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Antimicrobial Activity of *Spilanthes acmella* and Its Chemical Composition

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Abstract: In present study an attempt has been made to study the antimicrobial activity and chemical composition of leaves and flowers of *Spilanthes acmella***.** The leaves and flowers of the plant were collected and subjected for study of their chemical composites and antimicrobial activity of their extracts. The samples were given the code SALS1, SALS2 and SALS3 and SAFS1, SAFS2 and SAFS3 respectively for the extracts of leaves and flowers. The crude extracts of leaves and flowers were prepared by using various solvents like Petroleum ether, Ethanol and double distilled water. All the extracts were used to study their antimicrobial activity against gram positive bacteria eg. *Bacillus subtilis*, gram negative bacteria eg. *E. coli* and *K. Pneumonia* and anti-fungal activity against *Aspergillus Niger*. The chemical composition of leaves and flowers like Dry matter, Total ash, Ether extract, Crude fibre, nitrogen, Crude protein, Total carbohydrates, Nitrogen free extract and Organic matter were studied. The results of antimicrobial activity revealed that all the crude extract samples of leaves were found to possess antibacterial property. Maximum inhibition was shown by all the three samples against *E. Coli* and *K. Pneumonia*. However the maximum inhibition of growth of *E. Coli* and *Bacillus subtilis* was shown by SAFS1 and SAFS2 respectively. All the crude extracts of samples shown antifungal activity against *Aspergillus Niger*. The samples of leaves were found to be more effective than flowers against the fungal strains of *Aspergillus Niger*. In present study it has been concluded that the leaves may be used against the infectious diseases caused by *E. Coli, K. Pneumonia* and *A. Niger* as herbal medicine.
Keywords: *Spilanthes acmella*, Akarkara, leaves and flowers extracts. Chemical composition, antibacterial activity and antifungal activity.

INTRODUCTION

Infectious diseases have been a leading cause of morbidity, disability and mortality in the world. Their control is a constant challenge that faces health workers and public health officials in both industrialised and developing countries. Nature has been a source of medical treatment for thousands of years and today plant based system continue to play an important role in the primary health care of 80% of the world's population [1, 2]. The medicinal plants are important resources for all major systems of medicine/ healthcare, neutraceuticals and cosmetics.

Plants besides providing nutrition, have always formed an important source of chemical compounds, which can be used for medicinal purposes. Human knowledge of the medicinal value of plants date back probably for more than five thousand years [3]. It has been reported that about 64% of the total global population remains dependent on traditional medicine and medicinal plants for provision of their health care needs [4]. Traditional treatment may provide valuable clues for the development of new oral hypoglycaemic agents and simple dietary adjimets. One of important plant *Urtica dioica* has been used for centuries for food and medical purposes [5].

The use of traditional medicines is expanding to newer horizons and plants still remain as the novel source of structurally important compounds that lead to the development of drugs [6]. Plants are the invaluable incredible and traditional sources for the curability of various diseases in the form of medicines [7]. Secondary metabolites of plants have been implicating for therapeutic activities [8].

Plants and its products are safe and as a result there is continuous use of plant product as a drug is found to be an alternative way to cure the patients [9]. Natural products of higher plants may give a new source of anti microbial agents with possibly novel mechanism of action contrary to synthetic drugs [10]. Demand for medicinal plants is increasing in both developing and developed countries due to growing

recognition of natural products, being non narcotic, having no side effects, easily available at affordable prices [11].

Spilanthes acmella is an important medicinal plant commonly known as Akarkara plant with rich source of therapeutic constituents. It has conical small yellow flowers.

Fig-1: Mature flowers of *Spilanthes acmella*

The whole plant is claimed to possess medicinal properties [12]. Very little or no work on chemical composition has been reported in literature, keeping this in view the present study has been undertaken.

MATERIALS AND METHODS

In order to evaluate the nutritive value of any feeding stuff the quantitative estimation of the various chemical constituents of plant parts is essential prerequisite were performed. The sample of leaves and flowers of *Spilanthes acmella* were collected from Hamirpur of Himachal Pradesh, India and given code SAL and SAF respectively.

