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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SOME MEDICINALLY IMPORTANT PLANTS

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ABSTRACT

The ethanolic extracts of different plant parts of *Alangium salviifolium* Linn, *Andrographis paniculata* Nees and *Spilanthus acemella* Murr showed significant antibacterial activity against *Escherichia coli*, *Pseudomonas aureginosa, Staphylococcus aureus* and antifungal activity against *Aspergillus flavus A. niger, A. fumigatus* and *Fusarium oxysporium*. *Alangium salviifolium* root extract and *S. acemella* leaf extract showed highest antibacterial activity against *E. coli* and *Staphylococcus aureus.* Root and stem extracts of *Alangium salviifoilium* recorded significant activity against all the test bacteria and fungi.

KEY WORDS : Antibacterial, *Alangium salviifolium*, activity index, bioactive compounds.

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INTRODUCTION

The people of India have a very long-standing tradition in the use of natural medicines and the local practices are still quite common in the treatment of diseases[1-2]. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[3]. The assessment of plants used in conventional medicines is anticipated to make available new antimicrobial agents [4-5]. Plants contain numerous biologically active compounds, many of which shown to have antimicrobial activities[6].

Plant derived medicines have been a part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases. Given the alarming incidence of antibiotic resistance of pathogenic microbes in particular, there is a constant need for discovering new and effective therapeutic agents[7-9].

Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[10-13].

In the present work *Alangium salviifolium* Linn, *Andrographis paniculata* Nees and *Spilanthus acemella* Murr plants were evaluated for their antibacterial and antifungal properties.

Alangium salviifolium belongs to Alangiaceae family and has various medicinal properties and used as laxative, astringent, pungent, purgative, alleviates spasms, anthelmintic, emetic, antiprotozoa, hypoglycemic.

Andrographis paniculata commonly known as “king of bitters” is used for its bitter tonic, stomachic, antipyretic and laxative properties in ayurveda. It is antifungal, antityphoid, hepatoprotective, antidiabetic and cholinergic.

Spilanthes acmella (L.) Murr. belongs to the family Asteraceae and is an important medicinal plant grown in tropics and subtropics. It has been well documented for its uses as antibacterial, antifungal, and antimalarial activity. Traditionally, plant is also used in treatment of toothache, flue, cough, and tuberculosis.

MATERIAL AND METHODOLOGY

Collection of plant material

Plant material collected from pot cultivated plants in Rajasthan University campus. Plant material was authenticated by Herbarium, Department of Botany, Rajasthan University, Jaipur, Rajasthan, India. Colleted plant parts (Root, stem and leaf) shade dried and grinded with pestle mortar.

Test organisms

Pure cultures of test bacteria, namely *Echerichia coli, Klebsella pnemoniea, Staphylococcus aureus, Psedumonas aeruginosa, Salmonella typhirium* were obtained through the courtesy of SMS medical college, Jaipur, India, while the test fungi, namely *Aspergillus flavus, A. niger, A. fumigatus, Fusarium oxysporum, Rizopus sps.* and *Penicillum sp.* were obtained from the Seed Pathology Laboratory, Department of Botany, University of Rajasthan, Jaipur, India which are maintained on Nutrient Broth and Potato Dextrose Agar respectively.

Ethanolic extraction

Leaf, stem and root powder of *Alangium salviifolium* L.*, Andrographis paniculata* Nees and *Spilanthus acemella* Murr. were homogenized with 80% ethanol separately and left overnight. Later, each of these homogenates was filtered, the residue re-extracted (3x) for complete exhaustion, the extract(s) were pooled individually and dried in vaccuo[14].

Each of these extracts/ fractions was stored in a refrigerator until screened against the selected test organisms. However, their final concentration (1g/ml) in the respective solvents was prepared, before use.

Media and Cultivation

For bacteria, Nutrient broth medium was prepared from 8% Nutrient Broth (Hi media ltd) in distilled water and agar, and autoclave (15 lbs psi) for 25-30min. The agar test plates were prepared by pouring ~15 ml of this medium into Petri dishes

under aseptic conditions. The peptone saline solution was prepared (by mixing 3.56g of KH_2PO_4, 7.23g of Na_2HPO_4, 4.30g of NaCl and 1 g peptone in 1L of distilled water followed by autoclaving) and the bacterial cultures were maintained on this medium

by periodic sub culturing and incubation at 37°C for 48h.

For fungi, Potato Dextrose Agar (PDA), medium was prepared (by mixing 1000ml of potato infusion to 20g each of agar and glucose followed by autoclaving as usual) and the test fungi were incubated at 27°C for 72h and cultures were maintained on the same medium by periodic sub culturing.

