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Screening of *invitro* antimicrobial activity of *Barlaria buxifolia* Linn. (Acanthaceae) stem extract

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ABSTRACT

This study was aimed to evaluate the antimicrobial activities of Barlaria buxifolia Linn. has been studied by agar diffusion method. The carbon tetrachloride, ethyl acetate, ethanol and methanol extracts were prepared using soxhlet apparatus. The result of antimicrobial assay revealed that the extracts showed significant inhibitory activity against all the tested pathogens compared with standard and significant activity was observed with ethanolic and methanolic extract. Ethanol and methanol extracts revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins and tannins. Thus, the results depict that stem extracts of B.buxifolia could be used as potential source of antibacterial and antifungal agents in the treatment of infectious diseases.

Key words: Antimicrobial, Agar diffusion method, *Barlaria buxifolia* L. Gentamycin, Minimum Inhibitory Concentration (MIC).

INTRODUCTION

Barlaria buxifolia Linn (Acanthaceae) is widely distributed throughout the greater part of India [1-4].This plant is found growing in abundance in the deccan and karnatic plains, especially on roadsides and waste lands. It is found in abundance in most of the plains often on hard and gravelly soil. This plant has usefull medicinal properties [5] and extracts of the roots and leaves of this plant are known to be used locally for curing cough and inflammation [6]. From the

phytochemical screening presence of flavonoids, phenols, saponins and tannins of ethanol and methanol extracts are of good inhibitory action against selected organisms. Thus it was thought worthwhile to explore this plant for antimicrobial activity. It is concluded that phytochemicals obtained from medicinal plants are the sole remedy to this emerging problem. Therefore an attempt has been made to study the antimicrobial activity of the extracts of *Barlaria buxifolia* Linn on selected microorganisms.

EXPERIMENTAL SECTION

Plant material

The fresh matured stem of *B.buxifolia* L. was collected in sufficient quantities from the local areas of Nelamangala taluk, Bangalore rural district, Karnataka, India during the month of May-June and was authenticated by Dr.Krishnegowda, Professor, Department of Botany, Director, Dayananda Sagar College of Biological Sciences, Bangalore, Karnataka, India.

Preparation of Extracts

The stem of *B.buxifolia* was washed under running tap water. This was dried in shade at room temperature for few days. The dried stem was chopped into small parts in a blender and pulverized to a fine powder using a mechanical grinder and stored in airtight container. The dried powder material of the stem was defatted with petroleum ether (60-80^{0}C) and subsequently extracted with four different solvents namely carbon tetrachloride, ethyl acetate, ethanol and methanol in a soxhlet apparatus. The solvent was completely removed under reduced pressure to obtain the extracts as solid residues. The freshly prepared extracts were used for chemical tests for the identification of chemical constituents using standard methods.

Phytochemical Screening

The freshly prepared extracts were chemically tested for the presence or absence of various primary or secondary metabolites using standard methods [7-8].

Test Microorganisms

The organisms selected for antimicrobial activity were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans, Gentamycin* and *Amphotericin* as standard.

Screening for Antimicrobial Activity

Antibacterial activity

Media used: Peptone-10g, Nacl-10g and yeast extract 5g, Agar 20g in 100 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37^0 C for 18 hours. The agar plates of the above media were prepared and wells made in the plate. Each plate was inoculated with 18 h old cultures (100 µl 10^{-4} Cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. The control wells with Gentamycin were also prepared along with plates for the analysis of inhibitory activity of solvents, each well filled with 100 µl of solvents. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted

Antifungal activity

Media used: The media used is Potato Dextrose Agar (PDA). 250 g of peeled potato were boiled for 20 min and squeezed and filtered. To this filtrate 20 g of dextrose was added and the volume was made up to 1000ml by distilled water.

Initially, the stock cultures of were revived by inoculating in broth media and grown at 27^0C for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 h old cultures (100 µl 10^{-4} Cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. The control plates with standard antibiotic were also prepared along with plates for the analysis of inhibitory activity of solvents, each well filled with 100 µl of solvents. All the plates were incubated at 27^0C for 48 h and the diameter of inhibition zone was noted.

Determination of Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of a compound/extract/drug that completely inhibits the growth of the microorganism in 24h [Aneja et. al., 2009]. The MIC for the carbon tetrachloride, ethyl acetate, ethanolic and methanolic stem extracts were determined by following the modified agar well diffusion method [Cappuccino and Sherman, 1995]. Different concentrations of each dilution were introduced into wells in the specific media agar plates already seeded with 100 µl of standardized inoculum (10^{-4} Cfu) of the test microbial strain. All test plates were incubated aerobically at 37^0 C for 24hours and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition, considered as the MIC, was recorded for each test organism.