Table-1: Details of codes used for various solvent extracts of different parts of plant

Plant part	Codes	Solvent used
Spilanthes acmella Flowers	SAFS1	Petroleum ether
	SAFS2	Ethanol
	SAFS3	Double distilled water
Spilanthes acmella Leaves	SALS1	Petroleum ether
	SALS2	Ethanol
	SALS3	Double distilled water

The respective samples of leaves and flowers were separated from the other parts of the plant and were subjected for the analysis of following chemical parameters.

Dry Matter (DM)

A known weight of the samples was taken in a pre-weighed aluminium moisture cup and dried at 100± 5ºC in hot air oven for about 24 hours and was again weighed after cooling the sample in a desicator. The loss in weight was recorded as moisture content of the sample, dry matter was calculated on a percentage basis. The moisture content of the sample was determined in order that the analytical results of various constituents studied could be evaluated on uniform DM or moisture free basis.

Total Ash (TA)

A known weight of the samples was taken in a pre-weighed silica crucible. It was desmaked on a heater and then ashed in a muffle furnace at 600 ºC for 24 hrs. After cooling the silica crucible in a desicator, the weight was recorded. The difference in initial and the final weight of the crucible gave the total ash content, which was expressed on a percentage basis.

Nitrogen Free Extract (NFE)

It comprises essentially the more soluble carbohydrates such as starch, sugars, the hemicelluloses and more soluble part of the cellulose and pentose. It was calculated by simply subtracting the sum of CP, EE, CF and TA from 100 and expressed as percentage.

Organic Matter (OM)

Organic matter was calculated by deducting TA and DM basis from 100 which represented the sum of CP, CF, EE and NFE of the sample.

Ether Extract (EE)

A known quantity of moisture free sample was extracted with petroleum ether for 8 hrs in soxhlet extractor fitted with weighed flasks for the collection of the ether extracts. After the excess of the solvent is recovered, the oil flasks containing the extractives were

dried in oven at 100± 5ºC for overnight to constant weight. The difference in weight of the oil flask before and after extraction gives the amount of ether extract and expressed as percentage on DM basis.

Crude Fibre (CF)

It was determined by digesting the moisture and fat free sample with 1.25% H_2SO_4 and subsequently with 1.25% NaOH for 30 minutes each and by filtering each time through muslin cloth through repeated washing with hot water to make it acid and alkali free respectively followed by drying in an electric oven at 45ºC. The weight loss during the ignition is the CF content of the sample.

Crude Protein (CP)

A known quantity of sample was digested in an adequate volume of the conc. H_2SO_4 in the presence of 10 gm digestion mixture and the digested material was made to a known volume. Suitable aliquot was used for distilling ammonia (NH_3) from the ammonium sulphate formed during digestion in presence of 40% sodium hydroxide (NaOH) in Tecator Kjeltec 1030 auto analyser. The liberated ammonical solution containing Tashiro's as indicator. The converted ammonium borate was titrated against 0.1N HCl.
1ml of 0.1N HCl = 0.0014gm N

The determination of the crude protein is based on the fact that protein on an average contains 16% nitrogen, the nitrogen content multiplied by 6.25, therefore was taken as the crude protein content.

Total Carbohydrates (TC)

Sum of percentage of crude fibre and NFE was expressed as total carbohydrates as percentage on DM basis.

TC% = CF% + NFE%

Assay Techniques

Various microbiological techniques are available to assay the antimicrobial activity of any compound eg. DISC Diffusion method, cup plate method, over lay assay techniques etc. Agar diffusion method (well diffusion method) or cylinder plate method was used for antibacterial activity as reported by [13, 14]. Wells were made in seeded agar and the test sample was then introduced directly in to these wells. After incubation, the diameter of the clear zones around each well was measured and compared against zone of inhibition of the known concentrations of the standard antibiotics.

The fresh plant materials collected were rinsed with water and kept under shade at room temperature for drying. After complete dryness, these plant parts were ground in to powder and were kept in sealed plastic bags dully labelled. Extraction of these plant parts were carried out one by one by simple maceration process [13, 14].

