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Extraction of Phytochemicals and Study of Their Antimicrobial and Antioxidant Activities of Leaves of Spilanthes Acmella L

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ABSTRACT

The pharmacological activities of any plant is because of the presence of primary metabolites, secondary metabolites. Spilanthes acmella could also be an important medicinal plant commonly mentioned as akarkara plant with rich source of therapeutic constituents. By chewing the leaves or flowers, it produces a numbing effect to the tongue and gums so it's called as toothache plant. In soxhlet extraction technique Extraction solvents was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. Gram positive bacteria Staphylococcus aureus NCIM 2079, Gram negative bacteria Escherichia coli NCIM 2109, Fungi (Yeast) Candida albicans NCIM 3471, Fungi (Mould) Aspergillus niger NCIM 545 are used as reference Strain for antimicrobial activity. Antioxidant activity was tested by DPPH method.

Extracts of leaves of Spilanthes acmella L. using Chloroform, ethyl acetate and methanol solvents exhibited potent antimicrobial activity against Escherichia coli, Staphylococcus aureus, Candida albicans and Aspergillus niger. The Chloroform extract showed highest antimicrobial activity against Escherichia coli with zone of inhibition of 25.24mm and 10.54 against Staphylococcus aureus. Potent activity against Aspergillus niger with zone of inhibition of 19.12 mm. Antioxidant activity was tested by DPPH method. The use of herbal crude drugs, in tracts and their remedies have significantly increased throughout the world. Phytochemical extraction by Soxhlet apparatus is very effective time saving and solvent saving Technique. The scientific and authentic researches can be done in order to exploit traditional knowledge of medicinal plants.

Keywords: Phytochemicals, Spilanthes acmella L., hot extraction, Soxhlet, Chloroform extract, antimicrobial, antioxidant activity, etc.

I. INTRODUCTION

Herbs have provided some of the important life savings drugs used in armamentarium of modern medicine. Among the estimated 4,00,000 plants species, only 6% have been studied for biological activity and about 15%

have been investigated phytochemically[1]. Although there is great advancement in medical science, plants are considered as important contributors in health care [2]. According to World Health Organization about 80% of population relies on traditional medicines for their primary health needs [3]. Plants have ability to synthesize secondary metabolites. These synthesized metabolites are aromatic substances, used by plants as defensive molecules against predation by microorganism, insects and other herbivores [4]. However, these defensive molecules give plants their medicinal value which is appreciated by human being.

According to WHO 11% of the basic and essential drugs are obtained from plant and number of synthetic drugs are also obtained from natural precursors. Phytochemicals possess antioxidants [5], antifungal [6], and antibacterial [7]. A huge number of plants are used as remedy against several ailments by tribal communities. Besides, the origin of most of the drugs available now a days, are from medicinal plants. Research on medicinal plants is increasing day by day due to high cost and possible side effects associated with the use of modern drugs. Treatment of ailments using medicinal plants is often cheaper and in almost all cases it lacks side effects[8]. It is very important to search for effective but of low cost and reliable traditional therapeutic agents, hence also the abuse of drugs for ailment is in high increase which motivated drug resistant organisms. This work is aimed at studying *Spilanthes acmella* L.

Introduction of *Spilanthes acmella* L.:

Spilanthes acmella L., an ayurvedic herb, is distributed widely in forest throughout India. *Spilanthes acmella* could also be an important medicinal plant commonly mentioned as akarkara plant with richsource of therapeutic constituents. By chewing the leaves or flowers, it produces a numbing effect to the toung and gums so it's called as toothache plant. Flower heads and roots are utilized in treatment of scabies, psoriasis, scurvy, and toothache, infection of gums and throats and paralysis of tongue [8, 9]. The plant has been used as anti-inflammatory and analgesic, anesthetic and antipyretic, bio-insecticides and as remedy for rheumatism, and infection of gums and as immunostimulant [10]. The flower heads are chewed to alleviate the toothache and other mouth related troubles. Leaves are
used externally in treatment of skin diseases. Root decoction is used as purgative.
Leaf decoction is used as diuretic and lithotriptic. Whole plant is used in treatment of dysentery [11].

