Antimicrobial Potential of *Eupatorium adenophorum* Spreng

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ABSTRACT

The antimicrobial activity of Petroleum-ether, Benzene, Chloroform, methanol and aqueous extracts of crude leaves of *Eupatorium adenophorum* Spreng were tested against the growth of *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes* and *Pseudomonas aeruginosa* by Paper disk diffusion technique. Nearly all the extracts were found to exhibit moderate to good antibacterial activity against the bacterial pathogens tested and the petroleum ether extract recorded largest zone of inhibition against *B.subtilis*. The positive results so obtained were compared with that of the reference standard antibiotic ciprofloxacin. Antifungal activity of the above extracts were tested on fungal strains *Aspergilus niger, Aspergilus candidus* and *Candida albicans* using Fluconazole as the standard drug. The result shows significant antimicrobial activity of extracts against tested fungi and bacteria.

Keywords: *Eupatorium adenophorum* Spreng, Antibacterial, Antifungal, Extracts.

INTRODUCTION

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Diseases can spread, directly or indirectly, from one person to another. Infectious diseases are the second leading cause of death worldwide. About one – fourth of all the medicines we use, come from rainforest plants. However, scientific have been conducted only to a limited extent with few medicinal plants[1,2]. The present study was designed to search for newer, safer and more potent antimicrobials components which may accomplish our present need. Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time tested and comparatively safe both for human used and for environment. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth[3,4].

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Eupatorium Linn (Fam Asteraceae) is a large genus of herbs, shrubs or undershrubs, distributed chiefly in tropical America, a few species occurring in Europe, Africa and Asia and India. Different parts of *Eupatorium adenophorum* Spreng are used in Ayurveda and other folk medicines for the treatment of cut and wounds. Leaves are used as an application to unhealthy ulcers. A decoction of the plant and the juice of the leaves are traditionally used as popular haemostatic remedy for various kinds of hemorrhage. Traditionally the leaves paste mix with mustard oil is useful for ulcer. Every part of the plant either alone or in combination has also been recommended for snake bite. *Eupatorium* adenophorum Spreng consist of various bioactive constituents like triterpenoids, flavanoides, sterols, saponins, triterpene alcohols and lactones[5-8].

MATERIALS AND METHODS

Collection and Authentication of Plant

Leaf samples of *E. adenophorum* Spreng were procured from the forest of Nagdhar Pokhari Chamoli (Uttarakhand) and identified Botanical Survey of India, Northern Regional centre, Dehradun (BSD) with the Accession number 1127802, 1127803. A voucher specimen has been preserved in the department for further verification.

Preparation of Plant Extracts

The air dried leaves of *E. adenophorum* Spreng as moderately coarse powders were completely extracted with petroleum ether (60-80), benzene, chloroform, alcohol (95% v/v) and water in soxhlet extractor apparatus. The extracts were concentrated under vacuum (50^0), dried and weighed. Percentage extractives by successive extraction were found to be 2.390, 1.029, 1.179, 2.189, 3.456 and 3.968 percent (w/w) respectively. These extracts were dissolved in Dimethyl sulfoxide (DMSO) containing 1000 µg/ml.

Microbial strains

In total 9 microbial strains used for the experiment were collected as pure cultures from I.F.T.M, Department of Microbiology, Moradabad, UP. Both gram positive, gram negative bacteria and fungi were taken for the test. The microorganisms were maintained on nutrient agar medium (HIMEDIA).

Antimicrobial Activity

Antibacterial and Antifungal activity of the crude extract was investigated against 9 bacterial and fungal strains by the paper disk diffusion technique[9] using 100µl of suspension containing 108 CFU/ml of bacteria spread on nutrient agar medium. Sterile 6mm diameter filter paper discs were impregnated with 400µg of each of the sterile test material (petroleum ether, benzene, chloroform, methanol and aqueous extract) and placed in nutrient agar medium. Ciprofloxacin (30µg/disc) disc were used as positive control to ensure the activity of standard antibiotic against the test organisms. The sample discs and the standard antibiotic discs were placed gently on the previously marked zones in the agar plates pre- inoculated with the test bacteria and fungi. The plates were than kept in a refrigerator at 4^0c for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were than inverted and kept in an incubator at 37^0c for 24 hours. The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gave clear zones of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale[10].

Minimum Inhibitory Concentration

MIC was determined using the broth dilution technique.[11,12] The minimum inhibitory concentration value was determined for the microorganisms that were sensitive to the extracts under study. A two- fold serial dilution of each extracts was made to using nutrient broth. MIC is defined as the lowest concentration where no visible turbidity was observed in the test tubes.