Sample Preparation

Petroleum ether, ethanol and double distilled water were used to extract bioactive products from leaves and flowers of *Spilanthes acmella*. The test bacterial isolates studied were *Bacillius subtilis* (MTCC-121), *Escherichia coli* (MTCC-1652) and *Klebsiella pneumonia* (MTCC-109), *Aspergillus Niger*. These cultures were maintained in culture collection centre of SBSPGI, Dehradun.

Test Culture Preparation

Test cultures were inoculated in nutrient broth (0.5gm NaCl; 0.5gm peptone; 0.3gm beef extract; 1000ml of water pH 7) and kept in incubator at 37 ºC for 24 hrs. The fungal strains were inoculated in fungal medium (0.4gm yeast extract; 1.5gm sucrose; 0.2gm $NaNO_3$; 0.001gm $FeSO_4.7H_2O$; 0.05gm K_2HPO_4; 0.05gm $MgSO_4$; 0.005gm KCl; 100ml distilled water; pH 5.0-5.5) kept in another incubator at 23 ºC for 48 hrs.

Cup Plate Method

Sample extracts were poured in to the wells drug in the petri-plates of solid agar medium and before filling the wells with sample supernatants the plates were swabbed with test organism cultures on the respective plates i.e. nutrient agar (Nutrient Broth with 1.5% agar) for bacterial test cultures and fungal agar medium (fungal medium with 1.5% agar for fungal culture *A. niger*).

RESULTS AND DISCUSSIONS

Spillanthes acmella, commonly known as toothache plant, is an important ornamental cum medicinal plant widely distributed to tropical and subtropical regions of the world.

Chemical Composition of *Spillanthes acmella*

Various phytochemical parameters viz. Dry matter, total ash, ether extract, crude fibre, nitrogen, crude protein, total carbohydrates, nitrogen free extract and organic matter were studied in the leaves and flowers sample of *Spillanthes acmella*.

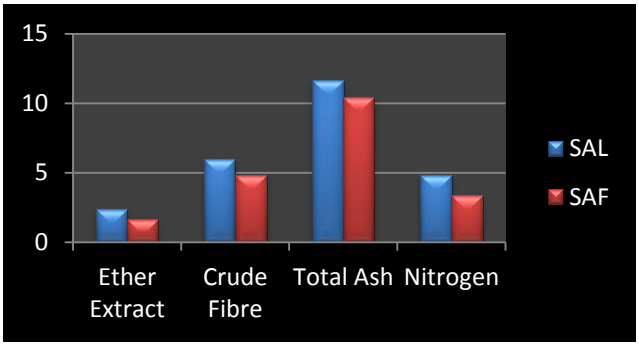
The observations of the present study reveal that the dry matter was 78.40% and 75.50% of flowers and leaves respectively. The total ash content in leaves was 11.64% whereas in flowers 10.39%. The ether extract or the presence of fat was 1.63% in flowers and 2.14% in leaves. The crude fibre was found to be 5.93% in leaves and 4.82% in flowers. The nitrogen was 4.78% in leaves and 3.37% in flowers. The crude protein was 29.87% in leaves and 21.06% in flowers. The observation of the present study also showed that the presence of total carbohydrates was 50.82% in leaves and 58.91% in flowers. The nitrogen free

extracts in flowers 62.10% and 50.12% in leaves. The organic matter was 88.36% in leaves and 89.61% in flowers (Table-2).

