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Antimicrobial activity of the leaves of *Myxopyrum serratulum* A. W. Hill.

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ABSTRACT
The antibacterial and antifungal activities of petroleum ether (40-60°C), benzene, chloroform, ethanol and water extracts of the leaves of *Myxopyrum serratulum* A. W. Hill. (*Oleaceae*) were tested for their antimicrobial activity in Agar diffusion assay. Significant antimicrobial activities were found against three gram positive bacteria, *Streptococcus faecalis*, *Baccillus subtilis* and *Baccillus cereus,* four gram negative bacteria; *Klebsiella aerogens*, *Escherichia coli*, *Pseudomonas aeruginosa, Proteas vulgaris* and two fungi, *Candida albicans*, *Asparagillus flavans* strains by using zone of inhibition assay. The activities were confirmed by Activity Index (AI). Activity Index values were found to be higher for ethanolic extract followed by the water extract. Among all the extracts tested; the ethanolic extract showed a good antimicrobial potential against microorganisms used in the study. The Minimum Inhibitory Concentration (MIC) for the ethanolic extract was also determined. The results support the ethnomedicinal use of *Myxopyrum serratulum* for the treatment of wounds and cuts.

Keywords: *Myxopyrum serratulum,* Ethnomedicine, Antimicrobial, Zone of inhibition, Minimum inhibitory concentration.

INTRODUCTION

Studying medicinal plants with ethno-botanical importance and folklore reputation has become the more important need in recent times in order to promote the use of herbal medicines and to determine them as source of new drugs. [1] In recent years, use of antimicrobial drugs in the treatment of infectious disease has developed multiple drug resistance [2] and with increases in production of new antibiotics, by pharmaceutical industry, resistance to these drugs has also increased. [3] Hence, scientists are shifting their attention to folk medicine in order to find new leads for better drugs against microbial infections. Plant materials are known as source of new antimicrobial agents, as a result search has been to discover new antibacterial drugs of plant origin. [1] The plant *Myxopyrum serratulum* commonly known as "Chaturamullai" in Tamil and "Chaturadharalata" in Sanskrit is reported to possess a number of medicinal values. [4] It is a large woody climbing shrub with quadrangular stems. It grows throughout Kerala and evergreen forests at altitudes of 600 m – 900 m. The roots are useful in scabies and prurigo in children. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, consumption, fever, otopathy, neuropathy and cuts and wounds. [4]

A perusal of literature revealed that *M. serratulum* has not been subjected to antimicrobial screening. In the present work, the effect of various solvent extracts of *M. serratulum* on three gram-positive bacteria *viz.*, *Streptococcus faecalis, Baccillus subtilis* and *Baccillus cereus* and four gram negative bacteria *viz.*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella aerogens* and *Proteas vulgaris* and two fungi *Candida albicans, Asparagillus flavans* by using zone of inhibition assay [5] has been reported. The corresponding solvents are used as solvent control. The antibiotics, Streptomycin 10µg/disc and Clotrimazole 10µg/disc are used as standards for bacteria and for fungi respectively.

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MATERIAL AND METHODS

Plant materials

The plant was collected in the month of September from Trivandrum, Kerala, India and was identified by Dr. V. Chelladurai, Research Officer (Botany). Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu, India. A voucher specimen (MSU/PHAR/HER-139) has been preserved in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli - 627 012.

Extraction of plant material

The leaves of *M. serratulum* were dried under shade and powdered. The dried powder (500 g) was successively extracted using petroleum ether (40-60°C), benzene, chloroform, ethanol and water by using Soxhlet apparatus. The last trace of solvent was removed under reduced pressure by rotary evaporator. The dried crude extracts were used for the study.

Micro-organisms used

Bacterial strains used for testing included *Streptococcus faecalis* (MTCC 459), *Baccillus subtilis* (MTCC 619), *Baccillus cereus* (MTCC 430), *Escherichia coli* (MTCC 443), *Proteas vulgaris* (MTC 1771), *Pseudomonas aeruginosa* (MTCC 741) and *Klebsiella aerogens* (MTCC 530). The fungi used were *Candida albicans* (MTCC 183), *Asparagillus flavans* (MTCC1973). These were obtained from Central Research Institute, Khashuli, Chandigarh, India. The stock culture was maintained on Mueller Hinton agar medium (Himedia chemicals) at 37^{o}C.

Preparation of the test organisms

The bacterial and fungal cultures were incubated for 24 h at 37°C in nutrient agar slants (Himedia, Mumbai, India) respectively. Before streaking, each culture was diluted (1:10) with fresh sterile nutrients broth. Plates were prepared by pouring 20 ml of freshly prepared No.1 medium (Himedia, Mumbai, India) into 20 mm × 100 mm Petri plates. Inoculam (5 ml) was poured directly over the surface of prepared plates to uniform depth of 4 mm and then allowed to solidify at room temperature.

