ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antibacterial Activity and Phytochemical Analysis of the Extracts of *Melastomamalabathricum* and *Holigarnaarnottiana*, Important Medicinal Plants of Western Ghats of Karnataka, India.

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ABSTRACT

Study was undertaken with the aim of identifying the antibacterial active compound of *Melastomamalabathricum* and *Holigarnaarnottiana*, the important medicinal plants of Western Ghats of Karnataka, India. Shade dried and powdered plant materials were subjected to polarity based successive solvent extraction with petroleum ether, chloroform, ethyl acetate, methanol and water. Known concentration of the extracts were tested against *Bacillus cereus* (MTCC1272), *Escherichia coli*(MTCC7410), *Klebsiellapneumoniae* (MTCC7407), *Pseudomonas aeruginosa* (MTCC424) and *Staphylococcus aureus* (MTCC7443). The bioactive extracts were subjected to phytochemical analysis to identify the active compounds. Ethyl acetate and methanol extracts of *H.arnottiana* and the methanol extract of *M.malabathricum* showed broad spectrum antibacterial activity. Phytochemical analysis of the active extract revealed the presence of alkaloids, flavonoids, terpenoids and tannins. The results justify the use of these plants by herbal healers and suggested for further work on the isolation and characterization of bioactive molecules.

Keywords: Antibacterial activity, Phytochemicals, human pathogenic bacteria, *Melastomamalabathricum*, *Holigarnaarnottiana*

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ISSN: 0975-8585

INTRODUCTION

Medicinal plants have been the mainstay of human healthcare since the dawn of civilization. Large section of the Indian population relies on medicinal plants for their primary healthcare [1]. Systematic study of the traditional medicine systems of various indigenous communities has provided new lead molecules for the cure of many human ailments [2].

India is blessed with a huge wealth of medicinal plants and a widely practiced tradition of herbal medicine. The Western Ghats of India are a treasure trove of medicinal plants, most of which are used in the traditional medicine of the local population [3]. It is home to about 500 medicinal plants of which many are endemic to the region[4]. However scientific validation of the curative properties of many plants of this region is yet to be done.

Human pathogenic bacteria are the cause of many infectious diseases like food poisoning, urinary tract infection, gastroenteritis, meningitis, hemolytic-uremic syndrome, pneumonia, respiratory disorders, tuberculosis, wound infection, secondary infections in immunocompromised individuals and sexually transmitted diseases. Although an array of antibiotics is available for their treatment, the problem of antibiotic resistance has effectively limited the treatment options. Thus it is necessary to discover new antibiotics to stem the tide of antibiotic resistance in bacteria [5].

Secondary metabolites of plants are an alternative to synthetic chemistry and microbial origin antibiotics. Screening of plants used in ethnomedicine for the cure of infectious diseases is considered as a suitable strategy as they are known to possess medicinal properties and are regarded as safe [6]. The first plant based antibacterial drug to be extensively studied was allicin isolated form *Aliumsativum*[7]. Since then a large number of medicinal plants have been studied for their antibacterial properties world over [8]. However, a large number of medicinal plants used by various local communities of the Western Ghats remain to be scientifically evaluated for antibacterial properties. Two such medicinal plants *Melastomamalabathricum* and *Holigarnaarnottiana* have been selected for this study.

MATERIALS AND METHODS

Test plants

Melastomamalabathricum L. (Melastomataceae) is an erect bristly shrub with elliptic leaves arranged oppositely. Flowers are large, red-purple in colour, terminal and solitary, pentamerous with ovoid calyx tube covered with paleacous scales. Stamens are 10 in number with alternately long and short filaments arranged opposite to the calyx lobes and have purple anthers[9]. Its leaves are used to treat dysentery, diarrhoea, piles, gastric ulcers, scar, pimple, and black spots on skin[10].

