculoskeletal Disorders:** Biophysical cues play a critical role in musculoskeletal health and disease, influencing bone remodeling, cartilage maintenance, and muscle function. Therapeutic interventions that modulate mechanotransduction pathways hold promise for treating musculoskeletal disorders such as osteoporosis, osteoarthritis, and muscular dystrophy. Engineered scaffolds, mechanical loading protocols, and pharmacological agents targeting mechanosensitive molecules can enhance tissue repair and functional recovery in musculoskeletal injuries and degenerative conditions.

G. **Neurological Disorders:** Mechanobiology contributes to neurological health and disease by regulating neuronal development, synaptic plasticity, and neural circuit formation. Understanding the biomechanical cues that govern nervous system function can inform the development of therapies for neurological disorders such as traumatic brain injury, spinal cord injury, and neurodegenerative diseases. Biomaterial-based approaches, neuroprotective agents, and neuromodulation techniques may promote neural regeneration and functional recovery in neurological conditions.

H. **Personalized Medicine:** Advances in mechanobiology have the potential to revolutionize personalized medicine by integrating biophysical data with genomic, transcriptomic, and clinical information to tailor therapeutic interventions to individual patients. Patient-specific modeling of mechanotransduction pathways and cellular responses can guide the selection of optimal treatments and predict patient outcomes,

paving the way for precision medicine approaches in diverse fields of healthcare.

VII. CONCLUSION

Mechanobiology provides a conceptual framework for understanding how mechanical forces regulate cellular behaviors and tissue morphogenesis. By elucidating the intricate interplay between cells and their microenvironment, researchers have uncovered fundamental principles governing mechanotransduction pathways and their implications for tissue development and disease progression. Future studies aimed at deciphering the biophysical mechanisms underlying mechanobiological phenomena will further advance our knowledge and inform the design of novel therapeutic interventions targeting mechanotransduction pathways in health and disease.

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Nanotechnology Applications in Forensic Analysis: A Review

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ABSTRACT

Nanotechnology has emerged as a promising field with vast applications across various disciplines, including forensic science. This review aims to explore the burgeoning role of nanotechnology in forensic analysis. By harnessing the unique properties of nanomaterials, forensic scientists can enhance analytical techniques, improve evidence collection, and augment investigative processes. This article provides an overview of nanotechnology applications in forensic analysis, including DNA analysis, fingerprint detection, drug analysis, trace evidence analysis, and crime scene investigation. Furthermore, challenges and prospects of integrating nanotechnology into forensic science are discussed.

Keywords: Nanotechnology, Forensic Analysis, DNA Analysis, Fingerprint Detection, Drug Analysis, etc.

I. INTRODUCTION

Nanotechnology, the manipulation of matter at the nanometer scale, has emerged as a transformative field with far-reaching applications across various domains, including medicine, electronics, and materials science. In recent years, its potential in forensic analysis has garnered significant attention, offering novel solutions to longstanding challenges in evidence collection, analysis, and crime scene investigation.Forensic science plays a crucial role in the criminal justice system by providing scientific evidence to support criminal investigations and legal proceedings. Traditional forensic techniques often rely on macroscopic observations and chemical analyses, which may have limitations in sensitivity, specificity, and reliability. However, the advent of nanotechnology has opened new avenues for enhancing forensic analysis through the precise manipulation and control of materials at the nanoscale.

The integration of nanotechnology into forensic science offers several distinct advantages. One of the most significant benefits is the ability to improve the sensitivity and specificity of analytical techniques used in evidence analysis. Nanomaterials exhibit unique physical and chemical properties that can be leveraged to enhance the detection and characterization of forensic evidence, including DNA, fingerprints, drugs, and trace materials. [1-3]

In DNA analysis, nanotechnology enables highly sensitive and rapid detection methods that surpass the capabilities of conventional techniques. Nanoparticles functionalized with DNA probes or fluorescent labels facilitate the detection of specific DNA sequences with unparalleled precision. Furthermore, nanoscale biosensors enable single-molecule DNA sequencing, revolutionizing genetic profiling in forensic applications.[4]

Fingerprint detection, another critical aspect of forensic analysis, has also benefited from advancements in nanotechnology. Traditional methods for fingerprint visualization often suffer from limitations such as low sensitivity and background interference. Nanoparticles functionalized with ligands that selectively bind to components of fingerprints offer improved contrast and visibility, enhancing the accuracy of fingerprint identification on various surfaces and substrates.[5]

Moreover, nanotechnology plays a pivotal role in drug analysis, enabling the detection and characterization of illicit substances and pharmaceuticals in forensic samples. Nanoparticle-based sensors offer high selectivity and sensitivity for the identification of drugs of abuse, enhancing the capabilities of forensic laboratories to analyze complex matrices and trace evidence.[6]

Trace evidence analysis, which involves the identification and characterization of microscopic materials found at crime scenes, is another area where nanotechnology holds great promise. Nanosensors integrated into forensic tools enable the detection of trace materials such as fibers, glass fragments, and gunshot residues with unprecedented accuracy and reliability. Functionalized nanoparticles enhance the specificity of detection, allowing forensic examiners to link suspects to crime scenes or victims based on minute traces of evidence.[7]

Furthermore, nanotechnology has revolutionized crime scene investigation by enabling rapid and precise analysis of forensic evidence in real-time. Portable nanodevices equipped with sensors and imaging capabilities facilitate the detection of biological fluids, explosives, and chemical residues at crime scenes, aiding forensic investigators in evidence collection and analysis.[7-8]

The nanotechnology offers a wealth of opportunities to enhance forensic analysis and improve the reliability of evidence-based investigations. By harnessing the unique properties of nanomaterials, forensic scientists can overcome traditional limitations and achieve unprecedented levels of sensitivity, specificity, and reliability in evidence analysis and crime scene investigation. Continued research and interdisciplinary collaboration are essential to unlock the full potential of nanotechnology in forensic science and advance the pursuit of justice in society.[9-10]

II. NANOTECHNOLOGY IN DNA ANALYSIS

Nanotechnology has revolutionized DNA analysis, offering novel approaches to enhance the sensitivity, specificity, and speed of forensic genetic profiling. In traditional DNA analysis methods, the detection and identification of specific DNA sequences often require large sample volumes and prolonged processing times, limiting their applicability in forensic investigations. However, nanotechnology-based approaches have overcome these limitations by leveraging the unique properties of nanomaterials to achieve highly sensitive and rapid DNA detection.[11-13]

One of the key advancements facilitated by nanotechnology is the use of gold nanoparticles and quantum dots in DNA analysis. These nanomaterials possess distinctive optical and electronic properties that make them ideal candidates for enhancing DNA detection techniques. Gold nanoparticles, for instance, can be functionalized with DNA probes or fluorescent labels, enabling the specific recognition and binding of target DNA sequences. The interaction between gold nanoparticles and DNA molecules leads to changes in their optical properties, such as

colorimetric shifts or enhanced fluorescence, which can be easily detected and quantified.[12-15]

Quantum dots, semiconductor nanoparticles with unique electronic properties, offer another promising avenue for sensitive DNA detection. By conjugating quantum dots with DNA probes, forensic scientists can achieve highly specific and multiplexed detection of DNA sequences. Quantum dots exhibit intense and stable fluorescence, allowing for the simultaneous detection of multiple DNA targets in a single assay. Moreover, the tunable emission spectra of quantum dots enable the development of customizable detection platforms tailored to the specific requirements of forensic DNA analysis.[16-17]

Furthermore, nanoscale biosensors have emerged as powerful tools for single-molecule DNA sequencing, revolutionizing genetic profiling in forensic applications. Nanopore-based sequencing technologies, for instance, utilize nanoscale apertures to capture individual DNA molecules and analyze their sequence in real-time. The translocation of DNA through nanopores generates characteristic electrical signals that can be decoded to infer the nucleotide sequence of the DNA molecule. This approach offers several advantages over traditional sequencing methods, including reduced sample requirements, shorter analysis times, and the ability to detect DNA modifications and structural variations.[18]

In addition to enhancing the sensitivity and specificity of DNA analysis, nanotechnology enables the development of miniaturized and portable devices for on-site forensic investigations. Nanoscale sensors and microfluidic systems integrated into handheld devices enable rapid and decentralized DNA analysis, allowing forensic scientists to perform genetic profiling directly at crime scenes or in field laboratories. These advancements have the potential to expedite forensic investigations, improve evidence collection practices, and enhance the overall efficiency of the criminal justice system.[19]

In conclusion, nanotechnology has revolutionized DNA analysis in forensic science, offering innovative solutions to overcome traditional limitations and enhance the accuracy and reliability of genetic profiling techniques. Continued research and development in nanotechnology-enabled DNA analysis are essential to realize its full potential in forensic investigations and contribute to the advancement of justice and security.[18-19]

III.NANOPARTICLES IN FINGERPRINT DETECTION

Nanotechnology has significantly advanced the field of fingerprint detection, addressed inherent limitations of traditional methods and provided forensic investigators with more reliable and sensitive techniques for identifying latent fingerprints. Conventional fingerprint detection methods often suffer from low sensitivity, background interference, and limited applicability to challenging surfaces. However, nanotechnology-based approaches offer innovative solutions to overcome these challenges and enhance the accuracy and effectiveness of fingerprint analysis.[20]

One of the key contributions of nanotechnology to fingerprint detection is the development of nanoparticles specifically designed to interact with components present in fingerprints. These nanoparticles, typically composed of materials such as gold, silver, or carbon-based materials, can be functionalized with ligands that selectively bind to amino acids, lipids, and other organic compounds found in fingerprints. By targeting specific molecular components of fingerprints, nanomaterials enhance the contrast and visibility of latent prints, enabling forensic examiners to visualize and analyze them with greater precision.[20-21]

Gold nanoparticles, for example, exhibit unique optical properties that make them ideal candidates for enhancing fingerprint detection techniques. Functionalized gold nanoparticles can selectively bind to the organic residues present in fingerprints, leading to localized changes in their optical properties. These

changes can be visualized using techniques such as dark-field microscopy or surface-enhanced Raman spectroscopy, allowing forensic examiners to identify and analyze latent prints on various surfaces and substrates.Carbon-based nanoparticles, including carbon nanotubes and graphene oxide, have also shown promise in fingerprint detection applications. These nanoparticles possess high surface area and adsorption capacity, enabling them to effectively capture and retain organic residues from fingerprints. Functionalized carbon nanoparticles can be dispersed in solvents or applied as thin films to enhance the contrast and visibility of latent prints, even on challenging surfaces such as paper, cardboard, or textured materials.Furthermore, quantum dots, semiconductor nanoparticles with tunable optical properties, offer another promising avenue for fingerprint detection. Quantum dots can be functionalized with ligands that selectively bind to fingerprint components, enabling the specific labeling and visualization of latent prints with high sensitivity and specificity. Moreover, the photostability and narrow emission spectra of quantum dots allow for long-term imaging and analysis of latent prints, facilitating forensic investigations and evidence documentation.[19-22]

Nanotechnology-based approaches have also facilitated the development of portable and handheld devices for on-site fingerprint detection. Miniaturized sensors and imaging systems integrated into portable devices enable forensic investigators to analyze latent prints directly quickly and accurately at crime scenes or in field laboratories. These advancements have the potential to expedite forensic investigations, improve evidence collection practices, and enhance the overall efficiency of fingerprint analysis in forensic science.The nanotechnology has revolutionized fingerprint detection in forensic science, offering innovative solutions to enhance the sensitivity, specificity, and portability of fingerprint analysis techniques. Continued research and development in nanotechnology-enabled fingerprint detection are essential to further advance forensic investigations and contribute to the advancement of justice and security.[19-22]

IV. NANOMATERIALS IN DRUG ANALYSIS

Nanotechnology has made significant strides in the field of drug analysis, offering novel approaches to improve the detection, identification, and characterization of illicit substances and pharmaceuticals in forensic samples. Traditional drug analysis methods often face challenges such as limited sensitivity, selectivity, and sample throughput, hindering their applicability in forensic investigations. However, nanotechnology-based approaches have emerged as promising solutions to overcome these limitations and enhance the capabilities of forensic laboratories in drug analysis.[23]

One of the key contributions of nanotechnology to drug analysis is the development of nanoparticle-based sensors and detection platforms. Nanoparticles, such as gold nanoparticles, quantum dots, and magnetic nanoparticles, offer unique properties that can be exploited to improve the sensitivity and specificity of drug detection assays. Functionalized nanoparticles can selectively bind to target drug molecules, facilitating their detection and quantification in complex forensic samples.[24]

Gold nanoparticles, for example, can be functionalized with ligands or antibodies that specifically recognize and bind to target drug compounds. The interaction between gold nanoparticles and target molecules leads to changes in their optical properties, which can be detected and quantified using techniques such as surface-enhanced Raman spectroscopy or colorimetric assays. These nanoparticle-based sensors enable rapid and sensitive detection of drugs of abuse, pharmaceuticals, and toxic compounds in forensic samples, enhancing the capabilities of forensic laboratories to analyze complex matrices and trace evidence. [22-26]

Furthermore, nanotechnology has facilitated the development of nanoparticle-enhanced chromatography and mass spectrometry techniques for drug analysis. Nanoparticles can be used as stationary phases in chromatographic separations or as ionization matrices in mass spectrometry, improving the separation and identification of drug compounds in forensic samples. analysis. [le-based chromatography columns offer higher resolution and faster analysis times compared to traditional chromatographic methods, enabling forensic scientists to achieve greater accuracy and efficiency in drug analysis. [25-26]

Moreover, nanomaterials have been integrated into microfluidic systems and lab-on-a-chip devices for on-site drug analysis in forensic investigations. Miniaturized and portable platforms equipped with nanoparticle-based sensors and detection modules enable rapid and decentralized analysis of drugs directly at crime scenes or in field laboratories. These advancements have the potential to expedite forensic investigations, enhance evidence collection practices, and improve the overall efficiency of drug analysis in forensic science. The nanotechnology has revolutionized drug analysis in forensic science, offering innovative solutions to enhance the sensitivity, specificity, and portability of analytical techniques. Continued research and development in nanotechnology-enabled drug analysis are essential to further advance forensic investigations and contribute to the detection and prevention of drug-related crimes.[26]

V. NANOSENSORS FOR TRACE EVIDENCE ANALYSIS

Nanotechnology has introduced groundbreaking advancements in trace evidence analysis, revolutionizing the detection, identification, and characterization of microscopic materials encountered in forensic investigations. Trace evidence, which encompasses a diverse range of materials such as fibers, glass fragments, gunshot residues, and soil particles, plays a crucial role in linking suspects to crime scenes, victims, or objects of interest. Traditional methods for trace evidence analysis often suffer from limitations such as low sensitivity, complex sample preparation, and limited applicability to challenging matrices. However, nanotechnology-based approaches offer innovative solutions to overcome these challenges and enhance the accuracy and reliability of trace evidence analysis in forensic science.

Nanosensors represent a key area of nanotechnology that has been extensively explored for trace evidence analysis. These nanoscale devices are designed to selectively detect and quantify trace materials based on specific interactions between the target analytes and the sensor surface. Functionalized nanoparticles, such as gold nanoparticles, carbon nanotubes, and quantum dots, serve as the building blocks of nanosensors, providing enhanced sensitivity and selectivity for trace material detection.[27]

Gold nanoparticles, for instance, can be functionalized with ligands or receptors that selectively bind to target trace materials, enabling their sensitive detection and quantification. The interaction between gold nanoparticles and trace analytes leads to changes in the nanoparticles' optical properties, which can be detected and quantified using spectroscopic techniques such as surface-enhanced Raman spectroscopy or colorimetric assays. These nanoparticle-based sensors offer high sensitivity and specificity for trace material detection, enabling forensic examiners to identify and characterize minute traces with unprecedented accuracy and reliability.[25-25]

Carbon-based nanomaterials, including carbon nanotubes and graphene oxide, have also shown promise in trace evidence analysis applications. These nanomaterials possess high surface area and adsorption capacity, allowing them to effectively capture and retain trace materials from complex forensic samples. Functionalized carbon nanoparticles can be integrated into sensor platforms for the

selective detection of trace materials, offering enhanced sensitivity and robustness for forensic applications. Furthermore, nanotechnology has facilitated the development of microfluidic systems and lab-on-a-chip devices for on-site trace evidence analysis in forensic investigations. Miniaturized and portable platforms equipped with nanosensors enable rapid and decentralized analysis of trace materials directly at crime scenes or in field laboratories. These advancements have the potential to expedite forensic investigations, improve evidence collection practices, and enhance the overall efficiency of trace evidence analysis in forensic science. [25-27]

In conclusion, nanotechnology has significantly advanced trace evidence analysis in forensic science, offering innovative solutions to overcome traditional limitations and enhance the sensitivity, specificity, and portability of analytical techniques. Continued research and development in nanotechnology-enabled trace evidence analysis are essential to further advance forensic investigations and contribute to the resolution of criminal cases.[25-27]

VI. NANOSENSORS FOR TRACE EVIDENCE ANALYSIS[27-30]

Nanotechnology has emerged as a transformative tool in crime scene investigation, facilitating rapid, precise, and comprehensive analysis of forensic evidence to aid law enforcement agencies in solving criminal cases. The integration of nanotechnology into crime scene investigation encompasses various subpoints and techniques, each contributing to the enhancement of evidence collection, analysis, and interpretation. Below are additional subpoints related to nanotechnology in crime scene investigation:

A. **Portable Nanodevices for On-Site Analysis:** Nanotechnology has enabled the development of portable and handheld devices equipped with sensors, imaging systems, and microfluidic platforms for on-site analysis of forensic evidence. These miniaturized devices empower forensic investigators to detect biological fluids, explosives, chemical residues, and other trace materials directly quickly and accurately at crime scenes, thereby expediting evidence collection and preserving the integrity of forensic samples.

B. **Nanomaterial-Based Imaging Techniques:** Advanced imaging techniques based on nanomaterials offer enhanced visualization and characterization of latent evidence at crime scenes. Nanoparticle-enhanced imaging modalities, such as fluorescence microscopy, atomic force microscopy, and scanning electron microscopy, enable forensic examiners to identify and document minute traces of evidence with high resolution and contrast. Moreover, nanoscale contrast agents and probes enhance the detection of biological fluids, DNA, and other forensic markers, aiding in crime scene reconstruction and evidence interpretation.

C. **Nanoparticle-Enhanced Luminol Detection:** Luminol, a chemiluminescent reagent commonly used to detect bloodstains at crime scenes, can be enhanced using nanoparticle-based approaches. Nanoparticles functionalized with catalysts or luminogenic compounds amplify the chemiluminescent signal produced upon the reaction of luminol with blood residues, increasing the sensitivity and detection limits of bloodstain analysis. Nanoparticle-enhanced luminol detection offers improved visualization of bloodstains on various surfaces and substrates, including fabrics, wood, and metals, enhancing the efficacy of bloodstain pattern analysis in forensic investigations.

D. **Nanoscale Sensors for Hazardous Material Detection:** Nanotechnology facilitates the development of nanoscale sensors capable of detecting hazardous materials, explosives, and chemical contaminants at crime scenes. These

sensors, based on nanomaterials such as carbon nanotubes, graphene, and metal oxides, offer high sensitivity and selectivity for detecting trace amounts of explosives, volatile organic compounds, and environmental pollutants. Nanoscale sensor arrays integrated into portable devices enable rapid screening of crime scenes for potential threats and hazardous materials, ensuring the safety of forensic investigators and law enforcement personnel.

E. **Smart Materials for Evidence Collection and Preservation:** Nanotechnology enables the design and fabrication of smart materials and coatings tailored for evidence collection and preservation at crime scenes. Nanomaterial-based substrates, such as superhydrophobic surfaces and molecularly imprinted polymers, enhance the capture, recovery, and preservation of forensic samples, including DNA, fingerprints, and trace materials. These smart materials prevent contamination, degradation, and loss of forensic evidence during collection, packaging, and transportation, ensuring the integrity and reliability of evidentiary materials for subsequent analysis and forensic examination.

Incorporating these subpoints into crime scene investigation practices harnesses the full potential of nanotechnology to enhance forensic capabilities, expedite criminal investigations, and contribute to the administration of justice. Continued research and development in nanotechnology-enabled crime scene investigation are essential to further advance forensic science and address evolving challenges in law enforcement and criminal justice.

VII. CHALLENGES AND FUTURE DIRECTIONS

Despite the promising applications of nanotechnology in forensic analysis, several challenges remain to be addressed. Standardization of nanomaterial synthesis, validation of analytical methods, and ethical considerations regarding privacy and data security are essential for the widespread adoption of nanotechnology in forensic science. Future research efforts should focus on developing integrated nanotechnology platforms for multiplexed analysis and enhancing the reliability and robustness of nanomaterial-based forensic techniques.

VIII. CONCLUSION

Nanotechnology holds immense potential to transform forensic analysis, offering innovative solutions for evidence collection, analysis, and crime scene investigation. By harnessing the unique properties of nanomaterials, forensic scientists can overcome traditional limitations and enhance the sensitivity, specificity, and reliability of analytical techniques. Continued research and development in nanotechnology are essential to realize its full potential in forensic science and criminal justice.

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Determination of Antibiotic Effects of Leaf and Latex Extracts of Calotropis procera

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ABSTRACT

The rapid emergence of resistant bacteria is occurring worldwide due to this the efficacy of antibiotics is at risk. The present study shows the antimicrobial effects of Calotropis procera leaf & latex extract which was prepared by using organic & aqueous solvents. Antibacterial activity of these extract was determined against E. coli, Bacillusspp.&A. Niger using agar-well diffusion and paper-disk diffusion methods. Calotropis procera latex exhibited the widest zone of inhibition, measuring 16mm against E. coli and 12mm against Bacillus spp. in agar well diffusion method while both organisms showed a 10mm inhibition zone in the disk diffusion method and leaf extract shows zone of inhibition measuring 10mm against E. coli, Bacillusspp. All the extracts of Calotropis procera were found least effective to inhibition the growth of A.niger. Future prospective of this work, to determine Calotropis procera latex activity for wound healing, anti- inflammation, anti-cancer properties.

Keywords: Calotropis procera, antimicrobial effects, latex, zone of inhibition

I. INTRODUCTION

A material that destroys or stops the growth of microorganisms like bacteria, fungus, or protozoa is known as an antimicrobial agent. Antimicrobial substances are either microbicidal—they kill bacteria—or microbiostatic—they stop them from growing. Medicinal plant's leaves, flowers, roots, and flavonoids have all been shown to be effective in treating a variety of illnesses[1].The shrub Calotropis procera, which belongs to the Asclepiadaceae family, is roughly six meters tall. The plant is tall, erect, heavily branched, perennial, and covered in milky latex. In Senegal, leprosy, ringworm, and syphilitic sores are among the skin conditions for which milky latex is locally used[2]. The plant utilized in traditional treatments, Calotropis procera, exhibits stronger antibacterial properties in its latex compared to its leaves. Calotropis procera's cystine peptidases Latex is essential for the management of fungal

microorganisms[3]. In vitro and in vivo, C. procera leaf extracts, chopped leaves, and latex have all demonstrated considerable potential as nematicides. There have also been reports on the possible applications of C. procera leaves in water treatment and their capacity to lower the total viable count [2].The development of antibiotic resistance and the emergence of unfavourable side effects from some medications has prompted researchers to look for novel antibacterial medicines, particularly those derived from medicinal plants. Indigenous plants serve as a storehouse for a variety of metabolites and an endless supply of important compounds with a range of biological characteristics[4]. Because natural plant products offer a consistent source of bioactive antimicrobial agents that are safe, effective, and have a broad spectrum with low toxicity and good pharmacokinetics for clinical use without chemical modification, more research should be done on these plants to gain a better understanding of their therapeutic qualities[5]. In the present study, we investigated the antibacterial and antifungal activities of different extracts of leaves and latex of C. procera.

II. MATERIALS AND METHODS

Collection and Processing of plant samples:

Leaves and latex of Calotropis procerawere collectedfrom local areas of Satara parisar, Chh. Sambhajinagar.Leaves were air dried for 1-2 days in hot air oven and blended into powder using electric blender. The samples were stored in air tight containers for further analysis. Latex was asceptically collected, one sample of latex was used directly, further three extracts were prepared.

Extraction of Plant Extracts:

Extraction of leaf and latex of Calotropis procera was donewith ethanol, chloroform and water. 05g of leaf powder were dissolved in 50mL of each solvent, 0.5mL of latex was dissolved in 0.5mL of each solvent. The suspended solutions were left to stand for 5 days and labelled accordingly. The extracts were filtered and stored at 40c.

Antibacterial test:

The antibacterial activities of aqueous, chloroform and ethanolic extracts and pure latex were determined by paper disc and agar well diffusion method. Two microorganisms used in study as test organisms which are obtained from biotechnology department Deogiri College.

Paper disc diffusion: The sterile filter paper discs (5mm diameter) were soaked in test extracts. Further microorganisms E.coliand Bacillus species were spreaded on EMB and Nutrient agar plates respectively. The soaked discs were placed on respective agar plates, the plates were incubated at 370c for 24 hrs.

Agar well diffusion: Firstly spreading of test organisms were done on respective agar plates, the wells was made with the help of sterile tips. 250µL of test extracts were filled into each well in respective agar plates.

Antifungal test: The antifungal test was performed against A.niger, using well diffusion and disk diffusion method as performed for antibacterial test.

III.RESULT AND DISCUSSION:

Antibacterial activity:

In the disc diffusion method 10,12,12,10,10,10,10,12mm zone of inhibition was observed using E.colias test microorganism for latex, petroleum ether latex extract, ethanol (latex), chloroform (latex), Water (latex), Ethanol (leaves) Chloroform (leaves) respectively.

Well diffusion method was performed for pure latex for E.coli microorganism 16mm zone was observed.

In the disc diffusion method, using Bacillus as test microorganism 10mm zone of inhibition was observed for all the extracts except for water (leaves) 11mm zone was identified.

Bacteria	E. coli		Bacillus	
	Disc	Well	Disc	Well

Latex	10	16	10	12
Petroleum ether(latex)	12	-	10	11
Ethanol(latex)	12	-	10	-
Chloroform(latex)	10	-	10	-
Water(latex)	10	-	10	-
Ethanol(lvs)	10	-	10	-
Chloroform(lvs)	10	-	10	-
Water(lvs)	12	-	11	-

Table 1: Antibacterial properties of Calotropis procera leaf and latex extracts using disc and well diffusion method (zone of inhibition in mm)

IV. ANTIFUNGAL ACTIVITY

Well diffussion method	Leaves extract(chloroform)	Leaves extract(ethanol)	Leaves extract(water)
Aspergillus niger	-ve	-ve	-ve

Table 2: Antifungal activity of Calotropis proceraleaf extract.

Zone of inhibition was not found effective for A.niger for all the extracts

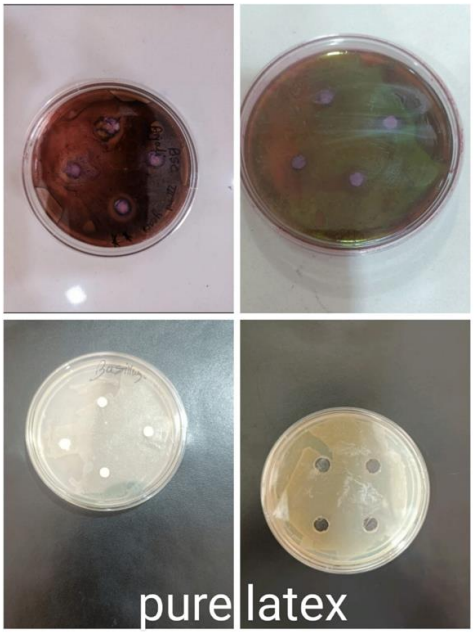

Figure1: Shows EMB (for E. coli) and Nutrient agar plates (Bacillus Sp.) for disc and well diffusion method for pure latex.

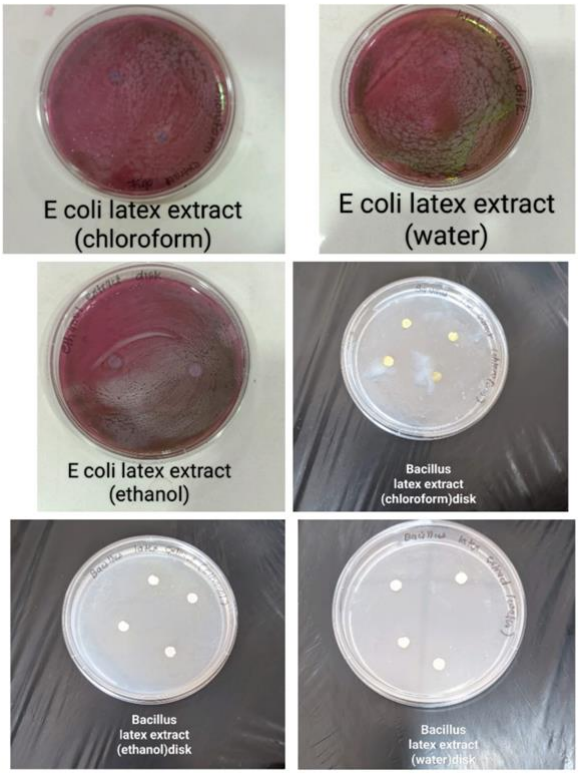

Figure2: Shows EMB (E.coli) and Nutrient agar (Bacilllus sp.) plates, for disc and well diffusion method for respective sample as labeled in figures.

