

Antimicrobial, Phytochemical and Dyeing Activities of Some Common Household Vegetables

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ABSTRACT

Any substance that is active against microbes is known as an antimicrobial. Vegetables owe their antimicrobial properties majorly to secondary metabolites such as alkaloids, glycosides, tannins and volatile oils present within & other properties like dyeing due to their coloured pigments. This study aims to understand these properties of some of the common vegetables and how they can be exploited. The plant extracts were prepared using two solvent- Ethanol and Distilled water in order to extract the bioactive compounds. The three household vegetables used were – Spinach (*Spinacea oleracia*), Beetroot (*Beta vulgaris*) & Radish (*Raphanus raphanistrum*). These extracts were then tested for their antimicrobial activity against two organisms *Escherichia coli* and *Staphylococcus aureus*, isolated from the sewage & meat sample respectively. After isolation and confirming the organism strain antimicrobial activity was evaluated by using Agar well diffusion method, using antibiotics as standards. Zones of inhibition were observed on sterile Muller Hinton's agar which was then recorded. Thus this study emphasizes that consumption of these vegetables can help to improve our immune system for better immunity towards some common pathogens and have various nutritional and pharmaceutical applications. Also presence of phytochemical constituents like Saponins, Tannins, Flavonoids, Phenol and Vitamin-C in vegetable samples were confirmed by specific phytochemical tests. Dyeing property of the extracts were also evaluated by dipping two sample pieces of cloth – cotton & styrofoam in the extracts for 24 hrs. after which it's colour was observed and recorded.

Keywords: - Vegetables, Extracts, Antimicrobial activity, Phytochemical, Dyeing

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I. INTRODUCTION

A renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes

with the recognition that plant-derived products have great potential as sources of pharmaceuticals. Although leaves, roots, flowers, whole plants, and stems were examined for useful phytochemicals in many research projects, few reports refer to

vegetables as, sources for pharmaceuticals.[1] The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization are of great importance in Indian Ayurveda.

At present, the development of resistance in microorganisms is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants. Most cures for common diseases are resolved by eating fruits or vegetables as they have tons of phytochemicals and other valuable substances.[1] Even today, plant materials play a major role in primary health care with maximum therapeutic and minimum side effects, in many developing countries.[2] The use of antimicrobials is a new technology by the food industry to increase the shelf life of food and overcome the issues of food quality and safety. These antimicrobials could be of natural or synthetic type, but natural antimicrobials are gaining much importance than synthetic ones. [20] Different vegetables have anti-microbial, anti-inflammatory and anti-oxidant properties& thus in a good diet, fruits and vegetables play an important role. [11] In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs is beyond the reach of three quarters of the third world's population, although many such countries spend 40-50% of their total wealth on drugs and healthcare.[1] In the near future with the growing knowledge about antioxidants, that their presence in everyday foods promote health, combined with the assumption that a number of common synthetic preservatives may have hazardous effects, will led to

multiple investigations in the field of search natural antioxidants source from vegetables.[1]

The term qualitative phytochemical analysis refers to the procedures involved in establishing and proving the identity of the phytochemical constituents present in the crude plant extract. The pharmacological actions of crude drugs are determined by the nature of their constituents. Thus, the plant species may be considered as a biosynthetic repository not only for the chemical compounds and constituents but also for a multitude of phytochemicals and substances including alkaloids, tannins, flavonoids, saponins, phenols, etc. which exert definite physiological effects. These phytochemicals and substances are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used. [10]

Today with global concern over the use of eco-friendly biodegradable materials, research is being undertaken around the world on the application of natural dyes in textile industry, coloured paints, crayons, rangoli, etc. Extracting dyes from plant (natural) sources avoids the environmental pollution. Also there is increasing demand for dyes from plant origin particularly for textile application due to consumer desire to replace synthetic chemicals by natural compound. It is unlikely that all dyes will be produced solely from plants but a percentage of everyday colours could be naturally derived. [4]

Spinach (*Spinacia oleracea L.*) is considered as a functional food due to its diverse nutritional composition and green pigmentation due to chlorophyll. Spinach-derived phytochemicals and bio-actives are able to scavenge reactive oxygen species and prevent oxidative damage, modulate genes involved in metabolism, proliferation, inflammation, and antioxidant defence and curb food intake by inducing secretion of satiety hormones. These

biological activities contribute to the anti-cancer, anti-obesity, hypoglycaemic, and hypolipidemic properties of spinach. [10]

Radish is low in calories and is a good source of vitamin C. Radishes contain enough vitamin C to protect against scurvy. The seed oil is used to make soap. It increases appetite, produces cooling effects, and prevents constipation.[18] Radish (*Raphanus raphanistrum*) possess major antioxidant and pro-oxidant potential due to the acylated pelargonidin derivatives and could promote the cleavage of plasmid DNA with Cu ions alone & damage could be inhibited by horseradish peroxidase and catalase present in it. This horseradish peroxidase is said to be a potential decolorizing agent.[14] Radish roots are considered to be good for patients suffering from troubles in liver gallbladder ailments, haemorrhoids (piles), jaundice, and enlarged spleen. The seeds are said to be carminative, diuretic, expectorant, and peptic.

