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PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY OF AN ETHNOMEDICINAL PLANT *MALLOTUS PHILIPPENSIS* (LAM.) MUELL. ARG. VAR. *PHILIPPENSIS* (EUPHORBIACEAE)

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A potential Indian ethnomedicinal plant, *Mallotus philippensis* (Lam.) Muell. Arg., var. *philippensis* was selected and the roots were analyzed for preliminary phytochemical screening and antimicrobial investigations. Preliminary phytochemical screening revealed the presence of the bioactive compounds such as carbohydrates, flavonoids, gums, oils and resins, phenolic groups, steroids, tannins and terpenoids in root. Analysis of antimicrobial activity of hexane, chloroform and ethanol extracts of root showed significant antimicrobial activity against the tested microorganisms. Moreover, hexane and ethanol extracts exhibited maximum activity against the tested human pathogens. The Bioactive compounds responsible for these antimicrobial activities should be isolated and identified to develop a new drug of pharmaceutical interest.

Keywords : Antimicrobial activity; Drug discovery; Ethnomedicine; Human pathogens; *Mallotus philippensis* var. *Philippensis;* Phytochemistry.

Introduction

Medicinal plants have always had an important place in the therapeutic armory of mankind. According to WHO[1], 80% of world populations rely on medicinal plants for their primary health care needs. Out of the 3, 50,000 plant species known so far, about 35,000 (some estimate up to 70,000) are used worldwide for medicinal purposes and less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential[2,3]. This green inheritance thus represents an enormous reservoir of putative lead compounds to be discovered for various diseases. At least 25% of the prescription drugs issued in the USA and Canada contain bioactive compounds that are derived from or modeled after plant natural products[4]. Medicinal plants would be the best source to obtain a variety of drugs and therefore such plants should be investigated to understand better about their properties, safety and efficacy[5]. Medicinal plants are major sources of obtaining antimicrobial drugs[6].

The genus *Mallotus* Lour., (Euphorbiaceae) comprises of about 150 species in the world, of which 20 species has been reported from India[7] and 11 species with 2 varieties are reported from Tamil Nadu state alone[8]. Medicinally, the root is used for skin diseases[9], rheumatism[10], tonic, spermatorrhea, bleeding and purgative[11]. The chemical constituent hydrocyanic acid was reported from root[10].

After scrutiny of published literature, so far no sufficient work has been done on antimicrobial activity of root extracts. The active principles of many drugs found in plants are secondary metabolites[12]. Hence the phytochemical and pharmacological investigations are very important for drug discovery. Hence in the present study, the preliminary phytochemical screening studies and antimicrobial activities against various human pathogens were studied.

Material and Methods

Collection of Plant Materials: The roots of *Mallotus philippensis* (Lam.) Muell, Arg. var. *philippensis* were collected from Marakanam Reserve forest near Pondicherry. The botanical identity was confirmed by comparing the fresh specimens with herbarium specimens at French Institute Herbarium, Pondicherry. The dry specimens were preserved at Bio-Science Research Foundation, Pondicherry for reference (Voucher no. ACTDKVJ42).

Preparation of the Extracts : The roots were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with organic solvents such as hexane,

chloroform and ethanol by Soxhlet method. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in vacuo and stored at 4°C. They were used for preliminary phytochemical screening and antimicrobial activity. The graded concentrations (100, 50, 25 and 12.5mg/ml) of different extracts were prepared for the bioassay.

Phytochemical Screening :Phytochemical screening of the different plant extracts was performed using the methods described[13, 14].

Antimicrobial activity

Test Organisms : All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh. These microbes include the Gram-negative bacteria, *viz. Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451) and *V. vulnificus* (MTCC 1145); the Gram-positive bacteria, viz *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Streptococcus pneumoniae* (MTCC 655) and *fungi viz., Aspergillus flavus* (MTCC 277), *A. fumigatus* (MTCC 343), *A. niger* (MTCC 1344) and *Candida albicans* (MTCC 227), respectively.