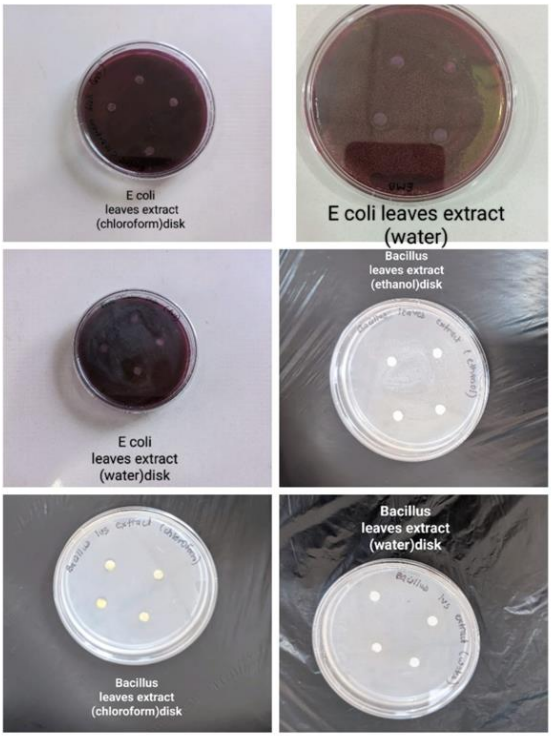

Figure3: Shows (E.coli) and Nutrient (Bacillussp.) plates, for disc and well diffusion method for respective sample as labeled in figures.

V. CONCLUSION

In the present study, we successfully determined the antibiotic effects of leaf and latex extracts of Calotropis proceraamongst which the pure latex showed largest zone of inhibition against E.coli microorganism comapried to the other extracts of calotropis leaf and latex extracts.

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Isolation of Nicotine Degrading Bacteria from the Oral Cavity of Tobacco Consumers

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ABSTRACT

The tobacco use is one of the greatest threats to global health today. Approximately one- third of the adult populations in the world use tobacco in some form. Chewing tobacco is a serious health risk that can cause severe oral disease and introduce pathogenic bacteria into the oral cavity. Nicotine is the addictive agent in tobacco products and it is present in sufficient quantities in all commercially available tobacco products to cause and sustain addiction in children and adults. Nicotine degradation by bacteria has received increasing attention in the past 50 years because bacteria have the potential to reduce nicotine levels in tobacco.

The efforts made in the present study were to isolate nicotine utilizing / degrading bacteria from the mouth of tobacco chewers. Fifty samples of saliva were collected from tobacco chewers of different types. The isolation of bacteria was done on liquid mediums having nicotine as the only carbon source. The bacteria isolated from sample study were found to be Pseudomonas sp. The study was also carried out to find out percentage utilization of nicotine by the isolated bacteria. It was found that maximum utilization of nicotine was upto 2% by the bacterial isolates. It was observed that an increased concentration of nicotine in the medium was inhibitory to the growth of bacteria in the medium.It was concluded that Pseudomonas sp. present in the mouth can utilize and break down the nicotine. Further, it should be noticed that an increased amount of nicotine has been found to be inhibiting for these nicotine utilizing bacteria.

Keywords : Nicotine, Tobacco chewers, Pseudomonas sp.

I. INTRODUCTION

Tobacco has always been a menace in developing countries like India and chewing tobacco apparently is widely prevalent in the state of Maharashtra. Unfortunately not much data is available pertaining to the prevalence of chewing forms of tobacco in the urban region of Marathwada especially Aurangabad, which is a rapidly developing industrial and tourist hub in the South-Central part of India. According to NFHS-III, in India, 55.8% male, 10.8% female in the age group of 12 to 60 years have been found to be consuming tobacco.Among males, 32.7% smokers while 36.5% tobacco chewers are reported, while among females; it is reported to be 1.4 and 8.4%, respectively[1]. Tobacco is a product prepared from the leaves of tobacco plants. Chewing tobacco is a serious health risk that can cause severe oral diseases and introduce pathogenic bacteria into the oral cavity [2]. Tobacco is an addictive drug that is harmful to one's health and its use could possibly lead to death [3]. Consumption of tobacco leads to halitosis, stained teeth, dental restorations. and serious diseases such as oral cancer (4) gingivitis, periodontitis [5], melanosis (increased pigmentation on the cheeks and gums), and leukoplakia (white patches or plaques on the lining of the oral mucosa) [6]

Nicotine, a principal pyridine alkaline, heterogenous group of organic nitrogen containing bases often with a heterocyclic ring [7] and is notorious for its significant contribution to tobacco addiction. Nicotine is colorless laevo rotatory liquid but its salts are dextrorotatory. Nicotine is very toxic to humans because it is easily absorbed in the body; its hydrophilic nature contributes to the environmental contamination [8] [9]. Nicotine is not a direct cause of most tobacco smoking-related diseases, but it is highly addictive (Benowitz [10]. Currently, regulatory strategies to counter the tobacco induced disease. epidemics are very much focused on nicotine.

Nicotine degradation by microorganisms has received increasing attention in the past 50 years because microbes have the potential to reduce nicotine levels in tobacco [11] [12]. Many bacteria capable of utilizing nicotine have been isolated and characterized [13] As an environment-friendly treatment; microbial degradation of nicotine has been considered as a promising method due to its low cost and high efficiency. However, the current understanding of nicotine metabolism in microorganisms is poor. Therefore the aim of the present investigation was to isolate nicotine degrading bacteria from the oral cavity of tobacco chewers and to study its effect on nicotine degradation.

II. MATERIALS AND METHODS

Sampling :

The Oral samples of tobacco chewers (saliva swab) were collected from fifty subjects from the age group of 18-35 years.(Table 1).The collected samples were inoculated in a test medium which contains pure nicotine.This nicotine was used as a carbon source with other ingredients like K_2HPO_4, KH_2PO_4, $MgSO_4$, $(NH_4)_2SO_4$, NaCl and distilled water.

Other media: Phenol red broth, Pseudomonas agar.

Isolation and Biochemical characterization :

The samples were inoculated in Test medium containing 2% nicotine. After incubation at 37°C for 48 hrs, turbidity observed is the suggestive of the organisms utilizing nicotine. These growth culture tubes are then identified by biochemical characterization using phenol-red broth with different carbohydrates.

The organisms were also cultivated on selective media like Pseudomonas agar for their confirmation. The isolated bacteria, were identified on the basis of morphological, cultural and biochemical characteristics [12]

Inhibiting Effect of Different Nicotine Concentrations on Isolated Bacteria:

Two sets of test tubes containing liquid medium with increasing nicotine concentration (Table 2) were used with 8 test tubes in each set. One set was inoculated with the isolated bacteria and the other was kept uninoculated. In each set, one tube containing the standard concentration of nicotine was considered as Blank. Both the sets of test tubes were incubated for 1-2 days at 37°C. After incubation, the tubes were observed for turbidity and compared to the uninoculated tube of the respective concentrations. Optical density was taken at 620 mm. The optical densities of the inoculated tubes were compared with optical densities of the uninoculated tubes observe the effect of increasing concentration of nicotine on the bacteria.[14].

Table 1.Isolates of nicotine utilizing bacteria from saliva of different tobacco chewers.

Sr. no	Saliva sample of tobacco chewers	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
1.	Tobacco (loose)	P_{n1}	P_{n1}	P_{n1}	P_{n1}	P_{n1}	P_{n1}		P_{n1}	P_{n1}	P_{n1}
2.	Gutka	P_{n2}	P_{n2}		P_{n2}		P_{n2}	P_{n2}	P_{n2}		P_{n2}
3.	Zafrani tobacco		P_{n3}	P_{n3}				P_{n3}	P_{n3}		P_{n3}
4.	Packed tobacco	P_{n4}	P_{n4}	P_{n4}		P_{n4}	P_{n4}		P_{n4}		P_{n4}
5.	Bidi tobacco	P_{n5}		P_{n1}	P_{n1}	P_{n1}		P_{n1}	P_{n1}	P_{n1}	

Legends-

Pn_1,Pn_2,Pn_3,Pn_4,Pn_5– Bacterial isolates

N1,N2,N3,N4,N5 – Sample numbers

Table 2.Percentage utilization of nicotine by different bacterial isolates.

Sr.no	Percentage of nicotine	Pn_1	Pn_2	Pn_3	Pn_4	Pn_5
1	0.50	–	–	–	–	–
2	1.00	–	–	–	–	–
3	1.50	+	+	+	+	+
4	2.00	+	+	+	+	+
5	2.50	+	+	+	+	+
6	3.00	+	–	+	–	–
7	3.50	+	–	–	–	–
8	4.00	–	–	–	–	–

Legends : + growth , – no growth

III.RESULTS AND DISCUSSION

A nicotine- degrading bacterial isolates were isolated from oral cavity samples obtained from tobacco chewers in Aurangabad. On the basis of biochemical reactions and cultural characteristics Pseudomonas sp was identified. Saliva is the first biological. fluid that is exposed to tobacco which is responsible for the changes in salivary $P_{H.}$ The bacteria could use nicotine as the sole carbon,nitrogen and energy source, [15]

which showed turbidity after incubation period indicative of utilization of nicotine

The Pseudomonas isolates were also tested for their maximum tolerance of nicotine percentage as shown in Table 2. There are references of Pseudomonas possessing NIC gene for biodegradation of Nicotine (alkaloid) [16].

The bacteria, especially Pseudomonas species, were found in the mouth of tobacco chewers which can utilize nicotine. Maximum number of Pseudomonas isolates were observed in the individuals chewing tobacco (loose) which have higher content of nicotine (Table 1.)

The percentage utilization of nicotine as the sole carbon source.was found to be the highest of 3.5%. However, Pseudomonas isolate could not be able to grow in medium with nicotine. percentage as low as 0.5%.

Nicotine utilizing organisms were also studied for nicotine degradation by some researchers[17]. In other studies normal oral flora were isolated as *Staphylococcus sp,Streptococcus sp,Proteus sp, Pseudomonas sp and Klebsiella sp* and reported that *Pseudomonas* has the ability to degrade nicotine[14]

IV. CONCLUSION

The present study suggested that the bacteria isolated from the oral swabs of tobacco consumers can utilize nicotine present in the tobacco and also decrease the hazardous effect that nicotine has on human health after continuous consumption. The bacteria isolated found to be the *Pseudomonas* sp.

It can therefore be concluded that the presence of *Pseudomonas* sp. in the mouth is an advantage itself. The utilization of nicotine by *Pseudomonas* sp. is beneficial to tobacco. chewers, because a part of the tobacco in the nicotine is degraded in the mouth Which might help in decreasing the risk associated with it. Hence it can be said that the species of *Pseudomonas* present in mouth can utilize and break down the nicotine. However, it should also be taken into consideration that an increased amount of nicotine has been found to be inhibitory for these nicotine utilizing bacteria also.

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Optimisation for Lime Soda Ash Water Softening Process with Low-Cost Adsorbent for Reducing the Total Hardness of Water

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ABSTRACT

Lime soda water softening is traditional method. Water hardness can cause many problems including scaling and excessive soap consumption. In present investigation, water softening experiments were conducted to observe the changes in total hardness with varying dosages of lime and soda ash for different time intervals and with use of RHP adsorbent of desired doses. Results indicated that an increase in lime & soda ash dosage with adsorbent caused decrease in total hardness of water samples. For 50 cm3 of synthetic water sample, use of 17.5 cm3 Lime with 12.5 cm3Soda ash dose with 0.500g RHP adsorbent dose gave 58.88% hardness reduction in 1hourin economical way.

Keywords: Water Hardness, Lime, Soda ash, adsorbent, water sample

I. INTRODUCTION

Water is essential for survival of human being. Groundwater is the main source of domestic, industrial and agriculture supplies in many regions of India. There is an increasing demand for groundwater due to surface water quality deteriorationand scarcity in rainfall. Many times, available groundwater is called as hard water, because Calcium (Ca^{+2}) and Magnesium (Mg^{+2}) ions are present in it. Hard water can cause corrosion and silting in industrial operations. Water hardness is a measure of the quantity of divalent ions such as calcium, magnesium and/or iron in water. Water hardness is identified in form of bicarbonates, Chlorides and Sulphates and should be below the BIS standard IS 10500:2012 for drinking water. Currently methods like chemical precipitation using lime, nanofiltration, carbonnanotubes, electrocoagulation etc are available for water softening, but are not suitable because these processesare energy consuming ,expensive and difficult to operate and cause large volumes of sludge. Hard water is very dangerous to human body especially on hair and skin. This gave need of search of environment friendly, low-cost, and low energy processes for water softening. So current investigations include use of lime & soda ash in optimized doses with *Rice Husk Powder*(*RHP*)as an adsorbent .

II. METHODS AND MATERIAL

The EDTA titration method was used to measure the total hardness of the ground water (Kasun T. Samarasiri, 2016) .Quick lime CaO, Slaked lime $Ca(OH)_2$, Soda ash $NaC0_3$ and Sodium hydroxide (NaOH, are common water softening reagents (Kasun T. Samarasiri, 2016).In present investigations, use of Lime & Soda ash combination is studied to reduce water hardness along with use of adsorbent.Different variables studied to optimize the process are given in following table-

Sr no	Variable parameters	Different levels used		
		1	2	3
1	Retention time	30minutes	60minutes	80minutes
2	Lime dose for 50 cm3 Water sample	10cm³	12.5 cm³	17.5 cm³
3	Soda ash dose for 50 cm3 water sample	5 cm³	7.5 cm³	12.5 cm³
4	pH	Neutral	9.6	10.6

Table 1- Different variables used for optimisation of lime sodaprocess

Four different treatments given to synthetic water samples are discussed below-

Treatment 1

In treatment 1, a sample of 50 cm³ synthetic water sample was taken in conical flask and 10 cm³ of lime and 5 cm³ of Sodaash is added and shaken in horizontal viabrator for 30 minutes. Thenit was filtered and the hardness was measured by EDTA titration method. The initial pH value was determined. The initial total hardness of the sample 340 ppm was reduced into 310 ppm after treatment. The percentage reductionof the total hardness is 8.82%.

Treatment 2

In treatment 2, 50cm³ synthetic water sample was taken in conical flask and 12.5cm³ of lime and 17.5 cm³ of soda ash is added to it and shaken in viabrator for 60 minutes.Then it was filtered and the hardness was measured by EDTA titration method.After the treatment,the initial total hardness of the sample is 340 ppm was reduced into 260 ppm .The percentage reductionof the total hardness is 23.53%.Furthur treatment should be given to reduce more water hardness,

Treatment 3

In treatment 3, a sample of 50 cm³ synthetic water sample was taken in conical flask and 17.5cm³ of lime and 12.5 cm³ of soda ash is added to it andis shaken in viabrator for 80 minutes. Thenit was filtered and the hardness was measured by EDTA titration method. The initial pH value was determined. The initial total hardness of the sample is 340ppm and after treatment, was reduced by 200 ppm. The percentage of the total hardness reduction of the treatment 3 was found 47.17%.

Treatment 4-

In treatment 4, sample of 50cm³ synthetic water sample was taken in conical flask and 17.5cm³ of lime and 12.5 cm³ of soda ash and 0.500 gms *RHP* adsorbent (*Rice Husk Powder*) are added and shaken in viabrator for 60 minutes . Then it was filtered and the hardness was measured by EDTA titration method. The initial pH value was determined. The initial total hardness of the sample is 340ppm and after treatment, the hardness was reduced to 150 ppm. It is below desirable level The percentage of the total hardness reduction of the treatment 4 is 55.88%.

Observed graphs for different treatments are as follows-

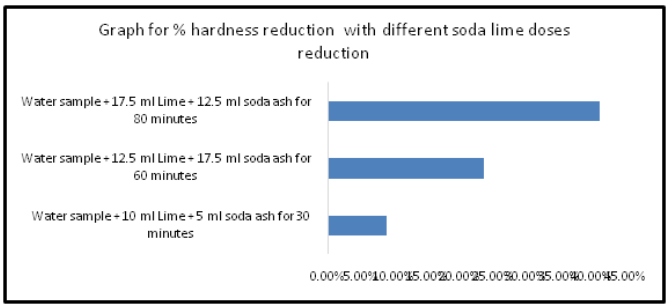

Figure1- Graph showing percent reduction in water hardness with different propotions of sodalime treatment to water sample

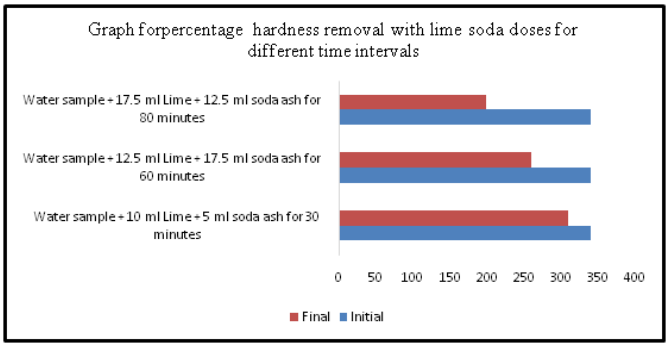

Figure2- Graph showing initial and final percent reductiion in water hardness with different propotions of sodalime treatment to water sample

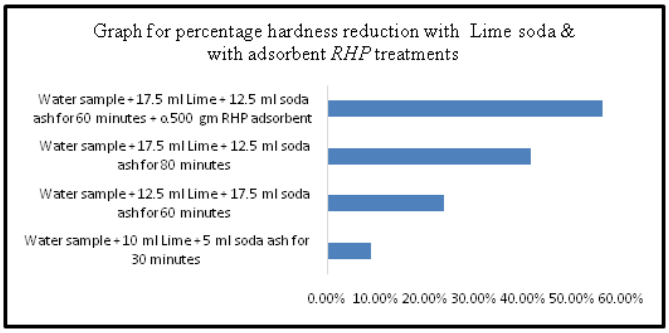

Figure3- Graph showing percent reduction in water hardness with different propotions of sodalime doses treatment to water sample and with 0.500 gms of *RHP* adsorbent .

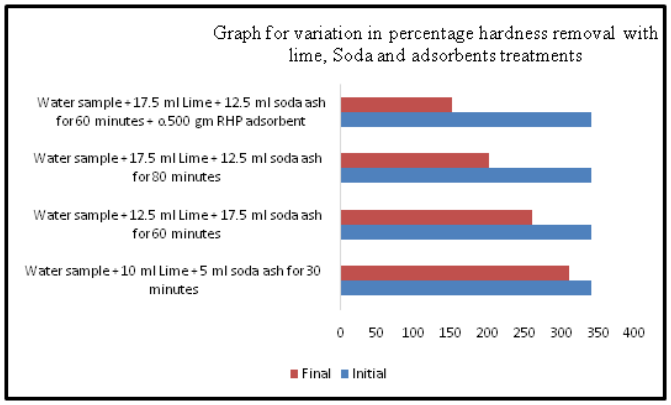

Figure 4- Graph showing initial and final percent reductiion in water hardness with different propotions of sodalime treatment to water sample and with 0.500 gms of *RHP* adsorbent

Similar type of treatments are given to well water by Kusum et, al, in 2016.These lime soda water softening methods are further improvedfor the purification process from different chemical treatments many researchers.

III.RESULTS AND DISCUSSION

The method is universal because waters of almost any composition may be treated with lime and soda.Following table2 shows the findings of four different treatments given to water sample in present investigations.

Sr. No.	Treatment	% Reduction Total Hardness
1	Water sample + 10 cm^3 Lime + 5 cm3 soda ash for 30 minutes	8.82%
2	Water sample + 12.5 cm^3 Lime + 17.5 cm3 soda ash for 60 minutes	23.53%
3	Water sample + 17.5 cm^3 Lime + 12.5 cm3 soda ash for 80 minutes	41.17%
4	Water sample + 17.5 cm^3 Lime + 12.5 cm3 soda ash	55.88%

	for 60 minutes + 0.500 gm RHP adsorbent	

Table2 -The percentage reduction in total hardness forfour different treatments given to water sample.

In this treatment, two reagents are being used namely lime and soda ash. Lime decreases the carbonate hardness, (Mg^{2+}) and removes $C0_2$ from the water. Soda on the other hand reduces the non - carbonate hardness, mainly due to Ca^{2+}, that showed after the liming and the reaction occurs after the addition of soda ash is as follows. (Darshak Vaniya, 2018)

Lime Precipitate $MgSO_4 + Ca(OH)_2 \text{ -- > } Mg(OH)_2 + CaSO_4$

Soda ash Precipitate $CaSO_4 + Na2CO_3 \text{ -- > } CaCO_3 + Na_2SO_4$

In present investigations, when different ratios of lime & water are used in soda lime process, as quantities of lime and Soda ash are increased, the reduction in water hardness is observed. But at 17.5 cm^3 lime 12.5 cm^3Soda ash with adsorbent is best removal 41.17% in time 80 minutes is observed but with for same dosage of lime & soda with adsorbent 0.500gms reductions is increased to 55.88% in 60 minutes. i.e. use of adsorbent decreased time required for process to 60 minutes due to adsorption process at adsorption sites. Similar results with optimum dose of same concentration of lime and soda ash removal of hardness is reported to maximum of 50.91 percent (Darshak Vaniya, 2018) for removal of ground water hardness.Similar studies were carried out by use *of wheat straw ash (WSA) & Rice Husk ash (RHA)* as adsorbents for removal of water hardness (Hari Lal Kharel, 2016) Similarly *DjamelGhernaout et al*, studied process of combination of liming & alum coagulation with alum for dam water with dosages alum = 15, lime = 100, NaOH = 100 mg/L.((Djamel Ghernaout 1, 2018). Similarly effect of pH was studied on precipitation ((Hari Lal Kharel, 2016). Tyler Smith ,2022 reported that Magnesium removal is not typically required in Florida due to the limited amount of magnesium in most source water (Tyler Smith, 2022).

IV. CONCLUSION

Lime soda water softening is efficient traditional, ecofriendly greener method with selection of proper doses of lime and soda ashes makes desirable quality of water.In present investigation, in watersoftening experiments, varying dosages of lime and soda ash for different time intervals and with use of desired dose of *RHP adsorbent* were successful to reduce the total hardness of water significantly till 55.88%.Resultsindicatedthatanincreaseinlime& soda ash dosage with adsorbent caused a decrease in total hardness of water samples. For 50 cm^3 of synthetic water sample, use of 17.5 cm^3 Lime with 12.5 cm^3 Soda ash dose with 0.500g *RHP* adsorbent dose gave 55.88% hardness reduction in 1hourin an economical way. So optimum conditions for Soda lime process for hardness reduction are-

Sr no	Variable parameters	Optimum to be used
1	Retention time	60 minutes
2	Lime dose	17.5 cm^3
3	Soda ash dose	12.5 cm^3
4	Adsorbent dose	0.500 gm
5	pH	Normal

Table3- Optimum conditions observed for Soda lime process for hardness reduction

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Exploring Feline Fur: The Medullary and Cuticle Indices of Cat Hair

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ABSTRACT

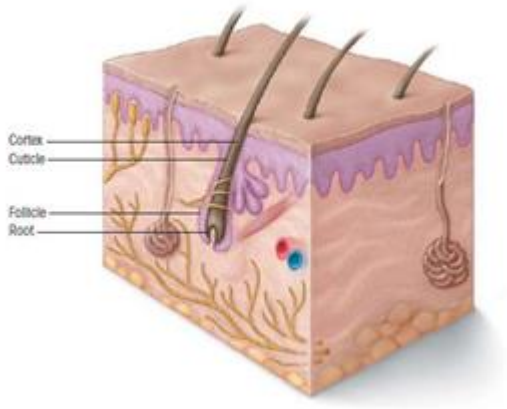
The purpose of this study is to examine cat hair's medullary index and cuticle index for forensic purposes. Even though it's frequently overlooked, cat hair is important for forensic investigations, especially when it deals with crime involving animals or interactions between humans and animals. This paper provides a thorough analysis of the structure and composition of cat hair, highlighting its unique features that differentiate it from the hairs of other mammals. A sample of cat hair was collected and prepared for light microscope analysis. There are a total of 43 samples taken, of which 21 are male and the remaining 22 are female. The cuticle index, which shows the quantity of cuticle scales per unit length of hair, and the medullary index, which shows the ratio of medulla diameter to hair shaft diameter, was estimated. Understanding the intricacies of cat hair morphology and composition is essential for accurate forensic analysis and can provide valuable evidence in criminal investigations. This research provides valuable insights into the microscopic characteristics of cat hair, enhancing the understanding of cat biology and aiding in forensic investigations involving animal hairs.

Keywords: cat hair's, medullary index, cuticle index, hair sample.

I. INTRODUCTION

The purpose of this study is to examine cat hair's medullary index and cuticle index for forensic purposes, cat hair is important for forensic investigations. A keratinous thread that emerges from the epidermis is called hair. It is mainly composed of keratinized, dead cells. Hair follicles are epidermal penetrations of the dermis where hair strands begin. The portion of hair that is not attached to the follicle is known as the hair shaft, and it is mostly visible at the skin's surface. The term "hair root" refers to the remaining hair, which is rooted in the follicle and remains underneath the skin's surface. At the hair

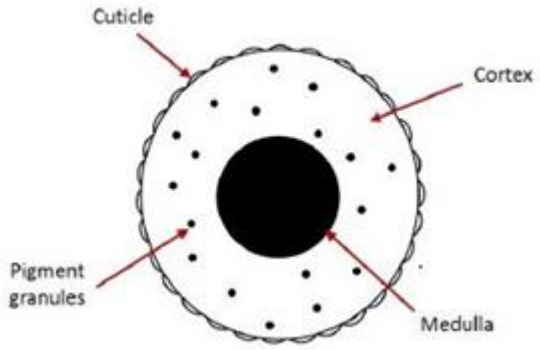
bulb, located deep below the dermis, the hair root terminates. The hair matrix is a layer of basal cells that are actively dividing during mitosis. The connective tissue-based hair papilla, which has blood capillaries within, is encircled by the hair bulb.



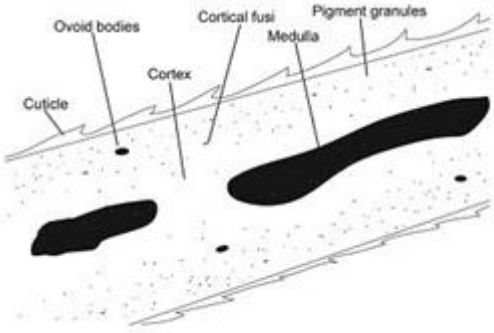
"Fig.1: Skin cross-section demonstrating hair emerging from the follicle, a tube like structure"

HAIR MICROSTRUCTURE:

It is possible to investigate variations between hairs from various populations by examining the microstructure of hair and its distribution within each layer. Hair shaft is consisted of three layers: cuticle, cortex and in certain cases medulla.



"Fig.2: Cross section of Hair Shaft"



"Fig.3: Diagrammatical view of Hair Shaft"

1. Cuticle:

Cuticle has also important protective properties and barrier functions against physical and chemical insults. The outermost covering of hair, known as the cuticle, is composed of 5 to 10 covered keratinized cells that resemble roof tiles and act as a barrier to prevent environmental and mechanical stress on the hair fiber. Under transmission electron microscopy, each cuticle cell can be shown to consist of three distinct layers: the A-layer, the exterior exocuticle, and the inside endocuticle. Cuticle cells are bound together by the CMC (Cellular Membrane Complex).

2. Cortex:

The cortex, which is the major body of a human hair, is made up of cells that are 2–5 μm in diameter and 100 μm long. The cells are bound together by a CMC and intermediate filaments (IF) that are bundled into macrofibrils. The primary structure of the cortex, which is evidently composed of a composite material, is composed of the 12 different types of keratins (which account for 40% of cortical proteins) and the over 100 different types of keratin-associated proteins (KAPs), which account for 60% of cortex proteins.

3. Medulla:

The medulla is found in the middle of the hair shaft and is best seen in fibers that are coarser. The medulla of hair has distinct structural proteins from other keratins in the hair and eosinophilic granules packed with citrulline, an amino acid, which eventually forms internal coatings in the membranes of mature cells. The medulla may be continuous, interrupted, fragmented, or absent. The medulla is a cellular column running through the center of the hair. The medullary index measures the diameter of the medulla relative to the diameter of the hair shaft. For humans, the medulla generally occupies less than one-third the diameter of the shaft, while for animals it is generally one-half or greater.