ANTIMICROBIAL ASSAY

The antimicrobial activity was determined by the Paper disc diffusion method. [6] A suspension of the organism was added to sterile nutrient agar medium at 45°C. The mixture was transferred to sterile Petri plates and allowed to solidify. Sterile disc of diameter 5 mm (made from whatmann filter paper previously sterilized in UV-lamp) which was dipped in test drug solution of each extract prepared by dissolving 10mg of the extract separately in 10 ml of the respective solvents. Then the sterile disc containing each test drug solution of the plant extract (200 µl) was placed over the seeded agar plates in such a way that there is no overlapping of zone of inhibition. Standards and a blank were placed on the surface of agar plate. [7] The antibiotics, Streptomycin 10µg/disc and Clotrimazole 10 µg/disc were used as standards for bacteria and for fungi respectively. The plates were kept at room temperature for 2 h to allow diffusion of the test drug into the agar; they were incubated for 24 h and 48 h at 37^{o}C for the bacterial and fungal strains respectively. After the incubation period was over, the plates were observed for Zone of Inhibition (ZI) measured in millimeters (mm). From the results (Table 1), the Activity Index (AI) and Proportion Index (P.I) (Fig. 1) were calculated using the following formulae:

$$\text{Activity index(A1)} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}}$$

$$\text{Proportion Index} = \frac{\text{Number of positive results obtained for individual extract}}{\text{Total number of tests carried out for each extract}}$$

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the ethanolic extract of *Myxopyrum serratulum* was determined in µg/ml. [8-9] The samples of the extract were prepared at seven different concentrations, 900, 750, 600, 450, 300, 200 and 100 µg/ml and the samples were loaded into each disc with 200 µl containing 180, 150, 120, 90, 60, 40 and 20 µg/ml respectively against the test organisms. 90% Ethanol was used as a solvent control. The plates were then incubated at 37°C for 24 h and the results were recorded. [10]

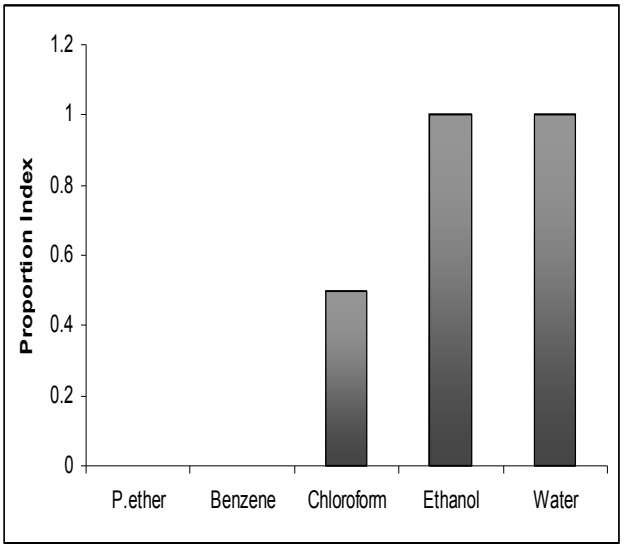


Fig. 1: Proportion Index of antimicrobial activity of various extracts of *M. serratulum* A. W. Hill

RESULTS AND DISCUSSION

The bioassay results for antimicrobial activity of the various solvent extracts of *M. serratulum* are presented in Table 1. From the results it is very clear that both the ethanol and water extracts inhibited the growth of all the tested strains of bacteria and fungi. The chloroform extract was effective in *Baccillus cereus, Klebsiella aerogens, Proteas vulgaris, Candida albicans* and *Asparagillus flavans*. But the petroleum ether and benzene extracts were resistant to all the tested strains of bacteria and fungi. Among the various extracts tested, the ethanol extract showed the maximum activity against all the tested strains used in the present study followed by water extract (Table 1).

The ethanol extract showed the highest activity in *P. aeruginosa* followed by *K. aerogens, E. coil*, *P. vulgaris* and *S. faecalis*.

When the Activity Index is considered (Table 2) the ethanol extract showed good antimicrobial potential than the water extract against the tested microorganisms. The difference in the activity may be due to the different secondary metabolites present in the ethanol and water extracts. Different solvents have various degrees of solubility for different phyto-constituents. [11] This indicates that the secondary metabolites act as an antimicrobial compounds, which either inhibit or kill the bacteria by different mechanisms.