Previous work on *Spilanthes acmella* L.:

Preliminanary studies have reported as diuretic17, antiinflammatory and analgesic18, vasorelaxant and antioxidant19. There shortly more data was found on its leaf and root phytochemical analysis [12]. In phytochemical investigations of the genus Spilanthes, Acmella ciliata, one of the closely related species to Spilanthes, was found to contain 20 amides [13].

II. METHODS AND MATERIAL

Sample Species Collection:

The sample Species were collected from Satpura ranges in Dhule Disrict. The collected sample species were observed and identified by taxonomist, as *Spilanthes acmella* L. Healthy plants were washed to remove clay, leaves were separated, and then separated dried leaves were crushed and ground to powder [22]. This powdered sample was used for next extraction.

Extraction:

Extraction is the basic step to separate the desired natural phytoconstituents from the plant material. The precise mode of extraction depends on texture and water content of plant material being extracted and type of substance which is to be isolated. Crude extract was taken from Soxhlet apparatus using Chloroform, ethyl acetate and water as a solvent [20]. These solvents show good solubility for maximum organic compounds, and due to low boiling point it can be easily recovered.

Soxhlet extraction or hot continuous extraction:

In this method, powdered sample was placed in thimble chamber of the Soxhlet apparatus. The extraction solvent Chloroform was heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reached the siphon arm, the liquid contents emptied into the bottom flask again and the process was continued [22].

About 200g of dried sample powder was weighed and extraction process is carried out by using 250 ml of Chloroform in Soxhlet apparatus for 48 hours. The extract was concentrated by evaporation at 80^0C for 8 hours and then dried. The concentrated extract was made in Gel form and stored at room temperature prior to phytochemical screening **[19, 20].**

Phytochemical screening

Preliminary Screening of secondary metabolites:

1. Saponin: 0.5g Extract was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth.

2. Steroids: 2ml of acetic acid added to 0.5g extract with the addition chloroform, heated, with the addition of 2ml H_2SO_4. The colour changes to orange indicates presence of steroids [16].

3. Flavanoids: 0.5g Extract was shaken with 1 ml of dilute ammonia solution. A yellow color indicates the presence of Flavanoids [19].

4. Tannins: 0.5g Exctract was boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride solution was added and brownish green or blue black colour indicated its presence [18].

5. Cardiac glycosides: 0.5g Extracts was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. Then 1ml of concentrated H_2SO_4 was added. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer, indicates the presence of cardiac glycosides [17].

6. Free Alkaloids: 0.5g extract was dissolved in 1.5 ml of 2% HCL and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract [19]

7. Alkaloids salts: The 0.5g aqueous extract of each organs of the plant (25ml) was stirred with 15ml of 10% HCl on a steam bath for 30 minutes. The mixture was extracted three times with dimethyl ether. 1 ml of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate was regarded as evidence for the presence of salts alkaloids in the extract [18].

8.Terpenoides: The presence of Terpenoides was determined as described for steroids except that red, pink or violet colour indicates the presence of Terpenoides.

9. Salkowskitest: 0.5g of extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of Terpenoides.

10. Anthracenosides: To 0.5g extract was added 15 ml of 10% HCl. The mixture was refluxed for 30 minutes. After cooling, the mixture is extracted three with 15 ml of diethyl extract. After evaporation of 8 ml of etheric layer, the residue was treated with 2 ml of hot water and some drops of 10% NH_4OH. Appearance of red orange colour revealed the presence of anthracenosides [17]

Material and method for antimicrobial study

Gram positive bacteria *Staphylococcus aureus* NCIM 2079, Gram negative bacteria *Escherichia coli* NCIM 2109, Fungi (Yeast) *Candida albicans* NCIM *3471*, Fungi (Mould) *Aspergillus niger* NCIM *545* are used as reference Strain.

Concentration used for anti microbial tests:

Stock solution [1000 microgram per ml] of each compound was prepared in Dimethylsulfoxide (100 % DMSO). Assay carried out by taking concentration 100 microgram per disk. Hi-media antibiotics disk: Chloramphenicol (10 microgram/disk, Amphotericin-B (100 units/disk) moistened with DMSO are used as standard [24,25].

