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IN VITRO ANTIMICROBIAL ACTIVITY OF LEAF EXTRACTS OF *BALIOSPERMUM MONTANUM* (WILLD.) MUELL. ARG., A RED LISTED MEDICINAL PLANT IN SOUTHERN WESTERN GHATS

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The present paper reports for the first time the *in vitro* antifungal activity of the methanolic leaf extracts of *Baliospermum montanum*, a Red-listed potent medicinal plant in Southern Western Ghats of peninsular India. Screening of antibacterial activity of leaf extracts (12.8 mg/ disc) of *B. montanum* was analyzed which showed a clear zone of inhibition against the bacterial strains *viz. Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Serratia marcescens.* The extract also showed antifungal activity against the fungal strains such as *Aspergillus niger, Neurospora crassa* and *Rhizopus nigricans.* So the plant can be effectively used as an antimicrobial agent and the results of *in vitro* studies can be further extended for the better utilization of this plant.

Keywords : Antifungal activity; *Baliospermum montanum;* Leaf extract; Red listed plant.

Introduction

Baliospermum montanum (Willd.) Muell. Arg. (Family Euphorbiaceae) is an important woody Red listed medicinal plant found in Southern Western Ghats of India. The various parts of the plant (root, stem, leaves and seeds) are used by different tribal communities for the treatment of a variety of ailments. The seeds are externally used as stimulant and rubefacient. The dried roots are considered antihelmenthic, diuretic and useful in treating enlarged spleen, abdominal tumors, etc.[1]. Auxillarenic acid is present in the seeds of *B. montanum,* while 12-deoxy-5βhydrophorbol-13-myristate, 13-palmitate, 12-deoxyphorbol-13-palmitate, baliospermin and montanin are reported to be present in the roots[2]. Presence of steroids, terpenoids and flavanoids is also reported from the plant[3]. Pharmacological screening of the plant revealed its antibacterial, hepatoprotective, anticancerous, free radical scavenging, immunomodulatory and anthelmintic effects[4]. Due to indiscriminate exploitation and unscientific harvesting the plant is found to be diminishing and is put under Red list category[5]. The crude extracts of leaves evaluated for its antimicrobial potential showed significant antibacterial activity but found ineffective against the fungal strain used in an earlier study[6]. So the present investigation targets the screening of both antibacterial and antifungal activities of leaf extracts of *B. montanum.*

Material and Methods

Baliospermum montanum plants collected from the forest segments of Palode, Thiruvananthapuram maintained in the greenhouse of Department of Botany, University College, Thiruvananthapuram served as the plant mateial for the present study.

Screening of antimicrobial activity: Antibacterial activity assays: Crude extract of leaves of *B. montanum* were tested to detect their antibacterial properties against four strains of bacteria by disc diffusion method[7]. The standard strains of pathogenic and industrially important bacteria *viz. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Serratia marcescens* were selected for antibacterial studies. They were procured from Department of Microbiology, Govt. Medical College, Thiruvananthapuram.

For the preparation of the media for subculturing the bacteria, peptone water was made by dissolving peptone in distilled water (13.6 gl^{-1}). The solution was then autoclaved 121 ^{0}C and 15 lbs pressure for 20 minutes. The spores of each bacterium from the isolated colony were introduced into the media taken in test tubes. The test tubes were kept in an incubator at a temperature of 37 ^{0}C for an hour for maximum growth[8].

Pure culture of each bacterium from the peptone water was spread evenly on the nutrient agar plates with sterile swabs under highly aseptic conditions. The different bacteria were inoculated in different petridishes. Discs of 6 mm diameter were cut from Whatmann No. 1 filter paper and the sterilized discs were then soaked in leaf extract