Bioassay for antimicrobial activity : Agar well-diffusion method15 was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8h old - broth culture of respective bacteria and fungi. Two wells (8mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

Results and Discussion

Preliminary phytochemical screening : The results of Phytochemical screening revealed the presence of flavonoids, phenolic groups, steroids, tannins, and terpenoids in all the three tested extracts. Carbohydrates and gums, oils & resins are present only in ethanolic extract. Alkaloids, amino acids, anthraquinones, catechins, coumarins, proteins, quinones and saponins are absent in all the three extracts (Table 1).

Antimicrobial activity : The results of antimicrobial activity of various extracts of the root showed concentration-dependent activity against all the tested bacteria and fungi and the zone of inhibition ranged from 12 to 31mm at various concentrations (Table 2). In hexane extract, the zone of inhibition ranged from 12 to 25mm against gram positive bacteria such as *B. subtilis* and *S. pneumoniae* and gram-negative bacteria such as *E. coli, P. vulgaris, S. typhi* and *V. parahaemolyticus* at various concentration. No activity observed against the fungi. In chloroform extract, the zone of inhibition ranged from 18 to 24mm against *B. subtilis* only. No activity observed against gram negative bacteria and fungi. In ethanol extract, the zone of inhibition ranged from 13 to 30 mm against gram positive bacteria, 15 to 31 mm against gram negative bacteria and 12 to 27 mm against fungi, respectively. Regarding antimicrobial activity, it was found that the ethanol extracts of roots exhibited maximum antimicrobial activity against the tested human pathogens and it may be due to the presence of secondary metabolites such as phenolic groups and steroids as reported[16,17]. The significant activity of the results against the fungus, *Candida albicans* provides additional confirmation to the phenolic compounds and steroidal compounds which are more effective in higher concentration inhibited the growth of all fungi[18,19]. Even in hospitals, majority of disinfectants such as phenols, lysol, cresols used are belonging to phenolic groups. At the same time, the present findings of antimicrobial activity against *S. pneumoniae, E. coli, P. aeruginosa, P. vulgaris, S. typhi, V. parahaemolyticus and V. vulnificus* revealed the medicinal potential value of ethanol extracts against pneumonial fever, septicaemia, ear infections caused by *S. pneumoniae*, urinary tract infections, abdominal pain, diarrhea, vomiting caused by *E. coli,* urinary tract infections, hospital-acquired wound infections, septicaemia caused by *P. vulgaris* and *P. aeruginosa,* the typhoid fever caused by S. typhi and diarrheal infections caused by *Vibrio* species, aspergillosis, otomycosis, caused by *Aspergillus* species and candidiasis, meningitis, skin diseases caused by *C. albicans,* respectively.

Thus from our findings, it was concluded that the bioactive principles responsible for the antimicrobial activities against these tested microorganisms should be isolated identified and elucidated its structure to develop a new lead of therapeutic interest to cure various human ailments.

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Table 1. Preliminary phytochemical screening of various extracts on roots of *Mallotus philippensis* var. *philippensis*.

Root extracts	Phytoconstituents Hexane	Chloroform	Ethanol
Alkaloids	-	-	-
Amino acids	-	-	-
Anthraquinones	-	-	-
Carbohydrates	-	-	+
Catechins	-	-	-
Coumarins	-	-	-
Flavonoids	+	+	+
Gums, oils and resins	-	-	+
Proteins	-	-	-
Phenolic groups	+	+	+
Quinones	-	-	-
Saponins	-	-	-
Steroids	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+

+ = present, - = absent

Table 2. Antibacterial activity of various extracts on roots of *Mallotus philippensis* var. *philippensis* aginst various microorganisms.

Tested Microorganisms	Hexane (mg/ml)				Chloroform extract (mg/ml)				Ethanol extract (mg/ml)			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
Gram positive bacteria												
B. subtilis	18	15	13	12	24	22	20	18	30	27	23	22
S. aureus	-	-	-	-	-	-	-	-	19	16	14	13
S. pneumoniae	16	15	14	12	-	-	-	-	25	24	20	20
Gram negative bacteria												
E. coli	24	22	20	19	-	-	-	-	31	29	27	26
P. aeruginosa	-	-	-	-	-	-	-	-	25	22	19	15
P. vulgaris	25	23	20	19	-	-	-	-	26	25	21	20
S. typhi	21	19	18	15	-	-	-	-	31	29	28	26
V. parahaemolyticus	25	23	20	20	-	-	-	-	28	26	24	22
V. vulnificus	-	-	-	-	-	-	-	-	28	26	24	23
Fungal pathogens												
A. flavus	-	-	-	-	-	-	-	-	22	21	19	18
A. fumicatus	-	-	-	-	-	-	-	-	24	22	21	20
A. niger	-	-	-	-	-	-	-	-	27	26	23	21
Candida albicans	-	-	-	-	-	-	-	-	19	18	15	12

(Measurement indicates the zone of inhibition in mm).