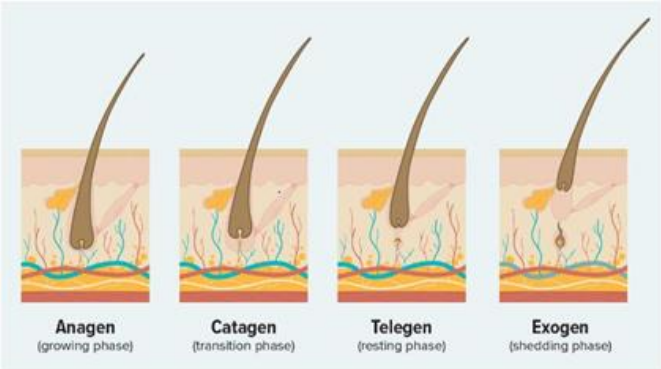
Pigmentation of hair follicle:-

Pigmentation of the hair shaft has several advantages, such as protection from UV rays, thermoregulation,

and enhanced sexual sensibility. Melanin, the color found in hair, is also a strong scavenger of free radicals. Melanosomes are located in the cuticle, cortex, and medulla; however, their predominant position is within cortical cells. Melanosome distribution and number determine hair color. Therefore, melanin synthesis within the active anagen hair bulb may aid in mitigating the effects of reactive oxygen species-induced cellular stress. Follicle melanogenesis is a cyclic event as opposed to the continuous melanogenesis seen in epidermal melanocytes. The anagen-catagen transition is stopped early, and then essential melanogenesis-related enzymes are downregulated, which is followed by melanocyte apoptosis in hair follicles.

Hair growth:-

Hair formation is a constant cyclic process. The mature follicles go through a growth cycle that includes phases of growth (anagen), regression (catagen), rest (telogen), and shedding (exogen). The length of each phase varies according to the individual's age, nutritional and hormonal health, and hair location.



"Fig.4: Stages of Hair Growth"

Different stages of hair growth include:

1. Anagen (active growth phase)

The initial growth phase during which the hair follicle actively produce hair. Anagen growth is the active phase in which the hair follicle take on its onion-like shape and works to produce the hair fibres. Almost 85–90% of all scalp hairs are in anagen. Six portion of the anagen stage is demonstrated. Hair stem cells multiply during anagen I–V, enclose the dermal papilla, grow to the skin's surface, and start to proliferate the hair shaft and IRS, respectively. The morphology of the hair shaft then starts to emerge as hair matrix melanocytes start to produce pigment; in anagen VI, the creation of the dermal papilla and hair bulb is realised, and the new hair shaft emerges from the skin. In hair follicles, this phase can persist for up to 6–8 years.

2. Catagen (transition phase)

A transition stage between the anagen and telogen phase of hair growth. Catagen lasts approximately 2 weeks in humans, regardless of the site and follicle type. During catagen the proximal of the hair shaft is keratinized and forms the club hair, whereas the roots of hair typically take on elongated appearance as the root bulb shrinks and is pushed out of the hair follicle. 5% of the hairs on your head are in the catagen phase.

3. Telogen (resting phase)

The final growth phase in which hair naturally falls out of the skin. In 2 to 3 months make up the telogen stage. 10 to 15% of the hair is at the telogen stage. The hair shaft changes into club hair during the telogen stage and eventually sheds. The follicle stays in this stage until the hair germ, which is sensitive to signals from the dermal papilla that initiate anagen, begins to exhibit increased transcriptional and proliferative activity in late telogen, that leads anagen to begin. One of the primary targets of the hair cycle, telogen is impacted by a number of modulatory substances, including thyroid hormones, prolactin, ACTH, retinoid, and androgens.

4. Exogen (Shedding phase)

However the patient defines it as the most major part in hair development, there is less interest in the mechanism behind hair shedding. Human telogen hairs are frequently maintained from multiple follicular cycles, which implies that anagen and exogen stages are separate. The shedding phase is known as exogen because it is thought to be an active

process that occurs independently of telogen and anagen.

II. METHODS AND MATERIAL

There are a total of 43 samples taken, of which 21 are male, and the remaining 22 are female, of which 32 are Ferals, 05 are Persian, and the remaining are cross-breeds. While collecting the samples, precautions were taken, such as gloves for covering hands, face masks, and plastic sterile zip-lock bags for collecting hair samples from cats. For examination, the sample was taken with the help of tweezers and placed on a petri dish with ethanol to remove excess dirt from the hair. Then the hair sample was taken on the slide and mounted with water by covering it with a cover slip, and then the sample was examined under a light microscope, magnification of 10X and objective lens 45X was used for studying was used for examination. For studying the medullary index and cuticle index of cat hair by using standard method for measure using scale and formula is

$$\text{Medullary index} = \frac{\text{Diameter of hair shaft}}{\text{Diameter of medulla}}$$

Calculate the medullary index.

For cuticle index use the formula

$$\text{Cuticle index} = \frac{\text{Thickness of cuticle}}{\text{Diameter of hair shaft}}$$

Calculate the cuticle index.

Observation of sample is shown below:-

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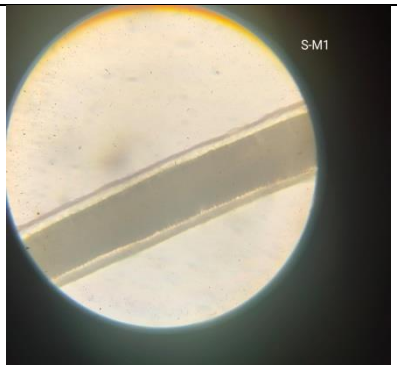
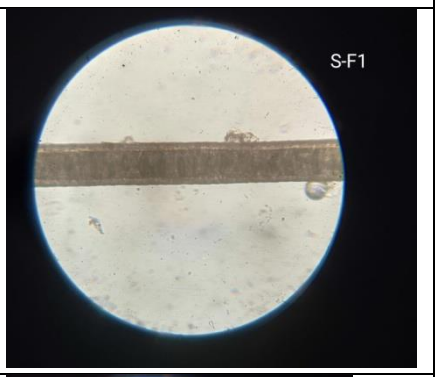
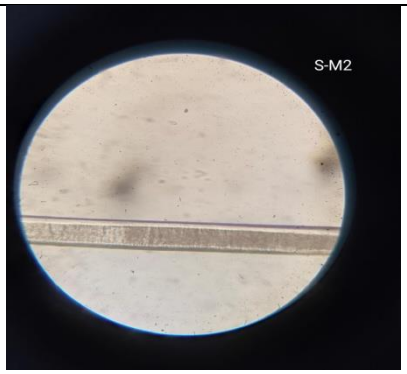
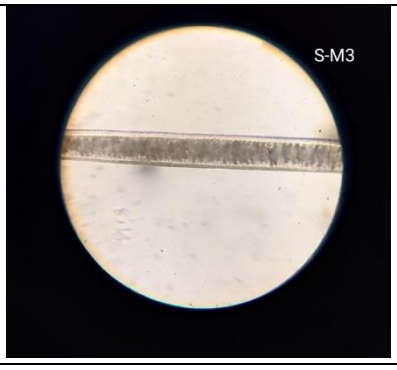
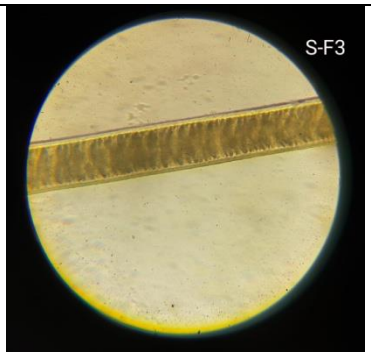
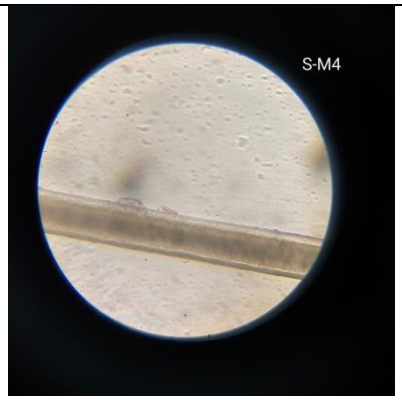
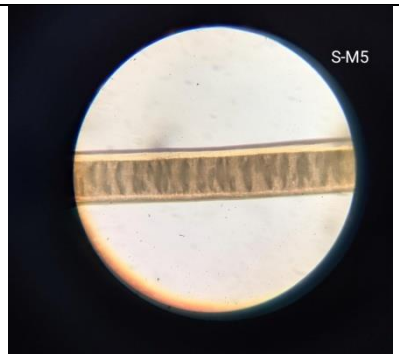
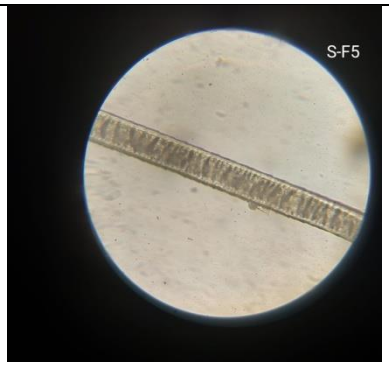
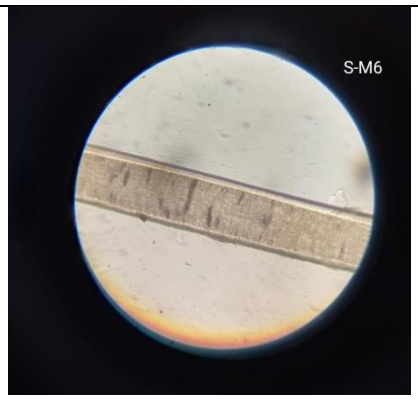
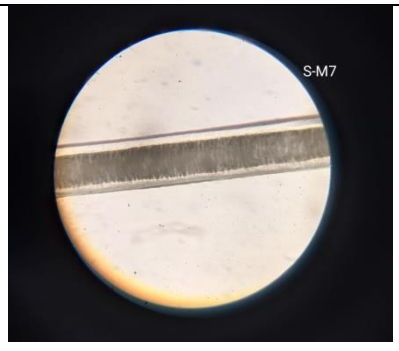
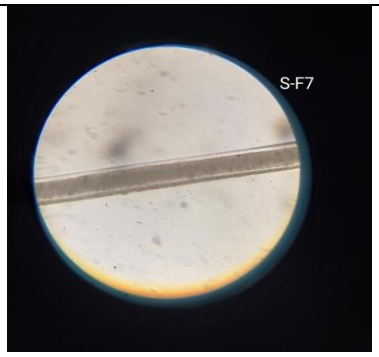
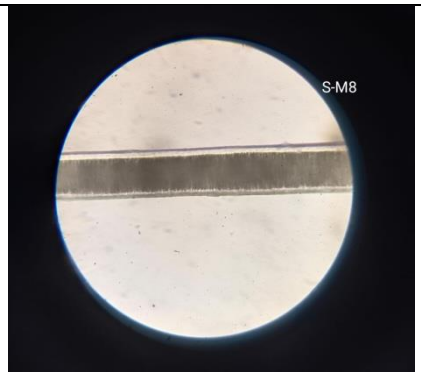
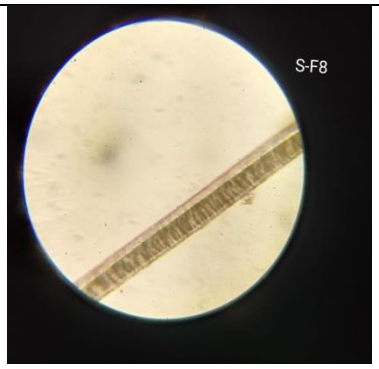
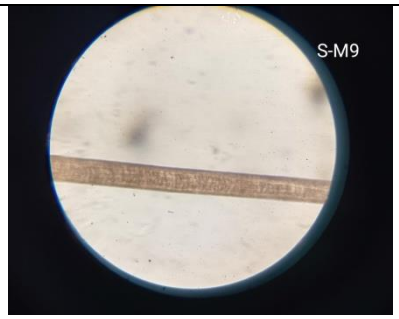
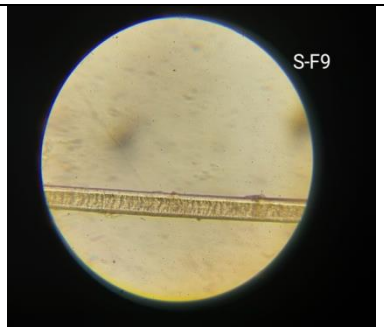
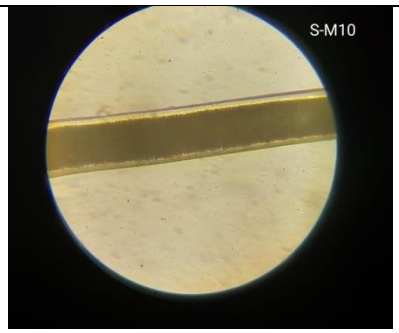
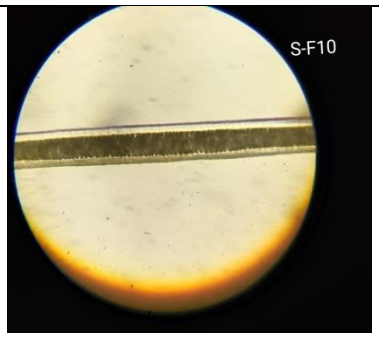
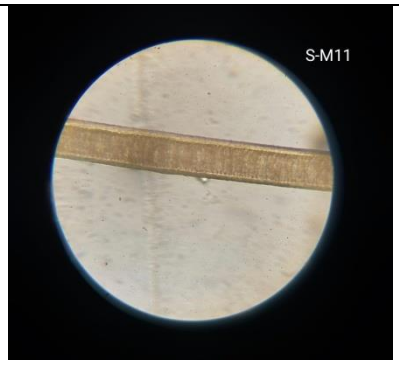
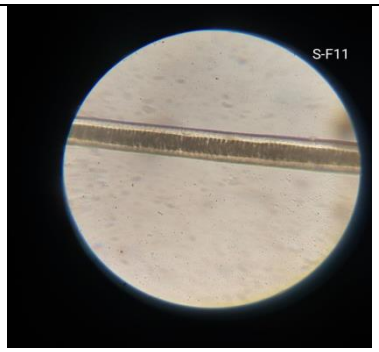
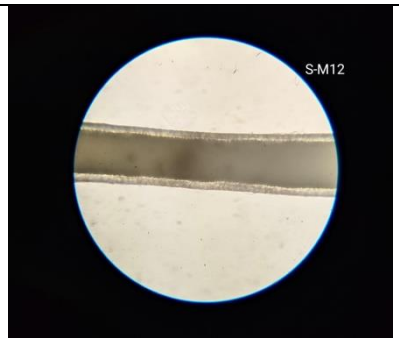
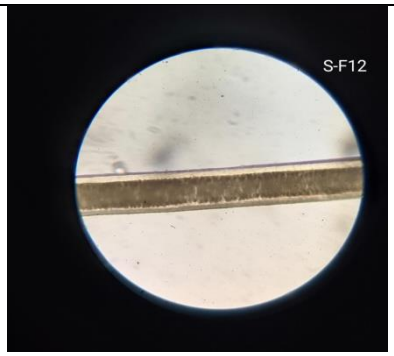
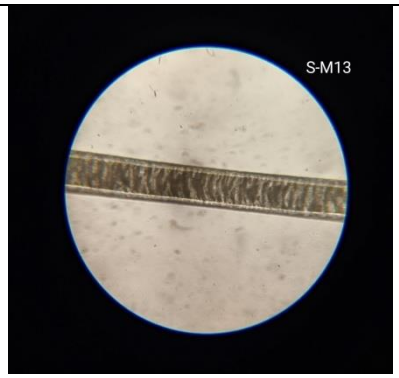
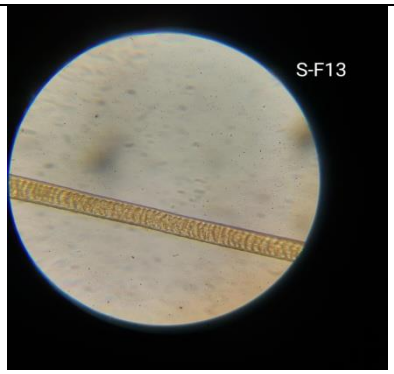
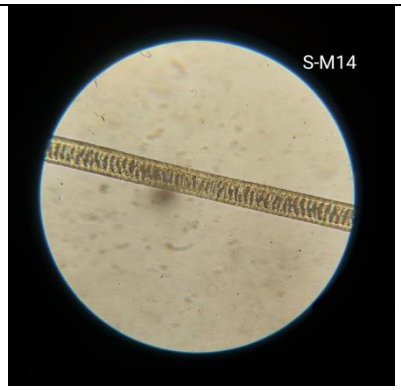
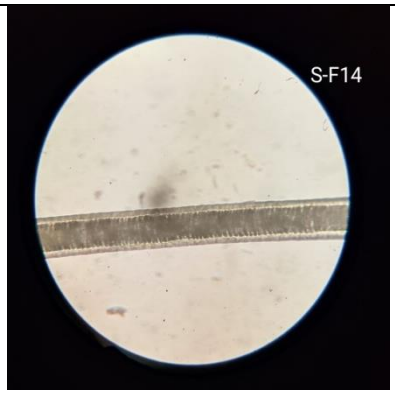
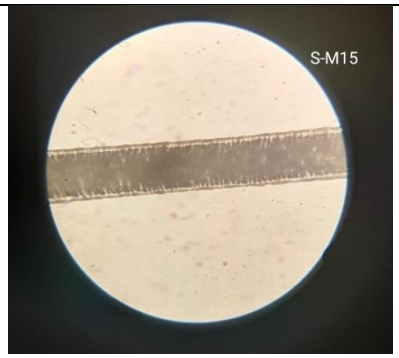
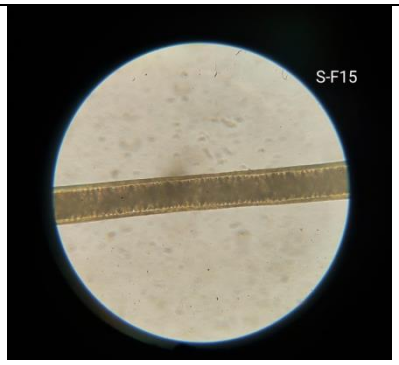
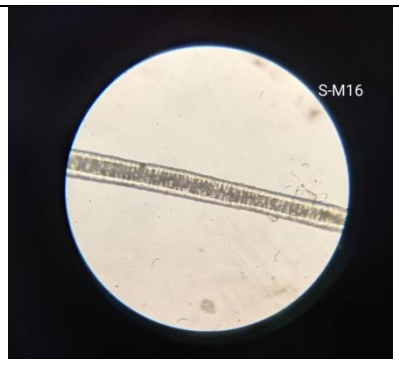
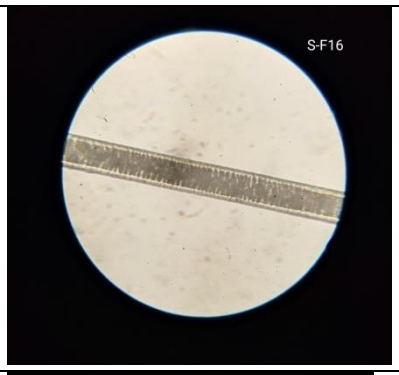
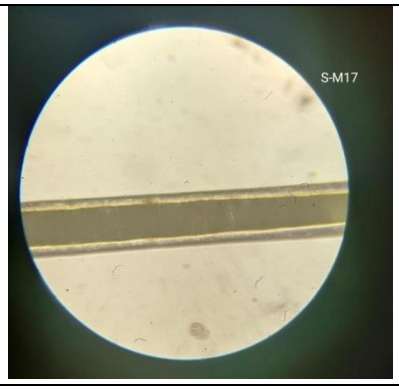
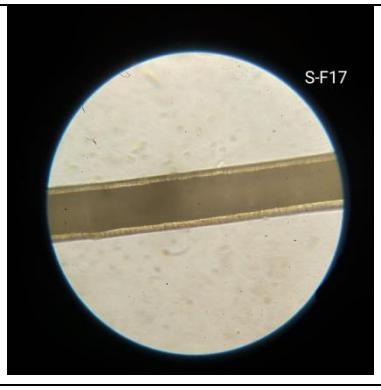
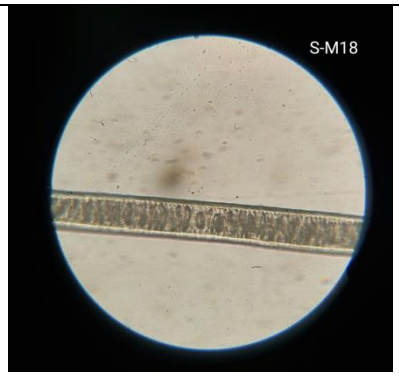
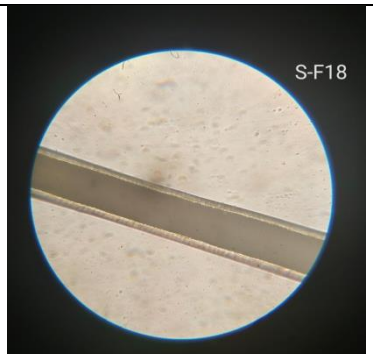
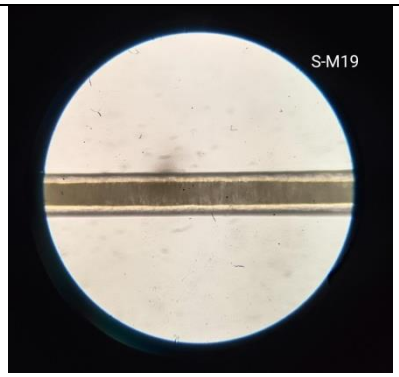
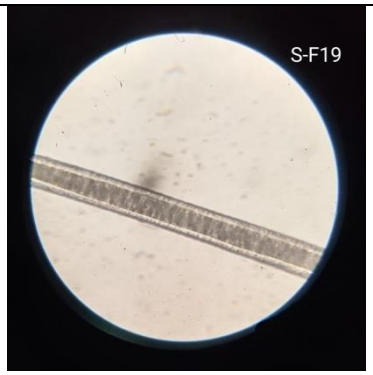
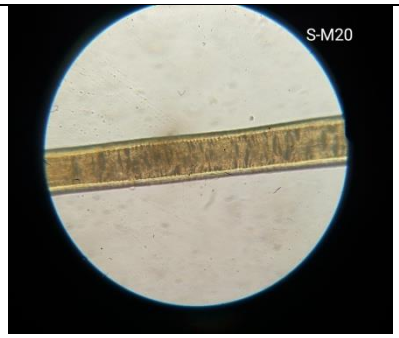
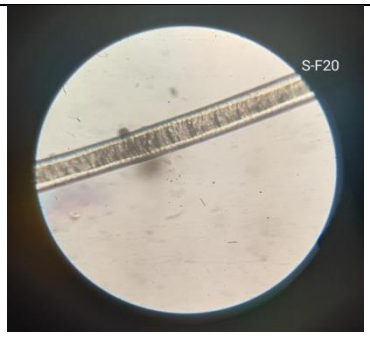
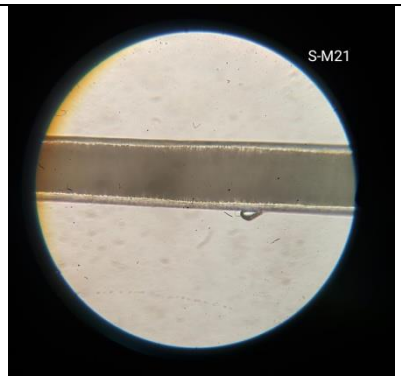
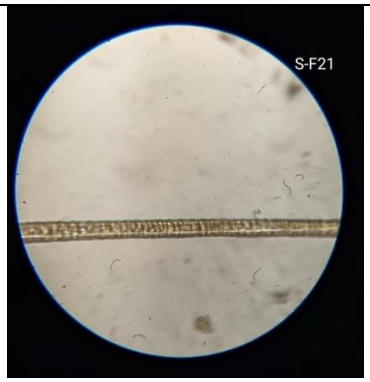
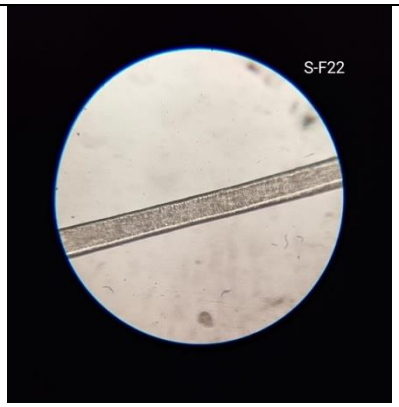
1.		
2.		
3.		

4.		
5.		
6.		
7.		

8.		
9.		
10.		
11.		
12.		

13.		
14.		
15.		
16.		
17.		

18.		
19.		
20.		
21.		
22.	NA	

III.RESULTS AND DISCUSSION

Result:-

In the world of feline fur, there are hidden secrets waiting to be revealed through scientific analysis. One of the key factors in understanding cat hair is examining the medullary and cuticle indices, which can provide insights into the structure and characteristics of different breeds. By delving into these indices, we can uncover the unique qualities that make each cat's fur distinct and fascinating. The innermost layer of hair, known as the medulla, was made up of pigments. Throughout the investigation, medulla was prevalent in every domestic animal species. The cuticle index, which shows the quantity of cuticle scales per unit length of hair, and the medullary index, which shows the ratio of medulla diameter to hair shaft diameter, were estimated. The Medullary Index can help distinguish between different types of hairs. Understanding the intricacies of cat hair morphology and composition is essential for accurate forensic analysis and can provide valuable evidence in criminal investigations. For scaling the pattern Cuticular and medullary scale patterns were considered as characteristic to species and subspecies which can be used in the identification. In present study guard hair collected from three different types of cat such as, feral, cross bread of feral and Persian and Persian cats which shows the medullary and cuticle indices. Two different characteristics, such as the cuticle index and medullary index of cat hair, were analyzed in 43 hair samples. During microscopic and optical examination of each hair sample, the mathematical ratio is provided in Table.1 contain male information and Table. 2 contain female information, and the male hair sample's **medullary index** value, which ranged from 0.55 to 0.86 in male samples and 0.53-0.77 in samples of females, and **cuticle index** value, which ranged from 0.10 to 0.23 in male samples and 0.2 to 0.28 in female samples as shown in tables.

TABLE NO.1 (MALE):-

SAMPLE NO.	BREED NAME	MEDULLARY INDEX	CUTICLE INDEX
1.	FERAL	0.70	0.16
2.	FERAL	0.78	0.17
3.	FERAL	0.7	0.15
4.	PERSIAN	0.66	0.16
5.	CROSS(F+P)	0.66	0.15
6.	CROSS(F+P)	0.76	0.10
7.	FERAL	0.66	0.17
8.	FERAL	0.73	0.15
9.	FERAL	0.76	0.14
10.	FERAL	0.77	0.11
11.	PERSIAN	0.71	0.13
12.	FERAL	0.75	0.13
13.	FERAL	0.66	0.14
14.	FERAL	0.55	0.23
15.	FERAL	0.86	0.71
16.	PERSIAN	0.6	0.36
17.	FERAL	0.6	0.17
18.	FERAL	0.6	0.22
19.	CROSS(F+P)	0.55	0.16
20	FERAL	0.56	0.19
21.	FERAL	0.82	0.14

TABLE NO.2 (FEMALE):-

SAMPLE NO.	BREED NAME	MEDULLARY INDEX	CUTICLE INDEX
1.	FERAL	0.65	0.14
2.	FERAL	0.70	0.15
3.	FERAL	0.64	0.09
4.	CROSS(F+P)	0.53	0.23
5.	CROSS(F+P)	0.71	0.15
6.	FERAL	0.77	0.09
7.	FERAL	0.66	0.26
8.	FERAL	0.61	0.2
9.	FERAL	0.64	0.2
10.	FERAL	0.55	0.19
11.	PERSIAN	0.53	0.22
12.	FERAL	0.66	0.15
13.	FERAL	0.71	0.08

14.	FERAL	0.63	0.15
15.	FERAL	0.77	0.11
16.	CROSS(F+P)	0.72	0.27
17.	PERSIAN	0.75	0.16
18.	FERAL	0.70	0.16
19	FERAL	0.57	0.22
20	FERAL	0.5	0.28
21.	FERAL	0.55	0.3
22.	FERAL	0.66	0.2

IV. CONCLUSION

This study provides valuable insights into the unique structure of cat hair and its potential applications in forensic science. The medullary and cuticle indices of cat hair provide valuable information for understanding the unique characteristics of feline fur. By analyzing these indices, researchers can gain insight into the structural composition of cat hair. A sample of 43 samples, 21 male and 22 female, was collected and prepared for light microscope analysis. The cuticle index, which shows the quantity of cuticle scales per unit length of hair, and the medullary index, which shows the ratio of medulla diameter to hair shaft diameter, was estimated range of Male medullary index is **0.55 to 0.86** and cuticle index is **0.10 to 0.36**, and Female samples have range of medullary index from **0.53 to 0.77** and cuticle index is **0.08 to 0.28**.

