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Phytochemical and antimicrobial studies on the leaves of *Spilanthes acmella*

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ABSTRACT

Modern pharmacopoeia still contain at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. The work is being pursued on the phytochemical, antimicrobial and antioxidant investigation on the leaves of Spilanthes acmella. Successive extraction of the crushed leaves of the plants has been done with various solvents and these extracts were tested quantitatively. These extracts were further tested for their antimicrobial activity against the bacterial strains of Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Micrococcus luteus, Klebsiella pneumonaie and against the fungal strains of Aspergillus niger, Penicillum chrysogenum, Rhizopus arrhigus, Rhizopus stolonifer. The results have shown that the ethyl acetate and methanol extracts can act as a standard drug against the becterial strain of Klebsiella pneumonaie and the water and ethyl acetate extract also showed very good activity against the fungal strains of Rhizopus stolonifer and Rhizopus arrhigus.

Keywords : *Spilanthes acmella,* Antibecterial activities, Antifungal activities.

INTRODUCTION

Medicinal plants sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India. System of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative application. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs [1]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of

action contrary to synthetic drugs [2]. Demand for medicinal plant is increasing in both developing and developed countries due to growing recognition of natural product, being non-narcotic, having no side-effects, easily available at affordable prices and sometimes the only source of health care available to the poor. Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs [3]. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. *Spilanthes acmella* is an important medicinal plant commonly known as akarkara plant [4] with rich source of therapeutic constituents. It is called the toothache plant because by chew the leaves or flowers, it produces a numbing effect to the toung and gums [5]. In ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, toothache, infection of gums and throats, paralysis of tongue and remedy for stammering in children. The leaves of the plants are reported to contain come important phytoconstituents such as alkamides (Spilanthol), which is responsible for the trigeminal and saliva inducing effects of products [6], isobutylamide derivatives, α- and β-amyrin esters, sigmasterol, triterpenoidal saponins, amino acids and alkaloids [7-9].The plant has been well documented for its uses as anti-inflammatory and analgesic [10], anesthetic and antipyretic [11], bio-insecticides [12] and as remedy for rheumatism [13,14], infection of gums[15] and as immiunostimulant [16]. In continuation of this the study is carried out the phytochemical and antimicrobial studies of *Spilanthes acmella*.

EXPERIMENTAL SECTION

Experimental work was carried out on the leaves of *Spilanthes acmella* under the two heads: Phytochemical investigation of extracts and Antimicrobial activity of leaves extracts.

1. Phytochemical investigation of extracts

1.1 Collection of plant material

The plant leaves was collected from outside the Institute campus Dolphin (PG)Institute of Biomedical and Natural Sciences, (DIBNS) Manduwala, Dehradun during the month of Oct. to Dec. Collected plant material was authenticated by Dr. S. Biswas, Head, Botany Division, Forest Research Institute, Dehradun and Dr. Rakesh Kumar Head, Department of Biosciences, DIBNS. The collected plant material was washed with water to remove mud and other undesirable material and dried under shade.

1.2 Preparation of extracts

Washed leaves of *Spilanthes acmella* were crushed and extracted with methanol by cold percolation method [17]. From total methanol extract, we prepared different extract by separation technique using non-polar to polar solvent viz. petroleum ether, chloroform, ethyl acetate and water.

1.3 Qualitative phytochemical test

The different extract of leaves of *Spilanthes acmella* were tested for various components by their specific tests viz. Mayer's test, Dragendroff's test, Wagner's test for alkaloids; Raymond's test, Legal's test, Bromine water test for glycosides; Gelatin test, Ferric chloride test, Vanillin hydrochloride test for tannins & phenolic compounds; Shinoda test (Magnesium hydrochloride

reduction test), Zinc hydrochloric reduction test, Alkaline test for flavonoids; Million test, Ninhydrin test, Xanthoproteic test for proteins and amino acids; Salkowski test, Sulfur powder test for sterols and triterpenoids; Molisch's test, Benedict's test, Barfoed's test, Bromine water test for carbohydrates; Spot test, Saponification test for fats and fixed oils and Foam test for saponins.

2. Antimicrobial activity of extracts

The antimicrobial activity of the leaves extracts of *Spilanthes acmella* were carried out. The leaves extracts were screened for antibacterial and antifungal activities.