Holigarnaarnottiana (Anacardiaceae) is a large tree (up to 35m tall) with finely fissured bark and alternately arranged simple leaves which are clustered at twig ends. Its flowers are small and found in axillary and terminal panicles, polygamous and greenish white in colour. The fruit is a drupe[9]. The leaves and bark are used to treat fever, cough, rheumatism, dysentery and skin diseases[11].

Plant collection

Holigarnaarnottiana and *Melastomamalabathricum* were collected from the Western Ghats in Sringeritaluk (13.42°N 75.25°E), Chikkamagalur district, Karnataka State, India. The leaves were collected during February-March and November. The identification of the plant was authenticated by expert taxonomist of the Department of Studies in Botany, University of Mysore. The herbarium specimens of the plants were deposited in the herbarium of Department of Studies in Botany, University of Mysore. The leaves of the test plants were washed in running tap water and then with distilled water, shade dried at room temperature, powdered and stored in airtight containers until further use.

Extraction

The powdered plant material was sequentially extracted with petroleum ether, chloroform, ethyl acetate, methanol and water in a Soxhlet apparatus. The extracts were dried and stored at 4˚C until further use [12].

Test microorganisms

Bacillus cereus (MTCC1272), *Escherichiacoli* (MTCC7410), *Pseudomonasaeruginosa* (MTCC424), *Staphylococcusaureus* (MTCC7443) and *Klebsiellapneumoniae* (MTCC7407) were the test bacteria. The stock cultures were maintained on Nutrient agar medium.

Antibacterial activity assay

Test extracts were prepared by dissolving the dried extract in their respective extraction solvents at a concentration of 0.1g/ml. Mueller Hinton agar was used as the test medium. Suspensions of the test bacteria in normal saline were prepared and their turbidity adjusted to 0.5 McFarland (1x106cells/ml). The bacterial suspension was swabbed on the agar surface with a sterile cotton swab. Filter paper disc (6mm) loaded with the extract (5mg) was placed on the inoculated plate. The solvent used for extraction served as the negative control (50µl/disc). Streptomycin (10µg) served as the positive control [13].

Determination of the Minimal Inhibitory Concentrations (MIC)

MIC of the bioactive extracts was determined by two fold serial dilutions in 96 well plates. Mueller Hinton Broth was used as the test medium. The concentrations of the extracts tested ranged from 100mg/ml to 10µg/ml. Streptomycin (10mg/ml to 1µg/ml) was used as the positive control. Bacterial suspension of turbidity equivalent to 0.5 McFarland was used as the test inoculum. TTC (2,3,5-Triphenyl-2H-tetrazolium chloride) was added to all the wells after incubation for 24h at 35±2˚C. The first well along the dilution gradient showing colour change to pink/red was taken as the MIC of the extract [14].

Phytochemical analysis

The extracts which possessed antibacterial activity were subjected to preliminary phytochemical analysis by the methods of Harborne[15]. Thetest included were for the detection of alkaloids, triterpenoids, steroids, saponins, flavonoids, tannins, glycosides, cardiac glycosides and anthraquinones.

Statistical analysis

The results were subjected to One-way ANOVA and Tukey's HSD analysis by the software SPSS ver.20.

RESULTS

The antibacterial activity of *M. malabathricum* and *H. arnottiana* are presented in table-1. Methanol extract of *M. malabathricum*was active against four of the test bacteria with zone of inhibition ranging between 12 and 15mm. The ethyl acetate and methanol extracts of *H. arnottiana* also inhibited four of the test bacteria with the zone of inhibition ranging between 10.6 and 17.6mm. *E. coli* was not inhibited by any of the test extracts. Petroleum ether, chloroform and aqueous extracts of both the plants did not show inhibitory activity. The largest zone of inhibition (17.6mm) was recorded against *B.cereus*by the ethyl acetate extract of *H.arnottiana*. Statistical analysis of the results revealed that the activity of the extracts against *P. aeruginosa*was significant and was comparable with that of Streptomycin ($P<0.05$).