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On first Zagreb index and forgotten topological index

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ABSTRACT

The first Zagreb index and forgotten topologial index are mostly studied degree based topological indices in molecular graph theory. The firstZagreb index is defined as$M_1(G)=\sum_{u\in V(G)} d_u^2=\sum_{uv\in E(G)} (d_u + d_v)$.The forgotten topological index is defined as $F(G)=\sum_{u\in V(G)} d_u^3=\sum_{uv\in E(G)} (d_u^2 + d_v^2)$ .In this paper whether we get the same result with triangular benzenoid system, crown graph CW_n,$TUC_4C_6(S)$ nanotubes and smart polymer SP(n)graphs by using vertex sets and edge sets is tested for first Zagreb index and forgotten topological index by classical formulas and topological polynomials.

Keywords: Crown graph,first Zagreb index,forgotten index,smart polymer,topological polynomials, triangular benzenoid.

I. INTRODUCTION

Let G be a simple, finite, connected graph with vertex set V(G) and edge set E(G). The degree of a vertex $u \in V(G)$ is denoted by d_u and is the number of vertices adjacent to u.The edge connecting the vertices u and v is denoted by uv.A molecular graph isrepresentation of the structural formula of a chemical compound in terms of graph theory whose vertices correspond to the atoms of compound and edges correspond to chemical bonds. A topological index is a numerical parameter mathematically derived from the graph structure;several such topological indices have been considered in theoretical chemistry and have found some applications in QSPR/QSAR study [1].The first Zagreb index M_1 can be defined as [2-4]

$$M_1(G)=\sum_{u\in V(G)} d_u^2. \quad (1)$$

The first Zagreb index can also be rewritten as [5-6]

$$M_1(G)=\sum_{uv\in E(G)} (d_u + d_v). \quad (2)$$

Second Zagreb index

$$M_2(G)=\sum_{uv\in E(G)} (d_u \times d_v). \quad (3)$$

The F-index of a graph is defined as the sum of cubes of the vertex degrees of the graph.Forgotten topological index is defined as [7-9]

$$F(G)=\sum_{u\in V(G)} d_u^3=\sum_{uv\in E(G)} (d_u^2 + d_v^2). \quad (4)$$

In terms of eccentricity of a graphthe first Zagreb index is defined as [10]

$$M_1^*(G)=\sum_{uv\in E(G)} (e_u + e_v), \quad (5)$$

where e_u is the eccentricityof a vertex u.

The multiplicativefirst Zagreb index $PM_1(G)$ is defined as [11-12]

$$PM_1(G)=\prod_{uv\in E(G)}(d_u + d_v). \quad (6)$$

Or multiplicative first Zagreb index is also defined as[13-14]

$PM_1(G) = \prod_{u \in V(G)} (d_u)^2$. (7)

The first Zagreb polynomial is defined as [15-18]

$M_1(G,x) = \sum_{uv \in E(G)} x^{d_u + d_v}$. (8)

The$M_1(G)$ can be calculated as the first derivative of first Zagreb polynomial at x = 1 as [19]

$M_1(G) = \frac{\partial M_1(G,x)}{\partial x}|_{x=1}$. (9)

First Zagreb index can be obtained from M-polynomial as [20-24]

$M_1(G) = (D_x + D_y)(M(G; x, y))|_{x=y=1}$. (10)

The forgotten polynomial of a graph G is defined as

$F(G,x) = \sum_{uv \in E(G)} x^{(d_u^2 + d_v^2)}$. (11)

The forgotten topological index from M-polynomial is obtained as [25]

$F(G) = (D_x^2 + D_y^2)(F(G; x, y))|_{x=y=1}$. (12)

We consider the graph of triangular benzenoid system G withn as the number of hexagons in the base graph oftriangular benzenoid system.It has $\frac{1}{2}n(n+1)$ hexagons, $n^2 + 4n + 1$ vertices and $\frac{3}{2}n(n+3)$ edges with degree either of 2 or 3[26-28].

M-polynomial for triangular benzenoid system is

$M(G;x,y) = 6x^2y^2 + 6(n-1)x^2y^3 + \frac{3}{2}n(n-1)x^3y^3$

and the first Zagreb index is $M_1(G) = (D_x + D_y)(M(G,x,y))|_{x=y=1} = 9n^2+21n-6$.

There are two different results obtained for forgotten topological index with triangular benzenoid system as [29]

$F(G) = \frac{39}{2}n^2 + \frac{177}{2}n - 60$

and $F(G) = 27n^2 + 51n - 30$[30].

So it is interesting to study the results obtained for the first Zagreb index and forgotten topological index by classical formulas of topological indices and polynomials given in terms of vertex sets and edge sets of triangular benzenoid system,crown graphCW_n,$TUC_4C_8(S)$nanotubes and smart polymerSP(n)- Dox loaded micelle comprising PEG-PAsp block copolymer with chemically conjugated Dox.

For more details on F-index we refer to the article [31-34].Zagreb indices and Zagreb polynomials have been studied in [35-37].In 2017 Nazi et al. defined first leap Zagreb index by taking 2-distance degree of vertices instead of vertex degree [38].Some topological indices of Dox–loaded micelle comprising PEG-PAsp block copolymer with chemically conjugated Dox SP(n)were investigated in [39-42].From Schultz index,modified Schultz index,it is easy to construct graph polynomials having the properties that their first derivative at x = 1 are equal to the Schultz and modified Schultz indices [43-44].Eccentric related indices of nanostar dendrimers have been studied in [45].Topological indices of $TUC_4C_8(S)$ were studied in [46-48].Crown graph (Sun) CW_n is a corona of form $C_n \circ K_1$,wheren≥3,that is helm with central vertex [49-50].Let $CW_n$is a crown graph having 2n vertices and 2n edgesgiven by $E_{13} = n$,$E_{33} = n$. Then the M-polynomialfor CW_n graph is

$M(CW_n;x,y) = nxy^3+nx^3y^3$. (13)

II. MATERIALS AND METHODS

In order to study chemical compound topologically the vertex set V(G) and edge set E(G) has to be obtained firstly. FirstZagreb index and forgotten topological index defined in the form of vertices and edges of molecular graphs are taken to validate results of these indices for some molecular graphs.A molecular graph or a chemical graph is a graph such that its vertices correspond to the atoms and edges to the bonds.The molecular graphs of triangular benzenoid system,crown graph$CW_6$are given in figure (1),(2) and (3) respectively.The first Zagreb index and forgotten topological index can be calculated as the first derivative of the corresponding topological polynomials at x = 1.In this paper first Zagreb index and forgotten index of triangular benzenoid system,crown graph CW_n,$TUC_4C_8(S)$ nanotubes and smart polymerSP(n)- Dox-loaded micelle comprising PEG-PAsp block copolymer with chemically conjugated Dox are investigated by vertex sets, edge sets and topological polynomials.

III.RESULTS AND DISCUSSION

Triangular benzenoid system

Theorem 1. First Zagreb index of triangular benzenoid system is $9n^2+21n-6$.

Proof. This theorem is proved by usingdifferent definitionsand topological polynomials of first Zagreb index.

(a) By using equation (1),vertex set and table (2), the first Zagreb index is obtained as

$M_1(G)= \sum_{u\in V(G)} d_u^2 = \sum_{u\in V_1} d_u^2 + \sum_{u\in V_2} d_u^2 =(3n+3)4+[n(n+1)-2]9= 9n^2+21n-6.$

(b) By using equation (2) and table (1) we have

$M_1(G)=\sum_{uv\in E(G)} (d_u + d_v) =(2+2)E_1+(2+3)E_2+(3+3)E_3$

$=4\times6+5(6n-6) +6[\frac{3}{2}n(n-1)]= 9n^2+21n-6.$

(c)$M_1(G,x) = \sum_{uv\in E(G)} x^{(d_u+d_v)}$

$= \sum_{uv\in E_1} x^{2+2}+\sum_{uv\in E_2} x^{2+3}+\sum_{uv\in E_3} x^{3+3}$

$= 6x^4+(6n-6)x^5+\frac{3}{2} n(n-1)x^6.$

$M_1(G) = \frac{\partial M_1(G,x)}{\partial x}|_{x=1}$

$= 6\times 4+(6n-6)\times 5+\frac{3}{2} n(n-1) \times6= 9n^2+21n-6.$

(d) Using equation (10) and table (1) we have M-polynomial for triangular benzenoid system

$M_1(G;x,y) = 6x^2y^2 + 6(n-1)x^2y^3 + \frac{3}{2}n(n-1)x^3y^3.$

$D_xM_1(G;x,y) =12x^2y^2 + 12(n-1)x^2y^3 + \frac{9}{2}n(n-1)x^3y^3.$

$D_yM_1(G;x,y) =12x^2y^2 + 18(n-1)x^2y^3 + \frac{9}{2}n(n-1)x^3y^3.$

$(D_x+D_y)M_1(G;x,y) = 24x^2y^2 + 30(n-1)x^2y^3 + 9n(n-1)x^3y^3.$

$M_1(G) =(D_x+D_y)(M_1(G;x,y))|_{x=y=1}= 9n^2+21n-6.$

(e)**Triangular benzenoid G(2) graph**

From figure (1)and table (2) we have$|V_2|=9,|V_3|=4$.

$M_1(G) = \sum_{u\in V_1} d_u^2 + \sum_{u\in V_2} d_u^2$

$=4\times9+9\times 4 = 72.$

Theorem 2. Forgotten topological index of triangular benzenoid system is $27n^2+51n-30$.

Proof. This theorem is proved by using different definitions and topological polynomials of forgotten topological index.

(a)Using equation (4) and table (2)

$F(G)=\sum_{u\in V(G)} d_u^3$

$=\sum_{u\in V_1}(d_u^3) + \sum_{u\in V_2}(d_u^3)$

$= (3n+3)\times 8+(n(n+1)-2)\times 27= 27n^2+51n-30.$

(b) Using equation (4) and table (1)

$F = \sum_{uv\in E(G)} (d_u^2 + d_v^2)$

$= \sum_{uv\in E_1}(2^2 + 2^2) + \sum_{uv\in E_2}(2^2 + 3^2) + \sum_{uv\in E_3}(3^2 + 3^2)$

$= 6\times 8+(6n-6)\times 13+\frac{3}{2} n(n-1) \times18= 27n^2+51n-30.$

(c)Using equation (11) and table (1)

$F(G,x)= \sum_{uv\in E(G)} x^{(d_u^2+d_v^2)} = \sum_{uv\in E_1} x^{(2^2+2^2)} + \sum_{uv\in E_2} x^{(2^2+3^2)} + \sum_{uv\in E_3} x^{(3^2+3^2)}$

$= 6x^8 + 6(n-1)x^{13} + \frac{3}{2}n(n-1)x^{18}.$

$F(G) = \frac{\partial F(G,x)}{\partial x}|_{x=1}$

$= 6\times 8+ 6(n-1) \times 13+\frac{3}{2}n(n-1) \times 18= 27n^2+51n-30.$

(d) M-polynomial for triangular benzenoid system

$F(G;x,y) = 6x^2y^2 + 6(n-1)x^2y^3 + \frac{3}{2}n(n-1)x^3y^3.$

$D_x^2 F(G;x,y) =24x^2y^2 + 24(n-1)x^2y^3 + \frac{27}{2}n(n-1)x^3y^3.$

$D_y^2 F(G;x,y) =24x^2y^2 + 54(n-1)x^2y^3 + \frac{27}{2}n(n-1)x^3y^3.$

$(D_x^2 + D_y^2)F(G;x,y) =48x^2y^2 + 78(n-1)x^2y^3 + 27n(n-1)x^3y^3.$

$F(G) =(D_x^2 + D_y^2)(F(G;x,y))|_{x=y=1}=27n^2+51n-30.$

(e)**For G(2)**

It is observed from figure 1,the vertices are $|V_2|=9,|V_3|=4$.

$F(G) = \sum_{u\in V_1} d_u^3 + \sum_{u\in V_2} d_u^3$

$=\sum_{u\in V_1} 2^3 + \sum_{u\in V_2} 3^3 =9\times8+4\times 27 =180.$

Theorem 3. Let G be a graph on n vertices of triangular benzenoid system, then

$F(G)\leq M_1(G)^2-2M_2(G)$.

Proof. This theorem is proved by using equations (1),(2), (3) and (4)for triangular benzenoid systems:G(n) and G(2) graphs.

Forgotten topological index $F(G)=\sum_{uv\in E(G)} d_u^3 =\sum_{uv\in E(G)} (d_u^2 + d_v^2)= 27n^2+51n-30.$

First Zagreb index $M_1(G)= \sum_{uv\in E(G)} d_u^2 = \sum_{uv\in E(G)} (d_u + d_v)= 9n^2+21n-6.$

Second Zagreb index $M_2(G)= \sum_{uv\in E(G)} (d_u \times d_v) = \frac{27}{2}n^2+22.5n-12.$

Then$(27n^2+51n-30) \leq (9n^2+21n-6)^2 - 2(\frac{27}{2} n^2+22.5n-12)$.

For G(2) graph$180\leq5010$.

Crown graphCW_n

Consider a molecular graph$G = CW_n$, then the edges are $E_{13}=n, E_{33}=n$ and vertices$V_1=n, V_3=n$.

Theorem 4. First Zagreb index of crown graph CW_n is 10n.

Proof. This theorem is proved by using different definitions and topological polynomials of first Zagreb index.

(a) By using equation (1), vertex set and table (2) the first Zagreb index is obtained as

$M_1(G)=\sum_{u\in V(G)} d_u^2$

$= \sum_{u\in V_1} d_u^2 + \sum_{u\in V_2} d_u^2 = n(1)+n(9)= 10n.$

(b)$M_1(G)=\sum_{uv\in E(G)} (d_u + d_v)$

$= n(1+3)+n(3+3)= 10n.$

(c)$M_1(G,x) = \sum_{\mathbf{uv\in E(G)}} x^{(d_u+d_v)}$

$= \sum_{uv\in E_1} x^{1+3}+\sum_{\mathbf{uv}\in E_2} x^{3+3} = nx^4+nx^6.$

$M_1(G) = \frac{\partial M_1(G,x)}{\partial x}|_{x=1}$

$= n\times 4+n\times 6= 10n.$

(d) M-polynomial for crown graph CW_n

$M_1(G;x,y) = nx\, y^3 + nx^3y^3$.

$D_xM_1(G;x,y) = nx\, y^3 +3nx^3y^3.$

$D_yM_1(G;x,y) =3n\, x\, y^3 + 3nx^3y^3.$

$(D_x+ D_y)M_1(G;x,y) = 4n\, x\, y^3 +6nx^3y^3.$

$M_1(G) =(D_x+ D_y)(M_1(G;x,y))|_{x=y=1}= 4n+6n=10n.$

Theorem 5. Forgotten topological index of crown graph CW_n is 28n.

Proof. This theorem is proved by using different definitions and topological polynomials of forgotten topological index.

a)$F(G)=\sum_{u\in V(G)} d_u^3$

$=\sum_{u\in \mathbf{V_1}}(d_u^3) + \sum_{u\in \mathbf{V_2}}(d_u^3)$

$= n\times 1+n\times 27= 28n.$

(b) $F(G) =\sum_{uv\in E(G)} (d_u^2 + d_v^2)$

$= \sum_{uv\in \mathbf{E_1}}(1^2 + 3^2) + \sum_{uv\in \mathbf{E_2}}(3^2 + 3^2)$

$= n(10)+ n(18)= 28n.$

(c)$F(G,x)=\sum_{uv\in E(G)} x^{(d_u^2+d_v^2)}$

$= \sum_{uv\in E_1} x^{(1^2+3^2)} + \sum_{uv\in E_2} x^{(3^2+3^2)}$

$= \sum_{uv\in E_1} x^{(10)} + \sum_{uv\in E_2} x^{(18)}= nx^{10} + nx^{18}.$

$F(G) = \frac{\partial F(G,x)}{\partial x}|_{x=1}$

$= n\times 10+ n\times 18= 28n.$

(d)M-polynomial for crown graph CW_n

$F(G;x,y) = nx\, y^3 + nx^3y^3.$

$D_x^2\, F(G;x,y) =nxy^3 + 9nx^3y^3.$

$D_y^2\, F(G;x,y) =9nxy^3 + 9nx^3y^3.$

$(D_x^2 + D_y^2)F(G;x,y) =10nxy^3 + 18nx^3y^3.$

$F(G) =(D_x^2 + D_y^2)(F(G;x,y))|_{x=y=1}= 28n.$

$TUC_4C_8(S)$ nanotubes

Consider a molecular graph$G = TUC_4C_8(S)$ then the edges are $E_{22}=2m$, $E_{23}=4m$ and $E_{33}=12mn-2m$ and with vertices $V_2=4m, V_3=8mn$.

Theorem 6. First Zagreb index of$TUC_4C_8(S)$nanotubesis 72mn+16m.

Proof. This theorem is proved by using different definitions and topological polynomials of $M_1(G)$.

(a) By using equation(1),table(2),vertex set and edge set,first Zagreb index is obtained as

$M_1(G)=\sum_{u\in V(G)} d_u^2$

$= \sum_{\mathbf{u}\in \mathbf{V_1}} d_u^2 + \sum_{\mathbf{u}\in \mathbf{V_2}} d_u^2$

$=4m(2^2)+8mn(3^2)= 72mn+16m.$

(b)$M_1(G)=\sum_{uv\in E(G)} (d_u + d_v)$

$= (2+2)E_1+(2+3)E_2+(3+3)E_3$

$= 2m\times 4+4m\times 5 +(12mn-2m) \times 6= 72mn+16m.$

(c)$M_1(G,x) = \sum_{\mathbf{uv}\in \mathbf{E(G)}} x^{(d_u+d_v)}$

$= \sum_{uv\in E_1} x^{2+2}+\sum_{\mathbf{uv}\in E_2} x^{2+3}+\sum_{\mathbf{uv}\in E_3} x^{3+3} = 2mx^4+4mx^5 +(12mn-2m)x^6.$

$M_1(G) = \frac{\partial M_1(G,x)}{\partial x}|_{x=1}$

$= 2m\times 4+4m\times 5+(12mn-2m) \times 6= 72mn+16m.$

(d)M-polynomial for$TUC_4C_8(S)$ nanotubes

$M_1(G;x,y) = 2mx^2y^2 + 4mx^2y^3 + (12mn - 2m)x^3y^3$.

$D_xM_1(G;x,y)=4mx^2y^2 + 8mx^2y^3 + (36mn - 6m)x^3y^3.$.

$D_yM_1(G;x,y)=4mx^2y^2 + 12mx^2y^3 + (36mn - 6m)x^3y^3$

$(D_x+ D_y)M_1(G;x,y)= 8mx^2y^2 + 20mx^2y^3 + 2(36mn - 6m)x^3y^3.$

$M_1(G) =(D_x+ D_y)(M_1(G;x,y))|_{x=y=1}=72mn+16m.$

Theorem 7. Forgotten topological index of $TUC_4C_8(S)$ nanotubesis 216mn+32m.

Proof. This theorem is proved by using different definitions and topological polynomials of forgotten topological index.

(a)$F(G)=\sum_{u\in V(G)} d_u^3$

$=\sum_{u\in V_1}(d_u^3)+\sum_{u\in V_2}(d_u^3)$

$=(4m)\times 8+8mn\times 27=216mn+32m.$

(b) $F(G)=\sum_{uv\in E(G)}(d_u^2+d_v^2)$

$=\sum_{uv\in E_1}(2^2+2^2)+\sum_{uv\in E_2}(2^2+3^2)+\sum_{uv\in E_3}(3^2+3^2)$

$=2m\times 8+4m\times 13+(12mn-2m)\times 18=216mn+32m.$

(c)$F(G,x)=\sum_{uv\in E(G)} x^{(d_u^2+d_v^2)}$

$=\sum_{uv\in E_1} x^{(2^2+2^2)}+\sum_{uv\in E_2} x^{(2^2+3^2)}+\sum_{uv\in E_3} x^{(3^2+3^2)}$

$=\sum_{uv\in E_1} x^8+\sum_{uv\in E_2} x^{13}+\sum_{uv\in E_3} x^{18}=2mx^8+4mx^{13}+(12mn-2m)x^{18}.$

$F(G)=\frac{\partial F(G,x)}{\partial x}|_{x=1}$

$=2m\times 8+4m\times 13+(12mn-2m)\times 18=216mn+32m.$

(d) M-polynomial for $TUC_4C_8(S)$ nanotubes

$F(G;x,y)=2mx^2y^2+4mx^2y^3+(12mn-2m)x^3y^3.$

$D_x^2F(G;x,y)=8mx^2y^2+16mx^2y^3+9(12mn-2m)x^3y^3.$

$D_y^2F(G;x,y)=8mx^2y^2+36mx^2y^3+9(12mn-2m)x^3y^3.$

$(D_x^2+D_y^2)F(G;x,y)=16mx^2y^2+52mx^2y^3+18(12mn-2m)x^3y^3.$

$F(G)=(D_x^2+D_y^2)(F(G;x,y))|_{x=y=1}=216mn+32m.$

Smart polymer SP(n)

Consider a molecular graphG(n) = SP(n)-Dox loaded micelle comprising PEG-PAsp block copolymer with chemically conjugated Dox,where n is step of growth of smart polymer,then the edges are $E_{12}=2n+1$,$E_{13}=9n+1$,$E_{14}=n$,$E_{34}=n$,$E_{22}=5n+4$,$E_{23}=18n-1$,$E_{24}=2n$ and $E_{33}=16n$ and with vertices $V_1=12n+2$,$V_2=16n+4$,$V_3=20n$ and $V_4=n$.

Theorem 8. First Zagreb index of Smart polymerSP(n)is 272n+18.

Proof. This theorem is proved by using different definitions and topological polynomials of first Zagreb index.

(a) By using definition and vertex set the first Zagreb index is obtained as

$M_1(G)=\sum_{u\in V(G)} d_u^2$

$=\sum_{u\in V_1} d_u^2+\sum_{u\in V_2} d_u^2+\sum_{u\in V_3} d_u^2+\sum_{u\in V_4} d_u^2$

$=(12n+2)\times 1+(16n+4)\times 4+20n\times 9+n\times 16=272n+18.$

(b)$M_1(G)=\sum_{uv\in E(G)}(d_u+d_v)$

$=(1+2)E_1+(1+3)E_2+(1+4)E_3+(3+4)E_4+(2+2)E_5+(2+3)E_6+(2+4)E_7+(3+3)E_8$

$=(3)(2n+1)+(4)(9n+1)+(5)n+(7)n+(4)(5n+4)+(5)(18n-1)+(6)2n+(6)16n$

$=272n+18.$

(c)$M_1(G,x)=\sum_{uv\in E(G)} x^{(d_u+d_v)}$

$=\sum_{uv\in E_1} x^{1+2}+\sum_{uv\in E_2} x^{1+3}+\sum_{uv\in E_3} x^{1+4}+\sum_{uv\in E_4} x^{3+4}+\sum_{uv\in E_5} x^{2+2}+\sum_{uv\in E_6} x^{2+3}+\sum_{uv\in E_7} x^{2+4}+\sum_{uv\in E_8} x^{3+3}$

$=(2n+1)x^3+(9n+1)x^4+nx^5+nx^7+(5n+4)x^4+(18n-1)x^5+2nx^6+16nx^6.$

$M_1(G)=\frac{\partial M_1(G,x)}{\partial x}|_{x=1}$

$=(2n+1)3+(9n+1)4+n\times 5+n\times 7+(5n+4)\times 4+(18n-1)5+(2n)6+(16n)6$

$=272n+18.$

(d)M-polynomial for smart polymer SP(n)

$M_1(G;x,y)=(2n+1)x^1y^2+(9n+1)x^1y^3+nx^1y^4+nx^3y^4+(5n+4)x^2y^2+(18n-1)x^2y^3+2nx^2y^4+16nx^3y^3.$

$D_xM_1(G;x,y)=(2n+1)x^1y^2+(9n+1)x^1y^3+nx^1y^4+3nx^3y^4+2(5n+4)x^2y^2+2(18n-1)x^2y^3+4nx^2y^4+48nx^3y^3.$

$D_yM_1(G;x,y)=2(2n+1)x^1y^2+3(9n+1)x^1y^3+4nx^1y^4+4nx^3y^4+2(5n+4)x^2y^2+3(18n-1)x^2y^3+8nx^2y^4+48nx^3y^3.$

$(D_x+D_y)M_1(G;x,y)=3(2n+1)x^1y^2+4(9n+1)x^1y^3+5nx^1y^4+4nx^3y^4+4(5n+4)x^2y^2+5(18n-1)x^2y^3+12nx^2y^4+96nx^3y^3.$

$M_1(G)=(D_x+D_y)(M_1(G;x,y))|_{x=y=1}=272n+18.$

Theorem 9. Forgotten topological index of smart polymer SP(n) is = 744n+34.

Proof. This theorem is proved by using different definitions and topological polynomials of forgotten topological index.

(a)$F(G)=\sum_{uv\in E(G)} d_u^3$

$=\sum_{u\in V_1}(d_u^3)+\sum_{u\in V_2}(d_u^3)+\sum_{u\in V_3}(d_u^3)+\sum_{u\in V_4}(d_u^3)$

$=\sum_{u\in V_1}(1)+\sum_{u\in V_2}(8)+\sum_{u\in V_3}(27)+\sum_{u\in V_4}(64)=744n+34.$

(b)$F=\sum_{uv\in E(G)}(d_u^2+d_v^2)$

$=\sum_{uv\in E_1}(1^2+2^2)+\sum_{uv\in E_2}(1^2+3^2)+\sum_{uv\in E_3}(1^2+4^2)+\sum_{uv\in E_4}(3^2+4^2)+\sum_{uv\in E_5}(2^2+2^2)+\sum_{uv\in E_6}(2^2+3^2)+\sum_{uv\in E_7}(2^2+4^2)++\sum_{uv\in E_8}(3^2+3^2)$

$= (2n+1)5 + (9n+1)10 + (n)17 + (n)25 + (5n+4)8 + (18n-1)13 + (2n)20 + (16n)18$

$= 744n+34.$

(c)$F(G,x)=\sum_{uv\in E(G)} x^{(d_u^2+d_v^2)}$

$= \sum_{uv\in E_1} x^{(1^2+2^2)} + \sum_{uv\in E_2} x^{(1^2+3^2)} + \sum_{uv\in E_3} x^{(1^2+4^2)} + \sum_{uv\in E_4} x^{(3^2+4^2)} + \sum_{uv\in E_5} x^{(2^2+2^2)} + \sum_{uv\in E_6} x^{(2^2+3^2)} + \sum_{uv\in E_7} x^{(2^2+4^2)} + \sum_{uv\in E_8} x^{(3^2+3^2)}$

$= (2n+1)x^5 + (9n+1)x^{10} + (n)x^{17} + (n)x^{25} + (5n+4)x^8 + (18n-1)x^{13} + (2n)x^{20} + (16n)x^{18}.$

$F(G) = \frac{\partial F(G,x)}{\partial x}|_{x=1}$

$= (2n+1)5 + (9n+1)10 + (n)17 + (n)25 + (5n+4)8 + (18n-1)13 + (2n)20 + (16n)18.$

$= 744n+34.$

(d)M-polynomial forsmart polymer SP(n)

$F(G;x,y) = (2n+1)x^1y^2 + (9n+1)x^1y^3 + nx^1y^4 + nx^3y^4 + (5n+4)x^2y^2 + (18n-1)x^2y^3 + 2nx^2y^4 + 16nx^3y^3.$

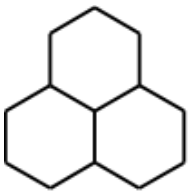
$D_x^2 F(G;x,y) = (2n+1)x^1y^2 + (9n+1)x^1y^3 + nx^1y^4 + 9nx^3y^4 + 4(5n+4)x^2y^2 + 4(18n-1)x^2y^3 + 8nx^2y^4 + 144nx^3y^3.$

$D_y^2 F(G;x,y) = 4(2n+1)x^1y^2 + 9(9n+1)x^1y^3 + 16nx^1y^4 + 16nx^3y^4 + 4(5n+4)x^2y^2 + 9(18n-1)x^2y^3 + 32nx^2y^4 + 144nx^3y^3.$

$(D_x^2 + D_y^2)F(G;x,y)$

$= 5(2n+1)x^1y^2 + 10(9n+1)x^1y^3 + 17nx^1y^4 + 25nx^3y^4 + 8(5n+4)x^2y^2 + 13(18n-1)x^2y^3 + 40nx^2y^4 + 288nx^3y^3.$

$F(G) = (D_x^2 + D_y^2)(F(G;x,y))|_{x=y=1} = 744n+34.$



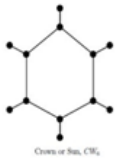


Figure 1.Figure 2.Figure 3.

