



PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF THE STEM OF *MALLOTUS PHILIPPENSIS* (LAM.) MUELL. ARG. VAR. *PHILIPPENSIS* (EUPHORBIACEAE)

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ABSTRACT

An ethnomedicinal plant, *Mallotus philippensis* (Lam.) Muell. Arg., var. *philippensis* was analyzed for chemical composition and antimicrobial activity. Preliminary phytochemical screening of various extracts of the stem revealed the presence of various classes of compounds such as amino acids, carbohydrates, flavonoids, gum, oil & resins, proteins, phenolic groups, saponins, steroids, tannins and terpenoids. Gas Chromatography-Mass Spectrometry (GC-MS) analysis showed the presence of ten compounds in ethanol extract. Bioassay of antimicrobial activity of hexane, chloroform and ethanol leaf extract showed significant activity against the human pathogens such as *Streptococcus pneumoniae* causing brain abscesses, pneumonia and septic arthritis, *Proteus vulgaris*, *Pseudomonas aeruginosa* causing urinary tract infections and septicemia, *Salmonella typhi* causing typhoid fever, *Vibrio* species causing diarrheal infections and the fungus *Candida albicans*. The antimicrobial activity of the hexane, chloroform and ethanolic stem extract showed concentration-dependent activity against all the tested bacteria with the zone of inhibition ranged from 12-26mm at various concentrations. But only the ethanol extract showed antimicrobial activity against the fungi *A. flavus* and *C. albicans* with the zone of inhibition ranged from 16-22mm at various concentrations.

Keywords: Ethnomedicine, *Mallotus philippensis* var. *philippensis*, phytochemistry, antimicrobial activity, human pathogens, drug development

INTRODUCTION

Medicinal plants have always had an important place in the therapeutic armory of mankind. According to WHO ¹, 80% of world population rely on medicinal plants for their primary health care needs. Out of the 350,000 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 products⁴. 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 efficacy⁵. Medicinal plants are major sources of obtaining antimicrobial drugs⁶.

The genus *Mallotus* Lour., (Euphorbiaceae) comprises of about 150 species in the world, of which 20 species has been reported from India⁷ and 11 species with 2 varieties are reported from Tamil Nadu state alone⁸. An ethnomedicinal plant, *Mallotus philippensis* (Lam.) Muell. Arg., var. *philippensis* locally known as *Kapilapodi* has been used medicinally for long time throughout India. Medicinally, the bark decoction is used for typhoid and meningitis⁹, stomach disorders such as diarrhea, dysentery, worms^{10,11} and stomachic effect¹². The chemical constituents such as betulin, friedelin, kamaladiol-3-acetate, lupeol, tannic acid, 3-hydroxy-D-A-friedoolean-3-en-2-one, 2 β -hydroxy-D-A-friedooleanan-3-one and 3 α -hydroxy-D-A-friedooleanan-2-one, were reported from the stem bark^{13,14,15,16}. Only one biological activity such an anti-tumour activity was reported for the compound 3 α -Hydroxy-D: A-friedooleanan-2-one isolated from the Stem Bark¹⁴.

After scrutiny of published literature, so far no sufficient work has been done regarding the antimicrobial activity on this selected plant. The active principles of many drugs found in plants are secondary metabolites¹⁷. Hence the basic phytochemical investigation on the extracts for their main phytochemicals is very vital. Hence in the present study the hexane, chloroform and ethanol extracts of stem of *M. philippensis* var. *philippensis* were screened for phytochemical constituents and the antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Plant material and preparation of the Extracts

The stem of *Mallotus philippensis* (Lam.) Muell, Arg. var. *philippensis* was collected from Marakanam Reserve forest near Pondicherry. Its identity was confirmed by comparing the fresh specimens with herbarium specimens at French Institute Herbarium, Pondicherry. The dry specimens were preserved at Bio-sciences Techno Park, Pondicherry for further reference (Voucher no. ACTDKVJ42).

The stem was chopped into small pieces, shade-dried and coarsely powdered by using a pulverizer. 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 analysis of the different plant extracts was performed using the methods described^{18,19}.

