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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *PLECTRANTHUS BOURNEAE* GAMBLE (LAMIACEAE)

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ABSTRACT

Objective: The aim was to investigate the antibacterial activity of *Plectranthus bourneae* Gamble (Lamiaceae) using different solvents.

Methods: Petroleum ether, chloroform, acetone, ethanol and aqueous leaves extracts at different concentration (25, 50, 75, 100 µg/ml) were evaluated for antibacterial activity using disc diffusion method against some Gram-positive species namely *Bacillus subtilis, Clostridium sporogenes, Staphylococcus aureus, Streptococcus mutans, Streptococcus mitis* and Gram-negative species *Salmonella typhi, Klebsilla pneumoniae, Entrobactor aerogenes, Citrobactor freundii, Escherichia coli*. The minimum inhibitory concentration (MIC) and minimum bacterial concentrations (MBC) were determined by serial dilution method.

Results: The antibacterial activity results of ethanol extract (50 µg/ml) showed maximum zone of inhibition (26 mm) against *S. aureus* and MIC value of 0.78 µg/ml and MBC of 1.56 µg/ml whereas aqueous extract was least effective on all the strains. The Gram-positive strains of *S. aureus* and *S. mutans* were found to be the most sensitive and resistance on all the extracts. Except *S. typhi*, Gram-negative bacteria used were less inhibitory effect to all the extracts.

Conclusion: The present study concluded that the ethanolic extracts of *P. bourneae* possess potential antibacterial activities against certain microorganisms.

Keywords: *Plectranthus bourneae,* Antibacterial activity, Disc diffusion method, Lamiaceae.

INTRODUCTION

The genus *Plectranthus* L'Herit belongs to the family Lamiaceae, subfamily Nepetoidea, tribe Ocimae, subtribe Plectranthinae and belongs to phenolics, and terpenoids classes [1,2]. *Plectranthus* species as medicinal plant was mostly used to treat digestive and skin infections; *Plectranthus ambonicus* is used to treat epilepsy and convulsions Caribbean [3]. The leaf extract of *Plectranthus tenuiflorus* is used to treat ear infections [4]. Plant essential oils and their components have been known to exhibit biological activities, especially antimicrobial activities [1]. Several *Pletranthus* species like *Plectranthus fruticosus* and *Plectranthus saccatus* were responsible for antimicrobial effect [5- 7]. Phytochemical studies indicated that many species of *Plectranthus* contain diterpenoids, steroids, phenolic compound and essential oils [2]. Medicinal properties attributed to the *Plectranthus* have been investigated by pharmacological assays covering anti-inflammatory, antitumor activities, anti-*Candida* activities, and fungitoxic activities [8-10].

Plectranthus bourneae is an endemic plant species distributed only in Pambar shola of Western Ghats of Tamil Nadu India [11,12]. It is a tender, sub succulent perennial herb. Literature survey on *Plectranthus* spp. revealed some therapeutic properties, but the therapeutic properties of *P. bourneae* have not been established so far. This present study focuses on phytochemical investigation of leaves of *P. bourneae* and their effect on antibacterial property.

METHODS

Plant material

The plants of *P. bourneae* were collected from Pambar Shola (Kodaikanal hill) in the Western Ghats of Tamil Nadu. The plant identity was confirmed with Botanical Survey of India, Southern Regional Circle, Coimbatore, Tamil Nadu, India. The plants were maintained in earthen pots in the glass house of Bharathidasan University, Tiruchirappalli, India.

Preparation of solvent extracts

The plant materials are washed in running tap water and then air dried in shade, the dried leaves were coarsely powdered. 100 g coarse powder of *P. bourneae* leaves were packed muslin cloth and the extraction was carried by soxhlet extraction technique. Different solvents were used successively with gradient polarity *viz*., petroleum ether, chloroform (non polar), acetone, benzene, ethanol and water (polar). For aqueous extract, the powdered leaves were extracted with water by boiling method. The extracts were completely evaporated by vacuum distillation and stored.

Preliminary phytochemical screening

Preliminary phytochemical screening and quantification of steroids, terpenoids, reducing sugars, alkaloids, tannins, flavonoids, saponins, phenolics, amino acids, anthraquinones, were evaluated using standard method [13,14].