Fig.1.Graph of triangular benzenoid G(2). Fig. 2.Graph of triangular benzenoid G(n). Fig. 3.Crown graphCW_6.

Table 1. The edge partition oftriangular benzenoid G(n).

(d_u, d_v)	(2,2)	(2,3)	(3,3)
Number of edges	6	$6(n-1)$	$\frac{3n(n-1)}{2}$

Table 2. The vertex partition of triangular benzenoid G(n)..

V_1	V_2	$\|V_1\|$	$\|V_2\|$
$\{u\| d_u = 2\}$	$\{u\| d_u = 3\}$	3n+3	n(n+1)-2

IV. CONCLUSION

The calculation of topological indices based on the formulas of first Zagreb index and forgotten topological index defined in terms of vertex sets, edge setsand topological polynomials of amolecular graph yield the same result for triangular benzenoid system,crown graph CW_n,$TUC_4C_8(S)$ nanotubes and smart polymer SP(n).

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Schiff Bases Derived From Carbonyl Oximes and Dithio Biurets

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ABSTRACT

The synthesis of some Fe(II) complexes with acid Schiff bases are reported here. These Schiff bases were derived by condensing carbonyl oximes and amines like Dithio biurets the characterization of the complexes was done on the basis of elemental analysis, molar conductivity, spectral studies like IR and Electronic and Anti-bacterial activity. On the basis of these analysis it was concluded that Co(II) complexes exhibit Octahedral binuclear geometry with $M_2L_2Cl_24H_2O$ stoichiometry.The molar conductivity data indicate that the complexes are non-electrolytic in nature. The metal complexes have been screened for their antibacterial activity.

Keywords: Fe(II) complexes, Schiff bases, Structural analysis, Antimicrobial studies

I. INTRODUCTION

The elemental analysis of the metal complexes of HPEPDTB and HPETDTB corresponds to 1:1 metal:ligand stoichiometry and suggests that the complexes may be represented by the general formula M2L2Cl2.xH2O where M =Fe and x = 4

II. METHOD AND MATERIAL

The complexes are coloured and non-hygroscopic in nature. They are insoluble in water and sparingly solube in common organic solvents though somewhat more soluble in dimethyl formamide (DMF) at room temperature. They give intense colour with dil. NaOH solution suggesting the presence of a free oxime group in the complexes.

III.RESULT AND DISCUSSION

All the complexes are thermally stable at least upto 120oC indicating fairly high thermal stability and hence a strong metal-ligand bond.

The values of molar conductance of the metal complexes in DMF solution at 10-3 M concentration are < 1.0 ohm-1 cm2 mole-1and are suggestive of the non-electrolytic nature of these complexes

The Fe(II) complexes of HPEPDTB and HPETDTB exhibit paramagnetic moments of 2.27 and 2.04 B.M. respectively at room temperature. The Fe(II) ion

shows little tendency to form four coordinate complexes. Most ferrous complexes are found to be octahedral showing either magnetism or paramagnetic corresponding to the presence of four unpaired electrons. The observed magnetic moments for Fe(III) complexes of HPEPDTB and HPETDTB are intermediate between the values expected for spin-free and spin-paired complexes. The possibility of such a magnetic moment arising due to the presence of Fe(II) ions in the complexes can be ruled out on the basis of the results of repeated elemental analysis and high stability of the complexes. Moreover, the complexes have been prepared in presence of a reducing agent like sodium thiosulphate. The Fe(III) species are therefore unlikely to be the cause for such an intermediate magnetic moment observed.

A possible explanation of the observed anomalous magnetic moments of these Fe(II) complexes could be based on a spin cross-over between 5T2gand 1A1g states. Alternatively, presence of a S=1 ground state can give rise to such intermediate moments. The Tanabe-Sugano diagram for a d6 configuration shows that in the neighbourhood of the 5T2g--- 1A1g crossover, a lowest lying triplet state (3T1) reaches its lowest energy relative to that of the ground state. In a field of symmetry lowest than Oh , a triplet state may in fact become the ground state. The complexes of the type FeL2(NCS)2.2H2O where L = phenanthroline, 5-chlorophenanthroline, 5-nitrophenan-throline etc. are found to have temperature independent moments typically of the order of 2.85 ± 0.5 B.M. “owing to the presence of a triplet ground state 3T

Another reason for intermediate magnetic moments for ferrous complexes can be due to a distortion of the six-coordinate complexes to such an extent that splitting of the orbital energies makes the occupation of more than one of them unfavourable. Investigation on the dihydrazone complexes of glyoxal, pyruvaldehydead benzil with Fe(II) have shown that such a distortion of the octahedral geometry of the Fe(II) leads to magnetic moments between 2.90-3.32 B.M.

The observed magnetic moments of 2.27 and 2.04 B.M. for the two Fe(II) complexes, though intermediate between those expected for spin-paired and spin-free Fe(II) complexes, are not quite in the range observed for the case discussed above. It is also interesting to note that such intermediate magnetic moments have also been observed or Fe(II) complexes of some carbonyl oximes as also for some pyridyl-pyrazole71 complexes of Fe(II). However, low temperature magnetic susceptibility measurements as well as Mossbauer spectral studies seem to be necessary to arrive at any definite conclusion in the present cases of Fe(II) complexes. The reflectance spectra of the two Fe(II) complexes diluted with MgCO3 exhibit three bands (Figs.5.70, 5.73) at 10,000-10,410 cm-1, 14,280-14,760 cm-1, and 22,220-26,660 cm-1 respectively.

In high-spin octahedral ferrous complexes, the electronic ground state 5D splits into 5T2g and 5Eg states; only one d-d transition (5T2g → 2Eg) band should therefore be observable. A distortion of a regular octahedron as evidence by the high intensities of observed transitions compounded with the intermediate magnetic moments obstained for the Fe(II) complexes of HPEPDTB and HPETDTB suggest that some mixing-in of 1A1g state with the 5T2g state may be responsible for the observed spectral characteristics.

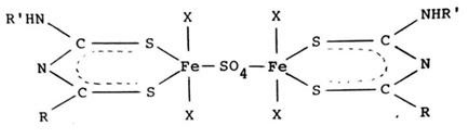
The PMR spectra of metal complexes can provide vital information on the mode of bonding in the metal complexes and a correlation can be derived between the observed chemical shifts of various proton resonance signals and the coordination behaviour of ligands with respect to different metal ions. However, due to limited solubility of these complexes in suitable solvents and/or paramagnetic nature of complexes of the transition metal ions, which lead to noisy base line and broadening of signals, the complexes did not yield PMR spectra of satisfactory quality. Only one of the complexes, namely Zn2(HPEPDTB)2.Cl2.4H2O, could therefore be studied.

The PMR spectrum of isonitrosoacetophenone is already discussed in section-I. For the sake of brevity the discussion on PMR in section-I and that on the ligands in the section is assumed wherever applicable and its repetition is avoided in the discussion that follows.The PMR spectrum of the complex shows oximino proton of the ligand as a singlet at ~ 12.2 δ which suggests that proton of =NOH is not replaced during complexation. A broad multiplet between 6.4-7.2 δ has its origin in the phenyl ring protons of the ligand.It may also be noted that the methine proton, which appears at 7.8 δ in the spectrum of HPEPDTB, is observed at nearly the same position(~7.5 δ) in the PMR spectrum of complex, indicating that the oxime group is not involved in bonding to the metal ion.The singlet for -SH proton, which appeared at 10.6 δ in ligand, however, disappears in the complex suggesting the removal of -SH proton during. A careful comparison of the spectra of the metal complexes with those of the ligands also reveals that bands due to vN-OH, and vN-H in the region around 3200-3300 cm-1 are observed for the complexes, which overlap in several cases making thier distinction difficult. The -N-H bending vibrations are observed near 1620-1600 cm-1, around the same position as in ligands, indicating that -NH group is not involved in coordination. The presence of free -NOH function in the complexes is also revealed by their solubility in dilute alkali solution.

IV. INFRARED SPECTRA

The FT-infrared spectra of the metal chelates (Figs.5.74-5.88) contain large number of bands of varying intensities and are quite complex. However, several structurally important bands, such as those due to v(O-H), v(C=N), v(N-OH), v(C=S) etc modes have been identified to derive information regarding the structural features of the ligands and their manner of bonding with the metal ions.The azomethine and oximino C=N stretching vibrations are relatively unaffected and are observed at ~1610-14 and 1563-1567 cm-1 respectively indicating that the azomethine or oxime nitrogen donors are not involved in bonding.The C=S stretching frequency of the ligands are found to undergo a negative shift on complexation suggesting that the ligands coordinate to the metal ions through sulphur donors. The C=S stretching bands are observed around 1220-1240 cm-1 in the spectra of the complexes.Another fairly strong band around 725-759 cm-1 due to C=S vibrations in ligands is also found to be shifted to lower frequencies between 686-692 cm-1 in complexes with medium intensity.The IR bands due to phenyl ring systems of the ligands observedaround 1497, 1450 and 1400 cm-1 are found to be almost unaffected in the spectra of the metal complexes

Absence of -SH band around ~2560 cm-1 it shows that the coordination occurs through sulfur atoms and larger low-frequency shift of the C=S stretching frequency may be attributed in part to the change in the symmetry of the ligand from Cs to C2v. Observed decrease in C=S frequencies together with negligible shift or slight increase in C=N stretching frequencies is suggestive of sulphur coordination of the ligands to the metal ion. Similar sulphur coordinated dithiobiuret complexes are reported in the literature.On the basis of the elemental analysis and results of various physicochemical studies, the bonding in the chloro metal complexes of HPEPDTB and HPETDTB can be represented as follows.



R = -N=C(C_6H_5)-CH(=NOH)
R'= phenyl/tolyl

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Forensic Limnology: an Overall View and It's Applications

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ABSTRACT

Diatoms are microscopic algae having cell wall made up of silica called as frustule, these frustule are transparent same as jewels, hence referred as jewel stone of water.Diatoms are tiny single celled organism that contribute to 20-25% of global oxygen production. Diatoms are around 200 genera around 10-12 thousand known species. They occur in diverse habitat such as deep in aquatic habitat, both marine and fresh water. In Drowning cases, the person enter the water while still alive, diatoms will find their way into their lungs in the event that they drown by inhaling water. After that, the diatoms are circulated to various organs like the kidneys, bone marrow, lungs. Diatoms cannot enter the body if the person is dead because there won't be any circulation in the death body. In the body, diatoms do not naturally occur. It could be strong evidence that drowning was the cause of death if diatoms from the same species that were discovered in the water where the body was collected are discovered in the corpse during laboratory testing. The bone marrow is considered a sacred organ, and if diatoms enter this tissue, they can indicate a drowning.and to identify the diatoms, the right procedure must be used to extract diatoms from tissues and water samples. The current study analyzed the literature on several techniques for extracting diatoms from postmortem and water samples.

Keywords: Diatoms, Drowning, Diatoms test, Bone marrow, Diatoms database, Applications.

I. INTRODUCTION

Diatoms are unicellular, photosynthetic, eukaryoticmicroalgae. There are more than 15000 species known of them.About 20-50% of the oxygen on Earth is produced by diatoms,through the process of photosynthesis, which is extremely for human respiration.They have a high growth rate, and exhibit a "boom and bust" lifecycle. The major type are phytoplankton ,which are basically plant type and are

autotropic in nature. Phytoplankton, Phytomeans "plants" and *plankton* means "to wander around" , which floats above the water surface.

- Domain:-Eukaryotes
- Phylum:-Gyrista
- Class:- Bacillariophyceae
- Order:- Centrals and Pennates.

The scientific word for diatom is Bacillariophyceae.

Diatoms are single-celled creatures that can coexist in colonies or alone. Their sizes and shapes vary, spanning from 2 to 200 micrometres.Diatoms can be found in vary habitat such as water bodies (like fresh water ,brackish water,sea water, ponds ,etc) soil, and some surfaces of damp. They also carry out carbon cycle and nitrogen metabolism or (OUC)ornithine urea cycle and photosynthesis. Pigments such as Chlorophyll a3 and c4 carry out photosynthesis. Diatoms stores food in the form of fats (oils) and leucosin (chrysolaminarin). They are not evenly distributed throughout the oceans. They tend to be more concentrated in particular zones that are vertical (up and down) and horizontal (side to side).

Diatoms have specialcell wall made up of silica known as frustule.This are transparent frustule which look like glass hence referred as "algae in glass houses".Frustules are silica made cell wall of diatoms and they are "bipartite" which means they have 2 valves. The larger valve known as "Epivalve" and smaller valve as "Hypovalve". The valve overlap like a lid of box in which the epivalve fits over hypovalve.

The frustules(Surface of diatoms)have pores, spines and elevations like sculptures and shapecalled as areolae. These structure help in exchange of gases and material within body and environment.

Diatoms are generally immotile except some male which consist flagella for locomotion and else all diatoms are immobile and stick to particular surface aur moisture containing area and rocks.

Various types of diatoms can be classified using varied patterns, pores, spines, and ridges seen on their walls, they are generally classified into 2 types as :-

A. centric (radial symmetry) -

The valves on top and bottom of them can slide under one another to let the cell expand. The cytoplasm of centric diatoms encircles a sizable central vacuole that is filled with a substance known as "cell sap" and lines the inside surface of the shell.

B. Pinnated (bilateral symmetry)-

They move by flowing cytoplasm via the slits along the raphes of their valves. Certain pennate diatoms possess a unique way of movement known as "gliding." They can move across surfaces by secreting sticky mucilage through a structure called the raphe.

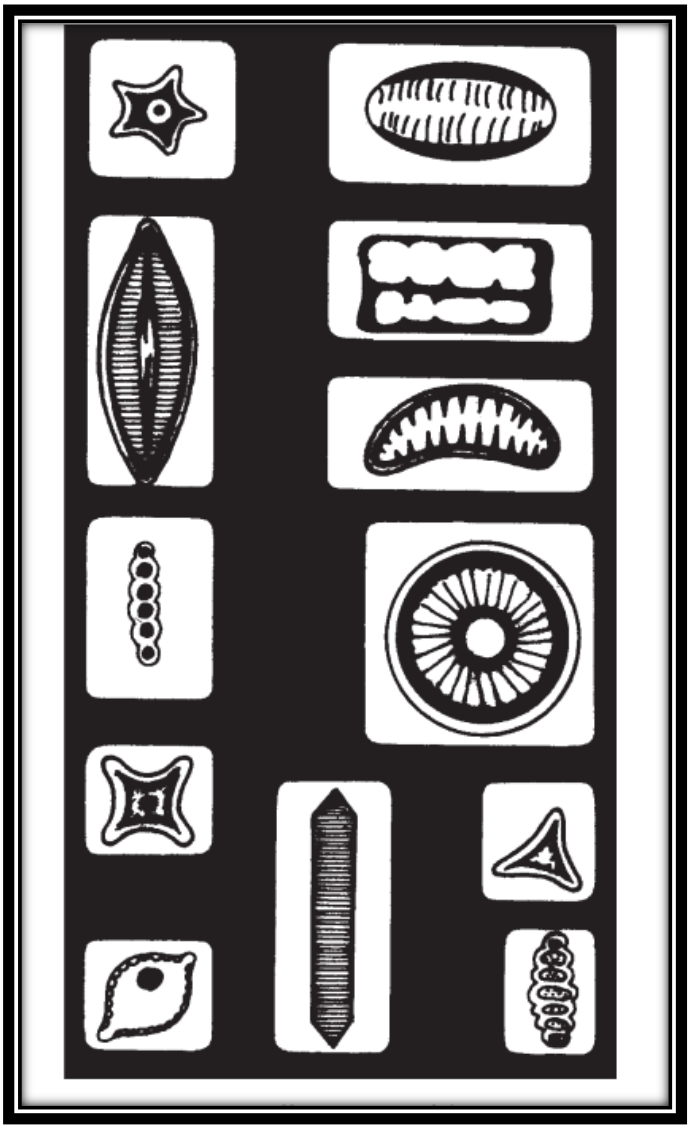

Fig: 1 different types of diatoms[40]

Diatoms reproduceAsexually through binary fission,producing two fresh diatoms with the same

genes. One of the two frustules—the silica cell walls—of the parent diatom is transferred to each new diatom. A smaller frustule is utilized to create a new, smaller frustule known as the hypotheca, while the bigger frustule becomes the epitheca. While the diatom with the smaller frustule shrinks, the one inheriting the larger frustule stays the same size as the parent. As a result, the population of diatoms shrinks on average.

Incze showed in 1942 that diatoms might enter the bloodstream through the lungs while drowning. Their existence becomes evident once the tissue is digested by acid in organs like the liver, brain, and bone marrow. The theory behind the use of diatoms as a diagnostic test for drowning is that unless the circulation is still active, diatoms will not penetrate the systemic circulation and be deposited in organs like the bone marrow, suggesting that the deceased was alive when submerged in water.

II. METHODS AND MATERIAL

Thomasand his associates described a technique for the detection of diatoms in tissues. 2–5 gm of the tissue or about 40 gm of the bone marrow from the shaft of a long bone or from the sternum may be taken by means of gynaecological curette. The marrow is placed in a Kjeldahl flask in which it is chemically digested by adding small quantities of concentrated nitric acid at a time. The contents are heated for about 1–2 hours. This yields a transparent yellow fluid with a supernatant disc of fat. The yellow fluid is next centrifuged. The centrifuged deposit (usually hardly visible to the naked eye) is to be poured on a slide and examined while still wet under a cover-slip.

There are some common acid digestion methods for detecting diatoms, they are as –

- **Acid digestion method:**

The Acid digestion method for diatoms extraction accepted worldwide. It is easy to perform and gives good results.

1) Nitric acid method:

1. Samples are collected from the suspected drowning victim. Care should be taken as to not contaminate the sample with foreign diatoms during the process.
2. Intact femurs, for example, are removed at autopsy and washed in distilled water. Femurs are longitudinally sectioned using a clean band saw, and the bone marrow about 50g is removed using a clean spatula and placed into a boiling flask.
3. Approximately 50 mL of concentrated nitric acid is added to the flask, and the marrow-acid suspension is boiled on a hot plate for approximately 48 hours-under a fume hood.
4. The suspension is then cooled and centrifuged, in some instances two separate times, with the supernatant discarded and the resulting acid-resistant material dropped onto clean microscope slides and the sediment is examined under the microscope.

2) Sulphuric acid method:

1. This has the advantage of not causing violent foaming. Check that all calcareous compounds have been removed first; otherwise the sample will become totally useless because gypsum crystals will form.
2. When sample has settled completely, discard supernatant.
3. Add concentrated sulphuric acid until the volume is twice that of the original sample.
4. Add potassium bichromate. In contrast to the H_2O, method, no special care is necessary as no violent reaction occurs. Just add enough bichromate to make for a saturated solution.

5. Let stand for 24 hours or more, or speed up the reaction in a water-bath 60 degrees. Even so, it may take several hours before the sample is clean. The sediment should look grayish and no plant fragments etc. should remain.
6. Let settle completely, discard supernatant and rinse several times as described above. The sulphuric acid method seems to remove resistant "dirt".

3) H2O2 Methods:[29]

Because hydrogen peroxide is less corrosive than acid, it is much kinder. It works best with samples that don't need to be cleaned too much and where rust shouldn't occur too much.

4) Hot H2O2 method:

1. Allow the diatom sample to settle for 24 hours
2. Decant the supernatant from the sample bottle taking care not to loose any of the diatom material;
3. Check the sample for the presence of calcium and decalcify the sample if necessary
4. Mix the diatom suspension and place 5 to 10 ml of the suspension in a beaker
5. Mark the beaker clearly with the sample number in several places; 1.3.4.6 Add 20 ml H2O2 and heat on a hot plate at 90°C for 1 to 3 hours
6. The beakers should be covered with a watch glass to prevent contamination between flasks if boiling becomes too vigorous and splashing occurs
7. Add a few drops of HCl and leave to cool
8. Rinse the samples as in method.

5) Cold H2O2 method:

1. Follow the procedure as in hot method ,except use of hotplate
2. Cover beaker with watch glass and leave for a minimum of four days
3. Rinse the samples as in method.

6) ENZYMATIC METHOD:

Tissue samples were treated with both chemical and enzymatic methods using concentrated nitric acid and Proteinase-K respectively. Enzymatic method was considered more convenient in terms of rapidity, safety and environmental protection than chemical test. In 1996 and 1999 Ludes et al., made use of hydrogen peroxide (130 vol. %) at 80oC for 12 hours for the treatment of the water samples. The solution was cooled at room temperature and second centrifugation was set for 15 minutes at 2500 rpm. A pellet containing diatoms was obtained by centrifuging the mixture with distilled water at 3000 rpm after discarding the supernatant. The sediment was air dried and mounted in Naphrax following the removal of the supernatant. Under a light microscope with an oil immersion objective (1000X), diatoms were studied.

Proteinase-K has also been recommended by Azparren et al. (1998), Kobayashi et al. (1993), Taylor (1994), and Quantin et al. (1994) for the extraction of diatoms from tissue samples of drowning victims.

Once they have entered the bloodstream, the blood stream distributes them throughout the body. Even though the experimentally drowned animals were only submerged for a brief time and were taken out of the drowning medium alive and gasping, they have been shown to enter into their organs.

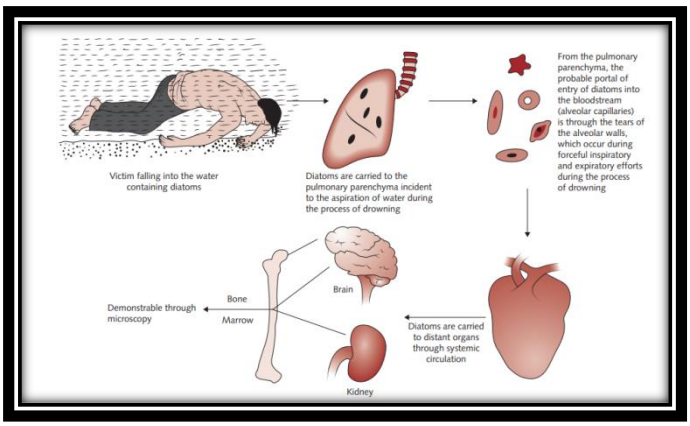

Fig2: the movement of diatoms based on presence an absence of circulation in drowned body[40]

7) Soluene-350 method:

The tissue needs to be sliced into tiny pieces for this process, and then it needs to be homogenized with distilled water. The precipitate is suspended in 8 volumes of Soluene-350 and incubated at 50°C for 2 hours, followed by an overnight stay at room temperature, following centrifugation at about 18,000 rpm for 10 mm. After centrifugation for 60 minutes at 3000 rpm, the precipitate is prepared for analysis.

8) Membrane filter:

This technique involved filtering using a membrane made of nitrocellulose with a 5 μm pore size. Ten milliliters of 5% sodium dodecyl sulphate (SDS) and five milliliters of blood were combined and gently swirled. A membrane filter with a diameter of 47 mm was used to filter the resultant haemolysate. The filter's pores blocked when the blood got severely clotted or putrefied. In these situations, a new filter required to be installed. Following filtration, the filters were digested for about ten minutes using about ten milliliters of fuming nitric acid. This solution was diluted 10–20 times with distilled water after chilling. Another membrane filter with a diameter of 25 mm was used to filter this dilution. After drying, the filter was examined under a microscope. The residue left over after nitric acid digestion was diluted with 150–200 ml of distilled water and passed through a membrane filter with a diameter of 47 mm while handling tissue samples. Petroleum ether or isopropyl alcohol were used alternately to totally breakdown any fat particles that remained on the filter. This approach was thought to be highly helpful in resolving issues that interfere with microscopic observations, such as the loss and destruction of diatoms and the emergence of inorganic crystals.

III.RESULTS AND DISCUSSION

Diatoms are used in many different contexts in both human culture and the natural world. Diatoms are utilized as fossils and as living things. Products such as food and biofuels can be made with living diatoms. It is possible to detect oil and gas deposits and reconstruct historical environmental conditions using fossil diatoms. Diatoms are an invaluable resource for scientific investigation as well. Diatoms have been utilized in research on many different subjects, such as palaeoecology, evolution, and climate change.Rather than forensic aspects diatoms are also applicable in different field such as:-

A. Applications:-

- **Nutraceuticals** are goods with both therapeutic and nutritional properties that can be made using diatoms.
  These substances may offer a number of health advantages. Diatom vitamins, for instance, can assist a variety of biological processes, diatom antioxidants can prevent cell damage, and diatom anti-inflammatory substances can lessen inflammation.[31]
- **Aquatic ecosystem**Diatoms have an impact on light penetration, which can change the purity of water. They aid in the dispersion and absorption of light, which has an impact on the ecosystem below the surface. Diatoms also aid in the intake of nutrients and the purification of water, which preserves the quality of the water.
- **Biofuels** as diatoms may be grown on non-arable land and have a high lipid content, making them a prospective source of biofuels. Biodiesel can be produced from diatom lipids via transesterification. Bioethanol can be produced by fermentation from diatom polysaccharides.
- Their silica cell wall is comprised of uniformly distributed nano-arrayed pores. In order to adsorb different trace metals, dyes, polymers, and medications that are harmful to both aquatic life and humans, they function as clever **nanocontainers**.[3]

- Diatoms, whether living or dead, have the ability to sense toxins at the nanoscale, which makes them smart nanocontainers capable of biosensing other pollutants and rich metals in wastewater.
- **Forensic** -Death by drowning is defined as a death caused by immersion in a liquid; hypoxemia and irreversible cerebral anoxia are the mechanisms behind acute drowning.Several tests have been proposed to confirm a victim's death by drowning because the diagnosis of drowning is one of the more challenging ones in forensic pathology.
- Diatoms generating several sustainable goods like biofuels, feed, bioactive compounds, and services like environment monitoring, diatoms are used in research to boost the sustainable economy.

- **Case study 1 –**

A thirty-year-old man's severely putrefied body was found in a canal. At the autopsy, no information on the cause of death was found. Research using nitric acid extracts from the femur, clavicle, and sternum revealed the existence of two species of diatoms (Cocconeis placentula and Cymbrella ventricosa). To locate the original drowning site, water samples were sent to the lab from various locations. The water sample from a location apart from the site where the body was retrieved had the same two types of diatom species. Additional police investigation confirmed that drowning was the cause of death.

- **Case study 2 –**

A 19-year-old boy's body was discovered immersed in a water tank. Upon autopsy, no signs of injuries were discovered. Navicula lanceolate, Navicula oblonga, and Gomphonema gracile are the three types of diatom species that were found in a nitric acid extract of the sternum, clavicle, femur, and lungs. The water sample from which the body was retrieved contained the same three species of diatoms. Thus, a cause of death was determined because of drowning

IV. CONCLUSION

The main agenda behind this review is to determine the various terminologies and the significance of the very most primitive type of organisms called as diatoms. Generally, in determining the cause of death of a drowning victim, diatoms discovered within the victim's body may provide supporting evidence. One can determine if the death was caused by a post-mortem or an ante-mortem. Diatoms are not always present in drowning instances, but when they are and are abundant in distant organs, they unquestionably offer compelling evidence for ante-mortem drowning. The primary objection to the diatom test's applicability in diagnosing drowning stems from the possibility of diatom penetration both before and after death, as well as the discovery of diatoms in the bodies of people who did not drown. Nonetheless, inconsistent and contradictory findings have been obtained from qualitative and quantitative research on diatoms in the organs of non-drowned people.

This study looked into a variety of diatom species and their vibrant applications. The results of this literature will be useful in the identification of diatoms, criminal investigations, and the location of crime scenes or drowning sites.

This study finds that when environmental conditions change, so do the types and species of diatoms.

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The Impact of Dopamine on Human Behavior

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ABSTRACT

Dopamine, a neurotransmitter associated with pleasure, motivation, and reward, plays a crucial role in shaping human behavior. In the present era, characterized by rapid technological advancements, social media, and changing lifestyles, understanding the impact of dopamine on human behavior is essential. This paper explores the multifaceted influence of dopamine on various aspects of human behavior, including addiction, decision-making, motivation, and social interactions. Through an examination of recent research findings and theoretical frameworks, this paper elucidates the intricate interplay between dopamine and human behavior in contemporary society. Additionally, implications for mental health, well-being, and societal dynamics are discussed, highlighting the need for further interdisciplinary research and interventions to navigate the complexities of dopamine-driven behaviors in the modern world.

Keywords: Dopamine, Human Behavior, Addiction, Decision-Making, Motivation, etc.