Statistical Analysis

The results are presented as diameter of inhibition zones in cm.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the four different solvent extracts revealed the presence of alkaloids, flavonoids, steroids and phenolic compounds as presented in **table-1**. The agar diffusion method for antibacterial and antifungal activities of the plant extracts showed significant results in terms of zone of inhibition. The results obtained showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extracts whereas significant activity was observed with ethanolic and methanolic extracts. The antimicrobial activity of *B.buxifolia* extracts on the agar plates varied in different solvents. It has been found that the ethanolic extract have significant activity against gram positive bacteria *Staphylococcus aureus* (0.9cm/09mm) at 2.0 mg more susceptible to the plant extract than the gram negative bacteria *Escherichia coli* (0.4cm/04mm) at 2.0 mg was least susceptible with the inhibition zone. The antibacterial activity of the plant extracts in terms of zone of inhibition was presented in **table-2**. There was no activity tested at 0.5 mg in all the organisms [9-12].The observed activity may be due to the presence of potent phytochemicals in the stem extracts. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids and phenolic compounds. [Cowan, 1999], [13].

Table-1. Phytochemical Screening of *Barlaria buxifolia* Linn.

Secondary Metaboites	Carbon tetra chloride	Ethyl acetate	Ethanol	Methnol
Alkaloids	+	-	+	-
Flavonoids	-	+	+	+
Steroids	-	-	+	+
Saponins	-	-	+	+
Tanins and phenolic comps.	-	-	+	+

Table-2. Antimicrobial Activity of Extracts of *Barlaria buxifolia* Linn.

Antibacterial Activity

Zone of Inhibition in cm								
Microorganism	**Carbon tetra chloride**				**Ethyl acetate**			
	0.5 mg	**1.0mg**	**2.0mg**	**MICmg**	**0.5 mg**	**1.0mg**	**2.0mg**	**MIC mg**
S.aureus	-	-	-	>2	-	-	0.5	2
B.subtilis	-	-	0.2	2	-	-	-	>2
E.coli	-	-	-	>2	-	-	0.2	2
P.aeruginosa	-	-	-	>2	-	0.2	0.3	1.0
	Ethanol				Methanol			
S.aureus	-	-	0.9	2	-	-	0.3	2
B.subtilis	-	-	-	>2	-	0.2	0.4	1
E.coli	-	-	0.4	2	-	-	0.3	2
P.aeruginosa	-	0.2	0.4	1	0.2	0.2	0.3	0.5
Gentamycin								
	200 µg	400 µg	800 µg	MIC µg	200 µg	400 µg	800 µg	MIC µg
S.aureus	2.5	2.7	3.4	25	2.5	2.7	3.4	25
B.subtilis	1.9	2.2	2.5	25	1.9	2.2	2.5	25
E.coli	2.6	2.8	3.1	25	2.6	2.8	3.1	25
P.aeruginosa	0.3	0.8	1.4	100	0.3	0.8	1.4	100

Antifungal Activity

Zone of Inhibition in cm								
Microorganism	**Carbon tetra chloride**				**Ethyl acetate**			
	0.5 mg	**1.0mg**	**2.0mg**	**MICmg**	**0.5 mg**	**1.0mg**	**2.0mg**	**MIC mg**
A.niger	-	-	-	>2	-	-	-	>2
C.albicans	-	-	-	>2	-	-	-	>2
	Ethanol				Methanol			
A.niger	-	-	-	>2	-	-	-	>2
C.albicans	-	-	-	>2	-	-	-	>2
Amphotericin								
	200 µg	400 µg	800 µg	MIC µg	200 µg	400 µg	800 µg	MIC µg
A.niger	0.3	0.5	0.7	100	0.3	0.5	0.7	100
C.albicans	0.9	1.3	1.5	50	0.9	1.3	1.5	50

CONCLUSION

The results of antimicrobial activity of different extracts of stem of *B.buxifolia* L. are reported for the first time. No previous report on the antimicrobial activity of this plant species could be found in the literature. In present findings both the ethanolic and methanolic extracts showed maximum antibacterial activity against all the selected bacteria, so this plant can be used to discover bioactive natural products that may serve as leads for the development of new

antibacterial drugs in the treatment of bacterial diseases such as cold, cough, chills, headache and nasal congestion.

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