Test microorganism

The following bacterial strains were employed in the screening, which was obtained from Department of Biotechnology, National College, Tiruchirappalli. Gram-positive *Bacillus subtilis* (NCBT 008), *Clostridium sporogenes* (NCBT 040), *Staphylococcus aureus* (NCBT 059), *Streptococcus mutans* (NCBT 062) and *Streptococcus mitis* (NCBT 060). Gram-negative *Salmonella typhi* (NCBT 058), *Klebsilla pneumoniae* (NCBT 018), *Entrobacter aerogenes* (NCBT 018), *Citrobactor freundii* (NCBT 041), *Escherichia coli* (NCBT 001).

Antibacterial assay

Antibacterial activity was determined by disc diffusion method using Nutrient agar (Hi Media) for bacteria. The bacterial strains were inoculated in the nutrient broth under aseptic condition and incubated at 37°C for 24 hrs. After incubation period, the test bacteria were inoculated on the nutrient agar plate using sterile cotton swab. The extracts were dissolved in the solvent. Sterilized discs (Hi Media, 6 mm), loaded in various concentrations of petroleum ether, chloroform, acetone, ethanol, and aqueous extracts (25, 50, 75, 100 µg/disc) were used to assess the dose-dependent activity of the extracts. These discs were applied over each of the culture plates and antibiotic discs of streptomycin (25 µg/disc) were used as positive and negative control was prepared using respective solvents. Then, the petri dishes were incubated at 37°C for 18-24 hrs. Zones of inhibition were measured, and the mean diameter was recorded. Each was assay performed in at triplicate and mean all the three experiments were taken.

Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)

The MIC was determined by comparing the various concentrations of plant extract which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition. The serial dilution method was used to determine MIC [15]. In this case four bacterial strains (*S. aureus, S. mitis, S. mutans,* and *S. typhi*) were selected to test the MIC of the plant extract in which higher zones of inhibition was exhibited.

The *P. bourneae* crude extracts were serial diluted in the range between 0.78 and 50 µg/ml in the test tubes containing 1 ml Muller-Hinton broth. Each test tube was added with 0.5 ml of standardized suspension of bacteria. Growth of bacteria was checked after overnight incubation at 37°C. MBC is usually an extension from the MIC, where the organisms are quantitatively subcultured from MIC tubes on antibiotic free agar medium to indicate the minimum concentration was no viable organism appears in the culture.

RESULTS

The percentage of yield from each fraction of *P. bourneae* is summarized in Table 1. The maximum yield was obtained from the ethanol extracts (41%), followed by petroleum ether (10%), acetone (13%), chloroform (16%), and aqueous extracts (18%).

Table 1: Data showing the weight of crude extract and yield of the crude of *P. bourneae*

Solvent	Weight of crude extract	Percentage yield
Petroleum ether extract	1.85 g	10.40
Chloroform extract	2.90 g	16.20
Acetone extract	2.40 g	13.40
Ethanol extract	7.50 g	41.80
Aqueous extract	3.25 g	18.20

P. bourneae: Plectranthus bourneae, All percentage were calculated from 17.0 g of the extract

The preliminary phytochemical investigation of petroleum ether, acetone, chloroform, ethanol and aqueous extracts of *P. bourneae* showed the presence of steroids, flavonoids, terpenoids, reducing sugars, tannins, flavonoids, saponins, amino acids, and phenolic compounds (Table 2).

Antibacterial activity of different solvent extracts of *P. bourneae* leaves against human pathogenic bacteria, *S. typhi, K. pneumoniae, E. aerogenes, C. freundii*, *E. coli, B. subtilis, C. sporogenes, S. aureus, S. mutans* and *S. mitis* were evaluated and compared by zone of inhibition in disc diffusion method. The ethanol, chloroform, and acetone extract exhibit significant antibacterial activity. The activities of various extracts were comparable to antibacterial agent of Streptomycin. The petroleum ether and aqueous extracts exhibit less antibacterial activity compared with other extracts. The chloroform extracts exhibited maximum activities of against five of the strains; *S. aureus* (12 mm), *S. mutans* (8 mm), *S. mitis* (6 mm), *S. typhi* (6 mm), *C. freundii* (8 mm). The acetone extract of maximum activities against five of the strains*; S. aureus* (12 mm), *S. mitis* (10 mm), *B. subtilis* (10 mm), *S. typhi* (8 mm), *E. aerogenes* (8 mm). The result of antibacterial activities is presented in Tables 3 and 4.