I. INTRODUCTION

Dopamine, a neurotransmitter renowned for its role in modulating human behavior, stands as a central protagonist in the intricate workings of the brain. Its influence permeates various facets of cognition, emotion, motivation, and reward processing, rendering it indispensable to our understanding of human behavior in the present era. As society traverses through rapid technological advancements, shifting cultural norms, and evolving lifestyles, the significance of dopamine in shaping human behavior becomes increasingly pronounced.At its core, dopamine is a catecholamine neurotransmitter synthesized in multiple regions of the brain, notably the substantia nigra and ventral tegmental area (VTA). This neurotransmitter operates within intricate neural circuits, projecting to diverse brain regions such as the prefrontal cortex, nucleus accumbens, and amygdala, where it orchestrates a symphony of physiological processes critical to human functioning.

Dopamine's involvement in the brain's reward system has garnered significant attention from researchers and clinicians alike. Through its modulation of

mesolimbic and mesocortical pathways, dopamine mediates responses to pleasurable stimuli, reinforces reward-seeking behaviors, and shapes our subjective experiences of pleasure and motivation. Furthermore, dopamine plays a pivotal role in reinforcement learning, facilitating the encoding of reward-related information and guiding adaptive decision-making processes.However, dopamine's influence extends far beyond the realms of pleasure and reward. Emerging research has highlighted its involvement in various cognitive functions, including attention, memory, and executive control. Dopaminergic dysfunction has been implicated in a myriad of neuropsychiatric disorders, ranging from addiction and depression to schizophrenia and attention-deficit/hyperactivity disorder (ADHD). Understanding the nuanced interplay between dopamine and human behavior is thus imperative for elucidating the underlying mechanisms of these disorders and informing targeted interventions.[1]

In the contemporary era, characterized by an unprecedented influx of information, ubiquitous digital technologies, and shifting social dynamics, the impact of dopamine on human behavior assumes heightened significance. The omnipresence of smartphones, social media platforms, and online entertainment avenues has revolutionized the way individuals seek and experience rewards, presenting both opportunities and challenges for dopamine regulation and mental well-being.Against this backdrop, this paper endeavors to delve into the multifaceted influence of dopamine on human behavior in the present era. By synthesizing insights from neuroscience, psychology, and sociology, we aim to unravel the intricate web of dopamine-driven behaviors, shedding light on their implications for mental health, societal dynamics, and human flourishing. Through an exploration of recent research findings and theoretical frameworks, we seek to navigate the complexities of dopamine's role in shaping human behavior, offering a nuanced understanding of its relevance in contemporary society.

As we embark on this journey of inquiry, it becomes evident that unraveling the mysteries of dopamine holds profound implications for our understanding of human nature and the challenges we face in the modern world. By illuminating the neurobiological underpinnings of dopamine-driven behaviors, we aspire to foster interdisciplinary dialogue, inspire innovative research endeavors, and pave the way for interventions aimed at promoting healthy dopamine regulation and enhancing human well-being in the present era.

This paper aims to explore the multifaceted influence of dopamine on human behavior in the context of the contemporary environment.[2]

II. DOPAMINE: NEUROBIOLOGY AND FUNCTION

Dopamine, a neurotransmitter belonging to the catecholamine family, serves as a fundamental mediator of neural communication within the central nervous system. Synthesized primarily in dopaminergic neurons located in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), dopamine exerts its influence across diverse brain regions, orchestrating a myriad of physiological and behavioral processes.Dopamine, a neurotransmitter belonging to the catecholamine family, serves as a fundamental mediator of neural communication within the central nervous system. Synthesized primarily in dopaminergic neurons located in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), dopamine exerts its influence across diverse brain regions, orchestrating a myriad of physiological and behavioral processes.[2-5]

A. Dopaminergic Pathways:

1. Mesolimbic Pathway: Originating in the VTA, the mesolimbic pathway projects to the nucleus accumbens, amygdala, and prefrontal cortex. This pathway is critically involved in reward

processing, reinforcement learning, and motivational behaviors.

2. Mesocortical Pathway: Arising from the VTA, the mesocortical pathway innervates the prefrontal cortex, where it modulates executive functions, working memory, and cognitive control.
3. Nigrostriatal Pathway: Emerging from the SNc, the nigrostriatal pathway extends to the dorsal striatum (caudate nucleus and putamen), regulating motor coordination, voluntary movement, and procedural learning.

B. Dopamine Receptors:

1. Dopamine exerts its effects through interaction with five distinct receptor subtypes: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4). These receptors are distributed heterogeneously throughout the brain and exhibit divergent signaling cascades, contributing to the complexity of dopamine-mediated neurotransmission.
2. D1-like receptors primarily couple to stimulatory G-proteins (Gs), activating adenylate cyclase and increasing cyclic adenosine monophosphate (cAMP) levels, thus promoting excitatory signaling cascades.
3. In contrast, D2-like receptors predominantly couple to inhibitory G-proteins (Gi/o), exerting inhibitory effects on adenylate cyclase activity and modulating neuronal excitability.

C. Functions of Dopamine:

1. *Reward and Reinforcement:* Dopamine plays a central role in the brain's reward system, mediating responses to pleasurable stimuli and reinforcing reward-seeking behaviors. Phasic dopamine release in response to unexpected rewards facilitates reinforcement learning and the encoding of reward-related information.
2. *Motivation and Arousal:* Dopamine is intricately involved in motivational processes, regulating incentive salience and energizing goal-directed behaviors. Dysregulation of dopaminergic neurotransmission can lead to motivational deficits, apathy, or anhedonia, characteristic symptoms of neuropsychiatric disorders.
3. *Motor Control:* In the nigrostriatal pathway, dopamine modulates motor function by exerting tonic inhibition on the indirect pathway and disinhibiting the direct pathway, thus facilitating smooth and coordinated movements.
4. *Cognitive Functions:* Dopamine modulates various cognitive processes, including attention, working memory, and executive control. Optimal dopamine levels are essential for maintaining cognitive flexibility, response inhibition, and goal-directed behavior.
5. *Emotional Regulation:* Dopamine contributes to the regulation of emotional responses and affective states, influencing mood, arousal, and stress responses. Dysregulation of dopaminergic neurotransmission has been implicated in mood disorders, anxiety disorders, and post-traumatic stress disorder (PTSD).

The dopamine serves as a versatile neurotransmitter with diverse neurobiological functions, ranging from reward processing and motivation to motor control and cognitive regulation. Its intricate interplay with dopaminergic pathways and receptor subtypes underlies the complexity of dopamine-mediated behaviors and underscores its significance in shaping human cognition, emotion, and behavior. Understanding the neurobiology of dopamine is essential for elucidating the pathophysiology of neuropsychiatric disorders and informing targeted therapeutic interventions aimed at restoring dopaminergic homeostasis and promoting optimal brain function.

III.DOPAMINE AND ADDICTION

Addiction, characterized by compulsive drug-seeking and drug-taking behaviors despite adverse

consequences, represents a complex interplay of genetic, environmental, and neurobiological factors. Central to the neurobiology of addiction is the mesolimbic dopamine system, which plays a pivotal role in the rewarding effects of drugs of abuse and the development of addiction-related behaviors.[3-6]

A. Dopamine's Role in Reward Processing:

1. Drugs of abuse, including stimulants (e.g., cocaine, amphetamines), opioids (e.g., heroin, oxycodone), and alcohol, exert their primary reinforcing effects by modulating dopamine neurotransmission within the mesolimbic pathway.
2. Acute administration of drugs of abuse results in robust increases in extracellular dopamine levels within the nucleus accumbens, a key component of the brain's reward circuitry. This surge in dopamine release is associated with feelings of euphoria, pleasure, and reinforcement.
3. Chronic drug exposure leads to neuroadaptations within the mesolimbic dopamine system, including alterations in dopamine receptor sensitivity, neurotransmitter release, and synaptic plasticity. These neuroadaptive changes contribute to the development of tolerance, dependence, and addiction.

B. Dopamine's Role in Reinforcement Learning:

1. Dopamine neurons exhibit phasic firing patterns in response to unexpected rewards or reward-predictive cues, encoding prediction errors and facilitating reinforcement learning processes.
2. Drugs of abuse hijack the brain's natural reward system, producing exaggerated dopamine responses to drug-related stimuli and disrupting the normal balance between incentive salience and inhibitory control.
3. Conditioned cues associated with drug use become potent triggers for drug-seeking behaviors, eliciting robust dopamine release and driving the compulsive cycle of addiction.

C. Neuroplasticity and Addiction:

1. Chronic drug exposure induces profound neuroplastic changes within the mesolimbic dopamine system, leading to long-lasting alterations in synaptic strength, dendritic morphology, and gene expression.
2. These neuroadaptive changes contribute to the transition from recreational drug use to compulsive drug-seeking behavior, characterized by the loss of control over drug intake and the emergence of drug cravings and withdrawal symptoms.
3. Neurobiological substrates implicated in addiction-related neuroplasticity include glutamatergic neurotransmission, opioidergic signaling, and cortico-striatal circuits involved in decision-making and impulse control.

D. Vulnerability Factors and Genetic Susceptibility:

1. Individual differences in susceptibility to addiction reflect a complex interplay of genetic, environmental, and developmental factors. Genetic polymorphisms in genes encoding dopamine receptors, transporters, and enzymes involved in dopamine metabolism have been implicated in addiction susceptibility.
2. Environmental factors, including stress, trauma, peer influence, and socioeconomic disparities, can modulate dopamine neurotransmission and contribute to the risk of addiction.

The dopamine plays a central role in the neurobiology of addiction, mediating the rewarding effects of drugs of abuse, reinforcing drug-seeking behaviors, and promoting the development of compulsive drug use patterns. Understanding the neurobiological mechanisms underlying dopamine's role in addiction is essential for elucidating the pathophysiology of substance use disorders and informing targeted interventions aimed at preventing and treating addiction-related behaviors. Integrative approaches combining pharmacotherapy, behavioral

interventions, and psychosocial support are crucial for addressing the multifaceted nature of addiction and promoting long-term recovery and well-being.

IV. DOPAMINE AND DECISION-MAKING

Decision-making is a complex cognitive process that involves evaluating alternatives, weighing potential outcomes, and selecting actions that maximize desired outcomes while minimizing costs and risks. Dopamine, a key neurotransmitter within the brain's reward circuitry, plays a critical role in modulating decision-making processes by integrating information about rewards, punishments, and the probability of different outcomes.[7-9]

A. Dopaminergic Modulation of Decision-Making:

1. Dopamine neurons exhibit phasic firing patterns in response to rewarding or aversive stimuli, encoding prediction errors and signaling the expected value of decision outcomes.
2. The mesolimbic dopamine system, originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens and prefrontal cortex, is centrally involved in reward processing and decision-making.
3. Dopamine release within the prefrontal cortex, a brain region critical for executive functions and cognitive control, modulates working memory, attentional processes, and behavioral flexibility, thereby influencing decision-making strategies and outcomes.

B. Reward Valuation and Risk Assessment:

1. Dopamine neurotransmission plays a key role in computing the expected value of decision outcomes, integrating information about rewards, punishments, and the probability of different outcomes.
2. Dopamine release signals the subjective value of decision options, biasing decision-making toward choices associated with higher expected rewards and lower potential costs or risks.
3. Dysfunction of the dopamine system can lead to aberrant reward processing, impaired risk assessment, and maladaptive decision-making behaviors, as observed in neuropsychiatric disorders such as addiction, schizophrenia, and attention-deficit/hyperactivity disorder (ADHD).

C. Temporal Discounting and Delayed Gratification:

1. Dopamine modulates temporal discounting, the tendency to devalue delayed rewards relative to immediate rewards, by influencing intertemporal decision-making processes.
2. Individuals with dysregulated dopamine neurotransmission may exhibit heightened impulsivity and preference for immediate gratification, predisposing them to addictive behaviors, financial impulsivity, and poor long-term planning.
3. Strategies aimed at enhancing dopamine regulation and promoting delayed gratification, such as cognitive-behavioral therapy (CBT), mindfulness-based interventions, and pharmacological treatments targeting dopaminergic neurotransmission, may help mitigate impulsive decision-making tendencies and promote self-control.

D. Neuroeconomic Perspectives on Decision-Making:

1. Neuroeconomic research integrates principles from neuroscience, psychology, and economics to elucidate the neural mechanisms underlying decision-making processes.
2. Functional neuroimaging studies have identified neural substrates associated with value-based decision-making, including the ventromedial prefrontal cortex, orbitofrontal cortex, striatum, and insula, which exhibit sensitivity to changes in reward magnitude, probability, and delay.
3. Computational models of decision-making, such as reinforcement learning and prospect theory,

provide insights into the cognitive algorithms and neural computations underlying human choice behavior, offering a framework for understanding the neurobiological basis of decision-making biases and deviations from rationality.

It plays a multifaceted role in modulating decision-making processes by influencing reward valuation, risk assessment, temporal discounting, and behavioral control. Understanding the neurobiological mechanisms underlying dopamine's influence on decision-making is essential for elucidating the cognitive and neural basis of human choice behavior and informing interventions aimed at promoting adaptive decision-making strategies and enhancing self-regulation in health and disease.

V. DOPAMINE AND MOTIVATION

Motivation, the driving force behind goal-directed behavior, encompasses a complex interplay of cognitive, emotional, and neurobiological processes that energize and sustain action toward desired outcomes. Dopamine, a neurotransmitter implicated in reward processing and reinforcement learning, plays a central role in mediating motivational states and modulating incentive salience.[10-11]

A. Dopaminergic Regulation of Motivation:

1. Dopamine neurotransmission within the mesolimbic and mesocortical pathways is critically involved in regulating motivational processes by encoding the subjective value of rewards and motivating goal-directed behaviors.
2. Phasic dopamine release in response to rewarding stimuli signals the anticipated pleasure or hedonic value of future rewards, promoting approach behaviors and energizing goal pursuit.
3. Dopamine neurons exhibit tonic firing patterns during the anticipation of rewards, maintaining arousal levels and sustaining motivational vigor over time.

B. Incentive Salience and Reward Prediction:

1. Dopamine plays a key role in attributing incentive salience to environmental stimuli associated with rewards, making them more attractive and attention-grabbing.
2. Pavlovian conditioning processes, through which neutral cues acquire motivational significance by being paired with rewards, are mediated by dopaminergic neurotransmission within the nucleus accumbens and other limbic structures.
3. Dysregulation of dopamine signaling can lead to aberrant incentive salience attribution, contributing to addictive behaviors, compulsive reward-seeking, and the persistence of maladaptive habits.

C. Role of Dopamine in Goal-Directed Behavior:

1. Dopamine facilitates goal-directed behavior by promoting the initiation and persistence of actions aimed at obtaining rewards and achieving desired outcomes.
2. Optimal dopamine levels are essential for maintaining task engagement, sustaining effort expenditure, and overcoming obstacles or delays encountered during goal pursuit.
3. Dysfunctional dopamine neurotransmission, characterized by hypo- or hyperdopaminergic states, can manifest as motivational deficits, anhedonia, or impulsivity, impairing goal-directed behavior and undermining goal attainment.

D. Dopamine and Reinforcement Learning:

1. Dopamine plays a crucial role in reinforcement learning processes by signaling prediction errors, the discrepancies between expected and actual rewards, and updating reward expectations based on prior experience.
2. Phasic dopamine release encodes the difference between the predicted and actual reward outcomes, facilitating associative learning and adaptive decision-making.

3. Disrupted dopaminergic signaling, as observed in neuropsychiatric disorders such as addiction, depression, and schizophrenia, can lead to aberrant reinforcement learning mechanisms, contributing to maladaptive behaviors and cognitive inflexibility.

As we know it serves as a central neuromodulator of motivational processes, regulating incentive salience, reward prediction, and goal-directed behavior. Understanding the neurobiological basis of dopamine-mediated motivation is essential for elucidating the mechanisms underlying human motivation and informing interventions aimed at promoting adaptive goal pursuit, enhancing task performance, and fostering well-being in health and disease. By unraveling the complexities of dopamine-driven motivation, researchers can gain insights into the neural circuitry of motivation and develop targeted therapies for motivational disorders and motivational deficits associated with psychiatric and neurological conditions.

VI. DOPAMINE AND SOCIAL INTERACTIONS

Social interactions are fundamental aspects of human behavior, encompassing a wide range of interpersonal relationships, affiliative bonds, and communicative exchanges. Dopamine, a neurotransmitter traditionally associated with reward processing and reinforcement learning, also plays a crucial role in modulating social behaviors and shaping social interactions.[7-11]

A. Dopaminergic Regulation of Social Reward:

1. Dopamine neurotransmission within the mesolimbic pathway, particularly within the nucleus accumbens, is implicated in the processing of social rewards, including social approval, affiliation, and cooperation.
2. Social interactions activate dopaminergic neurons in the ventral tegmental area (VTA) and elicit dopamine release within the nucleus accumbens, promoting positive affective states and reinforcing social behaviors.
3. Genetic and pharmacological manipulations of dopaminergic signaling in animal models have demonstrated the importance of dopamine in social reward processing and the formation of social bonds.

B. Dopamine and Empathy:

1. Empathy, the ability to understand and share the emotional experiences of others, involves complex neural circuits encompassing regions such as the prefrontal cortex, anterior cingulate cortex, and mirror neuron system.
2. Dopamine modulates empathic responses by influencing the salience of social cues, empathic accuracy, and emotional contagion.
3. Dysfunction of the dopamine system has been implicated in deficits in social cognition and empathy observed in neuropsychiatric conditions such as autism spectrum disorder (ASD) and schizophrenia.

C. Social Learning and Imitation:

1. Dopamine plays a role in social learning processes, including observational learning and imitation, by encoding the value of social information and facilitating the acquisition of social skills and behaviors.
2. Dopaminergic neurotransmission within the striatum and prefrontal cortex mediates reinforcement learning mechanisms underlying social imitation and the acquisition of socially adaptive behaviors.

D. Dopamine and Social Hierarchies:

1. Social hierarchies, characterized by dominance relationships and social status, involve complex interactions between individuals within a group and are modulated by dopaminergic neurotransmission.
2. Dopamine signaling within the mesolimbic system mediates social dominance behaviors, territorial aggression, and the pursuit of social status and prestige.

3. Dysregulation of dopaminergic signaling can lead to disruptions in social hierarchies, maladaptive dominance behaviors, and social aggression observed in certain psychiatric disorders and substance use disorders.

E. Dopamine and Social Bonding:

1. Social bonding, characterized by enduring affiliative relationships and attachment bonds, is facilitated by dopaminergic neurotransmission within the mesolimbic and mesocortical pathways.
2. Oxytocin, another neuropeptide implicated in social bonding and attachment, interacts closely with dopamine signaling to regulate pair bonding, maternal behavior, and social attachment in mammals.

The dopamine serves as a key neuromodulator of social behaviors and social interactions, regulating social reward processing, empathy, social learning, and social bonding. Understanding the neurobiological basis of dopamine-mediated social behaviors is essential for elucidating the neural circuits underlying human sociality and social cognition and informing interventions aimed at promoting social skills, enhancing social functioning, and fostering prosocial behaviors in health and disease. By unraveling the complexities of dopamine-driven social interactions, researchers can gain insights into the mechanisms underlying human social behavior and develop targeted therapies for social deficits associated with neuropsychiatric conditions and social dysfunction.

VII. IMPLICATIONS FOR MENTAL HEALTH AND WELL-BEING

The intricate interplay between dopamine and human behavior has profound implications for mental health and overall well-being. Dysregulation of dopaminergic neurotransmission has been implicated in a wide array of neuropsychiatric disorders, including addiction, depression, anxiety disorders, schizophrenia, bipolar disorder, and attention-deficit/hyperactivity disorder (ADHD). Understanding the role of dopamine in these conditions offers insights into their pathophysiology and informs therapeutic approaches aimed at restoring dopaminergic balance and promoting mental health.[9-12]

A. Addiction:

1. Dysfunctional dopamine signaling underlies the development and maintenance of addiction to drugs of abuse, gambling, and other compulsive behaviors.
2. Targeted interventions, such as pharmacotherapy, behavioral therapy, and cognitive-behavioral interventions, aim to restore dopaminergic homeostasis and mitigate addictive behaviors.

B. Depression and Apathy:

1. Dysregulation of dopamine neurotransmission has been implicated in the pathophysiology of depression and apathy, characterized by motivational deficits, anhedonia, and reduced reward sensitivity.
2. Antidepressant medications targeting the dopamine system, such as selective serotonin and norepinephrine reuptake inhibitors (SSNRIs), may enhance dopaminergic activity and alleviate depressive symptoms.

C. Anxiety Disorders:

1. Altered dopamine neurotransmission is implicated in anxiety disorders, including generalized anxiety disorder (GAD), panic disorder, and social anxiety disorder.
2. Anxiolytic medications that modulate dopaminergic neurotransmission, such as benzodiazepines and certain antipsychotic agents, may help alleviate symptoms of anxiety.

D. Schizophrenia and Psychotic Disorders:

1. Dysregulated dopamine transmission, particularly hyperactivity within the mesolimbic pathway, is a hallmark feature of schizophrenia and other psychotic disorders.

2. Antipsychotic medications targeting dopamine D2 receptors help mitigate positive symptoms of psychosis, such as hallucinations and delusions, by blocking excessive dopaminergic activity.

E. Bipolar Disorder:

1. Imbalances in dopamine neurotransmission contribute to the manic and depressive episodes characteristic of bipolar disorder.
2. Mood stabilizers and antipsychotic medications that modulate dopaminergic signaling, in conjunction with psychotherapy and lifestyle interventions, are used to manage symptoms and stabilize mood fluctuations.

F. Attention-Deficit/Hyperactivity Disorder (ADHD):

1. ADHD is associated with dysregulated dopamine neurotransmission within frontostriatal circuits, impairing attention, impulse control, and executive functions.
2. Stimulant medications, such as methylphenidate and amphetamines, increase dopamine availability in the brain and improve ADHD symptoms in many individuals.

G. Well-Being and Quality of Life:

1. Optimal dopamine regulation is essential for promoting emotional well-being, resilience, and overall quality of life.
2. Lifestyle factors, including diet, exercise, sleep hygiene, and stress management, can influence dopaminergic function and contribute to mental health and well-being.

The understanding the implications of dopamine dysregulation for mental health and well-being is essential for developing targeted interventions aimed at restoring dopaminergic balance and promoting optimal psychological functioning. By elucidating the neurobiological underpinnings of dopamine-related disorders and addressing the complex interplay between dopamine and human behavior, researchers and clinicians can advance our understanding of mental illness and enhance therapeutic approaches to promote mental health and well-being.

VIII. CONCLUSION

Although a conclusion may review the main points of the paper, do not replicate the abstract as the conclusion. A conclusion might elaborate on the importance of the work or suggest applications and extensions. Authors are strongly encouraged not to call out multiple figures or tables in the conclusion—these should be referenced in the body of the paper.

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Phytotransformation and Phytoremediation of Heavy Metals Using Pithophora Oedogonia A Weed Algae

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ABSTRACT

The ascent of industrialization throughout the years has prompted a critical expansion in how much effluents are released into the climate. This has turned into a central issue as these effluents can defile water bodies, dirty the air, and mischief the encompassing with biological system. Amongst all the industry the textile industry give significant contribution. Effluent release from the textile business has been a central issue for quite a long time, with various examinations and reports featuring its unfavourable impacts. The primary contaminations in textile effluents incorporate dye, heavy metals viz. chromium and copper and other harmful synthetic compounds utilized in production. Chromium and Copper sulphate are commonly used in the production of dyes, mordant and other chemicals, and it is been linked to various health problems, including neurological disorders and cancer. Algae absorb heavy metals from wastewater sources, for example, chromium, copper and iron. These heavy metals are micro nutrient for algal development, making algal species a promising contender for tackling to ecological contaminant. Incubated string algae test when tested with heavy metal for phytotransformation and Biosorption it was found that it phytotransform Potassium dichromate (K2Cr2O7) into Potassium chromate (K2CrO4), Copper sulphate (CuSO4) into Copper (I) sulphate (Cu2SO4). In this study, it was seen that Pithophora oedogonia phytotransform the heavy metal into comparatively less harmful transformant and thus can serve the purpose of phytoremediation. For this reason, Pithophora oedogonia can be utilized as a putative candidate for ETP of heavy metal containing effluents.

Keywords: Algae, Biosorption, Chromium, Copper, ETP, Phytoremediation, Phytotransformation, Pithophora oedogonia, Textile industry, Toxic heavy metals.

I. INTRODUCTION

Industrial effluents mention to the waste waters and different contaminants that are created because of industrial cycles. They might contain unsafe synthetic compounds, heavy metals, and different contamination, which can adversely affect both the climate and human health. Heavy metals are characterized as metallic components that have a generally high density compared with water (Paul Tchounwou *et. al.* 2012). The most regularly found heavy metals in squander water incorporate Chromium, Copper, Arsenic, Cadmium, Lead, Nickel and Zinc, all of which cause threat for human Well being and the climate (Blessy B. Mathew*et. al.* 2014). Chromium exists in the climate basically in two valence states, trivalent chromium (Cr III) and hexavalent chromium (Cr VI). Chromium III is significantly less lethal than chromium (VI). Chronic exposure to chromium (VI) in people brings about impacts on the respiratory system, with break and ulcerations of the septum, bronchitis, diminished pneumonic capability, pneumonia, asthma, and nasal tingling and touchiness announced (www.epi.gov.) Exposure to $CuSO_4$ can happen through inhalation, ingestion, or skin contact. When breathed in, it can aggravate the respiratory framework, causing splutter, trouble breathing and chest pain. Ingestion of enormous sums can prompt sickness, nausea, vomiting, abdominal pain, and diarrhea. Skin contact with $CuSO_4$ can cause irritation, redness, and burns (Royer & Sharman, 2023).

Pithophora oedogonia of family *Pithophoraceae* is a filamentous weed alga. Through its intricate root-like structures, known as rhizoids, *Pithophora oedogonia* can trap and bind heavy metal ions in the waters where it grows and prevents the spread and metal uptake of other organisms (Davis *et al.*, 2003). Research has shown that *Pithophora oedogonia* has the ability to absorb heavy metals through its cell walls and accumulate them in its tissues can actively transform and detoxify certain heavy metals, rendering them less harmful to the surrounding environment (Kapahi and Sachdeva, 2019), (Mahlangu *et. al.*, 2024). This dual capability of absorption and transformation makes *Pithophora oedogonia* a valuable candidate for phytoremediation applications in polluted water bodies and soil.

The potential of *Pithophora oedogonia* in environmental cleanup extends beyond its role in heavy metal removal. Its ability to enhance nutrient uptake and oxygen production in aquatic ecosystems can contribute to overall ecological restoration and water quality improvement (Samal *et al.*, 2020). This study aims to further explore the role of *Pithophora oedogonia* in the removal of heavy metals and its potential applications in environmental remediation.

II. METHODS AND MATERIAL

Collection and cleaning of algal sample

Algal Specimens were collected from local water reservoir from Chhatrapati Sambhajinagar, Maharashtra, India (19°51'55.6"N 75°18'54.5"E). The algal sample was washed under running tap water to remove dirt and attached epiphytes.

Authentication and identification

The algal sample was authenticated by Herbarium I/C, Dr. B. A. M. U., Chhatrapati Sambhajinagar and Department of Botany, GIS, Chhatrapati Sambhajinagar. Authenticated algal species was used for further experiment.

Isolation and selective cultivation

The cleaned algal sample was observed under microscope and hypae were isolated. The isolated hype were inoculated and cultivated in sterile sourced water (SSW) in well aerated and high intensity sunlight in a rectangular transparent jar. This lab cultivated algal sample (LCAS) were used for further experiments.

Bioaccumulation assay

String test

Heavy metal stock solution was prepared as (10mg/ml). Sterile thread was inoculated to the heavy

metal sample tube and incubated till 5 day. Visible accumulation of metal particles was observed on thread.

Biosorption

10 ml of heavy metal stock solution (10mg/ml) was added in a test tube with 0.1g of LCAS. Sample tubes were incubated and observed for declining absorbance of potassium dichromate and Copper sulfate at their respective absorption maxima. The constant optical densities were recorded at 350 nm and 600 nm for potassium dichromate and copper respectively.

Phytotransformation

10ml Stock solution of heavy metal (10mg/ml)was added in test tube with 0.1gm of LCAS. The reaction mixture was incubated for further consecutive days and optical density (O.D.) was measured daily was measured at 350nm for potassium dichromate and 600nm for copper sulfate.

Phytoremediation of potassium dichromate

Heavy metal solution was prepared by dissolving the Potassium dichromate ($K_2Cr_2O_7$) to distilled water. The algal strain were transferred to the heavy metal containing solution and incubated for next 5 days. Daily absorption was observed on spectrophotometer. O.D. was taken at 350 and 375 nm for dichromate and chromate respectively. Different parameters were recorded. All the procedures were carried out in triplicates.

