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Research Article

ANTIMICROBIAL ACTIVITY OF FEW SELECTED MEDICINAL PLANTS

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ABSTRACT
The petroleum ether, chloroform, methanol and aqueous extracts of leaves of *Ageratum conyzoides* Linn (Fam: Asteraceae), *Argemone mexicana* Linn. (Fam: Papaveraceae), *Heliotropium indicum* Linn (Fam: Boraginaceae) and stem barks of *Alstonia scholaris* (L.) R. Brown (Fam: Apocynaceae) were screened for their antimicrobial activity against *Bacillus subtilis*, *Stapphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* respectively. The results indicated that the chloroform, methanol and aqueous extracts of all tested plant materials are active against both Gram-positive and Gram-negative bacteria at the tested concentration. The spectrum of activity observed in the present study may be an indicative of the presence of broad spectrum antimicroial compounds in the extracts. Among the tested extracts, methanol extracts of all selected plant materials were found to be more effective than the other extracts under study. Preliminary phytochmical screening of the methanol extracts of selected plant materials primarily revealed presence of alkaloids, tannins and flavonoids. The present work justifies the use of these plant materials for antimicrobial activity as claimed in the folklore remedies.

KEYWORDS: Antimicrobial, Minimum inhibitory concentration, Zone of inhibition

INTRODUCTION
Search for antimicrobial factors from plants remains a potential area of investigation. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants[1]. However, plants used in traditional medicine are still understudied, particularly in clinical microbiology[2]. In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethnomedicinal plants may still be needed[3,4]. The knowledge and usage of herbal medicine for the treatment of various ailments among various tribes of Odisha is still a major part of their life and culture. It was learnt that the tribes inherit rich traditional knowledge about the medicinal uses of flora and apply this knowledge for making crude phytomedicines to cure number of diseases and ailments since time immemorial. Traditional knowledge forms the basis for origin of not only alternative medicine but also paved way to evolution of a gamut of new and novel modern medicines. Several plants were known to possess antimicrobial properties in the literature. In the present paper, we have selected four important folk medicinal plants claimed to possess promising antimicrobial effects on the infected wounds by the local tribes. The selected plants materials include leaves of *Ageratum conyzoides* Linn. (Fam: Asteraceae), *Argemone mexicana* Linn. (Fam: Papaveraceae), *Heliotropium indicum* Linn (Fam: Boraginaceae) and stem barks of *Alstonia scholaris* (L.) R. Brown (Fam: Apocynaceae) respectively.

MATERIALS AND METHODS

Plant Material

The plant materials were collected from the forests of Phulbani district of Odisha during November 2009 and authenticated by the taxonomists of Botanical Survey of India, Howrah. Voucher specimens *Ageratum conyzoides* Linn. (Sp. No: CNH/ I-I / (333)/2009Tech.II/375), *Argemone mexicana* Linn. (Sp. No: CNH/ I-I / (333)/2009Tech.II/375), *Heliotropium indicum* Linn (GKD/820/07) and stem barks of *Alstonia scholaris* (L.) R. Brown (Sp. No: CNH/ I-I / (333) / 2009 Tech.II/375) have been kept in our laboratory for future reference. The collected plant materials were separately washed, shade dried and pulverized to coarse powder.

Extraction

The powdered plant materials (500 g each) were separately and successively extracted with petroleum ether (40-60^0 C), chloroform, methanol and water for 48 h in a soxhlet extractor. Following extraction, the liquid extracts were concentrated under vacuum to yield dry extracts. The extractive values were determined with respect to the dried plant material. The colour, consistency and extractive values of different extracts are presented in Table I. Standard methods[5,6] were used for preliminary phytochemical screening of the different extracts to know the nature of phytoconstituents present within them.

Microrganisms used

Antimicrobial screening of the selected extracts was performed on selected microorganisms that cause wound infection in humans. The test was performed on *Bacillus subtilis* ATCC6633, *Stapphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC4563 and *Aspergillus niger* ATCC4563 respectively. Suitable strains of these microorganisms were procured from the microbiology department of our institute. (The microbial strains studied are identified strains and were obtained from National Chemical Laboratory (NCL), Pune, India.)

Screening for Antimicrobial activity

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of different extracts with respect to different test microorganisms were determined by broth dilution method[7]. Mueller Hinton broth (HiMedia, Mumbai) was used for the antibacterial study and Sabouraud dextrose broth (HiMedia, Mumbai) for the antifungal screening. All the extracts dissolved in 1 per cent of DMSO were first diluted to highest concentration (200 mg/ml) to be tested, and then serial two-fold dilution were made in a concentration range from 0.39 to 200 mg/ml in sterile water. For broth dilution, 0.1 ml of standardized suspension of a strain (10^6 CFU/ml) separately was added to each tube containing various extracts at concentrations of 0 (control), 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200mg/ml in the broth medium. The tubes were incubated at 37°C for 24 h for bacterial strains and 48 h for fungal strains, and observed for visible growth after vortexing the tubes gently. The lowest concentration of test extract in a tube that failed to show any visible macroscopic growth was considered as its MIC. Inhibition of proliferation was assessed by optical density measurements (625 nm). The results of the study are reported in Table II.