The intense red colour of beetroots (*Beta vulgaris*) derives from high concentrations of betalain, used as natural colorants by the food industry, but have also received increasing attention due to possible health benefits in humans, especially their antioxidant and anti-inflammatory activities. The betalain that are mainly found in beetroot are betacyanins and betaxanthins. [12]

Selection of resistant strains occurs so rapid for some bacteria that clinical usefulness of the antibiotics is lost over a period of time. The emergence and spread of microbes resistant to cheap and effective first-line drugs has become a common occurrence. At present most clinical isolates of *Staphylococcus aureus* are multi-drug resistant. *Escherichia coli* with related resistance profiles were also isolated in the same setting and even highly successful multidrug-resistant clades in different pathogens have made this a global challenge.[7] *Staphylococcus aureus* is an opportunistic pathogen which is Gram positive in nature has the ability to colonize the skin and mucous

membranes of humans and different animal species. It may cause mild to severe diseases, ranging from superficial wound infections or food poisoning to bacteraemia and other systemic infections.[8] *Escherichia coli* is a Gram-negative bacterium found in environment, food, intestine of people and animals. Most of its types are not harmful but some produces toxins which leads to mild to severe illness. [17]

II. MATERIALS & METHODS

A. Isolation of Organisms

Escherichia coli & *Staphylococcus aureus* were the two organisms used to test antimicrobial activity isolated from sewage & meat samples respectively. Serial dilutions of the samples was carried out and bulk seeded on St Nutrient agar plates. After incubation under aerobic conditions at 37°C, appropriate colonies were selected, purified, sub-cultured, stored for further studies and subjected to identification tests.

B. Identification of isolates

Organisms were selected on the basis of colony characteristics on St. Nutrient agar and plated on selective and differential media. The identification of the selected isolates was confirmed by Gram staining and various biochemical tests such as sugar fermentation test, catalase test as outlined in Bergey's Manual of Systematic Bacteriology..

C. Culture medium and Conditions

Following the isolation of organisms, a 0.1 ml culture (with its OD adjusted to 0.5) was inoculated in 100ml of St. Nutrient agar broth was incubated at temperature 37°C for 48-72 hrs. This inoculum was further used for the study as master culture. Un-inoculated St. Nutrient agar broth was used as a control.

D. Preparation of vegetables extracts

Fresh plant samples of Spinach (*Spinacea olerasia*), Beetroot (*Beta vulgaris*) and Radish (*Raphanus raphanistrum*) were randomly procured from various local markets in Mumbai. The edible parts of the

plant were washed with St Distilled water and cut into small pieces.

50 g of each sample was soaked in 150 ml of ethanol and distilled water each and were kept in dark for a period of 48h. The solution was then boiled until the solvent volume was halved and filtered using Whatman filter paper. The filtered solution obtained was stored in dark and wrapped with aluminium foil to avoid direct sunlight until further use.

E. Antimicrobial studies

The antimicrobial activity of plants extracts was screened using St Muller Hinton's medium. 18-24h old *E coli* and *S aureus* culture of 0.5 OD was used. Using a St. Cork borer 9mm wells were made and each well was filled with 0.5µl of prepared plant extracts. Appropriate solvents and antibiotic standards were maintained as control. Further the plate was incubated for 24h at 37°C. After 24 hrs. results were observed and zones of inhibition were measured and recorded.

F. Phytochemical tests

Test For Tannins – 1ml of extract added with 2-3 drops of 0.1% Ferric Chloride. [16]

Test For Flavonoids – 3 ml of 1% Aluminium chloride solution were added to 5 ml of each extract. [16]

Test For Saponins – 2.5ml of extract added with 10 ml distilled water. Shake vigorously. [16]

Test For Phenols – 1-2 ml of extract added with 5ml Folin Ciocalteu reagent & 4ml of sodium bi carbonate. Kept in water bath at 40°C for 30 mins. [16]

Test For Vitamin C – 0.5 ml DCPIP added with few drops of extract. [16]

G. Dyeing property of extracts

Different types of textile materials like cotton, Styrofoam which are commercially available were selected for the experiment. Each material was cut into equal size of 3cm² and were soaked in different extracts for 24h and dried at room temperature. Further the dyeing property and colour retention property of the fabrics was tested.