Viability analysis of phytoremediating algae:

The viability of algae was observed per day in terms of color, oxygen bubble formation and luxuriant visible growth. On the day 1 the P*ithophora oedogonia* was very weak after phytotransformation was determined and gradually the biological growth start to observe after 7 days the algae get fully reviewed.

III.RESULTS AND DISCUSSION

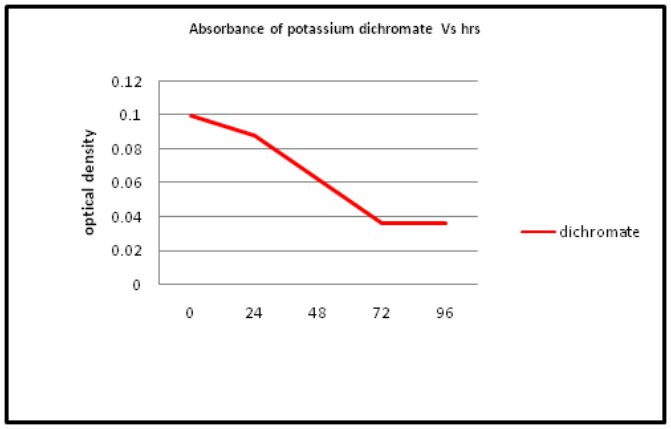
In the initial step of phyto-identification, fully grown algal specimen was acquisized, cleaned and its hypae ware confined on a clean grease free glass slide. LCAS were confirmed by Department of Botany, Government Institute of Sciences, Chhatrapati Sambhajinagar and the authenticated with accession no. 01020 by at Herbarium centre, Dr. B. A. M. U., Chhatrapati Sambhajinagar. It was observed to be of *Pithophora oedogonia* (Mart.) Wittrock. Algae were successfully cultivated with the isolated hypae by replacing the water and staying aware of the optimum condition.

In the next steps, Biosorption capacity of algae was observed. In which the direct relation between incubation time and amount of heavy metal was noticed. As with increasing time of incubation was seen, the amount of heavy metal was found to be diminished accordingly.

The adsorption readings of the different sample are as per the following:

Graph1

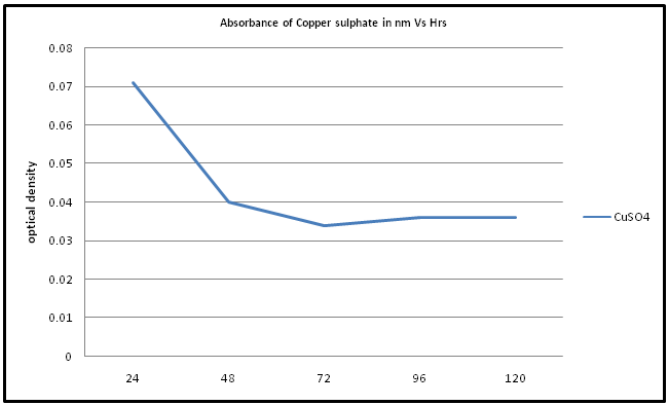
Absorbance of potassium dichromate (350 nm)



Initially potassium dichromate was present 100% (10mg/ml) in the test sample tube which show decrease with incubation time as after 24 hours 88%, after 48 hours 62.00% was left and after 72 hours 36.00% was remained. After 96 hours no considerable decrease was observed as the absorbance was approximately same as of 36.00%.

Graph 2

Absorbance of copper sulfate (600 nm)



When biosorption of copper sulfate was studies it was seen that on day 1 the copper sulfate is in abundant amount in the sample which was decreased to 56.33% followed by day 2. A decrease of 47.88% was found on day 3 which shows slight increase of 2.9% and on day 4 also remain 50.70% same as of day 3. As the copper results were not satisfactory, further phytoremediation was examined for the potassium dichromate (graph 3).

Phytotransformation was clearly visible as an observable color change when Potassium dichromate (orange) gets converted into potassium chromate (yellow) and copper sulfate (bright blue) into copper (II) sulfate (colorless).

As, in the Biosorption better results were observed of potassium dichromate than of copper sulfate.

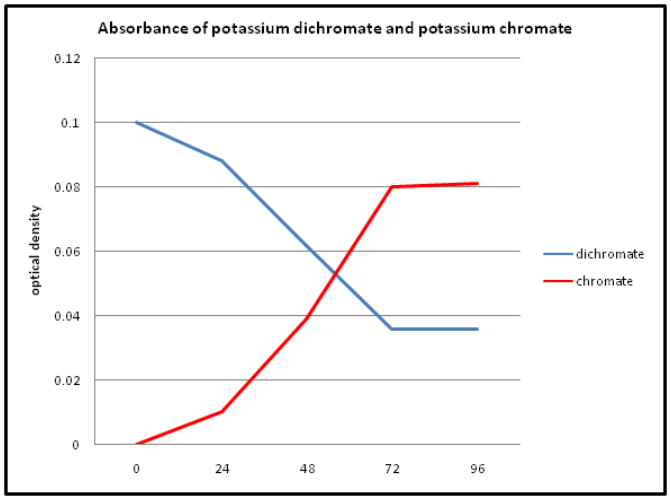
As per the observation of Graph 1, initially Potassium dichromate was available in high sum and no potassium chromate were seen in the sample however as the progression of time amount of potassium dichromate get reduced and measure of potassium chromate increments (Graph 3) as on 1st hour there was 0.00% potassium chromate which increase to approximately 12% after 24 hours of incubation on 48 hour it become 40% and after 72 and so on.

This concludes that on the 24 hrs incubation, 12% of potassium di chromate was absorbed by the algae and converted into 10% of potassium chromate. After 48 hrs of incubation 38% of potassium dichromate is degraded into 40% of potassium chromate and so on.

This calculation also indicated the sorption capacity of algae. As algae is found to absorb Potassium dichromate in its cells and then phytoremedaites it followed by leaking it out in the form of potassium chromate. The time lag and cumulative unequal percentages of concentrations of Potassium dichromate and potassium chromate justifies the fact of Biosorption.

Graph: 3

Absorbance of potassium dichromate and potassium chromate (350 nm and 375 nm respectively)



Scientists also recognize the ability of algae to transform chromium into less toxic forms. A study demonstrated that the microalgae *Chlorella pyrenoidosa* could reduce hexavalent chromium, the most toxic form of chromium, to trivalent chromium, which is less toxic and more easily immobilized in the environment. This transformation is facilitated by enzymes and metabolites produced by algae, which can convert toxic forms of chromium into non-toxic ones. (Gupta & Rastogi, 2009)

Bioremediation of chromium is a perplexing interaction that has collected the interest of researchers from different fields. One of the noticeable perspectives on this point comes from ecological microbiologists who underline the job of microbial networks in the remediation of chromium-tainted sites (Fernández *et al.*, 2018). These specialists feature the capability of native microorganisms to change and detoxify chromium through cycles like bioreduction and biomethylation (Karigar and Rao, 2011, Hazen and Tabak, 2005). Furthermore, biosorption studies show the effective adsorption capacity of dry SH-1 raw biomass and the high

recovery of Cr (VI) in a short time compared to the process of Cr (VI) extraction by SH-1 cells ((Hossan*et al.*, 2020).

A study has also conducted extensive research on the bioremediation of chromium that studies, has focused on the use of fungi to remove chromium from contaminated soil and water. They found that certain species of fungi, such as *Aspergillus* and *Trichoderma*, can effectively reduce the concentration of chromium in contaminated sites. They also believe that the use of fungi is a cost-effective and eco-friendly method of bioremediation (Dixit *et al.*, 2015).

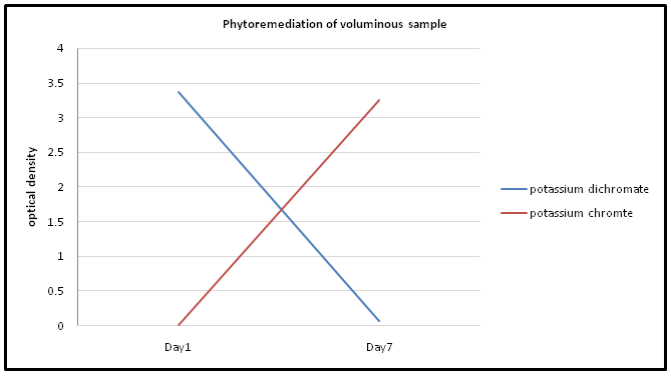
Then again, natural chemists offer an alternate point of view, zeroing in on the molecular systems underlying chromium take-up and change in plants. Their examination underlines the hereditary and metabolic pathways engaged with phytoremediation, where plants are utilized to eliminate chromium from the environment (Deshpande, 2016).

One more viewpoint on chromium bioremediation comes from specialists in the field of phytoremediation. Phytoremediation uses the inherent capacity of specific plants to retain and amass pollutants like chromium. A few scientists have zeroed in on the utilization of explicit plant species, including particular sorts of green growth, which have shown promising outcomes in the expulsion of chromium from sullied conditions. The capacity of these life forms to take-up and sequester chromium presents a harmless to the ecosystem and manageable way to deal with remediation (Ahemad, 2015).

When A large contaminated sample (10mg/ml) was phytoremediated, it was observed that initially 100% dichromate was present in the solution which after 7 days of incubation only 20.8% was left on the other hand on initially only 1% of potassium chromate present in the sample get increase gradually in higher concentration (Graph 4).

Graph 4

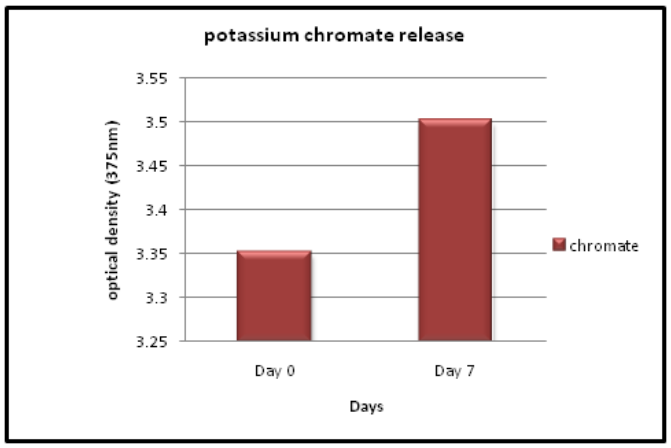
Phytoremediation of voluminous sample (1/100 g/ml)



When this above treated sample was again inoculated in SSW the potassium chromate show gradual increase from day 1 to day 7 it has approximately 15% increase in chromate release was found (Graph 4, 5).

Graph 5

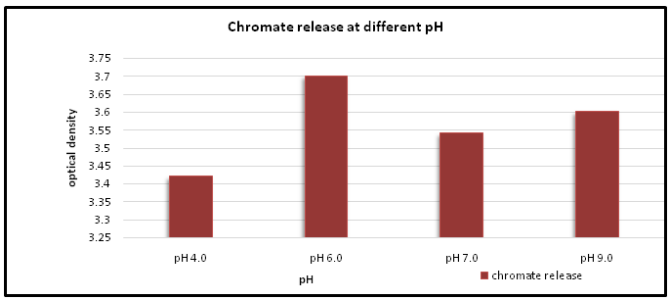
Chromate release after Phytoremediation into SSW



When inoculated in different pH SSW tubes with $K_2Cr_2O_7$, maximum release of potassium chromate was observed in pH 6 (Graph 6). It was detected that Potassium chromate concentration was highest at slightly acidic pH.

Graph 6

Release of potassium chromate at different pH



IV. CONCLUSION

Various researchers have different opinions about the biological remediation of chromium. But they agree that this is essential strategy to remove this harmful metal from the water in every view. Phytoremediation is seen to be a promising, sustainable and cost-effective way to clean up contaminated environments. It reduces hexavalent chromium to less toxic trivalent structures, reducing environmental harm *Pithophora* algae's rapid development rate, high metal absorption limit, and flexibility to different natural environments make it a putative candidate for the remediation of copper and chromium-containing waste water. Phytotransformation of liquid effluent by phytoremediation as well as recycling of phytoremediating algae biomass are the strategy making designs which may improve the environmental health. The Further innovation in this field will lead to more effective and efficient strategies for the biological remediation of chromium as mentioned in this study.

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Effect of Crude Blue Green Algal Extract on the Trigonell Foenum – graecum L.

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ABSTRACT

Fenugreek (Trigonella foenum-graecum L.), is a commonIndian spice that are particularly recognized for its culinary, medicinal, and aromatic properties and is consumed on large scale. In the present study the effect of crude extract of Blue Green Algae (BGA) in varying concentration was studied over the T. foenum-graecum L. Five treatments viz. (1%, 2%, 3% 4% & 5%) concentrations of crude BGA extract were sprayed on the leaves at an interval of 15 days was compared with the control i.e. untreated plants. Marked response was observed in morphological parameters and biochemical constituents of the test plants. Following morphological parameters like seed germination percentage, plant height, leaf numbers, leaf size, number of flowers, pod numbers, pod length, seeds per pod, node and internode distance, plant weight, leaf weight (dry and wet) and stem diameter was studied. Biochemical constituent's such as total chlorophyll, total proteins, carbohydrates, crude lipids and ascorbic acid contents were analyzed. The experimental results confirm increase in numbers of leaves, flowers, pods and seeds per pod along with plant height and weight. Early maturity was attained by the test plants as compared to the controlled plants.

Keywords: BGA, Foliar spray, fenugreek, Trigonella foenum-graecum L.

I. INTRODUCTION

Fenugreek herb (*Trigonellafoenum-gracum*) is an annual leguminous bean, and belongs to Fabaceaefamily. Fenugreek is routinely known as 'methi' which occupies an important place among the various spices grown in country with respect to area as well as production. Leaves and seeds of fenugreek are included in normal diet of family, especially diet of growing kids, pregnant ladies, puberty reaching girls

and elder members of family because of their haematinic value. Legumes are functional foods having therapeutic properties and promoting good health (Basch,2003). Fenugreek seeds are economic values and important nutritional source which can be used as human food supplement,as it contains importantphenolics, antioxidant (Randhir,2004)and having antimicrobial activity also (SitaKumari, 2016,Randhir,2004). It showspresence of carbohydrates, protein, fat and amino acids.The phytochemical constituents of fenugreek seeds contains phytosterols, flavonoids, alkaloids, amino acids, proteins, carbohydrates, glycosides, phenolic compounds, tannins, terpenoids, saponins, oil and fats (Nazhan, 2017).Blue green algae is an ancient group of unique prokaryotic microorganisms with the ability to performmutually compatible functions like nitrogen fixationand photosynthesis (Selvi,2012).BGA are highly recommended for very beneficial bioremediation applicationsfor *in-situ* and off-site removal of pollutants(Maheswari 2017). Theeffectiveness of algal extracts for enhancing the seed germination in *Phaseolus vulgaris w*as observed by Bhosle et al (1975), similarly on *Vignaunguculata* by Sekar et al (1995). Increase in vitamin-C concentration was recorded by Khemnar and Chaugule (2000) in *Trigonella* on treatment of *Ulva lactuca* L. Recently effect of algal extract on the yield of *Solanum melanogenum* L is demonstrated by Salve (2019).

II. METHODS AND MATERIAL

2.1 EXPERIMENTALCROP: *Trigonillafoenum-graecum* L. Variety Deepak was selected for the present study which is commonly cultivated in the region. 18 raised beds of 1Mt X 1Mt. size were prepared in the field. Prior to bed preparation the soil was ploughed and allowed to dry in the summer month, followed by the weeding and other cultural practices. The sowing and all the field experiments was carried out during the Kharif season of 2020 and 2021 at Dhoksal Tal. Mantha Dist. Jalna (M.S.) India.

2.2 ALGAL SAMPLES: The experimental algae *Anabaena fertilissima* C.B.Rao was collected from Chhatrapati Sambhajinagar Dist. of Maharashtra state and identified using standard monograph (Deshikachary 1959) The algal sample was washed in running tap water and twice with distilled water. The sample was dried in shade for 3-4 days. The dried sample was crushed in grinder and fine powder was made. 0.1gram powder was dissolved in 100ml D.W. for further process. It is filtered through a muslin cloth and final volume of the filtrate was made up to 100ml with distilled water. This aqueous extract was further diluted with distilled water to achieve various concentrations ranging from 1% to 10% (w/v).The experiment was set in triplicate, one set without treatment was considered as control experiment.

2.3 FOLIAR SPRAYING: The aqueous extract was sprayed on the test crop with the help of sprayer (50ml per plot) at an interval of 15 days and the observations were taken at the interval of 1 week (i.e. seven days).

2.4 PHYTOCHEMICAL TESTING: The test plants were tested for the various phytochemical constituents such as protein, lipids vitamins and chlorophyll contents by using standard biochemical methods (Sadasivam and Manickam 1992)

III.RESULTS AND DISCUSSION

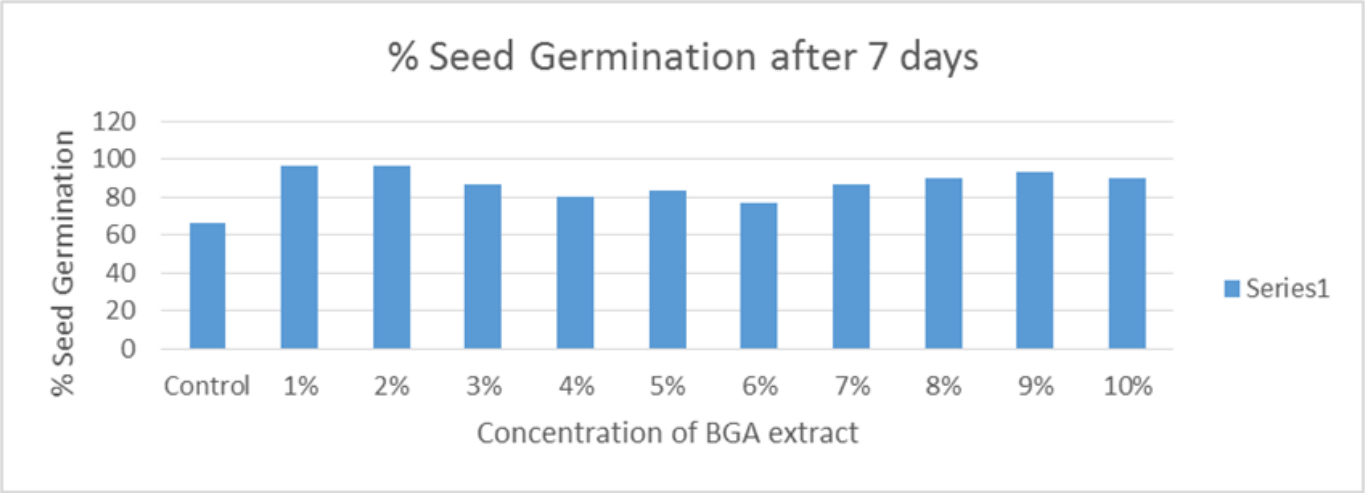


Fig. No.1 Effect of BGA extract on the seed germination of *T. foenum-graecum* L.

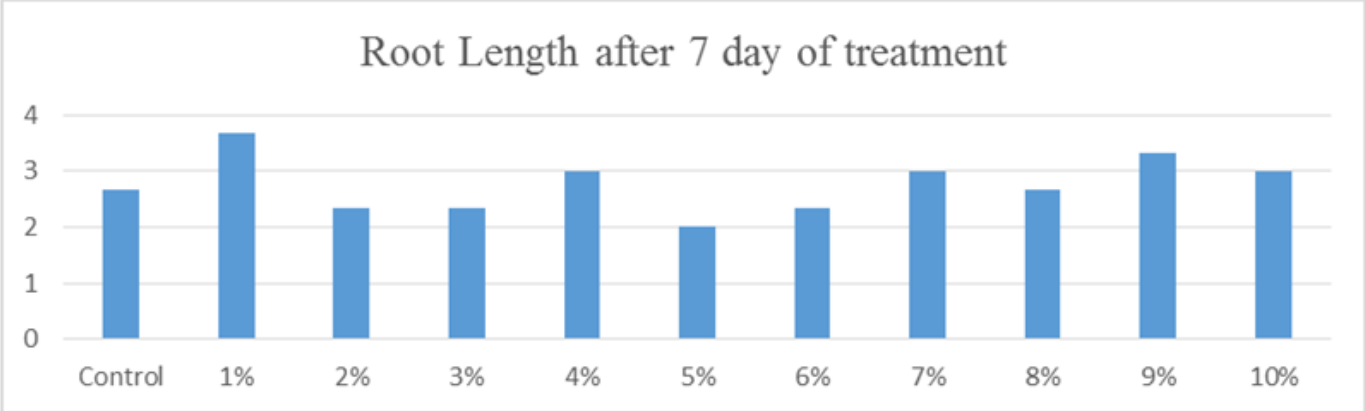
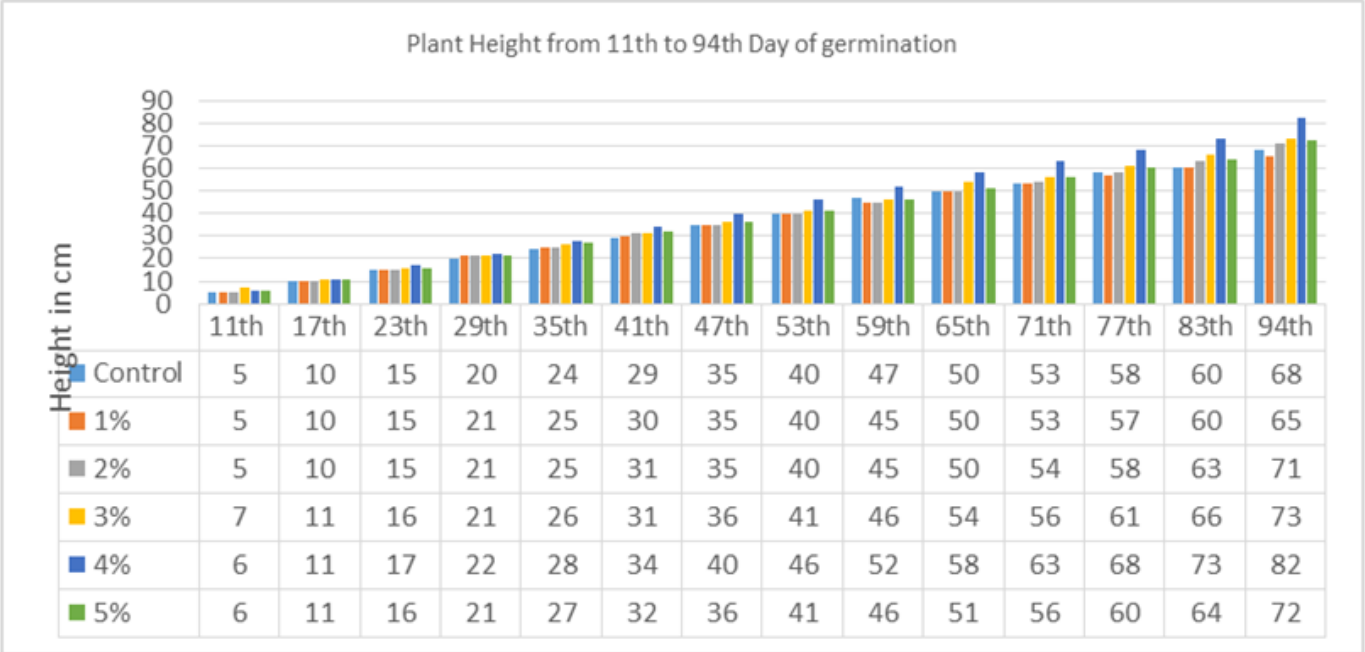


Fig. No.2 Effect of BGA foliar spray (1%-10% conc.) on the root length (cm) of *T. foenum graecum* L.[* all the values in cm]



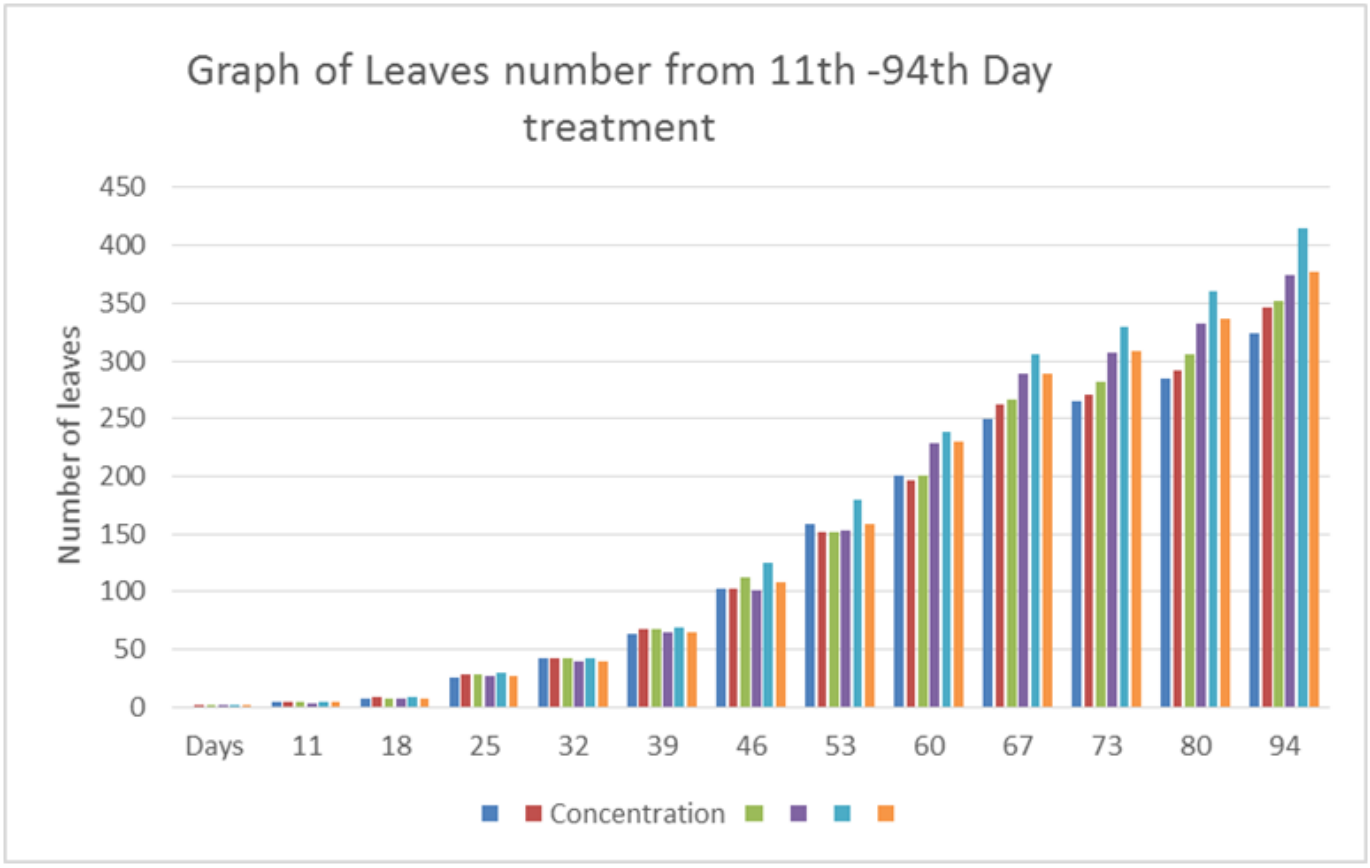
	11th	17th	23th	29th	35th	41th	47th	53th	59th	65th	71th	77th	83th	94th
Control	5	10	15	20	24	29	35	40	47	50	53	58	60	68
1%	5	10	15	21	25	30	35	40	45	50	53	57	60	65
2%	5	10	15	21	25	31	35	40	45	50	54	58	63	71
3%	7	11	16	21	26	31	36	41	46	54	56	61	66	73
4%	6	11	17	22	28	34	40	46	52	58	63	68	73	82
5%	6	11	16	21	27	32	36	41	46	51	56	60	64	72

Table No. 1: Effect of BGA foliar spray on the plant height of *T. foenum-graecum* L.
[* mean values in cm]

Figure No 3: Differences in plant height (control and treatments 1% - 5% foliar concentration)

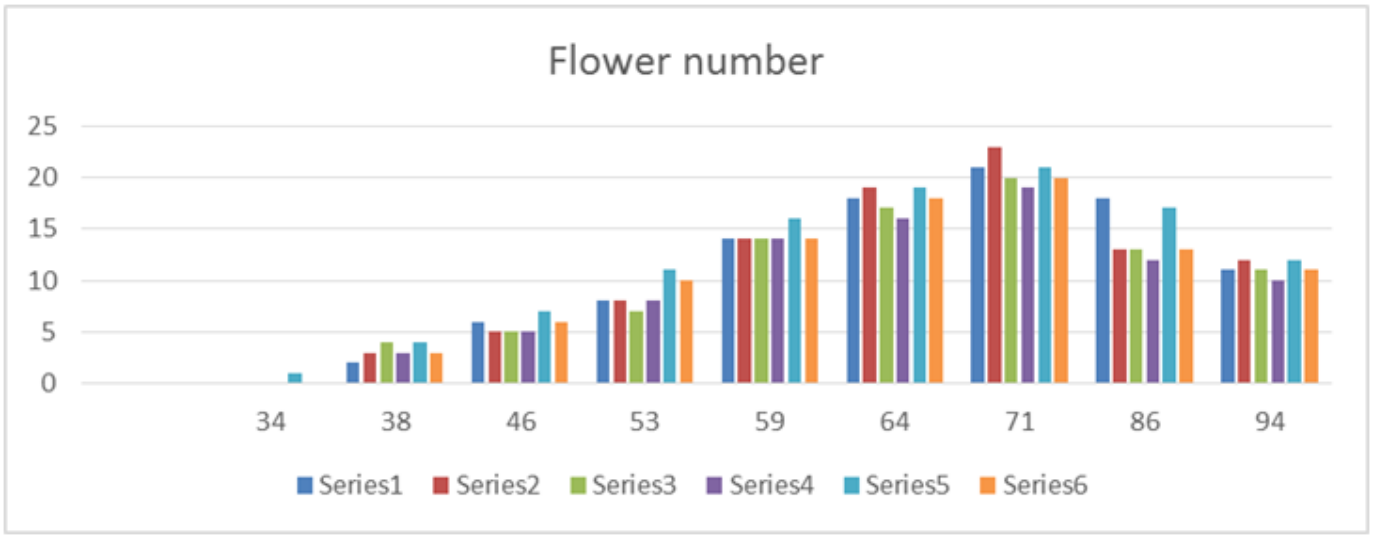
Table no.2: Effect of BGA foliar spray on leaf number of *T. foenum-graecum*L.