The results of Minimum Inhibitory Concentration (MIC) in µg/ml for the ethanolic extract of *M. serratulum* are presented in the Table 3. The MIC values varied from 200 to 300µg/ml for various organisms. The ethanolic extract of *M. serratulum* was more active against the gram-negative bacteria, *P. aeruginosa* (200µg/ml) when compared with that of the other organisms used in the present study.

Table 1: Antimicrobial activity of the various extracts of *Myxopyrum serratulum* A. W. Hill

S. No.	Name of the Organisms	Zone of Inhibition (mm)					
		Petroleum Ether (40-60°C) extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract	Standard
1.	*B. cereus*	-	-	5	18	17	21[a]
2.	*B. subtilis*	-	-	-	18	18	21[a]
3.	*S. faecalis*	-	-	-	19	17	21[a]
4.	*E. coli*	-	-	-	20	19	22[a]
5.	*K. aergens*	-	-	4	21	19	22[a]
6.	*P. aeruginosa*	-	-	-	22	20	22[a]
7.	*P. vulgaris*	-	-	6	20	18	22[a]
8.	*C. albicans*	-	-	8	18	16	24[b]
9.	*A. flavans*	-	-	6	19	18	25[b]

(a) - Streptomycin; (b) - Clotrimazole; (-) - No inhibitory effect

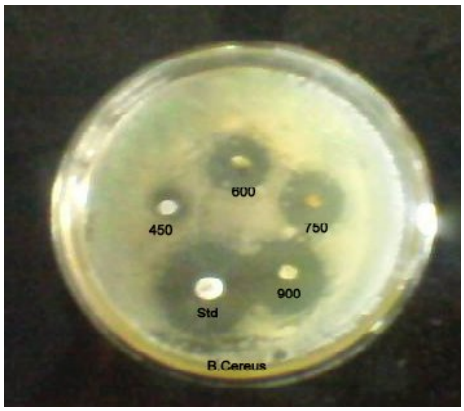
Table 2: Activity Index (AI) of the various extract of *M. serratulum* A. W. Hill

S. No.	Name of the Organism	Activity Index				
		Petroleum Ether (40-60°C) extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract
1.	*B. cereus*	0	0	0.23	0.85	0.80
2.	*B. subtilis*	0	0	0	0.85	0.85
3.	*S. faecalis*	0	0	0	0.90	0.80
4.	*E. coli*	0	0	0	0.90	0.86
5.	*K. aergens*	0	0	0.20	0.95	0.86
6.	*P. aeruginosa*	0	0	0	1.00	0.90
7.	*P. vulgaris*	0	0	0.27	0.90	0.80
8.	*C. albicans*	0	0	0.30	0.70	0.60
9.	*A. flavans*	0	0	0.20	0.76	0.68

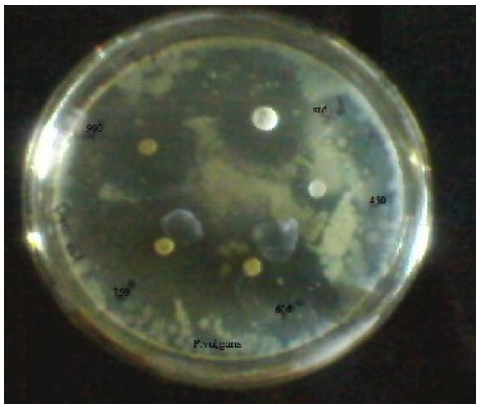
Table 3: Minimum Inhibition Concentration (MIC) determination (100 µg/ml to 900 µg/ml) of ethanolic extract of *M. serratulum* A.W.Hill

S. No.	Name of the organism	Zone of Inhibition (mm)						
		100µg/ml	200µg/ml	300µg/ml	450µg/ml	600µg/ml	750µg/ml	900µg/ml
1.	*B. cereus*	-	-	5	7	11	18	19
2.	*B. subtilis*	-	-	10	11	12	16	18
3.	*S. faecalis*	-	-	-	7	13	19	20
4.	*E . coli*	-	-	11	13	16	20	20
5.	*K. aergens*	-	-	11	13	17	20	21
6.	*P. aeruginosa*	-	5	12	14	16	21	22
7.	*P. vulgaris*	-	-	9	12	14	18	20
8.	*C. albicans*	-	-	6	9	14	17	18
9.	*A. flavans*	-	-	12	12	14	18	19

