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Diversity in antimicrobial activity of some medicinal plants of High Altitude area: *Achyranthes aspera, Thalictrum foliolosum, Valeriana wallichii, Hedychium spicatum, Woodfordia fruticosa, Acorus calamu, Eupatorium cannabium*

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ABSTRACT

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. In the present study extract of seven plants from high altitude area were evaluated for antimicrobial activity against hospital isolates(bacterial and fungal) bacteria viz. Staphylococcus aureus and Streptococcus pyogens, Escherichia coli, Salmonella typhimurium, and Pseudomonas fluorescence and Fungal viz. Aspergillus flavus, Aspergillus niger, Microsporum gypseum, Trichophyton mentagrophytes, and Trichophyton rubrum . The in vitro antimicrobial activity was performed by agar well diffusion method. Amongst the plant species studied, Woodfordia fruticosa showed broad spectrum antimicrobial activity.

Key words: Multiple drug resistance, Antibacterial, High altitude, Hospital isolates, *Woodfordia fruticosa*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; with more than 50% of chemotherapeutic agents currently in use having been derived from natural products [7]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants [28]. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [8]. Medicines obtained from plants are relatively safer than synthetic alternative [12, 10].

This plant-based, traditional medicine system continues to play an essential role in health care, they play dual role in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases [11]. Therefore, such plants should be investigated to better realize their properties, safety and usefulness [5].

In recent years, multiple drug resistances in human pathogenic microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. These undesirable side effects of certain antibiotics and the emergence of previously uncommon infections [14, 20] have forced scientists to control the use of antibiotic and develop research to better understand the genetic

mechanisms of resistance, and to continue studies to explore new antimicrobial compounds from various sources like medicinal plants. The ultimate goal is to offer appropriate and efficient antimicrobial drugs (either synthetic or natural) to the patient. Natural products, either as pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [17].

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents [1, 23, 16, and 3].
A screening of 56 extracts and from seven medicinal plants of high altitude for their antimicrobial (antibacterial and antifungal) properties has been carried out.

MATERIALS AND METHODS

Achyranthes aspera, Thalictrum foliolosum, Valeriana wallichii, Hedychium spicatum, Woodfordia fruticosa, Acorus calamus and Eupatorium cannabium collected from various high altitude regions as described below:

Name of the Plant	Family	Place	Month
Achyranthes aspera	Amaranthaceae	Phagli	October
Thalictrum foliolosum	Ranunculaceae	Phagli	July
Valeriana wallichii	Valerianacea	Jakhu	April
Hedychium spicatum	Zinziberaceae	Kufri	September
Woodfordia fruticosa	Lythraceae	Kandaghat	April
Acorus calamus	Araceae	Phagli	July
Eupatorium cannabium	Asteraceae	Darjeeling	October

Extraction of plant material
The shoots and roots of each plant were used separately to prepare different extracts. The plant material of each plant was washed and shade dried at room temperature. The dried material was crushed to coarse powder and weighed. 100 g of the dried plant material was subjected to soxhlet extraction for 18 h. The order of solvents used was petroleum ether (60^{o}C- 80^{o}C), Chloroform, Methanol and Distilled water. The extracts so obtained were concentrated by evaporation under reduced pressure at 50-60^{o} ± 5^{o} C. Hence, in total 56 extracts were prepared which were stored at 4^{o} C until used.

Microorganisms
Bacterial Cultures (Hospital isolates)

Gram-positive bacteria: *Staphylococcus aureus* and *Streptococcus pyogens*,

Gram-negative bacteria: *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas fluorescence*

The cultures were grown in Erlenmeyer flasks (250 ml) containing 100 ml Nutrient broth and were maintained in nutrient agar medium and preserved on the same medium at 4^{o} C. A loop full of bacterial cultures were inoculated individually in the medium and incubated under agitation (150 rpm) at specified temperatures.

Fungal Culture (Hospital isolates)
Aspergillus flavus, *Aspergillus niger*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*. The fungal cultures were maintained on potato dextrose agar (PDA) and preserved on the same medium at 4^{o}C. The cultures were subcultured periodically (5-7 days) under stationary condition on the same medium at 28 ±2^{o} C.