	Concentration					
Days	Control	1%	2%	3%	4%	5%
11	4	4	4	3	4	4
18	8	9	8	8	9	8
25	26	28	28	27	30	27
32	42	42	43	40	43	40
39	63	68	68	65	69	65
46	102	103	112	101	125	108
53	159	152	152	153	180	158
60	200	197	201	229	239	230
67	249	262	267	289	305	289
73	265	270	282	307	330	309
80	285	292	306	332	360	337
94	324	346	352	374	415	377



FigureNo.4: Effect of BGA foliar spray on leaf number of *T. foenum-graecum*L.

Table No.3: Effect of BGA foliar spray on flower number of *T. foenum-graecum*L.



FigureNo.5: Effect of BGA foliar spray on flower number of *T. foenum-graecum*L

Table No.4: Effect of BGA foliar spray on the number of pods after flowering in the test crop *T. foenum-graecum* L.

Days	Control	1%	2%	3%	4%	5%
45th	0	0	1	0	1	1
52th	9	9	11	9	13	9

59th	9	10	12	11	14	11
66th	18	18	22	20	22	20
73th	26	26	28	27	29	27
80th	29	31	34	36	36	34
94th	32	38	38	39	41	39

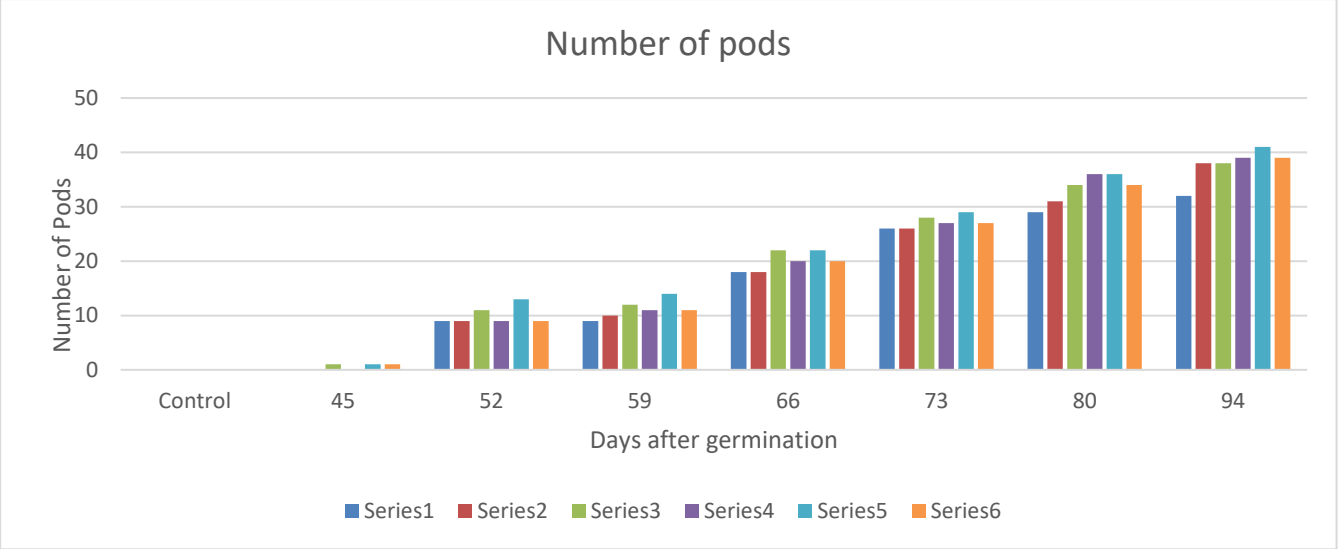


Fig No.6: Effect of BGA foliar spray on Pod number of *T. foenum-graecum L.*

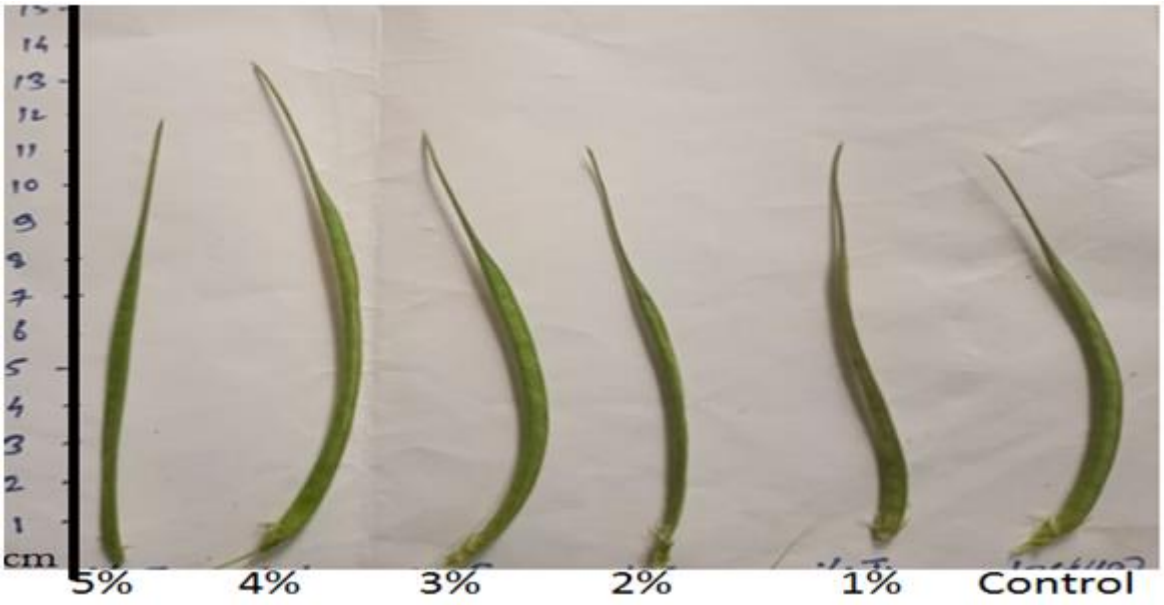


Fig. No 7: Difference in pod length after [1-5% foliar spray and control plants] after 94th day of seed germination

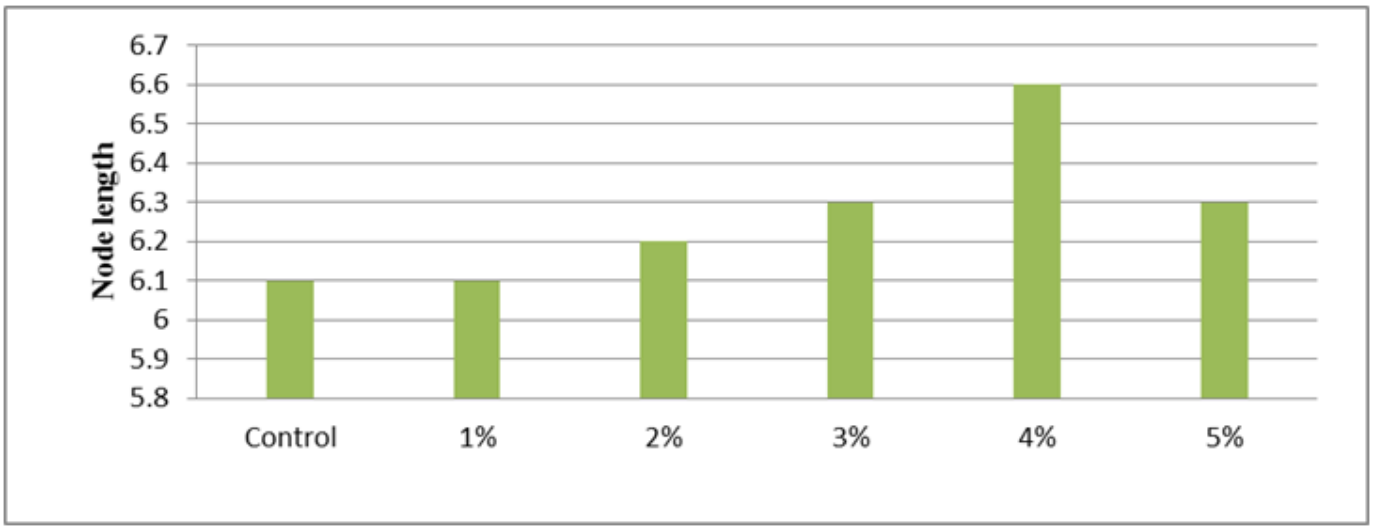


Fig.No.8: Effect of BGA foliar spray on node length of *T. foenum-graecum* L.

Fig. No.9: Differences in internodal length on 94th day old plant against controland 1-5% treatment foliar spray treatments.

Table No.5: Effect of BGA foliar spray on Chlorophyll constituents of *T. foenum-graecum* L.

Sample	Chlorophyll -a mg/gm	Chlorophyll b mg/gm	Total Chlorophyll mg/gm
Control	1.107	0.443	1.563
1%	0.97	0.414	1.385
2%	1.367	0.571	1.941
3%	1.03	0.425	1.455
4%	1.187	0.449	1.636
5%	1.265	0.507	1.772

(*mean values of 3 readings)

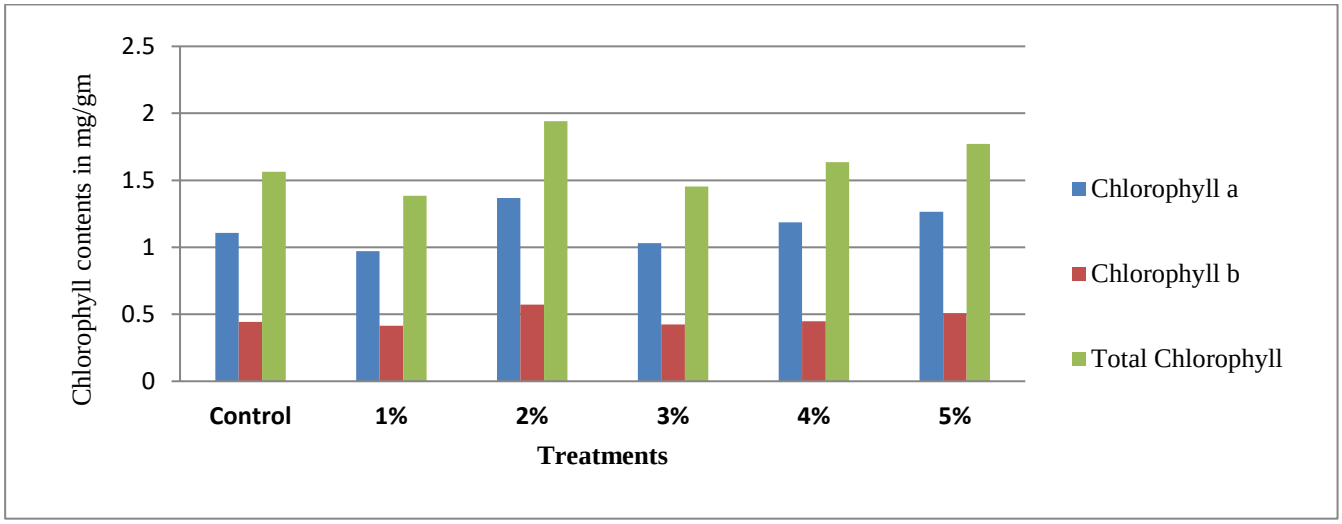


Fig.No.10: Effect of BGA foliar spray on Chlorophyll contents in test plant *T. foenum-graecum* L.

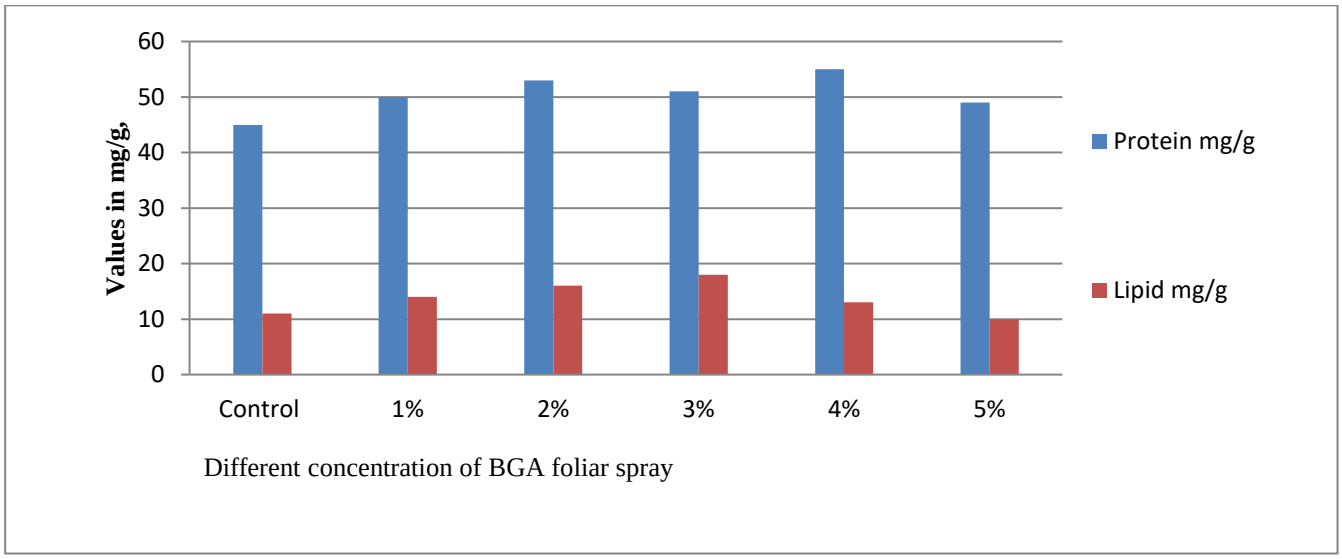
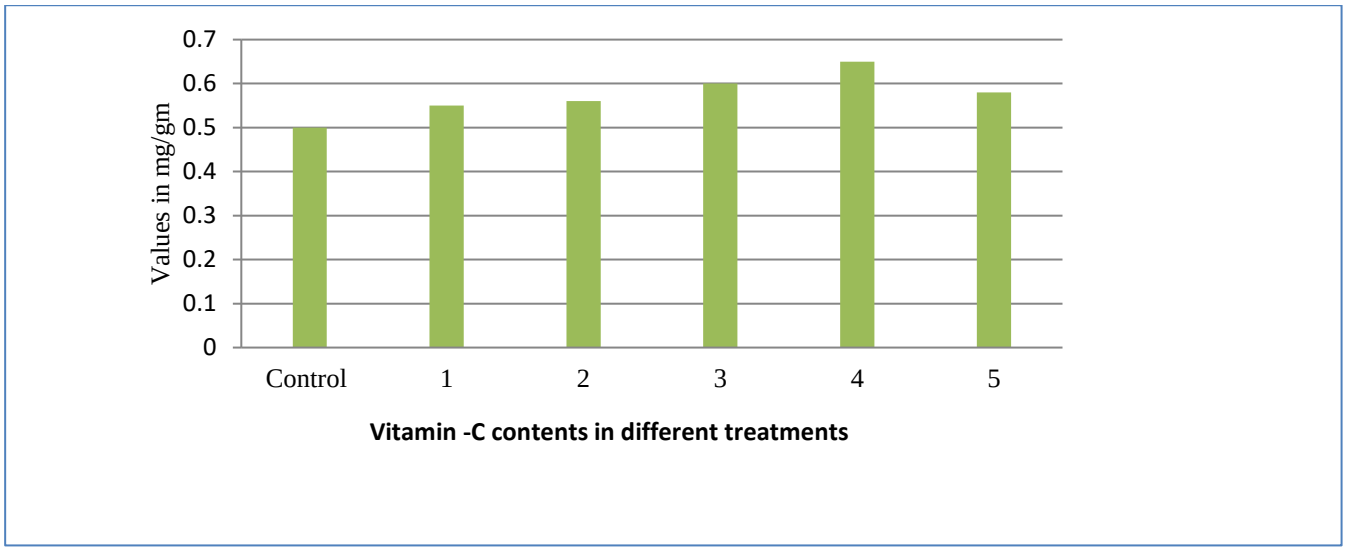


Fig.No.11: Effect of BGA foliar spray onprotein and lipid contents in leaves of *T. foenum-graecum* L.



FigureNo.12: Effect of BGA foliar spray onVitamin-C contents of *T. foenum-graecum* L.

The present work shows that algal foliar spray is most effective for enhancing the yield of *T. foenum-graecum* L. BGA foliar spray used in different concentration which showed that there is enormous growth in seed germination, plant height, leaf number, flower number, pod number, pod length, seed number, node and internode height and plant leaf weight etc.The effect of BGA application on seed germination is found to be maximum (i.e. 96.6%) in 1% and 2%treatments.While in nontreated seeds 67.% seed germination was observed on 8th day. The root lengthwas found to be maximum (3.66 cm) in lower concentration (1%) concentration.The plant height observed to be highest at 4% concentration of BGA on 94th day of treatment.The number of leaves increased at 4% concentration of BGA on 94th day. The number of flowers is maximum on 71st day at 4% concentrationof BGA.The number of pods increased on 94th day at 5% concentration of BGA. The height of pod and node length found to be increase on 94th day at 4% concentration of BGA. The weight of dry and fresh leaf is more at 2% and 4% respectively. Thus it is concluded that 4% concentration of BGA is found to be effective in increasing the seed germination, number of leaves per plant, number of flowers and pod length, and internodal distance. Even the phytochemical constituents of *T. foenum-graecum*L.i.e chlorophyll pigments, protein, lipids and vitamin contents are also found to be maximum in lower concentration (2%) of BGA foliar spray.

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Diversity of Forensically Important Flies in the Doodh Bazar Province of Nashik

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ABSTRACT

For medico-legal backing, insects can contribute to the investigation of criminal cases and establish the time of death by determining the colonization time of blowflies on dead bodies, which can be used to indicate Post mortem interval (PMI). The presence of specific blowflies highly build-up on their temperature and habit Forensic importance of flies of families Calliphoridae and Sarcophagidae were found in the Doodh Bazar zone of Nashik. For the collection of flies, insect nets were used and the very finest files were collected by narcotizing them with Chloroform and later picking them with forceps. However, this is the first forensic entomological report presenting such a peculiar diversity of species of blowflies. These findings are the very first observation in the whole Nashik district making the effort more valuable for future studies of forensic entomology.

Keywords: medico-legal, Blowflies, Post mortem interval (PMI), Calliphoridae, Sarcophagidae, Doodh Bazar

I. INTRODUCTION

The members of order Diptera are special kinds of files that are abundantly present in nature known as "true flies". They transmit disease to humans and domestic animals. They are also important as they act as bio-control for insect pests and help in decomposing the organic matter [1]. With-it the world of science about the legal backing of forensic science necrophagous forensic insects can provide detailed information about criminal cases like murder, suicide and wildlife crimes [2].The application of forensic entomology was established in the first half of the twentieth century, where taxonomists paved

interest in forensically important flies [3]. The flies that belong to the order Diptera are of forensic significance as they provide the traceable indication of the crime scene and help in establishing the time of death by determining the minimum Post mortem interval (m-PMI) while harvesting nutrients from decomposing carcasses [4]. The (m -PMI) is indicated by studying the development of the larvae on the carcasses. The dead body delivers constant physical, chemical and biological support with suitable protection from humidity, temperature and acts as a highly nutrient resource [5]. The order Diptera shows richness in the species near to 161,000 defined species worldwide [6]. The specimens of Calliphoridae and Sarcophagidae emerge in all stages of carcasses and help scientists determine body decomposition. Adult flies of these families are attracted to the carcasses due to the strong odour of rotten meat [7]. The bulk of Calliphoridae flies mostly corresponds to the metallic impression while the colour ranges from green or blue to bronze -shiny black [8]. Sarcophagidae has the colour impression of greyish and has lengthwise darker and lighter stripes [9]. These conclusions are the first remarks in the field of the medico-legal sector in the whole Nashik District making it a valuable contribution for further studies.

Objectives of the study:

The study aims to identify species of blowflies belonging to the families Calliphoridae and Sarcophagidae in the Doodh Bazar zone of Nashik in order to calculate the PMI (time of death) in criminal cases.However, there have been no reported cases of forensically important flies from the Doodh Bazar region of Nashik. Therefore, a primary investigation was carried out to identify and document forensically important blow flies in order to strengthen the forensic insect database.

II. METHODS AND MATERIAL

The Doodh Bazar province of Nashik is located near the Shalimar area of Nashik city. It is one of the major diversified areas as it has several slaughter shops. The chance was great to find blowflies because the leftover goats, sheep and meats and other decomposing materials of vegetables were also dumped in that particular area. The compilation of flies was done by using the definitive method. The files were captured by a hand insect net by the sweeping method as they flocked around the carcasses [10]. The small fine flies were narcotized by chloroform using the syringe. Later the files were picked up by the forceps wearing surgical gloves. The files were stored in 70 % alcohol in glass bottles and labelled with sample number and date of collection for further taxonomic examination.These collected flies were transported to the laboratory for further morphological and microscopical identifications.

III.RESULTS AND DISCUSSION

The order Diptera shows richness in the species near to 161,000 defined species worldwide.Such extremely important Dipteran blowflies are found in abundance in India. This is the firstrecordofforensicallyimportantblowfliesfromNashikdistrictastheywerestudiedandidentified.Their role in the medico-legal sector is numerous and such important blowflies were finallylearnedintheDoodhBazarofNashik.30-50specimensofflieswerecollectedandexaminedforidentification.

Calliphorids are a crucial constituent of ecosystem due to their role in decomposition. Common man refers to them as Blue/green bottle flies. They are characterized by metallic and shiny attractive bodies. These initial arrivals and settlers at the site of death utilize carrion to expand their offspring. Calliphorids play a crucial role in forensic entomology, particularly

in decomposition process and establish a unique faunal succession.

Below listed are the recorded flies with their characteristics.

The blue bottle flies are flies of genus *Calliphora* and green bottle flies are from genus *Chrysomya.* At the Doodh bazar slaughter station, we have observed significant abundance of members of genus *Chrysomya* in our recordings. The identification was solely reliant on morphology and literature review.

The key to identification of *Chrysomya* species

i. *Chrysomyamegacephala*

1. Anterior thoracic spiracle blackish brown to dark.
2. In males, eyes are touching with enlarged upper eye facets and sharp demarcation from the lower facets.
3. In females, fronto orbital plate is dark and frontal stripe is broader.
4. Lower calypter is brownish in colour with the dark rim.
5. Parafaciala is brilliant orange with golden hairs.
6. Post gena and genal dilation orange.

ii. *Chrysomyarufifacies*

1. Anterior thoracic spiracle white.
2. Outer vertical setae present in males, eyes are separated and Parafrontalia narrowed and contiguous.
3. Proepisternal setae present.
4. White hairs present on epistome.
5. Lower calypter is white.
6. Female possesses dorsal cleft in the posterior end of fifth tergite.

iii. *Chrysomyabezziana*

1. Anterior thoracic spiracle white to yellowish.
2. Eyes in males are close but not touching and absence of demarcation between the upper and lower facet.
3. In females, frons consists reddish fronto orbital plate.
4. Thorax is bluish green and anteriorly lightly silver dusted.
5. Abdomen is green to bluish purple in colour.
6. Lower calypter is uniform white.

iv. *Chrysomyaalbiceps*

1. Anterior thoracic spiracle is white.
2. In males, frons is narrowed and eyes are closed to each other whereas in females frons are dark in color and fronto orbital plate is whitish.
3. Outer vertical setae are present.
4. Proepisternal setae are absent.
5. Lower calypter is whitish.
6. Third segment of antenna is fully dark grayish.

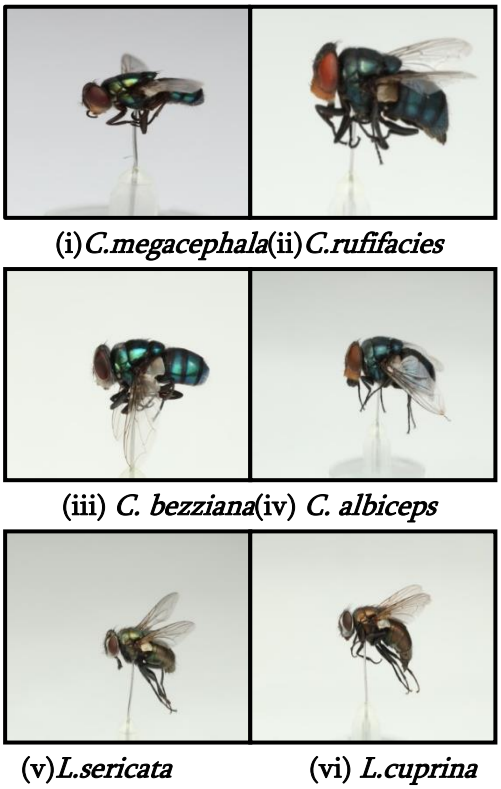

Figure: Blowflies from the Calliphoridae and Sarcophagidae families collected at the Doodh Bazar area.

The key to identification of *Lucilia* species

i. *Luciliasericata*

1. Anterior thoracic spiracles are blackish to brown.
2. Antennae are dark brown.
3. Parafrontalia covered with hairs, fronto orbital plate in females is wide and blackish.
4. Gena and genal dilation grayish to silvery in

colour.

5. Humeral callus bearing 6 to 8 hairs.
6. Basicosta bright yellow, thorax and abdomen has metallic greenish shining.
7. Lower calypter is whitish to yellow.

ii. *Luciliacuprina*

1. Anterior thoracic spiracles are black to brown.
2. In male Parafrontalia covered with fine hairs alongside with frontal bristles.
3. Frontal vitta is shorter, reddish to black in colour in female.
4. Humeral callus consisting 0 to 4 hairs.
5. Basicosta pale yellow, thorax and abdomen shining green to coppery in colour.
6. Lower calypter is white to yellowish.

Calliphoridae species have adopted different feeding habitats they feed on the decaying matter, decomposing vegetables wastes and carrions [18]Calliphoridae species surf different ecological niches. They act as bioindicators and help in determining the hygienic conditions of the environment [19]. Flies of the family Calliphoridae and Sarcophagidae are often the first to arrive at the corpus making it an effective breeding site and a significant source of Post mortem interval studies [20]. The observed flies are synanthropic where flies have adapted to live in a close association with human habitat [21].Forensically important blowflies were reported in the Doodh Bazar province of Nashik district. There are two families of Diptera flies the flies of the Calliphoridae family and the flies of the Sarcophagidae family.Both have distinctive features and functions as bioindicators and help in decomposing carcasses.

IV. CONCLUSION

Forensically significant blowflies belonging to the Calliphoridae and Sarcophagidae families are identified for the first time in the Doodh Bazar region of Nashik by this study. Documentation of species such as *Chrysomyamegacephala*, *Chrysomyarufifacies*and*Luciliasericata highlights their significance* in establishing environmental hygiene and the post mortem interval.

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