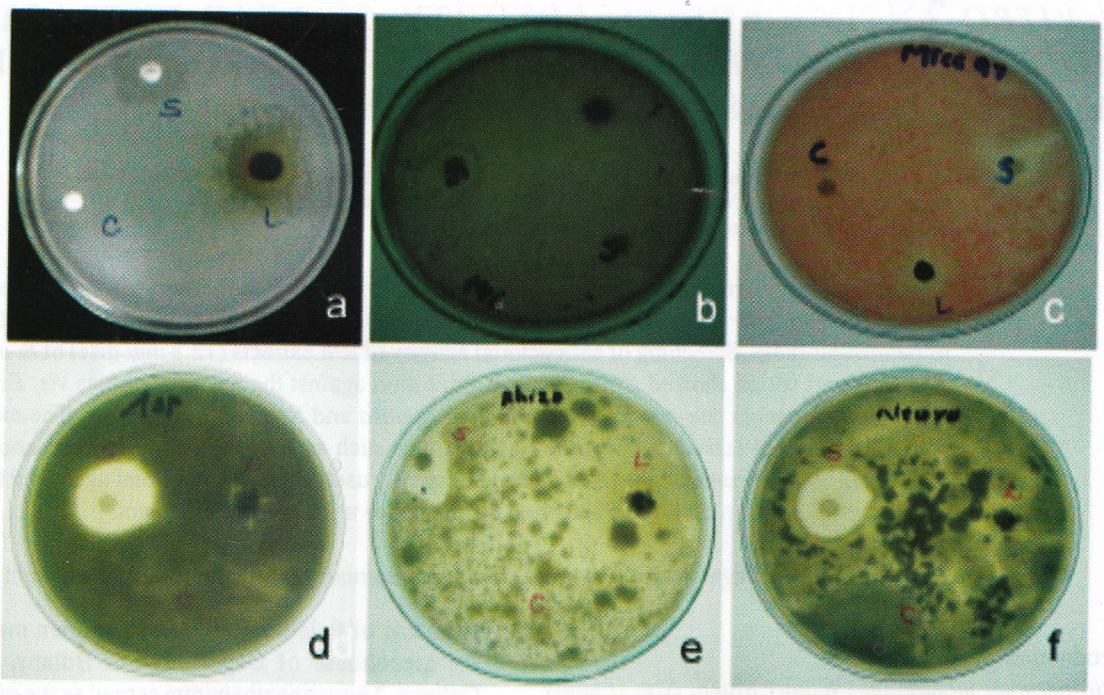

Fig.1. Antimicrobial activity of methanol extract of leaves of *B. mantanum.*
a-c : Antibacterial activity of methanol extract of leaves.; a. *Escherichia coli;* b. *Pseudomonas aeruginosa;* c. *Serratia marcescens;* d-f : Antifungal activity of methanol extract of leaves;; d. *Aspergillus niger;* e. *Rhizopus nigricans;* f. *Neurospora crassa.*
S - Standard; L - Leaf extract; C - Control.

Table 1. Antibacterial activity of methanol extract of leaves of *B. montanum.*

Test Organisms	Zone of inhibition (mm)*		
	Leaf extract	Standard	Control
Escherichia coli	16.0±0.20^{c}	24.1±0.36^{e}	NIL
Serratia marcescens	15.0±0.20^{bc}	17.2±0.32^{cd}	NIL
Pseudomonas aeruginosa	10.8±0.48^{a}	17.9±0.36^{d}	NIL
Staphylococcus aureus	9.3±0.42^{a}	20.9±0.36^{e}	NIL

Standard: Ampicillin; Control: Methanol; Leaf extract: 12.8 mg/disc
* Values are mean ± SE, means followed by the same letter in the superscript do not differ significantly based on ANOVA and t-test at $p \leq 0.05$.

Table 2. Antifungal activity of methanol extract of leaves of *B. montanum.*

Test Organisms	Zone of inhibition (mm)		
	Leaf extract	Standard	Control
Rhizopus nigricans	16.1±0.36^{c}	22.1±0.36^{c}	NIL
Neurospora crassa	12.6±0.48^{b}	20.3±0.42^{b}	NIL
Aspergillus niger	10.0±0.40^{a}	15.7±0.42^{a}	NIL

Standard: Miconazole; Control: Methanol; Leaf extract: 12.8 mg/disc
* Values are mean ± SE, means followed by the same letter in the superscript do not differ significantly based on ANOVA and t-test at $p \leq 0.05$.

till it was completely saturated. Control discs were prepared by using equal volume of solvent only[8]. Prepared disc containing the concentrated extract were placed over each bacterial colony. The inoculated petridishes were labelled and kept in an incubator at 37 °C for 24 hours. Antibacterial activity observed was recorded as zone of inhibition in millimeters.

Antifungal activity assays: The present study conducted antifungal activity studies against common phytopathogenic forms of fungi with crude leave extract in methanol at definite concentration by incorporating crude extract in the media[9]. All the fungi used here *viz. Aspergillus niger, Rhizopus nigricans* and *Neurospora crassa* were isolated from the Botanical garden of our Department.