Negative controls did not inhibit the test bacteria. Streptomycin (10µg) inhibited the test bacteria and the zones of inhibition ranged between 17 and 25mm.

Table-2 presents the MIC of all the three active extracts *viz.* methanol extract of *M. malabathricum*, ethyl acetate and methanol extracts of *H. arnottiana*. Methanol extract of *M. malabathricum*recorded highest inhibitory activity against *Staph*. *aureus* with an MIC of 48.82µg/ml. Ethyl acetate extract of *H. arnottiana*

showed significant inhibitory activity (97.65µg/ml) against *Staph. aureus* and *Kleb. pneumoniae*. Moderate inhibitory activity (195.31µg/ml) was observed in ethyl acetate extract of *H. arnottiana* against *B. cereus*, *P. aeruginosa*. Methanol extract of *H. arnottiana* also showed moderate inhibitory activity against *B. cereus* and *Kleb. pneumoniae*.

Table 1: Antibacterial activity of solvent extracts of *Melastomamalabathricum*and *Holigarnaarnottiana*

Plant name / Positive control	Solvent	*Bacillus cereus*	*Escherichia Coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	Negative Control
		Zone of inhibition (mm)					
Melastoma malabathricum	Petroleum ether	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
	Chloroform	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
	Ethyl acetate	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
	Methanol	15± 0.0[b]	0± 0.0[a]	12± 0.0[b]	15± 0.0[b]	14± 0.0[b]	0± 0.0[a]
	Aqueous	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
Holigarna arnottiana	Petroleum ether	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
	Chloroform	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
	Ethyl acetate	17.6± 1.3[b]	0± 0.0[a]	10.6± 4.2[b]	15.3± 0.9[b]	14± 0.4[b]	0± 0.0[a]
	Methanol	17.3± 0.9[b]	0± 0.0[a]	15 ± 0.0[b]	15 ± 1.75[b]	16 ± 0.94[b]	0± 0.0[a]
	Aqueous	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]	0± 0.0[a]
Streptomycin	Aqueous	25± 0.0[c]	20± 0.0[b]	17± 0.0[b]	20± 0.0[c]	22± 0.0[c]	0± 0.0[a]

Note: The values are mean of three replicates in mm ± Standard Error. The values with different superscript are significantly different from one another at P<0.05 (Tukey's HSD analysis).

Table 2: Minimal Inhibitory Concentrations of the active extracts of *Melastomamalabathricum*and *Holigarnaarnottiana*

Bacteria	MIC value (µg/ml)			
	Melastomamalabathricum	*Holigarnaarnottiana*		Streptomycin
	Methanol	Ethyl acetate	Methanol	Aqueous
Bacillus cereus (MTCC1272)	585.93	195.31	195.31	3.00
Pseudomonas aeruginosa (MTCC424)	585.93	195.31	12500.00	3.00
Staphylococcus aureus (MTCC7443)	48.82	97.65	6250.00	3.00
Klebsiellapneumoniae (MTCC7407)	390.62	97.65	195.31	3.00

Note: The values are the mean of three replicates

The phytochemical analysis (Table-3) revealed the presence of triterpenes, saponins, flavonoids and cardiac glycosides in the methanol extracts of *M. malabathricum* and *H. arnottiana*. The ethyl acetate extracts of *H. arnottiana*was found to be comprised of steroids, flavonoids and tannins.

Table 3: Phytochemical analysis of the active extracts of *Melastomamalabathricum*and *Holigarnaarnottiana*

Phytochemical	Plant name and extract		
	Melastomamalabathricum	*Holigarnaarnottiana*	
	Methanol	Ethyl acetate	Methanol
Alkaloids	-	-	-
Triterpenes	+	-	+
Steroids	-	+	-
Saponins	+	-	+
Flavonoids	+	+	+
Tannins	-	+	-
Glycosides	-	-	-
Cardiac Glycosides	+	-	+
Anthraquinones	-	-	-

(-) absent; (+) present.