Antibacterial activity
The antibacterial activity of the Petroleum ether, Chloroform, Methanol and Aqueous extracts were determined by the agar well-diffusion method on Nutrient agar (Hi Media, India) medium. Using a cork borer, wells (5 mm in diameter) were made in the agar medium (one in the center) and inoculums containing 10^{6}CFU/ml of the test bacteria were spread plated onto the surface of the medium with a sterile spreader [2]. 50µl of the extract was pipetted into the wells, whilst 50µl of DMSO served as a control. The agar plates were incubated at 37ºC for 24 h and the diameter of the zone of inhibition surrounding the wells was measured. The diameters of zone of inhibition due to extracts were compared with those produced by the commercial control antibiotics, tetracyclin and Chloramphenicol.

Anti-fungal activity
Anti fungal activity of each plant extract against different strains was checked by well plate assay. A lawn was made on PDA by mixing fungal inoculum in PDA. After solidification wells were punched out with sterile borer and 50µl

of the extract was pipetted into wells. Plates were incubated at 28° ± 2 ° C for 5-7 days to observe the zone of inhibition around the well.

Phytochemical analysis

The extract showing broad spectrum antimicrobial activity was subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycolsides in accordance with Trease and Evans & Harborne with little modification. [29, 9]

RESULTS

Antimicrobial activity

The bacterial cultures used for study were *Staphylococcus aureus, Streptococcus pyogens, Escherichia coli, Salmonella typhimurium, Pseudomonas fluorescence a*nd fungal cultures used for study were *Aspergillus flavus, Aspergillus niger, Microsporum gypseum, Trichophyton mentagrophytes, Trichophyton rubrum.* Table 1 shows the ZOI for the extracts which shows maximum zone of inhibition against the microbial cultures (whole data not shown). Then the percentage degree in inhibition by most effective antimicrobial extract was compared with standard antibiotics, tetracycline and Chloramphenicol as antibacterial and Ketoconazol as antifungal antibiotic as shown in Table 2 and 3 and it was found that MRWF (Methanol root extract of *Woodfordia fruticosa*) showed 33.3 times more activity than that of tetracycline against *E .Coli* and MSWF(Methanol shoot extract of *Woodfordia fruticosa*) showed 8.33 times more antibacterial activity than Chloramphenicol against *P.aeruginosa*.

Table 1: Diverse Antimicrobial activity of Plant Extracts of high altitude area

-		Maximum Zone of Inhibition(mm)					
		W. fruticosa	***A.aspera***	***A.calmus***	***T.foliolosum***	***V.wallichii***	***H.spicatum***
Bacterial cultures	*E. coli*	MRWF (20)	ASAA (15)	PRAC (13)	MSTF (14)	MSVW (14)	CRHS (15)
	P.aeruginosa	MSWF (26)	MSAA (16)	CRAC (25)	CRTF (13)	MSVW MRVW (15)	CSHS (15)
	S.typhimurium	MSWF (23)	MSAA (14)	MRAC (15)	MRTF (14)	PSVW (12)	PSHS (14)
	S.aureus	MSWF (21)	PSAC (P)	NI	CRTF (16)	PRVW (17)	PRHS (14)
	S.pyogens	MRWF (16)	PSAC PRAC CRAC (P)	NI	CRTF (17)	PRVW (13)	PSHS CRHS (14)
Fungal cultures	*A.flavus*	PRWF CRWF(22)	PSAA(31)	PSAC (24)	MSTF (22)	CSVW (28)	PSHS (28)
	A.niger	MSWF(23)	MSAA CRAA(20)	PSAC (20)	PSTF (21)	MSVW (18)	PSHS MRHS(21)
	M.gypseum	MSWF(21)	CSAA MRAA(28)	MSAC MRAC (19)	CRTF (22)	PRVW (25)	PRHS(30)
	T.mentagrophytes	MSWF(22)	NI	NI	MRTF (16)	NI	MRHS(P)
	T.rubrum	MSWF(23)	PSAA(24)	MSAC (25)	MSTF (11)	CSWV (37)	NI