Media used

Microbiological media used for bacteria (*Staphylococcus aureus, and Escherichia coli)* is **Nutrient agar** (Himedia)

Composition (gL-1): Sodium chloride, 5.0; Beef extract 10.0; Peptone 10.0 (pH 7.2)

Microbiological media for fungi (*Aspergillusniger*) is **Potato dextrose agar** (Hi-media)

Composition (gL-1): Potatoes infusion, 200.0; Dextrose 20.0 (pH 5.2)

Microbiological media for yeast (*Candida albicans*) is **MGYP** (all ingredients of Hi media)

Composition (gL-1): Malt extract, 3.0; Glucose, 10.0; Yeast extract, 3.0; Peptone, 5.0 (pH6.4) [24, 25]

Antioxidant activity:

DPPH radical scavenging assay

DPPH radical scavenging activity was done using the reaction mixture containing 1 ml of DPPH solution (2.0 mm /L, in 95% methanol v/v) with 3 ml extract was shaken and incubated for 30 min at room temperature in dark. After the incubation absorbance was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH in triplicates and calculated using the following equation [25, 27]:

$$Effect\ of scavanging(\%) = \left[\frac{1 - A\ sample}{Acontrol}\right] \times 100$$

Effect of scavenging(%) =[(1-A sample)/Acontrol] ×100

III. RESULTS AND DISCUSSION

The results Phytochemical Screening are mentioned in table no. 1

Table1: Phytochemical Screening of extracts of Leaves of *Spilanthes acmella* L. [4].

Test	Chloroform extract	Ethyl acatate extract	Methanol extract
Alkaloid	+	+	+

Steroid	-	-	+
Terpenoid	-	+	+
Flavanoids	+	+	+
Polyphenols	-	-	-
Tannins	-	-	-
Cardiac glycosides	-	+	-
Saponins	-	+	+

+ = Presence, - = absence

The results obtained for antimicrobial activity are presented in table no. 2 and figure no. 1 as below.

Table 2: An antimicrobial activity (diameter in mm) of Chloroform extract of parts of *Spilanthes acmella* L. [19].

Extract in different solvents	Bacteria		Fungi	
	E. coli	S. aureus	C. albicans	A. niger
Chloroform	25.24	10.54	-	-
Ethyl acatate	11.24	-	11.24	-
Methanol	13.00	-	-	19.12
C	30.32	29.82	-	-
A	-	-	18.55	18.13

E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, C. albicans = Candida albicans, A. niger = Aspergillus niger, C = Chloramphenicol (Standard antibacterial), A = Amphotericin B (Standard antifungal)

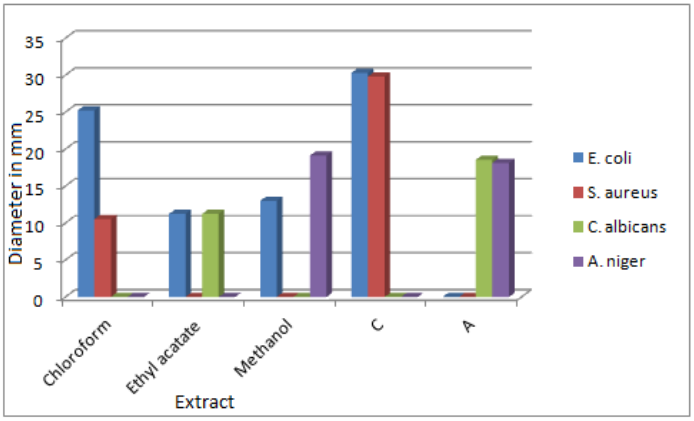

Fig. no. 1 An antimicrobial activity (diameter in mm) of Leaves extracts of *Spilanthes acmella* L. [24].

The results of antioxidant study were obtained in terms of percent scavenging activity. The percent scavenging activity for each extract is shown in table no. 3 and figure no. 2 as below.

Table 3: %Antioxidant activity of leaves extracts by DPPH method [26, 27].