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References

1. WHO 2001, *General guidelines for methodologies on research and evaluation of Traditional Medicine,* World Health Organisation, Geneva, pp 1-74.
2. Hostettmann K and Marston A 2002, Twenty years of research into medicinal plants: Results and perspectives. *Phytochem. Rev.* **1** 275-285
3. Shasany AK, Shukla SPS and Khanuja 2007, *Medicinal and Aromatic Plants.* (ed.) Kole C, Springer-Verlag Berlin Heidelberg, Germany.
4. Farnsworth NR 1984, *The role of medicinal plants in drug development.* (eds) Krogsgaard-Larsen P, Christensen SB and Koford H,. Balliere, Tindall and Cox. London, pp 8-98.
5. Nascimento GGF, Lacatelli J, Freitas PC and Silva GL 2000, Antibacterial activity of plant extracts and Phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.* **3** 886-891
6. Sofowora A 1986, *Medicinal plant and traditional medicine in Africa II,* John Wiley Chiechester, p 178.
7. Santana H and Henry AN 1973, *A Dictionary of Flowering Plants in India.* Council of Scientific and Industrial Research, New Delhi, pp 1-198.
8. Henry AN, Kumari GR and ChitraV 1987, *Flora of Tamil Nadu, India.* Series **1** Analysis. Botanical survey of India, Coimbatore, Vol. **2**, pp. 1-260.
9. Khan IA and Khanum A 2005, *Ethnomedicine and Human Welfare.* Ukaaz Publications, Hydcrabad, p 68.
10. Kirtikar KR, Basu BD and An ICS 1987, *Indian Medicinal Plants.* (eds.) Blatter E, Caius JF and Mhaskar KS, International Book Distributors, Dehra Dun vol. 4, pp 2266-2270.
11. Kadavul K and Dixit AK 2009, Ethnomedicinal studies of the woody species of kalrayan and Shervarayan hills, Eastern ghtas, *Tamil Nadu. Indian J. Traditional Knowledge* **8** 592-597
12. Cragg GM and Newman DJ 2005, Biodiversity: A continuing source of novel drug leads. *Pure. Appl. Chem.* **77** 7-24
13. Harborne JB 1998, *Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis.* Chapman and Hall, London, pp 1-288.
14. Trease GE and Evans WC 1989, *Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine.* Bailliere Tindall, London, pp 1-832.
15. Perez C, Paul M and Bazerque P 1990, Antibiotic assay by agar-well diffusion method. *Acta Biol. Med. Exp.* **15** 113-115
16. Pereira AP, Ferriera ICFR, Marcelino F, Valentao P, Andrade PB, Seabra R, Estevinho L, Bento A and Pereira JA 2007, Phenolic Compounds and antimicrobial activity of Olive *(Olea europaea* L. cv. *cobrancosa)* leaves. *Molecules* **12** 1153-1162
17. Lauro Figueroa V, Guillermo Ceballos R, Cedillo FD, López MDC R, Rosa MA, Magaña E and Fuentes LVG 2008, Evaluation and characterization of antimicrobial properties of pregnenolone-derivatives on *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli. Microbiol.* **50** 13-18
18. Winkelhausen E, Pospiech R and Laufenbera G 2005, Antifungal activity of phenolic compounds extracted from dried Olive Pomace. *Bull. Chem. Tech., Macedonia* **24** 41-46
19. Subhisha S and Subramoniam A 2005, Antifungal activities of a steroid from *Pallavacinia lyellii,* a liverwort. *Indian J. Pharmacol.* **37** 304-308