For culturing the fungus, nutrient broth solution was made by dissolving nutrient broth (13 gl^{-1}) in double distilled water. The solution was then steam sterilized in an autoclave. The spores of each fungus from the isolated colony were introduced into the media taken in test tubes. The test tubes were kept at room temperature for 2 days for maximum growth.

Potato Dextrose Agar media was used for fungal culture. The media was prepared by dissolving Potato Dextrose Agar (39 gl^{-1}) and agar-agar (10 gl^{-1}) in distilled water followed by heating. The media was sterilized in an autoclave as described earlier. It was then cooled at 50°C and about 20 ml of molten medium was poured into sterile petridishes and allowed to solidify under aseptic conditions.

Pure cultures of each fungus from the nutrient broth solution were spread evenly on the agar plates with sterile swabs under flame and highly aseptic conditions. The fungal strains were inoculated in different petridishes. Prepared discs containing the concentrated extract were placed over each fungal colony and covered with lid. The inoculated petridishes were labelled and kept in an incubator at 37 °C for 24 hours. Antifungal activity was observed as zone of inhibition in millimetres.

Statistical analysis: The experiments consisted of ten replicates and were repeated thrice. The observations were recorded as mean ± SE. The results were analyzed by ANOVA following arc sine transformation with mean separation analysis by LSD multiple't' test.

Results and Discussion

The results of the study revealed significant antimicrobial activities from the methanolic leaf extracts of *B. montanum*.

Antibacterial activity: The crude methanolic extract of leaves of *B. montanum* was evaluated for its antimicrobial potential by disc diffusion method. The extracts (12.8 mg/disc) were screened using *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus*. Ampicilline trihydrate (1.0 $mgml^{-1}$) served as standard for the study. The extract showed significant antibacterial activity against all the four bacterial strains comparable to standards (Fig. 1a-c). Zone of inhibition in 9-24 mm was observed (Table 1). No zone of inhibition was noticed in controls. The zone of inhibition recorded in *E. coli* and *Serratia marcescens* (16.0±0.20 mm and 15.0±0.20 mm, respectively) were more compared to *Pseudomonas aeruginosa* and *Staphylococcus aureus* (10.8±0.48 mm and 9.3±0.42 mm, respectively) .

In earlier studies, the crude ethanolic extract of leaves of *B. montanum* evaluated for its antimicrobial potential by disc diffusion method showed significant antibacterial activity as similar to the observations in the present study. The various concentration (10, 20, and 40 $mgml^{-1}$) of extract prepared in DMSO were screened using *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*. Ampicilline trihydrate (1 $mgml^{-1}$) and Fluconazole (0.5 $mgml^{-1}$) served as reference control. In another report, methanol and acetone extract of aerial parts of *B. montanum* were tested for antimicrobial activity against five strains of gram positive bacteria and seven gram negative bacteria[6]. The assay was performed by agar disc diffusion method using amikacin (30 µg/disc), amphotericin-B (100 units/disc) and Fluconazole (10 µg/disc) as standard antibiotics. The activity of both the extract were found less against gram positive and negative bacteria when compared to the standard used in the bioassay. All these findings in addition to the present report suggest that the methanolic extracts of leaves of *B. montanum* is a potent antibacterial agent compared to the standard.

Antifungal activity: In the present study, crude methanol extract of *B. montanum* leaves showed inhibition of fungal growth at a concentration of 12.8 $mgml^{-1}$ against three fungal strains *viz. Aspergillus niger, Rhizopus nigricans* and *Neurospora crassa*. The controls revealed no zone of inhibition (Table 2). Better zone of inhibition was recorded in the case of *Rhizopus nigricans* (16.1±0.36 mm) followed by *Neurospora crassa* (12.6±0.48 mm) and *Aspergillus niger* (10.0±0.40 mm). The antifungal activity assays conducted in the present study showed that the leaf extract of *B. montanum* has antifungal potentialities against *Rhizopus nigricans, Aspergillus niger* and *Neurospora crassa* compared to the standards (Fig.1d-f). This is in contrast to earlier report, where the methanol and acetone extract of aerial parts of *B. montanum* tested for antifungal

activity found to be ineffective against three fungal strains used in the study[10]. However, the antifungal activity assays conducted in the present investigation shows the antifungal potentiality of the leaf extracts of *B. montanum* and is the first report regarding this aspect.

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