Table 2: Comparison of antibacterial activity of most effective extract and standard antibiotics

		ZOI(mm)in presence of			% in inhibition as compared to standard antibiotics	
		Extract	T	C	T	C
Bacterial Culture	*E. coli*	20 (MRWF)	15	25	**33.33** ↑	20.00 ↓
	P.aeruginosa	26 (MSWF)	26	24	=	**8.33** ↑
	S.typhimurium	23 (MSWF)	29	36	20.68 ↓	36.11 ↓
	S.aureus	21(MSWF)	29	30	27.58 ↓	30.00 ↓
	S.pyogens	17(CRTF)	27	32	37.03 ↓	46.87 ↓

T- Tetracycline, C-Chloramphenicol

	Table 3: Comparison of antifungal activity of most effective extract and standard antibiotics			
	ZOI(mm)in presence of			% in inhibition as compared to standard antibiotics
		Extract	K	K
Fungal Culture	*A.flavus*	31(PSAA)	38	18.42
	A.niger	23(MSWF)	41	43.9
	M.gypseum	30(PRHS)	40	25.00
	T.mentagrophytes	22(MSWF)	43	48.8
	T.rubrum	37 (CSWV)	-	100

K-Ketoconazol

Table 4: Phytochemicals in Methanolic shoot extract of *Woodfordia fruticosa*								
Reducing sugars	Proteins	Fats and oil	Steroids	Cardiac glycosides	Anthraquinone	Flavonoids	Alkaloids	Tannins & phenolics
+	-	-	+	+	+	+	+	+

Phytochemicals:
The result of the phytochemical screening is presented in Table 4. This reveals moderate concentration of alkaloids, carbohydrate, cardiacglycosides, flavonoids, saponins, terpenes and steroids.

DISCUSSION

The screening of plants usually involves various approaches; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study. Traditionally plant parts, extracts, infusions etc were used for treatment of various diseases. The data shows that each plant extract shows different degree of ZOI (Zone of inhibition) against different microorganisms (bacterial & fungal isolates). Extrinsic and intrinsic parameters mainly affect the ZOI. The extrinsic parameters (like pH of the medium, period and temperature of incubation, volume of the well, concentration of plant extracts and size of inoculums) pose no much error in the results as they were fixed and standardized during experiment, However, intrinsic factors (Nature of medicinal plants including its phytochemicals, solubility and diffusing property) might be responsible for variability in diffusion of extract which result in variable ZOI [21]. From the tables it is obvious that MSWF (Methanolic shoot extract of *Woodfordia fruticosa*) shows broad spectrum of antimicrobial (antibacterial & antifungal) activity, A review of the literature on the antimicrobial activity of different plant extracts shows that methanol extracts have a high level of activity. The crude methanol extract of *Woodfordia fruticosa* contains certain constituents such as tannins with significant antibacterial properties [18], which enable the extract to overcome the barrier in Gram-negative cell wall [24]. Methanol extracts were more active than aqueous extracts for all 12 plants studied. Methanol provided more consistent antimicrobial activity compared to those extracted in water. These activities might depend on the compounds being extracted by each solvent, the polarity of the solvents, and their intrinsic bioactivity [19]. Plant methanol extracts contain many chemicals such as alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids [13], some of which have been associated to antibacterial activities and thus have curative properties against pathogens [15].

It is assumed that the traditional healers were able to obtain the active compounds by boiling large quantities of material in order to unlock the active compounds. The results of the present study on the effect of antibacterial and antifungal activity corroborate the results of previous screenings of medicinal plants for antibacterial activity, where most of the extracts of various plant species showed activity against gram-positive strains only [4, 6, 22, 25, 27].

CONCLUSION

It is concluded that methanol extract of *Woodfordia fruticosa* have a strong and broad spectrum of antibacterial activity against many bacterial and fungal isolates and further pharmacological evaluation will be carried out. The future prospects of present research work includes isolation and purification of the therapeutic antimicrobials from the active extract and there further pharmacological evaluation by several method such as – NMR , MS , GC-M S , TLC, HPLC.

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