Table-2: Chemical Composition of leaves and flowers of *Spillanthes acmella*

Parameters	SAL	SAF
Ether Extract (Fat) % w/w	2.41%	1.63 %
Crude Fibre % w/w	5.93 %	4.82 %
Nitrogen % w/w	4.78 %	3.37 %
Crude Protein % w/w	29.87 %	21.06 %
Dry Matter (Moisture % w/w)	75.5 %	77.40 %
Total Ash % w/w	11.64 %	10.39 %
Total Carbohydrates % w/w	50.82 %	58.91 %
Organic Matter % w/w	88.36 %	89.61 %
Nitrogen Free Extract % w/w	50.12 %	62.10 %

The results of the present study are in accordance with [15] reported the proximate composition of the root crops of onion, garlic, ginger and carrot the crude protein, fat, total ash and total carbohydrates is 25.74±2.40%, 63.55±0.04%, 23.54±2.335% and 85.11±0.88%; 1.61±0.06%, 0.54±0.00%, 6.36±1.12%, 3.12±0.24%, 4.83±0.71%, 3.47±0.07%, 6.84±0.52%, 6.39±0.02; 67.81±2.37%, 32.43±0.03%, 63.26±2.67% and 5.66±1.48% respectively [16] reported that the water extract of turmeric powder (*Curcuma longa L.*) found to contain moisture content 4.37%, carbohydrates 51.29%, protein 10.65%, volatile oil content 4.54%, crude fibre 2.5%, ash value 5.26% respectively [17] reported the physio-chemical composition in different varieties of ginger i.e. phenol 5.69±0.06%, oleoresin 2.93±0.02%, lipids 0.7±0.00%, dry matter 23.6±3.03%, crude fibre 1.2±0.04%, ash 0.9±0.00% in sample UG117GY25. The results of the present study reveal that fat, crude fibre and crude protein 2.14%, 5.93% and 29.87% respectively higher than flowers.



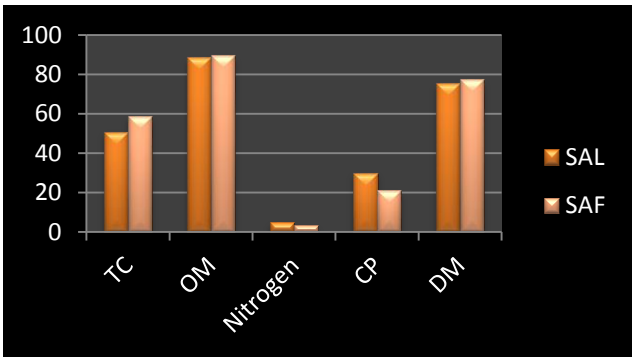


Fig-2 & 3: Comparison between proximate principles of leaves and flowers of *Spillanthes acmella*

However the carbohydrates, organic matter and nitrogen free extract 58.91%, 89.61% and 62.10% in flowers respectively were found to be higher than leaves 50.82%, 88.36% and 50.12% respectively (Fig. 2 & 3) [18] reported that crude fibre aids digestion, absorbs water and makes stool larger and softer, so preventing constipation.

Antimicrobial Activity

In-vitro antimicrobial activity of solvent extract against microbial cultures gram positive and gram negative bacteria and fungi were used. The test bacterial isolates studied were *Basicillus subtilis* (MTCC-121), *Escherichia coli* (MTCC-1652), and *Klebsiella pneumonia* (MTCC-109), *Aspergillus Niger*. The results depict in-vitro preliminary activity in terms of zones of inhibition in millimetre around the agar wells. The antibiotic Erythromycin (1-0mg/ml) was used as positive control while respective solvents were used as negative control in comparison to the plant extracts.

The sample SALS1 showed the 3mm, 15mm and 15mm inhibition zone against all the three bacterial strains. It was also higher in case of SALS3 that is 2mm, 14mm, 15mm in all the three bacterial strains (Fig-4). The inhibition zone was highest for *K. Pneumonia* that is 15mm in both the sample SALS1 and SALS3. However the sample SAFS2 showed 5mm, 12mm and 10mm zone of inhibition against all the three bacterial strains (Fig-5).

The growth of fungus *A. niger* was inhibited maximum 18mm by SALS1 then SALS3 and SALS2. The sample of flowers extracts SAFS1 showed growth inhibition of all the three bacterial cultures and the zone of inhibition were found of the 4mm i.e. in the *Bacillus subtilis*, it was less in gram negative bacteria i.e. 1mm. The sample SAFS3 showed growth inhibition around the agar wells was found to be 4mm and 3mm in both the gram negative bacteria in sample of SAFS1.