Gas chromatography-Mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC Clarus 500 Perkin Elmer equipment. Compounds were separated on Elite-1 capillary column (100% Dimethylpolysiloxane). Oven temperature was programmed as follows: isothermal temperature at 50°C for 2 min, then increased to 200°C at the rate of 10°C/min, then increased up to 280°C at the rate of 5°C/min held for 9 min. Ionization of the sample components was performed in the EI mode (70 eV). The carrier gas was helium (1ml/min) and the sample injected was 2 μ l. The detector was Mass detector turbo mass gold-Perkin Elmer. The total running time for GC was 36 min and software used was Turbomass 5.2. The individual constituents were identified by comparing their mass spectra with the spectra of known compounds stored in the spectral database, NIST of version year 2005.

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 method by Perez et al.²⁰ 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 (10mm 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

The results of Phytochemical screening revealed the presence of carbohydrates, flavonoids, phenolic groups and steroids in all the three tested extracts. Aminoacids and proteins were present only in hexane extract, terpenoids are present both in chloroform and ethanol extracts. Gum, oil & resins, saponins and tannins are present in ethanol extract only. Alkaloids, anthraquinones, catechins, coumarins and quinones are completely absent in all the three extracts (Table 1).

The compounds identified by GC-MS analysis from ethanol extract were enumerated with molecular formula, retention time, molecular weight and peak area % (Table 2). GC-MS analysis of an ethanol extract showed the presence 10 compounds. Among these, 2 compounds were belonged to aromatic groups and 8 compounds were belonged to aliphatic groups. 2 compounds of organic esters were identified under aromatic groups while 8 compounds of fatty acids were identified under aliphatic groups. Among organic esters, diethyl phthalate was found to be present as major constituents with highest peak area of 94.47 % and retention time 11.29 followed by methyl salicylate with peak area of 1.26% and retention time 6.33. Among fatty acids, ricinoleate was found to be present as major constituents with highest peak area of 3.72% and retention time 21.95 followed by methylricinoleate with peak area of 0.16 and retention time 20.93 respectively.

Table 1: Shows the preliminary phytochemical screening of various extracts of the stem of *Mallotus philippensis* var. *philippensis*.

Phytoconstituents	Stem extracts		
	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: Shows the GC-MS analysis of ethanol extract of the stem of *Mallotus philippensis* var. *philippensis*.

S. No.	Name of the Compounds	Molecular formula	Retention time	Molecular weight	Peak area %
(i) Aromatic groups					
(a) Organic ester					
1	diethylphthalate	C ₁₂ H ₁₄ O ₄	11.26	222	94.47
2	methylsalicylate	C ₈ H ₈ O ₃	6.33	152	1.26
(b) Aliphatic groups					
(ii) Fatty acids					
3	ricinoleic acid	C ₁₈ H ₃₄ O ₃	21.95	298	3.72
4	methylricinoleate	C ₁₉ H ₃₆ O ₃	20.93	312	0.16
5	1,2-benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	24.65	390	0.11
6	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	16.47	256	0.10
7	9,12-octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	18.99	280	0.08
8	9-octadecenoic acid, ethyl ester	C ₂₀ H ₃₈ O ₂	19.08	310	0.05
9	n-hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	16.47	284	0.03
10	octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	19.45	312	0.02

The results of antimicrobial activity (Table 3) of the hexane, chloroform and ethanolic stem extract showed concentration-dependent activity against all the tested bacteria with the zone of inhibition ranged from 12-26mm at various concentrations. But only the ethanol extract showed antimicrobial activity against the fungi *A.*

flavus and *C. albicans* with the zone of inhibition ranged from 16-22mm at various concentrations. The solvents used for extraction were used as control and all the solvent control did not show any activity. Standard antibiotics were also used along with the extracts for comparison as given in the Table 3.

Table 3: Shows an antibacterial activity of various extracts of the stem of *Mallotus philippensis* var. *philippensis*.