RESULTS AND DISCUSSION

Preliminary antibacterial studies were conducted on dried plant extracts of *E. adenphorum* Spreng using *Bacillus subtilis* (ATCC-6633), *Bacillus cereus (ATCC 11778) Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 11229), Klebsiella aerogenes (NCTC 418) and Pseudomonas aeruginosa* (ATCC 35032) at concentration of 100µl/disc by disc diffusion method. The significant antibacterial activity was determined by measuring the diameter of zone of inhibition and compared with the standard drug ciprofloxacin. From the data Table 1, it was found that all the crude extracts (400µgm/disc) exhibited moderate to good antibacterial activity against the bacterial pathogens tested herein and the petroleum ether extract of *E. adenophorum* Spreng recorded largest zone of inhibition (17 and 15 mm in diameter) against *B.subtilis* and *E.* coli. Antimicrobial antibiotic ciprofloxacin (30µgm/disc) was found to be active against all the bacteria tested herein. From the results of

Table 1: Antibacterial activity of crude leaf extracts from *E. adenophorum* Spreng

Bacteria	Diameter of zone of inhibition(mm) (Crude extract 400µ gm/disc)					Ciprofloxacin (30µ gm/disc)
	PEE	CE	BE	AE	Aq.E	
Gram positive						
B.subtilis	17	12	12			
B.cerus	12	11	11	14	13	22
S.aureus	11	11	11	15	10	20
				13	12	17
Gram negative						
E.coli	15	13	11	13	14	19
K. aerogenes	11	10	10	12	10	17
P.aeruginosa	12	10	10	12	10	16
DMSO	-	-	-	-	-	-

the MIC presented in Table 2 the petroleum ether extracts exhibited the lowest MIC value (120 µ gm / ml) against *B. subtilis* and the chloroform extracts of *E. adenophorum* Spreng exhibited the lowest MIC value (120 µ gm/ml) against *E.coli*.

The result of antifungal activity observed and reported in Table 3 and Table 4. The antifungal activity of dried plant extracts was done by disc diffusion method against two typical pathogen namely *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404) and *Aspergillus candidus* (NCIM 883) using Fluconazole as standard drug. The effect of the different extracts of *E. adenophorum* Spreng on the pathogenic fungi was carried out at concentration (400µgm/disc) at 25±2°C for 48 hrs of incubation. The extracts were found to have moderate antifungal activity. The petroleum ether extract shows largest zone of inhibition (16 mm in diameter) against *Candida albicans*. Antifungal antibiotic Fluconazole (30µgm/ml) was also found to be active against all the fungal species tested. The petroleum ether and alcoholic extracts exhibited the lowest MIC value (250µgm/ml) against *Candida albicans*.

This study confirmed that the petroleum ether extract (PEE) of leaves of *E.adenophorum* Spreng exhibit antimicrobial activity and the effects are attributable due to the presence of triterpenoids in the plant.

In conclusion, the fact that the extracts (PEE and AE) produced inhibitory activities but less when compared to reference drugs against almost all the test bacteria and fungi provides some scientific basis for some of the uses in traditional medicine like treatment of boils and scabies and as antiseptic. We therefore suggest the isolation and possible characterization of the active constituent(s) from the extracts of this plant species as possible antibacterial agents.

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Table 2: MIC of crude leaf extracts from *E. adenophorum* Spreng

	MIC(Crude extract µ gm/ml medium)					
Bacteria	**PEE**	**CE**	**BE**	**AE**	**Aq.E**	**Ciprofloxacin**
B.subtilis	120	250	500	250	500	5
B.cerus	250	500	750	250	500	5
S.aureus	800	820	750	750	800	5
E.coli	500	120	500	500	250	5
K. aerogenes	250	250	800	250	750	5
P.aeruginosa	250	500	500	250	500	5

Key: PEE = Petroleum ether extract, CE = Chloroform extract, BE = Benzene extract, AE = Alcoholic extract, Aq.E = Aqueous extract, B.subtilis = Bacillus subtilis, E.coli = Escherichia coli, S.aureus = Staphylococcus aureus, - = No growth

Table 3: Antifungal activity of crude leaf extracts from *E. adenophorum* Spreng

	Diameter of zone of inhibition(mm) (Crude extract 400µgm/disc)					Fluconazole (30µgm/disc)
Fungi	**PEE**	**CE**	**BE**	**AE**	**Aq.E**	
C. albicans	16	12	12	15	13	20
A. niger	13	11	12	13	11	16
A. candidus	13	12	11	13	10	18
DMSO	-	-	-	-	-	-

Table 4: MIC of crude leaf extracts from *E. adenophorum* Spreng against fungi.

	MIC(Crude extract µ/ml medium)					
Fungi	**PEE**	**CE**	**BE**	**AE**	**Aq.E**	**Fluconazole**
C. albicans	250	750	750	250	500	5
A. niger	500	800	820	500	850	5
A. candidus	500	750	1000	500	1000	5

Key: PEE = Petroleum ether extract, CE = Chloroform extract, BE = Benzene extract, AE = Alcoholic extract, Aq.E = Aqueous extract, MIC = Minimum inhibitory concentration, - = No growth.

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