III. RESULTS & DISCUSSION

A. Isolation of Organisms

Collected samples were serially diluted and plated on St. Nutrient agar plates at 37°C for 24 hrs. Further, based on colony morphology of *E.coli* and *S.aureus* on St. Nutrient Agar a colony was selected from each sample plate and plated on specific selective & differential media.

Isolated pink coloured nucleated colonies of *E.coli* were observed on MacConkey Agar by utilization of lactose indicated by Neutral red colour change to pink. *Staphylococci* produced yellow coloured pinpoint colonies by utilization of Mannitol, which is indicated by phenol red colour change to yellow on Mannitol Salt Agar plate.

For further confirmation biochemical tests were performed such as Catalase test, Oxidase test, Indole production test, Methyl red test, Vogues-Proskauer test, Citrate Utilization test, Triple sugar iron test, Urease test, Nitratase, Coagulase & Sugar fermentation tests as detailed in *Table 1*. All the results obtained were compared to the standard results in Bergey's Manual of systematic Bacteriology, according to which the isolates were identified to be *Escherichia coli* & *Staphylococcus aureus*.

Test	<i>S. aureus</i>	<i>E. coli</i>
Sugar Fermentation test		
Glucose	++	++
Sucrose	--	++
Mannitol	++	++
Xylose	--	+-
Lactose	++	+-
Indole test	N.A	+
Methyl red test	N.A	+
Vogues proskauer	N.A	-
Urease test	+	-
Citrate utilization	N.A	-

TSI	N.A	Yellow slant Yellow butt
Catalase test	+	+
Oxidase	N.A	-
Nitratase test	+	N.A
Coagulase test	+	N.A

Table 1:-Biochemical tests

[+] = Positive result, [-] = Negative result

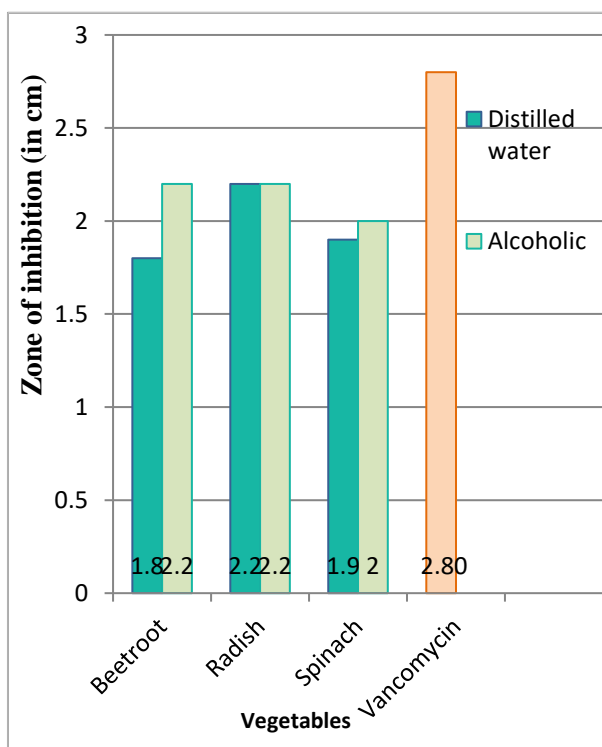
[++] = acid and gas production

[--] = no acid and no gas production

[+-] = acid production without gas production

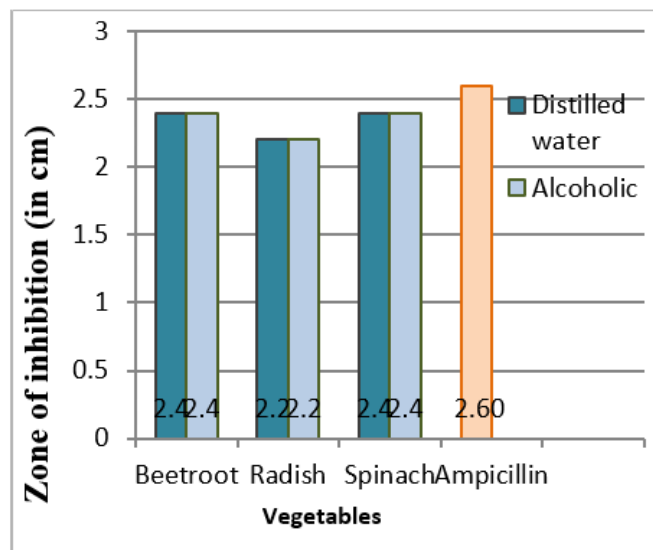
B. Antimicrobial activity

Agar well diffusion method was performed. 0.5µl of extract was poured in St. MH agar wells using micropipette. Standard antibiotics Ampicillin and Vancomycin (Conc 1mg/ml) were used as controls against *E.coli* and *S.aureus* respectively. Zone of inhibition for extracts was measured in centimeter (cm) to compare effective antimicrobial activity.