DISCUSSION

Ravi and Saj[16] have assessed the antioxidant potential of the bark of *H. arnottiana* and have reported that its ethanol extract has highest antioxidant activity. Further the GC-MS based phytochemical investigation of the bark extract has revealed the presence of 20 major compounds and the major constituent was identified to betetradecene. Other compounds reported by them include alkene hydrocarbons (6 compounds), aliphatichydrocarbons (1 compound), esters (2 compounds) fatty acid (2 compounds)and 1, 2 dihydroxy benzene which is the allergenic compound Urushiol[17].The antibacterial potential of the leaves of

H.arnottiana against a number of shrimp pathogenic bacteria were evaluated by Manilal and Idhayadhulla[18] and have reported that the ethyl acetate extract is active against all the test bacteria. Further, the GC-MS analysis of the ethyl extract revealed the presence of 3,7,11,15- tetramethyl- 2- hexadecen-1-o1 (42.1%) and 1-iodo-2- methylundecane (34.5%) followed by squalene (11.1%), vitamin E (8.5%) and heptadecane, 2,6,10,14-tetramethyl (3.7%)[18].

The results of the present study reveal that ethyl acetate and methanol extracts of the leaves of *H. arnottiana* show broad spectrum antibacterial activity. Much of the earlier work related to the biological activity studies of *H. arnottiana* are on the antioxidant activity and on the antibacterial activity against shrimp pathogenic bacteria. In the present study diverse range of human pathogenic bacteria were evaluated for their sensitivity to the solvent extracts. The results have revealed that ethyl acetate extract is highly potent in inhibiting four of the test bacteria suggesting broad spectrum antibacterial activity. Based on the phytochemical analysis and the antibacterial activity assay it can be concluded that flavonoids could be the possible antibacterial compound. The results of the present studysuggest that *H. arnottiana* is an important candidate plant for further work on isolation and characterization of the active principle.

Joffry*et al*[10] have compiled a comprehensive review on the ethnomedicinal uses, chemical constituents and pharmacological properties of *M. malabathricum*. The antibacterial activities of the various parts of the shrub have been reported earlier [19-21]. The present study has reported significant antibacterial activity in the methanol extract of the leaves of *M. malabathricum*, as reported by Grosvenor *et al*[19], Wiart*et al*[20] and Maji*et al*[21].

*P. aeruginosa*is a pathogen commonly implicated in nosocomial infections and is inherently resistant to most first line antibiotics. Identification of the active compounds present in methanol extract of *M. malabathricum* and ethyl acetate and methanol extract of *H. arnottiana* could provide new lead molecule(s) active against this pathogen. The activity of these extracts against *B. cereus*, *Staph. aureus* and *Kleb. pneumoniae* indicate that these extracts could be used in the treatment of diseases caused by these bacteria such as food poisoning, skin diseases, pneumonia, fever, dysentery etc.

The phytochemical analysis of the active extracts reveals that flavonoids are the common group in all the three extracts. Many flavanoids with antibacterial activity have been isolated from various plant sources by earlier workers [21]. The presence of triterpenoids, steroids and saponins which belong to the broad group terpenoids, may also be responsible for the antibacterial activity as these molecules are known for membrane disruption property [8]. Based on the earlier reports and the present study, we propose that the active principle in these extracts could be either flavonoids or terpenoids. However, previous studies of the phytochemical composition of these plants have not reported similar compounds. Hence, these extracts have to be subjected to activity guided separation to detect and characterize the antibacterial bioactive molecules.

We conclude that the ethyl acetate extract of *H. arnottiana*is the most potent extract followed by the methanol extracts of *M. malabathricum*and *H. arnottiana*. The findings of the study have validated the use of these plants by herbal healers to treat bacterial infections. The active extracts are a good source for the isolation of bioactive molecules which could provide new leads.

ACKNOWLEDGEMENTS

The authors wish to thank theInstitution of Excellence Program (MHRD), University of Mysore for financial assistance.

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