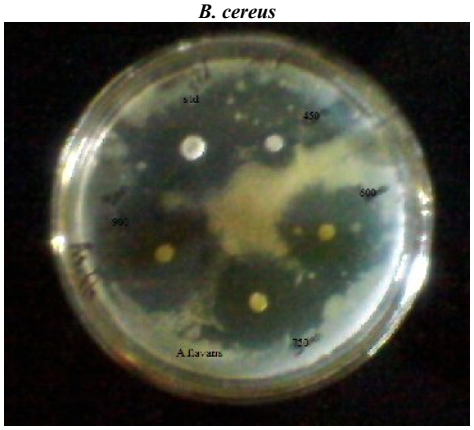
B. subtilis



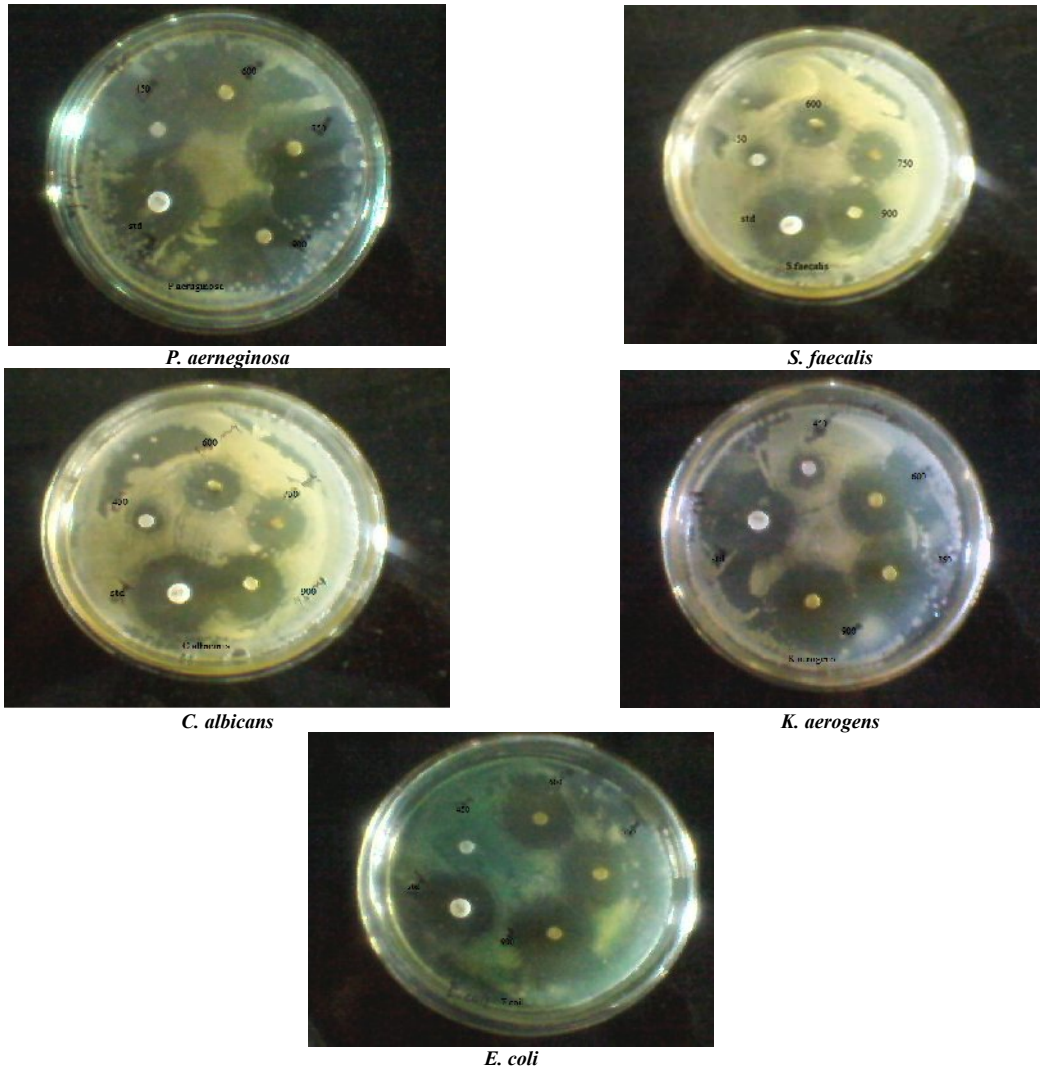
B. cereus



P. vulgaris



A. flavans



P. aerneginosa ***S. faecalis***

C. albicans ***K. aerogens***

E. coli

Fig. 2: Antimicrobial activity of the ethanolic extract of *M. serratulum* A. W. Hill Leaves

From the present investigation, it is concluded that the ethanol extract of *M. serratulum* has significant *in vitro* antimicrobial activity. The results obtained provide support for the traditional use of the plant for curing wounds and cuts.

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