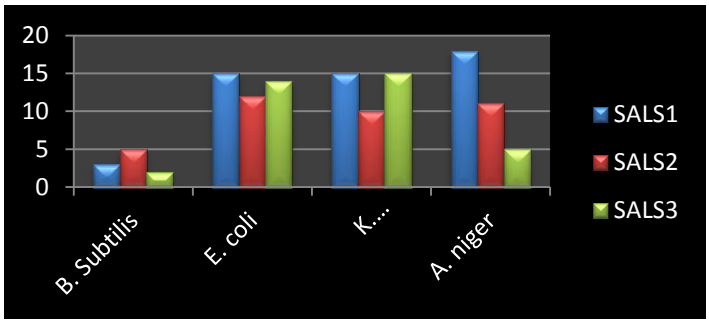


Fig-4: Comparison between antimicrobial activities of different solvent extracts of Leaves of *Spillanthes acmella.*

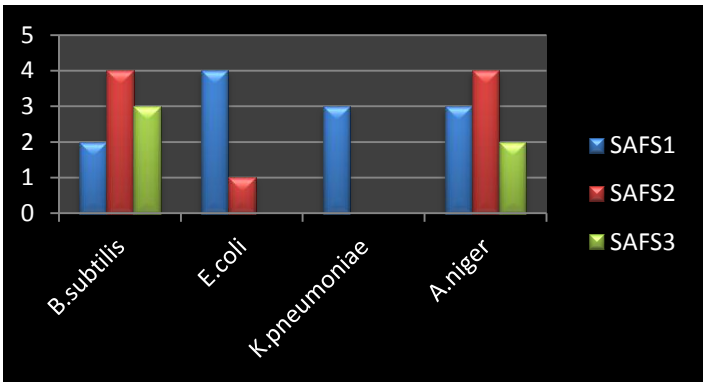


Fig-5: Comparison between antimicrobial activities of different solvent extracts of Flowers of *Spillanthes acmella*

The growth of fungus *A. niger* was inhibited maximum 18mm, 17mm and 11mm by SAFS1 followed by SAFS3 and SAFS2 respectively. Maximum growth of inhibition was shown in the sample SAFS1. Similarly various workers from different parts of the world reported antimicrobial activity of various plant extracts against various bacterial and fungal strains viz. [19] because of the outer phospholipids membrane with the structural lipopolysaccharide components, which prevent the antimicrobial agents to pass through the cell wall. [20, 21] reported that large numbers of phytochemicals belonging to several chemical classes have shown inhibitory effects on all types of microorganisms in –vitro and some plant extracts have shown activity on both gram positive and gram negative bacteria. [22] reported in-vitro antibacterial activity of some folklore medicinal plant used by people of India. All the extracts showed antibacterial activity against the gram positive strains *S. aureus, S. pyogenes, B. Cereus* and also the gram negative strains *K. Pneumonia, S. typhi, E. Coli, P. Aeruginosa* and *P. Mirabilis* causing serious infections in human beings and animals [23] reported that among the two extracts of *Aloe vera* such as ethyl acetate and ethanol exhibited maximum antibacterial activity against the gram positive and gram negative bacteria.

In the present study the observation reveals that the order of antimicrobial activity showed by different crude extracts of flowers was SAFS 2 >

SAFS3 > SAFS1 against *B. Subtilis* whereas no activity was shown by SAFS3 against *E. coli* and *K. Pneumonia*. However SAFS1 showed maximum antibacterial activity against *E. coli* and *K. pneumonia*. The order of antifungal of all the three crude extract of flowers was SAFS2>SAFS1>SAFS3 (Fig-6). The order of antimicrobial activity showed by different crude extract of leaves was SALS1>SALS2>SALS3 against *B. subtilis* whereas SALS1 showed maximum activity also against *K. pneumonia*.