Extract	Concentration		
	200µg/ml	400µg/ml	600µg/ml
Chloroform	30.09%	43.96%	67.82%
Ethyl acetate	80.02%	91.81%	97.08%
Methanol	100.13%	100.55%	101.24%
Ascorbic acid	81.27%	83.49%	96.67%
Control	00	00	00

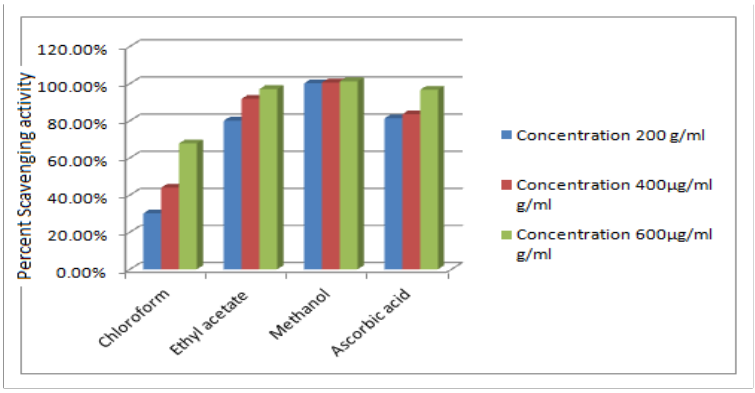

Fig no. 2 %Antioxidant activity of leaves extracts by DPPH method [28].

Discussion:

In our study phytochemical screening of leaves of *Spilanthes acmella* L. using chloroform, ethyl acetate and methanol as a solvent was carried out. Results obtained as shown in Table no. 1. Secondary metabolites of plant are mainly responsible for different pharmaco-logical properties and their therapeutic benefits [29]. Extracts of leaves in different solvents were tested against the pathogenic bacteria and fungi. Antioxidants have inhibitory capacity to oxidative stress induce cellu-lar damage and their main mechanism underlying this property is to scavenge free radicals due to their redox capacity [30,31]. In present work, we observed that the investigated leaves extracts of *Spilanthes acmella* L. possess good DPPH free radical scavenging potential. Chloroform extracts shows presence of alkaloids and flavanoids. Observed results of microbial activity as inhibitory zone formation are shown in table no. 2 and also clarified by Figure no. 1. The results obtained in antioxidant activity as % scavenging activity as shown in table no. 3 and clarified in figure no. 2. Alkaloids and flavanoids present in choloform extract shows activity against bacteria *Escherichia coli* with forming diameter of 25.24 mm and *Staphylococcus aureus* with forming diameter of 10.54mm. Alkaloids and flavanoids present in Choloform extract shows 30.09%, 43.96%, and 67.82% scavenging activity at 200 µg/ml, 400 µg/ml and 600 µg/ml. Ethyl acetate extract shows presence of alkaloids, terpenoides, flavanoid, cardiac glycosides and saponins shows activity against bacteria *Escherichia coli* with forming diameter of 11.24 mm and fungi *Candida albicans* with forming diameter of 11.24 mm. These secondary metabolites shown 80.02%, 91.81%, and 97.08% scavenging activity at200 µg/ml, 400 µg/ml and 600 µg/ml respectively. Methanol extract shows presence of alkaloids, steroids, terpenoides, flavanoid, and saponins. Alkaloids and other secondary metabolites present in leaves extract shows activity against bacteria *Escherichia coli* with forming diameter of 13.00mm and *Aspergillus niger* with forming diameter of 19.12mm. This is more potent antifungal activitis than standard (Amphotericin B). These secondary metabolites show 100.13%, 100.55%, and 101.24% scavenging activity. These free radical scavanging activities are more potent than standard used. The leaves extracts of different solvents can be considered as good source of anti bacterial, antifungal and anti oxidant agent due to presence of alkaloids, terpenoides, steroids, cardiac glycoside and saponins. However further studies are suggested to isolate the individual components using chromatographic techniques and to study different antifungal and anti cancer activities[32].

IV. CONCLUSION

Phytochemical extraction by Soxhlet apparatus is very effective time saving and solvent saving Technique. Specific phytochemical can be extracted using solvents with varying polarities. With this work I want to

conclude that plants have lots of useful phytoconstituents that can be used as medicine[32]. Leaves extracts shows activity against bacteria and fungi *Escherichia coli*, *Staphylococcus aureus* and fungi *Candida albicans* and *Aspergillus niger*, it also shows scavenging activity at three concentrations 200 µg/ml, 400 µg/ml and 600 µg/ml. Further purification and characterization will definitely give the more efficient antimicrobial and antioxidant drug for mankind [32].

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