Determination of Zone of Inhibition (ZOI)

The zone of inhibition of the test samples was performed by disc-diffusion method as suggested by Sassi *et al*., 2008[8]. The dried plant extracts were dissolved in 5% Dimethylsulphoxide (DMSO) and then in sterile water, to reach a final concentration of 30 mg/ml and sterilized by filtration by 0.22 µm Millipore filters. The media used were Mueller Hinton Agar (HiMedia) for the bacteria and Sabouraud Dextrose Agar (HiMedia) for the yeasts. The discs (6 mm in diameter) were impregnated with 10 µl of the extracts (300 µg/disc) at a concentration of 20 mg/ml and placed on the inoculated agar (10^6 Cfu/ml). Tetracycline (30 µg/ml) and Amphotericin B (30 µg/disc) were served as positive reference standards to determine the sensitivity of the tested microbial strains[9]. Control tests with the solvent DMSO (5%) employed to dissolve the plant extracts were performed for all assays and showed no inhibition of microbial growth. The inoculated plates were incubated at 37°C for 24 h for bacterial strains and 48 h for yeasts strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. The results of the study are presented in Table III.

RESULTS AND DISCUSSION

The colour, consistency and extractive values of various extracts of the selected plant materials are reported in Table 1. The preliminary phytochemical screening of *A. conyzoides* revealed presence of steroids and sterols, triterpenoids, alkaloids, flavonoids, tannins and phenolic substances, gums and mucilages, carbohydrates and proteins. Presence of steroids and sterols, triterpenoids, alkaloids, flavonoids, saponins, tannins and phenolic substances, gums and mucilages, carbohydrates and proteins were identified with *A. mexicana*. Various extracts of the barks of *A. scholaris* revealed presence of steroids and sterols, triterpenoids, alkaloids, saponins, flavonoids, tannins and phenolic substances, gums and mucilages, carbohydrates and proteins. The preliminary phytochemical screening of *H. indicum* leaf extracts showed presence of steroids and sterols, triterpenoids, alkaloids, flavonoids, saponins, tannins and phenolic substances, gums and mucilages, carbohydrates and proteins respectively.

In the present investigation, all test extracts showed some degree of activity against one or more of the bacterial and fungal strains. The antimicrobial activity profile indicated that the chloroform, methanol and aqueous extracts of all tested plant materials are active against both Gram-positive and Gram-negative bacteria at the tested concentration. The spectrum of activity observed in the present study may be an indicative of the presence of broad spectrum antimicroial compounds in the extracts. Among the tested extracts, methanol extracts of all selected plant materials were found to be more effective than the other extracts under study. Preliminary phytochmical screening of the methanol extracts of selected plant materials primarily revealed presence of alkaloids, tannins and flavonoids. Tannins are reported to be effective against a wide range of bacterial and fungal strains[10, 11]. They can be toxic to bacteria, yeasts and filamentous fungi[12]. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and they also complex with polysaccharide[13]. Flavonoids and alkaloids were also known to be synthesized by plants in response to microbial infection[14]. It should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity was probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls[15]. Methanol extracts of *A. conyzoides*, *A. mexicana, A. scholaris* and *H. indicum* possess antimicrobial activities and contained tannins, flavonoids and moreover alkaloids which are known to have antimicrobial activity[16].

The present work justifies the use of these plant materials for antimicrobial activity as claimed in the folklore remedies.

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Table I: Data showing the color, consistency and extractive values of different extracts of the selected plants.

Sl. No.	Plant	Parts used	Extract	Colour	Consistency	Yield % w/w
1	*Ageratum conyzoides*	Leaves	Petroleum ether	Pale green	Waxy	1.8
			Chloroform	Dark green	Greasy	3.2
			Methanol	Greenish brown	Sticky	8.6
			Aqueous	Brown	Sticky	10.7
2	*Argemone mexicana*	Leaves	Petroleum ether	Pale green	Waxy and oily	2.1
			Chloroform	Green	Greasy	3.2
			Methanol	Greenish brown	Sticky	7.2
			Aqueous	Pale brown	Sticky	9.4
3	*Heliotropium indicum*	Leaves	Petroleum ether	Pale green	Waxy	1.78
			Chloroform	Dark green	Greasy	2.54
			Methanol	Deep green	Greasy	6.4
			Aqueous	Pale brown	Sticky	11.2
4	*Alstonia scholaris*	Barks	Petroleum ether	Pale yellow	Waxy	2.4
			Chloroform	Dark brown	Greasy	3.6
			Methanol	Brown	Sticky	7.2
			Aqueous	Pale brown	Sticky	10.7