However SALS2 show maximum activity against *E. coli*. In case of *K. Pneumonia* maximum activity was shown by SALS1, the order of activity by different crude extract of leaves SALS1>SALS3>SALS2. The order of antimicrobial activity showed against *E. coli* was SALS2>SALS1>SALS3. The order of antifungal activity of all the three crude extracts of leaves was SALS3>SALS1>SALS2 (Fig-7). The results of the present study are in consonance with studies reported earlier [24] reported that antimicrobial activities of the crude methanolic extracts of different plant parts of 13 selected medicinal plants against four gram positive, five gram negative bacterial strains and four fungal strains.

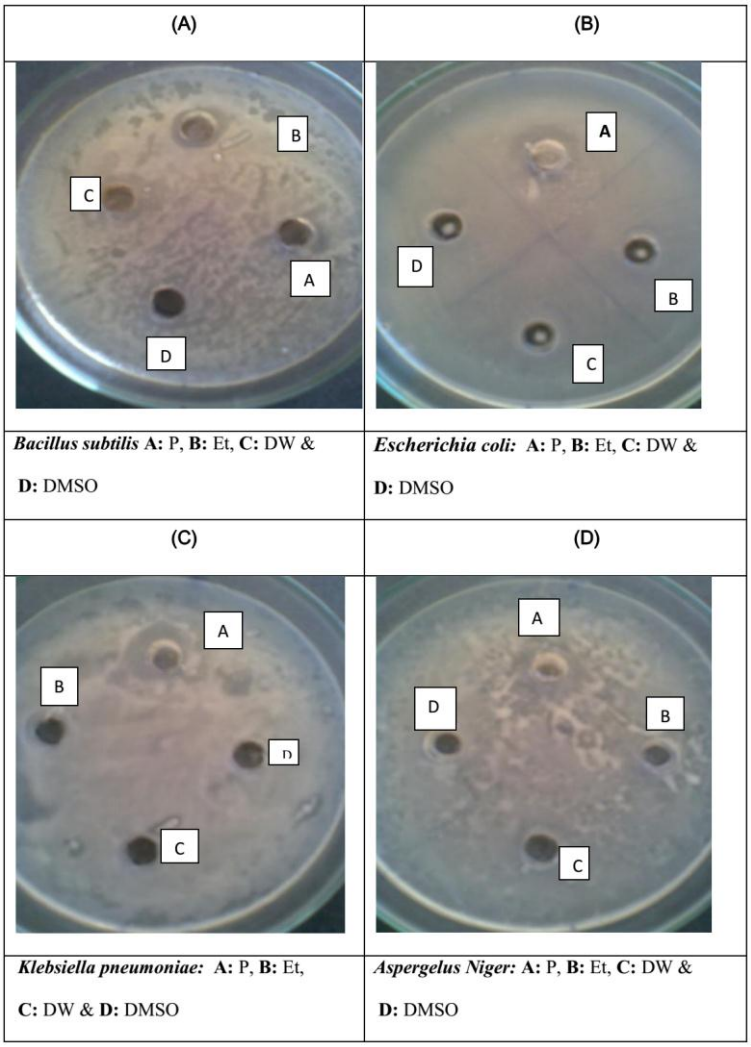


Fig-6: (A) Antimicrobial activity of *Spilanthes acmella* flower (Petroleum, Ethanol & DDW) against (A) *Bacillus subtilis (B) Escherichia coli (C) Klebsiella pneumoniae & (D) Aspergillus niger*