2.1 Antibacterial activity of leaves extracts

The bacterial cultures used in the study were *Escherichia coli, Klebsiella pneumonaie, Pseudomonas aeruginosa, Bacillus cereus, Micrococcus luteus.* These bacterias were provided by Department of Microbiology, Dolphin Institute of Biomedical and Natural Sciences, Manduwala, Dehradun and checked for purity by convention biochemical methods. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture were obtained by transferring a loop full of culture into nutrient broth and then incubated at 37ºC overnight. To test antibacterial activity, the well diffusion method was used.

2.1 (i) Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Nutrient Broth (NB). They were prepared and sterilized at 121ºC at 15 psi for 15-30 minutes in autoclave.

(ii) Plate preparation

25ml of pre autoclaved Mueller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri plates. These petri plates were allowed to solidify at room temperature.

(iii) Well diffusion method

After the plates solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spreaded over the Mueller-Hinton agar (MHA) media using L shaped sterilized glass spreader separately under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 8 mm diameter. In these well, about 0.1 ml of each leaves extracts individually was loaded. This method depends upon the diffusion of leaves extracts from hole through the solidified agar layer of petri dish to such an extent that the growth of added micro organism is prevented entirely in a circular area or zone around the hole containing leaf extract. Petri plates were incubated for 24 hrs at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well or holes were measured in mm and compared with standard drug.

2.2 Antifungal activity of leaves extracts

In this study, the antifungal activity was studied against the microorganism viz. *Aspergillus niger, Rhizopus stolonifer, Rhizopus arrhigus and Penicillium chrysogenum.* These cultures were obtained from the standard cultures maintained in the Microbiology Department of DIBNS,

Dehradun. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25ºC for about 72-96 hours and then stored at 4ºC as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cultures into sabouraud dextrose broth and then incubated at 25ºC for 72 hrs. To test antifungal activity, the well diffusion method was used. Here culture media preparation in sabouraud dextrose agar (SDA) and incubation period is 72 hours at 25ºC rest the method is same as that of antibacterial activity.

RESULTS AND DISCUSSION

The various extracts of the leaves of *Spilanthes acmella* were prepared and studied for phytochemical investigation and antimicrobial activity.

3. Phytochemical investigation of extracts

In petroleum ether extract yield was highest while in chloroform we got the least yield. The extracts of the leaves of *Spilanthes acmella* undergoes various qualitative chemical test. They showed their presence and absence in the different solvent systems. We find out that methanol extract and ethyl acetate were contains alkaloids, Glycosides, Tannins and phenolic compounds, Flavonoids and carbohydrates. Next is petroleum extract that contains Tannins and phenolic compounds, Flavonoids, Protein and amino acids, Triterpenoids and sterols and fats and fixed oil. Again the water extract that contains alkaloids, glycosides, Tannins and phenolic compounds, flavonoids and carbohydrates.

4. Antimicrobial activity of extracts

The leaves extracts (Methanol, petroleum ether, chloroform, ethyl acetate and water) were screened for antibacterial and antifungal activities.

Table 1. Antibacterial activity of different extracts of *Spilanthes acmella* and standard drug doxycycline

S. No.	Test organism	Inhibition zone in mm				
		Methanol	**Pet. ether**	**Ethyl acetate**	**Water**	**Standard drug doxycycline**
1.	*Escherichia coli*	14	18	30	-	38
2.	*Bacillus cereus*	-	12	-	-	24
3.	*Pseudomonas aeruginosa*	-	15	-	20	25
4.	*Micrococcus luteus*	10	10	18	-	-
5.	*Klebsiella pneumonaie*	**15**	-	**30**	-	15

Key: 1. (-)=Absent 2. Pet. Ether= Petroleum ether

4.1 Antibacterial activity

The antibacterial activity of different extracts of *Spilanthes acmella* and standard drug Doxycycline were tested for different strains of bacteria and zone of inhibition was recorded in millimeter. In table 1 antibacterial activities of different extracts against tested microorganism are shown. With *Escherichia coli*. Methanol and ethyl acetate showed weak activity but ethyl acetate showed comparable activity with standard drug. In case of *Bacillus cereus* none of the extract showed very good activity but against *Pseudomonas aeruginosa* water extract showed very good activity. In case of *Micrococcus luteus* standard drug doxycycline was inactive but

methanol, petroleum ether and ethyl acetate extract showed some activities. Lastly with *Klebsiella pneumoniae* methanol extract showed comparable with standard drug and ethyl acetate showed excellent activity as it showed more inhibition zone as compared to doxycycline.