Table II: Minimum inhibitory concentrations (mg/ml) of various extracts against microbial strains

Plant species	Extract	Minimum inhibitory concentration (mg/ml)					
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
A. conyzoides	Pet. ether	-	100	200	100	-	200
	Chloroform	200	50	50	50	50	100
	Methanol	50	12.5	6.25	12.5	25	25
	Aqueous	100	25	50	25	100	50
A. mexicana	Pet. ether	200	100	-	200	100	200
	Chloroform	50	50	200	100	50	100
	Methanol	12.5	6.25	50	12.5	12.5	25
	Aqueous	25	25	100	50	25	50
A. scholaris	Pet. ether	200	100	200	100	200	100
	Chloroform	50	50	50	25	100	50
	Methanol	12.5	12.5	6.25	12.5	25	12.5
	Aqueous	25	25	12.5	25	50	25
H. indicum	Pet.ether	-	100	-	200	-	100
	Chloroform	50	25	100	50	200	50
	Methanol	12.5	6.25	25	12.5	50	12.5
	Aqueous	25	12.5	50	25	25	25
Tetracycline		0.78	0.39	0.78	0.78	-	-
Amphotericin B		-	-	-	-	3.125	1.56

Table III: Antimicrobial activities of various extracts in disc diffusion assay

Plant species	Extract	Growth inhibition zone diameter (mm)					
		B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. niger***
A. conyzoides	Pet. ether	8.3 ± 0.4	9.1 ± 0.4	8.2 ± 0.0	10.3 ± 0.6	7.6 ± 0.6	9.2 ± 0.3
	Chloroform	11.6 ± 0.2	17.0 ± 0.6	13.3 ± 0.7	17.3 ± 0.1	9.2 ± 0.6	8.1 ± 0.0
	Methanol	16.3 ± 0.7	28.3 ± 0.8	18.6 ± 0.5	31.2 ± 0.2	19.6 ± 0.3	25.3 ± 0.4
	Aqueous	11.8 ± 0.3	15.0 ± 0.7	10.3 ± 0.4	20.2 ± 0.4	15.3 ± 0.5	23.7 ± 0.2
A. mexicana	Pet. ether	9.0 ± 0.4	8.2 ± 0.6	10.1 ± 0.4	8.3 ± 0.5	8.6 ± 0.8	9.2.0 ± 0.7
	Chloroform	12.6 ±0.4	15.6 ± 0.5	14.0 ± 0.3	16.2 ± 0.7	13.7 ± 0.9	13.0 ± 0.6
	Methanol	22.7 ± 0.6	26.7 ± 0.2	25.0 ± 0.6	24.0 ± 0.0	20.3 ± 0.9	21.5 ± 0.6
	Aqueous	15.7 ± 0.9	18.2 ± 0.7	21.0 ± 0.8	20.3 ± 1.4	18.3 ± 0.9	17.7 ± 0.5
A. scholaris	Pet. ether	8.8 ± 0.7	8.4 ± 0.2	9.1 ± 0.6	10.5 ± 0.8	10.6 ± 0.8	11.2 ± 0.4
	Chloroform	11.3 ± 0.2	20.3 ± 0.7	10.7 ± 0.4	18.0 ± 0.3	12.2 ± 0.6	16.1 ± 0.0
	Methanol	27.8 ± 0.6	30.6 ± 0.9	22.6 ± 0.5	28.2 ± 0.3	21.2 ± 0.6	25.3 ± 0.8
	Aqueous	18.0 ± 0.3	26.8 ± 0.2	19.6 ± 0.5	21.3 ± 0.4	17.1 ± 0.3	20.9 ± 0.6
H. indicum	Pet.ether	7.8 ± 0.7	10.1 ± 0.2	8.21 ± 0.6	11.5 ± 0.8	10.4 ± 0.8	11.2 ± 0.3
	Chloroform	14.1 ± 0.4	18.6 ± 0.9	15.3 ± 0.3	19.6 ± 0.0	13.2 ± 0.9	15.1 ± 0.6
	Methanol	21.3 ± 0.4	27.1 ± 0.6	30.2 ± 0.7	28.2 ± 0.4	20.2 ± 0.3	23.3 ± 0.3
	Aqueous	18.0 ± 0.7	22.8 ± 0.3	19.6 ± 0.3	23.3 ± 0.9	17.1 ± 0.6	18.9 ± 0.2
Tetracyclin		35.0 ± 0.3	34.6 ± 0.2	35.0 ± 0.7	34.3 ± 0.8	-	-
Amphotericin B		-	-	-	-	23.3 ± 0.9	28.0 ± 0.4

Note: The control disc used for solvent had no zone of inhibition, so this data is omitted from the above data. Inhibition zones including the diameter of the paper disc (6 mm). Results are expressed as the mean ± SEM of triplicate measurement