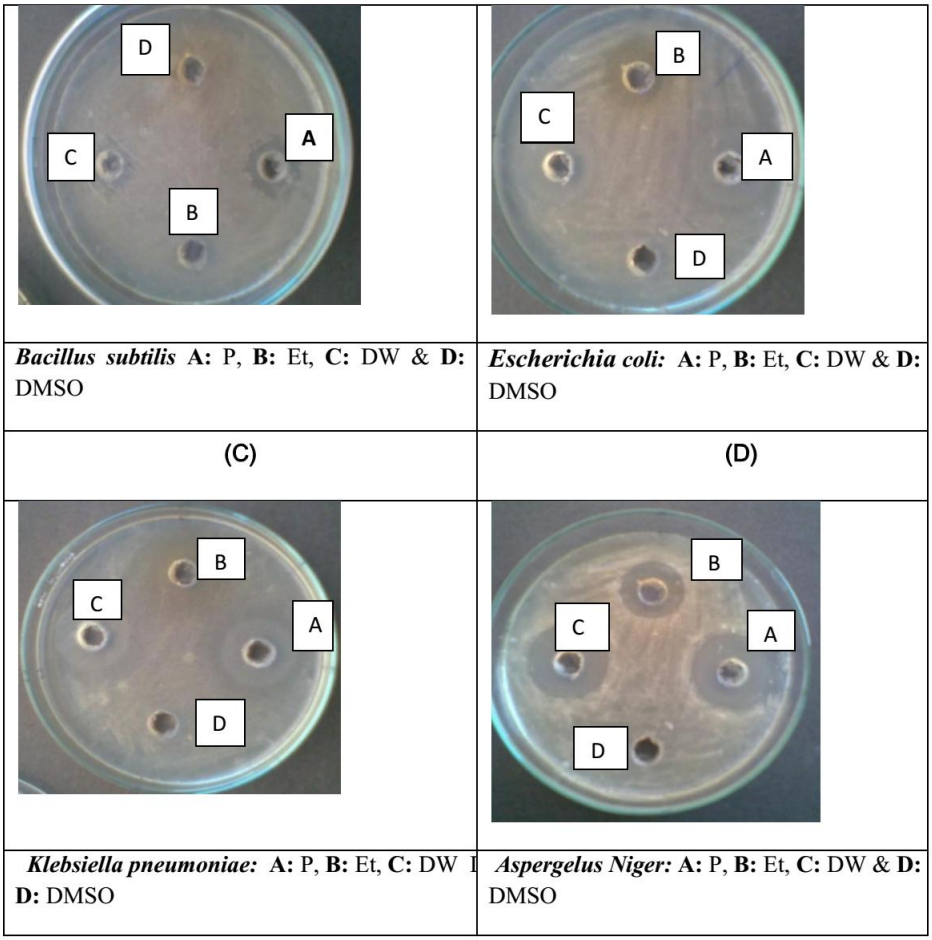


Fig-7: (A) Antimicrobial activity of *Spilanthes acmella* leaf (Petroleum, Ethanol & DDW) against (A) *Bacillus subtilis (B) Escherichia coli (C) Klebsiella pneumoniae & (D) Aspergillus*

The results showed *Woodfordia fruiticosa, Chenopodium ambrosoides, Viburnum cotinifolium, Euphorbia hirta, Vitex negundo* and *M. Rubicaulis* exhibited higher antibacterial activity against *E. coli*. Maximum antifungal activity against *A.niger* was exhibited by *C. Grata* followed by *E. hirta* and *V. Cotinifolium* respectively.

In present study *Spilanthes acmella* has shown antibacterial activity against gram positive bacteria i.e. *Bacillus subtilis* and gram negative bacteria *Escherichia coli* and *Klebsiella pneumonia* as well as it also showed antifungal activity against *Aspergillus Niger*.

CONCLUSION

The observations of present study indicate that flower parts of the plant may be used against bacterial infection caused by *K. Pneumonia,* and *E. coli* as well. The extract of plants may also be used against fungal diseases caused by the *Aspergillus Niger*.

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REFERENCES

1. Das, B., & Yadav, J. S. (1998). In "Role of biotechnology in medicinal and aromatic plants" IA Khan & A. Khanum. Ukaz publications, India 1, 13.
2. Duraipandiyan, V., Ayyanar, M., & Ignacimuhu, S. (2006). Antimicrobial activity of some ethnomedicinal plantsused by Paliyar tribe from Tamilnadu, India. *BMC Comp. Alter. Med*, *6*(1), 35-54.
3. Sofowara, A. (1982). Medicinal plants and traditional medicines in Africa. John Willey and Sons, New York: 256.
4. Cotton, C. M. (1996). Ethanobotany: Principle and application. John Willey and sons, New York: 339.
5. Mehri, A., Hasani, R. S., & Larijani, B. (2011). A systematic review of efficacy and safety of Urtica dioica in the treatment of diabetes.
6. Anilkumar, M. (2010). 10. Ethnomedicinal plants as anti-inflammatory and analgesic

agents. *Ethnomedicine: A source of complementary therapeutics*, 267-293.