4.2 Antifungal activity

Antifungal activity of different extracts of *Spilanthes acmella* and standard drug Fluconazole were tested for different strains of fungus and recorded the zone of inhibition in millimeters (Table-2). For *Aspergillus niger* only ethyl acetate extract showed weak activity and for *Penicillum chrysogenum* the methanol, petroleum ether and ethyl acetate showed some activity. In case of *Rhizopus arrhigus*, the methanol and petroleum extract showed weak activity but ethyl acetate extract showed more inhibition zone as compared to standard drug. At last, with *Rhizopus stolonifer*, the methanol extract showed weak activity but water extract showed excellent activity having more inhibition zone than the standard drug fluconazole.

Table-1 Antifungal activity of different extracts of *Spilanthes acmella* and standard drug Fluconazole

S. No.	Test organism	Inhibition zone in mm				
		Metha-nol	Pet. ether	Ethyl acetate	Water	Standard drug fluconazole
1.	*Aspergillus niger*	-	-	16	-	25
2.	*Penicillum chrysogenum*	12	15	14	-	32
3.	*Rhizopus arrhigus*	14	10	**23**	-	22
4.	*Rhizopus stolonifer*	15	-	-	**25**	23

Key: 1. (-)=Absent 2. Pet. Ether= Petroleum ether

CONCLUSION

All the extracts of *Spilanthes acmella* are rich in various phytoconstituents. The methanol and ethyl acetate extract can act as standard drug against bacterial strain *Klebsiella pneumonaie* as it showed more inhibition zone than the standard drug Doxycycline. In fungal strains the ethyl acetate and water extract can act as standard drugs against *Rhizopus arrhigus* and *Rhizopus stolonifer* respectively as it showed more inhibition zone than standard drug Fluconazole.

The presence of phytoconstituents in methanol and ethyl acetate extract such as flavanoids, tannins and other phytoconstituents are known to anti-microbial agent. The difference in susceptibility of various test bacteria towards the extracts as observed in the study could be due to the nature of the antimicrobial agents present in the extracts and their mode of action on the different test bacteria[18].

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REFERENCES

[1] F Jahan, R Lawrence, V Kumar, M. Junaid. *J. Chem. Pharm. Res.*, **2011**, 3(4), 777-789.
[2] B Kiran, V Lalitha, KA Raveesha. *J. Chem. Pharm. Res.*, **2011**, 3(4), 542-550.
[3] S Saboo, R Tapadiya, Is.S Khadabadi, UA Deokate. *J. Chem. Pharm. Res.*, **2010**, 2(3), 417-423.
[4] RN Chopra, SL Nayar, IC Chopra. Spilanthes: Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi, **1956**.
[5] RK Jansen. *Systematic Botany Monographs*, **1985**, 8, 1-115.
[6] RS Ramsewak, AJ Erickson, MG Nair. *Phytochemistry*, **1999**, 51(6), 729-732.
[7] WD Ratanasooriya, KPP Pires. *Pharmaceutical biology*, **2005**, 43(7), 614-619
[8] VG Gokhale, BV Bhide. *Journal of Indian Chemical Society*, **1945**, 22, 250-252.
[9] P Supaluk, AW Saowapasuphapong, L Ratana, R Smosak, P Virapong. *Molecule*, **2009**, 14, 850-867
[10] A Chakraborty, RKB Devi, S Rita, KH Sharatchandra, ThI Singh. *Indian Journal of Pharmacology*, **2004,** 36(3), 148-150.
[11] A Chakraborty, BRK Devi, R Sanjebam, S Khumbong, IS Thokchom. *Indian Journal of Pharmacology*, **2010,** 42(5), 277–279.
[12] AB Haw, LK Chan. *Journal of Applied horticulture*, **2003**, 5(2), 65-68.
[13] SC Sinha. 1st Ed., Manipur Association for Science and Society (MASS), Imphal, **1996**.
[14] KR Kirtikar, BD Basu. 2nd Ed. Indian Medicinal plants International Book distributors, Dehradun, **1987**.
[15] WD Ratnasooriya, KPP Pieris, U Samaratunga. *Journal of Ethnopharmacology*, **2004,** 91(2-3): 317-320
[16] RV Savadi, R Yadav, N Yadav. *Indian Journal of Natural Products and Resources*, **2010,** 1(2), 204-207.
[17] SS Agarwal, M Paridhavi, 1st Ed, Herbal Drug Technology, University Press Pvt. Ltd. **2009,** 323-324.
[18] SMD Rizvi, M Zeeshan, S Khan, D Biswas, OA Al-Sagair, JM Arif. *J. Chem. Pharm. Res.*, **2011,** 3(2):80-87.