To perform the antibacterial testing, a fresh suspension of test bacteria was prepared in saline solution from a freshly grown agar; while for antifungal testing a uniform spreading of the test fungi was made using sterile swab.

Antibacterial and Antifungal Assay

The antibacterial activity of different plant species was evaluated by agar disc diffusion method[15-16] The different organisms were pre-seeded separately using a sterile swab or spreading over previously sterilized culture medium plates and the zones of inhibition growth were observed around sterilized dried discs of Whatman No.1 paper (diameter 6mm) containing 4mg of plant extracts/ fraction or control (0.4ml of the respective solvent) or gentamycin (10mcg/ml) or mycostatin (100 units/ml) as reference compounds separately. Such treated discs were air dried at room temperature to remove any residual solvent (which might interfere in the determination). Before incubation, these plates were placed at low temperature for 1h to allow the maximum diffusion of the compounds from the tests discs into the agar plate. These were incubated at 37°C for 20-24h in case of bacteria and 48-72h for fungi, after which the zones of inhibition could be easily observed. Five replicates of each tests extracts/fractions were examined and the mean values are presented (Table 1-2). Activity index was calculated with reference to standard antibiotic drugs gentamycin for bacteria and mycostatin for fungi.

Statistical Analysis

Data were expressed as mean ± standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

RESULT

All the selected plant species have shown variable antibacterial activity ranging maximum (AI = 0.95) in root of *Alangium salviifolium* and minimum (AI = 0.61) in root of *Spilanthus acemella* against *E. coli*. In all the tested bacteria ethanolic extracts of *Alangium salviifolium* (root and leaves) has showed maximum activities when compared to plant parts of *Andrographis paniculata* Nees and *Spilanthus acemella* Murr.

The ethanolic extract of *Alangium salviifolium* (root) showed maximum activity against *A. flavus* (AI=0.91) and minimum against *Rizopus sps* (AI=0.81). In *S. acemella* the maximum activity was observed in root extract against *A. fumigatus and Rizopus sps* (AI=0.80) and lowest in leaf extract against *A. niger* (AI=0.60). *Andrographis paniculata* extracts has showed less activities against all the tested fungi. (Table 1-2 and Figure 1)

The highest antibacterial and antifungal activity was showed by *Alangium salviifolium* among the selected plant species. *Staphylococcus aureus* was the most susceptible bacteria and *Aspergillus fumigatus* was most susceptible fungi amongst all the bacterial strains investigated in the present work.

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants[17-22]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human

beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection.

In our earlier studies preliminary phytochemical investigations of selected plant suggest that they have most of the secondary metabolites in their ethanolic extract[23-25].

CONCLUSION

From our investigation of screening different plant species, the results obtained confirm the therapeutic potency of some plants used in traditional medicine. The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial and antifungal properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extract of *Alangium salviifolium* L., *Andrographis paniculata* Nees and *Spilanthus acemella* Murr. can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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Table 1

Antibacterial activity of ethanolic extracts of various plant parts of Alangium salviifolium Linn., Andrographis paniculata Nees and Spilanthus acemella Murr.

Plant parts	*Escherichia coli*		*Pseudomonas aureginosa*		*Klebsella pnemoniea*		*Salmonella typhirium*		*Staphylococcus aureus*	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
A. salviifolium										
Root	11.8±0.02	0.95	8.6±0.01	0.89	9.2±0.01	0.94	7.9±0.02	0.93	10.2±0.03	0.94
Stem	9.4±0.03	0.76	8.2±0.03	0.84	8.6±0.04	0.88	7.5±0.02	0.88	9.8±0.01	0.91
Leaf	8.5±0.02	0.68	7.2±0.02	0.74	8.5±0.02	0.87	7.2±0.02	0.85	8.7±0.02	0.80
A. paniculata										
Root	7.4±0.03	0.60	7.1±0.02	0.73	7.3±0.03	0.74	6.4±0.04	0.75	8.1±0.01	0.75
Stem	6.9±0.01	0.56	7.3±0.03	0.75	6.4±0.01	0.65	6.5±0.02	0.76	7.4±0.01	0.68
Leaf	7.7±0.02	0.62	6.5±0.02	0.67	6.2±0.02	0.63	6.2±0.01	0.73	6.8±0.02	0.63
S. acemella										
Root	7.6±0.01	0.61	7.7±0.03	0.79	8.0±0.02	0.82	6.7±0.01	0.79	7.8±0.03	0.72
Stem	8.4±0.02	0.68	7.3±0.01	0.75	7.5±0.01	0.76	7.2±0.04	0.85	8.3±0.01	0.77
Leaf	8.6±0.02	0.69	6.9±0.02	0.71	6.6±0.03	0.67	7.1±0.02	0.83	8.6±0.02	0.80

Values of IZ are the mean of five replicates (± SE) at 4 mg/disc concentration
Diameter of zone of inhibition formed by Standard antibiotics
IZ = Diameter of zone of inhibition (in mm) including the diameter of disc (6 mm)
AI = Activity Index
Standard Gentamycin (10mcg/ml)

Table 2
Antifungal activity of ethanolic extracts of various plant parts of Alangium salviifolium Linn., Andrographis paniculata Nees and Spilanthus acemella Murr.