7. Guerra, R. N. M., Pereira, H. A., Silveira, L. M. S., & Olea, R. S. G. (2003). Immunomodulatory properties of Alternanthera tenella Colla aqueous extracts in mice. *Brazilian Journal of Medical and Biological Research*, *36*(9), 1215-1219.
8. Timothy, O., Idu, M., Falodun, A., & Oronsaye, F. E. (2008). Preliminary Phytochemistry and Antimicrobial Screening of Methanol Extract of Baissea axillaris Hau. Leaf. *J Biol Sci*, *8*, 239-241.
9. Archana, S., Jatawa, R. Paul., Tiwari, A. (2011). Indian medicinal plants: A rich source of natural immunomodulator. *Intnl. J. Pharmacol, 7,* 198-205.
10. Kiran, B., Lalitha, V., & Raveesha, K. A. (2011). In vitro evaluation of antifungal activity of *Psoralea corylifolia* L. (seeds) and its different fractions on seed borne fungi of maize. *Journal of Chemical and Pharmaceutical Research*, *3*(4), 542-550.
11. Arora, S., Vijay, S., & Kumar, D. (2011). Phytochemical and antimicrobial studies on the leaves of Spilanthes acmella. *J. Chem. Pharm. Res*, *3*(5), 145-150.
12. Sinha, S. C. (1996). *Spillanthes acmella* medicinal plants of Manipur. Imphal: Manipur. *Association of Sci. And Soc. (MASS), 1,* 196.
13. Kavanagh, F. (1963). Analytical Microbiology. Academic Press, London 125-141.
14. Leven, M., Vanner Berghe, D. A., Mertens, F. (1979). Medicinal plants and its importance in antimicrobial activity. *J. Planta. Med, 36,* 311-321.
15. Odebunmi, E. O., Ogunsakin, E. A., & Ilukhor, P. E. P. (2002). Characterization of CRUDE OILS and petroleum products:(i) elution liquid chromatographic separation and gas chromatographic analysis of crude oils and petroleum products. *Bulletin of the Chemical Society of Ethiopia*, *16*(2), 115-132.
16. Surwase, V. S., Laddha, K. S., Kale, R. V., Hashmi, S. I., & Lokhande, S. M. (2011). Extraction and isolation of turmerone from turmeric. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *10*(5), 2173-2179.
17. Eleazu, C. O., & Eleazu, K. C. (2012). Physico-chemical properties and antioxidant potentials of 6 new varieties of ginger (Zingiber officinale). *Am. J. Food Technol*, *7*(4), 214-221.
18. Ayoola, P. B., Adeyeye, A. (2009). Proximate analysis and nutrient evaluation of some Nigerian pawpaw seeds varieties. *Sci. Focus, 14,* 554-558.
19. Kambezi, L., Afolyan, A. J. (2008). Extracts from *Alloe ferox* and *Withania sominifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. Afr. J. *Biotechnol, 7*(1), 012-015.
20. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev, 12*(4), 564-582.
21. Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian journal of microbiology*, *31*(4), 247-256.
22. Khan, A. B., Khan, A. A. (2008). Ethenomedicinal use of *Eclipta prostrates Linn. Ind. J. Tradi. Know, 7*(2), 316-320.
23. Thiruppathi, S., Ramasubramanian, V., Sivakumar, T., & Thirumalaiarasu, V. (2010). Antimicrobial activity of Aloe vera (L.) Burm. f. against pathogenic microorganisms. *J Biosci Res*, *1*(4), 251-258.
24. Khan, A. M., Qureshi, R. A., Gilani, S. A., & Ullah, F. (2011). Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad, Pakistan. *Journal of Medicinal Plants Research*, *5*(18), 4665-4670.