Test Microorganisms (10 µg/ml)	Hexane (mg/ml)			Chloroform (mg/ml)			Ethanol (mg/ml)			Standard drug
	100	50	25	100	50	25	100	50	25	
	Gram-positive bacteria									
<i>B. subtilis</i> (A)	-	-	-	-	-	-	16	14	-	32
<i>S. aureus</i> (A)	-	-	-	-	-	-	16	12	-	33
<i>S. pneumonia</i>	12	-	-	15	12	-	15	12	-	32 (C)
Gram-negative bacteria										
<i>E. coli</i>	19	14	-	19	15	-	23	19	13	35 (A)
<i>P. aeruginosa</i>	26	13	-	17	15	-	18	15	-	32 (A)
<i>P. vulgaris</i>	24	17	-	-	-	-	12	-	-	34 (Cl)
<i>S. typhi</i>	24	17	-	16	-	-	17	14	-	32 (Cf)
<i>V. parahaemolyticus</i>	18	15	-	20	16	-	25	19	12	33 (K)
<i>V. vulnificus</i>	20	-	-	-	-	-	16	12	-	35 (K)
Fungi										
<i>A. flavus</i>	-	-	-	-	-	-	21	19	16	32 (P)
<i>A. fumigatus</i>	-	-	-	-	-	-	-	-	-	33 (P)
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	32 (P)
<i>C. albicans</i>	-	-	-	-	-	-	22	19	18	34 (P)

(Measurement indicates the zone of inhibition in mm); A – Ampicillin; Cl – Clotrimazole; Cf – Ciprofloxacin; K – Kanamycin; P – Penicillin

Hexane extract showed the maximum zone of inhibition ranged from 20 to 26mm against gram-negative bacteria such as 26mm against *P. aeruginosa*, 24mm against *P. vulgaris*, 24mm against *S. typhi* and 20mm against *V. vulnificus* at 100mg/ml concentration. Chloroform extract showed the maximum zone of inhibition as 20mm against *V. parahaemolyticus*. Ethanol extract showed the maximum zone of inhibition ranged from 21 to 25mm against gram-negative bacteria such as 25mm against *V. parahaemolyticus* and 23mm against *E. coli* 100mg/ml concentration and against the fungi such as 21mm against *A. flavus* and 22mm against *C. albicans* 100mg/ml concentration respectively.

Phytochemical screening of the hexane, chloroform and ethanol extracts of stem of *Mallotus philippensis* var. *philippensis* showed the presence of secondary metabolites such as flavonoids, phenols, saponins, steroids, tannins and terpenoids. The presence of secondary metabolites in plants produces some biological activity in man and animals and is responsible for their use as herbs in ailments⁶. The active components usually interfere with growth and metabolism of microorganisms in a negative manner²¹. All the 10 compounds identified by GC-MS analysis possess various pharmaceutical applications. The major compound diethyl phthalate is used medicinally for the preparation of 67 consumer formulations including bath preparations (oils, tablets, and salts), eye shadow, toilet waters, perfumes and other fragrance preparations, hair sprays, wave sets, nail polish and enamel removers, nail extenders, bath soaps, detergents, aftershave lotions, and skin care preparations^{22,23} and also as a component in insecticide sprays, mosquito repellents and camphor substitute²⁴. Thus the compound identified by GC-MS analysis provide a proof to show the medicinal potential of an ethanol extract to treat various infectious diseases.

From the results of antimicrobial activity, it was found that the hexane and ethanol extracts exhibited maximum antimicrobial activity against the tested human pathogens. In our study, the maximum zone of inhibition against gram negative bacteria such as *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. typhi* and *V. parahaemolyticus* and against the fungi such as *A. flavus* and *C. albicans* may be due to the presence of secondary metabolites such as flavonoids, phenolic groups and steroids as suggested by previous reports^{25,26,27}. The significant activity of the results against the fungi, *A. flavus* and *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^{26,28,29}. Even in hospitals, majority of disinfectants such as phenols, lysol, cresols

used are belonging to phenolic groups. Thus recent findings of antimicrobial activity against *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *V. parahaemolyticus* and *V. vulnificus* revealed the medicinal potential value of hexane and ethanol extracts against abdominal pain, diarrhea, fever, nausea, septicaemia, urinary tract infections and vomiting, caused by *E. coli*, hospital-acquired wound infections, septicaemia and urinary tract infections by *P. vulgaris* and *P. aeruginosa*, typhoid fever by *S. typhi* and diarrheal infections by *Vibrio* species, skin related diseases by *C. albicans* and Aspergillosis and respiratory tract infections by *A. flavus* respectively.

CONCLUSION

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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