Plant parts	*Aspergillus flavus*		*A. niger*		*A. fumigatus*		*Penicillium sps.*		*Rizopus sps.*		*Fusarium oxysporum*	
	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI	IZ	AI
A. salviifolium												
Root	13.8±0.04	0.91	12.6±0.04	0.86	8.7±0.03	0.89	11.9±0.05	0.86	8.3±0.03	0.81	9.9±0.03	0.82
Stem	11.4±0.05	0.75	10.2±0.01	0.69	8.2±0.01	0.84	10.5±0.03	0.76	7.8±0.01	0.76	9.4±0.05	0.78
Leaf	10.5±0.02	0.69	9.2±0.03	0.62	8.6±0.03	0.88	9.2±0.02	0.67	7.2±0.03	0.71	9.3±0.03	0.77
A. paniculata												
Root	8.4±0.02	0.55	7.5±0.03	0.51	7.5±0.01	0.77	7.4±0.01	0.54	7.8±0.03	0.76	6.5±0.04	0.54
Stem	7.9±0.03	0.52	7.8±0.01	0.53	6.6±0.03	0.66	7.1±0.01	0.51	7.3±0.01	0.72	6.2±0.01	0.52
Leaf	7.8±0.01	0.51	7.1±0.03	0.48	6.2±0.04	0.63	6.3±0.03	0.46	7.0±0.03	0.69	6.2±0.02	0.52
S. acemella												
Root	9.6±0.01	0.63	9.7±0.04	0.66	7.8±0.05	0.80	8.5±0.05	0.62	8.2±0.03	0.80	8.4±0.03	0.70
Stem	10.4±0.03	0.68	9.4±0.01	0.64	7.2±0.03	0.73	7.7±0.03	0.56	8.2±0.01	0.80	8.3±0.02	0.69
Leaf	9.2±0.02	0.60	8.7±0.02	0.59	6.7±0.03	0.68	7.3±0.02	0.53	7.7±0.02	0.75	8.5±0.03	0.71

Values of IZ are the mean of five replicates (± SE) at 4 mg/disc concentration
Diameter of zone of inhibition formed by Standard antibiotics
IZ = Diameter of zone of inhibition (in mm) including the diameter of disc (6 mm)
AI = Activity Index
Standard Mycostatin (100 units/ml)

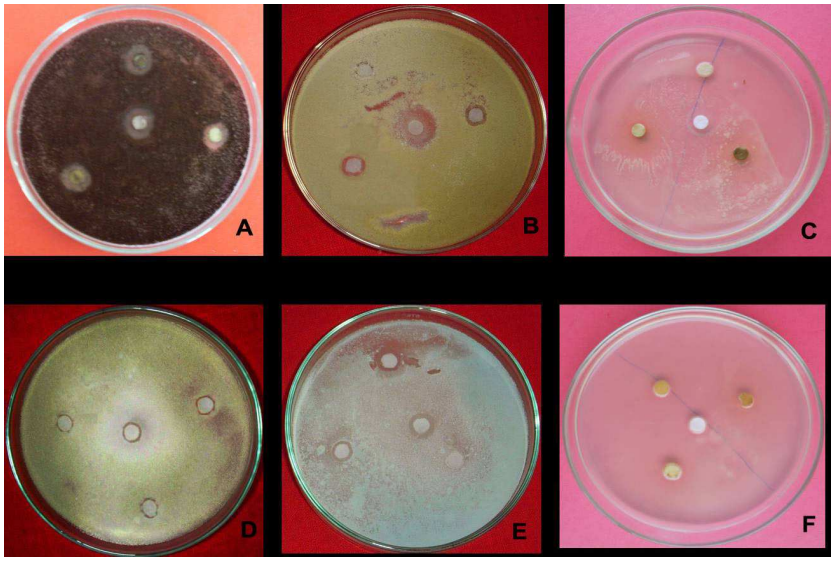

Figure 1
Antimicrobial activity of some medicinal plants ethanolic extracts

A. Alangium salvifolium activty against Aspergillus niger
B. Andrographis paniculata activity against Aspergillus fumigatus
C. Spilanthus acemell activity against Pseudomonas auregenosa
D. Spilanthus acemell activity against Aspergillus flavus
E. Andrographis paniculata activity against Penicillium sps
F. Alangium salvifolium activty against E coli

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