The ethanol extract showed maximum antibacterial activity against all test strains used in the study. On the other hand, the lowest antibacterial activity showed by aqueous extracts against all test strains. Though, the extracts showed prominent antibacterial activity against Gram-positive (*C. sporogenes, S. aureus, S. mutans*) and Gram-negative (*E. aerogenes, C. freundii, S. typhi*) bacteria, among all these strains *Klebsiella* sp. appeared to be very less zone of inhibition. Among the tested plant extracts ethanolic extract showed highest activity of 26 mm (50 µl) inhibition zone against *S. aureus* this was followed by 20 mm *S. mutans,* 18 mm *B. subtilis,* 15 mm *S. mitis* and 15 mm inhibition zone against *S. typhi.* Gram-positive bacteria were more susceptible toward this extract than Gram-negative bacteria except *S. typhi* (Table 4). The *S. aureus* strain was mostly resistance to various solvents of extracts and *S. mutans* was moderately resistance. When compared to the standard antibiotic it was seen that ethanol extract was effective than streptomycin against *S. aureus* and *S. mutans* whereas this antibiotic was resistant to this bacteria. The least activity of extract is 3 mm against *E. coli* and *S. typhi*, whereas 3 mm *C. freundii* and *E. aerogenes* at 50 µl was recorded by aqueous extracts. At lower concentration of extracts showed more significant zone of inhibition and higher concentration of extracts was observed limited zone of inhibition. The MIC value was 0.78 µg/ml for *S. aureus* and MBC of 1.56 µg/ml (Table 5).

DISCUSSION

In the present investigation, different extracts of *P. bourneae* was evaluated for exploration of their antibacterial activity against certain Gram-positive and Gram-negative bacteria, which was regarded as human pathogenic microorganism. Screening of bioactive agents from

Table 2: Preliminary phytochemical screening of the various extract of *P. bourneae*

Phytochemicals	Solvents				
	Aqueous	Ethanol	Acetone	Chloroform	Petroleum ether
Steroids	+	+	-	-	+
Terpenoids	+	+	-	+	-
Reducing sugars	+	-	+	+	+
Alkaloids	-	+	-	+	-
Tannins	-	-	+	+	-
Flavonoids	-	+	-	+	-
Saponins	-	+	-	-	-
Phenolic compounds	+	+	+	-	-
Amino acids	+	-	-	-	-
Anthroquinones	-	-	-	-	-

+: Present, -: Absent, *P. bourneae: Plectranthus bourneae*

Table 3: Zone of inhibition (mm) of Gram-positive bacterial agents at various concentrations of different test extracts of *P. bourneae* and the standard streptomycin

Extracts	Concentration µg/ml	Zone of inhibition (in mm)					
		B. subtilis	*C. sprogenes*	*S. aureus*	*S. mutans*	*S. mitis*	Streptomycin (control) 25 µg/disc
Petroleum ether	25	-	-	-	-	-	20.5
	50	-	-	-	-	-	
	75	-	-	6.0	6.5	6.5	
	100	5.0	8.0	6.5	-	6.0	
Chloroform	25	-	-	-	-	-	30.0
	50	-	-	5.0	-	-	
	75	5.0	-	12.0	8.0	6.0	
	100	5.5	5.5	8.0	7.5	6.0	
Acetone	25	-	-	6.0	-	-	30.0
	50	-	-	-	-	-	
	75	10.0	6.0	6.5	7.0	10.0	
	100	-	7.5	12.0	7.5	6.5	
Ethanol	25	-	-	8.0	6.0	6.0	24.5
	50	18.0	-	26.0	20.0	15.0	
	75	8.0	10.0	18.0	12.0	-	
	100	5.0	7.0	10.0	-	-	
Aqueous	25	-	-	-	-	-	30.0
	50	-	-	-	5.0	-	
	75	-	-	6.0	-	-	
	100	5.0	-	8.0	8.0	-	

-: No zone of inhibition, *B. subtilis: Bacillus subtilis, C. sprogenes: Clostridium sporogenes, S. aureus: Staphylococcus aureus, S. mutans: Streptococcus mutans, S. mitis: Streptococcus mitis, P. bourneae: Plectranthus bourneae*

Table 4: Zone of inhibition (mm) of Gram-negative bacterial agents at various concentrations of different test extracts of *P. bourneae* and the standard streptomycin