Graph 1:- Antimicrobial activity against *E.coli*

Against *E.coli*, maximum antimicrobial activity of 2.4cm zone of inhibition was shown by distilled water and alcoholic extracts of Beetroot & Spinach. (Graph1)



Graph 2:- Antimicrobial activity against *S. aureus*

Against *S.aureus*, maximum antimicrobial activity of 2.2cm zone of inhibition was shown by alcoholic extract of Beetroot & alcoholic and distilled water extract of Radish. (Graph 2)

C. Phytochemical tests

The Ethanol and Distilled water (aqueous) extracts of Radish, Spinach and Beetroot were screened for the presence of phytochemical constituents such as tannins, flavonoids, phenols, saponins and vitamin-c. (Table 2)

	Spinach		Radish		Beetroot	
	E	D/W	E	D/W	E	D/W
Tannins	+	+	-	-	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenol	+	+	+	+	+	+
Vitamin-C	+	+	+	+	+	+

Table 2:-Phytochemical tests

[+] = phytochemical present

[-] = phytochemical absent

D. Dyeing property of extracts

Sample pieces of cloth- cotton and Styrofoam were submerged in the ethanol and D/W extracts of radish,

beetroot and spinach for 24hrs. With beetroot extracts red coloration was washed away while spinach extracts retained green colouration. Green pigments of spinach showcased better retention than that of beetroot pigments. Whereas radish extracts showed no colouration on both sample cloth pieces. There was better pigment retention in ethanolic extract dipped cloth pieces than aqueous extract dipped cloth pieces.

IV. DISCUSSION

The above experimental study was carried out on a three vegetable extracts - spinach (*Spinacea oleracia*), beetroot (*Beta vulgaris*) & radish (*Raphanus raphanistrum*). Antimicrobial activity of extracts against two common infectious organisms– *E.coli* & *S.aureus* was studied. These organisms were isolated from sewage water sample & meat sample respectively & confirmed by biochemical tests analysis.

Extracts were prepared in two solvents – ethanol and distilled water. Both ethanolic and distilled water extracts showed moderate activity against both test organisms. But more effective antimicrobial activity was observed against *Escherichia coli* than *Staphylococcus aureus*.

Further these extract were subjected to various phytochemical analysis. From phytochemical analysis we can conclude that all these extracts were rich in tannins, flavonoids, saponins, phenols & vitamin c; except radish which showed no tannin activity. Presence of these phytochemicals thus also improve their nutritional & physiological importance in human diet along with their antimicrobial potential.

Extracts were also subjected for studying the dyeing property. In this study two fabric pieces- cotton & Styrofoam were submerged in the extracts. Only the alcoholic spinach extract show ability to be retained on the fabrics even after washing, whereas beetroot alcoholic extract was washed off, and radish showed no color. Only alcoholic extracts showed dyeing property. This activity can be enhanced by using mordents for better retention.

Thus study of all these properties of the extracts helped us to understand the underlying pharmacological potential of these extracts as antimicrobials, bio-preservatives. Also dyeing properties can be further enhanced and used at industrial scale for natural dye production.

V. CONCLUSION

It was found out that both alcoholic and aqueous extracts of Beetroot, Spinach and Radish show considerable antimicrobial activity against the test organisms. The aqueous and ethanolic extracts of vegetables exhibited larger zone of inhibition against *Escherichia coli* than *Staphylococcus aureus*. Thus ethanolic extracts have better antimicrobial potential compared to distilled water extracts.

Flavonoids and tannins are phenolic compounds, such plant phenolics are a major group of compounds detected in sample extracts that act as a free radical scavengers. The extracts also were positive for the presence of saponins and vitamin c content. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. Whereas vitamin C is required for growth, development and repair of body tissues and in the proper functioning of immune system.

Alcoholic extract of Spinach had the best retention of colour on cotton as well as styrofoam and so should be further tested for formulation of naturally derived dye. Beetroot extracts exhibited dyeing activity but did not retained on the fabric. Radish comprises of peroxidase and its activity can be exploited as an effective decolourizing agent.

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