Extracts	Concentration µg/ml	Zone of inhibition (in mm)					
		S. typhi	*K. pneumoniae*	*E. aerogenes*	*C. freundii*	*E. coli*	Streptomycin (control) 25 µg/disc
Petroleum ether	25	-	-	-	-	-	27.0
	50	4.0	-	5.5	-	-	
	75	4.0	-	8.0	5.0	-	
	100	-	-	-	-	6.5	
Chloroform	25	-	3.5	-	-	-	30.0
	50	-	4.0	8.0	-	-	
	75	6.0	4.8	5.5	-	5.0	
	100	-	-	-	8.0	5.0	
Acetone	25	-	-	-	-	-	30.0
	50	8.0	5.0	6.0	-	5.5	
	75	6.0	3.5	8.0	-	-	
	100	-	4.0	-	6.0	-	
Ethanol	25	6.0	4.0	5.0	-	5.0	30.0
	50	18.0	4.5	-	-	-	
	75	10.0	-	6.5	5.0	6.5	
	100	5.0	3.5	-	6.5	-	
Aqueous	25	-	-	-	-	-	30.0
	50	3.0	-	3.0	-	-	
	75	-	-	-	-	3.0	
	100	-	-	-	3.0	-	

-: No zone of inhibition, *S. typhi: Salmonella typhi, K. pneumonia: Klebsilla pneumoniae, E. aerogenes: Entrobactor aerogenes, C. freundii: Citrobactor freundii, E. coli: Escherichia coli, P. bourneae: Plectranthus bourneae*

Table 5: MIC and MBC values of ethanolic extract of *P. bourneae* for bacterial strains

Microorganism	MIC (µg/ml)	MBC (µg/ml)
S. aureus	0.78	1.56
S. mutans	6.25	25.0
S. mitis	12.5	50.0
S. typhi	25.0	>50.0

P. bourneae: Plectranthus bourneae, MIC: Minimum inhibitory concentration, MBC: Minimum bacterial concentration, *S. aureus: Staphylococcus aureus, S. mutans: Streptococcus mutans, S. mitis: Streptococcus mitis, S. typhi: Salmonella typhi*

plants is one of the most intensive areas of natural productive research today, yet the field is far from exhausted. Only 10% of all the plants have been investigated in detail for bioactive agents [16]. A significant proportion of pharmaceutical products in current use are designed from plants [17,18]. The preliminary investigation of this study showed all that extracts of petroleum ether, acetone, chloroform, ethanol, and aqueous (water) are active against human pathogens like Gram-positive and Gram-negative bacteria. The medicinal properties of the plant could be attributed to the presence of one or more of the detected plant natural products [19,20]. The present finding reveals that petroleum ether, chloroform and ethanol extracts were positive for steroidal compounds, which are known to be important in pharmacy for sex hormones [21]. The alkaloids and saponins were observed in ethanol, chloroform and aqueous extracts, which compounds significant for the treatment of syphilis and other venereal diseases [22]. Flavonoids were observed in ethanol extract which has contained antioxidant properties.

The terpenoids are considerable to be the significant compound for the antimicrobial and antioxidant activities observed by many *Plectranthus* species [23]. Aqueous, ethanol and chloroform extracts of *P. bourneae* contain terpenoids compound. The inhibitory effect of *P. bourneae* ethanolic leaf extract, particularly *S. aureus* showed the fact that this plant may be effective in scalp and skin disorders, which are frequently caused by *S. aureus*.

From the result obtained, it shows that the antibacterial activity of all the extracts is more significant activity on Gram-positive strains, because Gram-negative bacteria were reported to be less susceptible to the action of antibacterial activity, since they possess an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [24,25]. The aqueous extract showed very less zone of inhibition and some pathogen did not inhibit any of the test concentration. Similar result was reported earlier for this aqueous extract [17]. The ethanolic extract of *P. bourneae* showed significant antibacterial activity against all microorganisms. It is found that various solvents depending upon the polarity and solubility toward various phytoconstituents they can be extracted [26]. Ethanol extract obtained in this study might have higher solubility for more of active antibacterial phytoconstituents, consequently displaying the highest relative antibacterial activity. The MIC values of the plant extract obtained in this study were lower than MBC values (Table 5). These results are first of its kind regarding the antibacterial properties of *P. bourneae,* an endemic species of the Western Ghats.

CONCLUSIONS

In present study crude leaf extract of *P. bourneae* plant material was tested with polar and non polar organic solvent against ten bacteria strains. All the extracts have significant antibacterial activity on most of the bacteria tested in this study. Ethanol extract had maximum inhibition activity as compared to chloroform, acetone, petroleum ether and aqueous extract. The crude extract of the leaves are rich in phytochemicals and secondary metabolites such as steroids, alkaloids, terpenoids, flavonoids and tannins, these compounds may have direct interaction with the bacterial strains as antibacterial substances. Further studies are necessary to evaluate the safety of the herb for pharmaceutical applications.

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