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Short Communication

Phytochemical screening and antibacterial evaluation of stem bark of *Mallotus philippinensis* var. Tomentosus

K. Moorthy[1], K. Srinivasan[2], C. Subramanian[2], C. Mohanasundari[3] and M. Palaniswamy[2]

[1]Department of Microbiology, Vivekanandha College of Arts and Science for Women, Elayampalayam, Tamilnadu S. India-637 205.
[2]Department of Biology, Eritrea Institute of Technology, Asmara, Eritrea, North East Africa.
[3]Department of Microbiology Kandaswami Kandars College-velur (Namakkal), Tamilnadu, S. India-638182.

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***Mallotus philippinensis* var. Tomentosus is a medicinal plant, which was tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis*. Phytochemical screening of the stem bark of *M. philippinensis* indicates the presence of secondary metabolites. From the results obtained, eluted fractions of chloroform and methanolic extracts showed excellent zone of inhibition comparable to the standard drug used. However, the hexane extract did not show any appreciable activity. The results of the study showed the justification of the use of the plant against the bacterial pathogens.**

Key words: Antibacterial activity, *Mallotus philippinensis*, stems bark extract.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganism, animals and plants. One of such resources is folk medicine. Systematic investigation of folk medicine may result in the discovery of novel effective compounds (Tomoko et al., 2002; Roja and Rao, 2000). Herbal-based and plant-derived products can be exploitted with sustainable comparative and competitive advantage. Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker et al., 1995; Cordell, 1995). Presently, many scientists and organizations are in search of traditional remedies as alternate medicine (Cortella and Pocheltino, 1999).

In developing countries, the World Health Organization (WHO) estimates that about three quarters of the population relies on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and to get remedy from a variety of ailments of microbial origin (Kameshwara Rao, 2000; Subramani and Goraya, 2003).

In the present study, the *in vitro* antibacterial activity of the separated fractions of methanolic extracts from the bark of *Mallotus philippinensis* is described.

MATERIALS AND METHODS

Collection of plant materials

The stem bark of *M. philippinensis* var. Tomentosus were collected from the Kolli Hills of Salem district of Tamil Nadu, South India. The plant was identified at Rapinat Herbarium, Tiruchirapalli. The collected barks were shade-dried and coarsely powdered by using pulvarisor. These coarse powders were then subjected to successive extraction in various solvents by gradually increasing the polarity such as chloroform and methanol by using Soxhlet apparatus. The collected extracts were then taken up for further investigations.

*Corresponding author. E-mail: shrieenivasan@yahoo.co.in.

Table 1. Preliminary phytochemical screening of the bark of *Mallotus Philippinensis*.

Constituents	Hexane	Chloroform	Methanol
Alkaloids	-	+	-
Aminoacids	-	-	-
Anthroquinone glycosides	-	-	-
Flavones	-	-	+
Phenolic groups	+	+	+
Quinones	-	-	-
Saponins	-	-	+
Steroids	-	+	+
Sugars	-	-	+
Tannins	-	-	+
Triterpenes	-	-	+

+ = Present; - = Absent.

Preliminary phytochemical screening

The crude hexane, chloroform and methanolic extracts were subjected to phytochemical screening (Harborne, 1998; Kokate, 2003).

Tested microorganisms

Microbial strains of human pathogenic bacteria tested include gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and gram-positive *Bacillus subtilis*. All the microbial cultures were procured from MIIT, Chandigarh.

Media used

Muller-Hinton Agar (Hi-media) was used respectively for testing the antibacterial activity.

Screening of antibacterial activity

Antibacterial activity was screened by cup-plate method (Anonymous, 1985). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 h old - broth culture of respective bacteria. The plates were inoculated with 15 min after preparing the inoculum with a wax pencil the plate was divided into section according to the number of standard and sample solutions to be used. Sterilized cotton swab was dipped into the nutrient broth; excess fluid was removed by rotating the swabs with firm pressure against the inside of the tube above third level. Using the sterile cork borer, the well about 3 mm wide was made. Test and control drugs were added into the cup plate by using micropipette. Then the plates were incubated at 37ºC.

100, 75 and 50 mg/ml concentration of methanol extracts with respective solvents as control and the fraction elution of methanol extract were collected through column chromatographic techniques and different concentrations such as 5, 2.5, 1.25 and 0.625 mg/ml were prepared and tested against the pathogens along with respective standard drugs. Diameter of the inhibition zones observed and its values noted. Triplicates were maintained and the experiment was repeated thrice and the average values were calculated for its antibacterial activity.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of chloroform and methanolic bark extracts of *M. philippinensis* var. Tomentosus is presented in Table 1. Hexane extract revealed the presence of phenolic groups; chloroform extract showed the presence of alkaloids, phenolic groups, steroids and methanol extract showed the presence of flavones, phenolic groups, saponins, steroids, sugars, tannins and triterpenes. In the phytochemical screening, on the basis of number of secondary metabolites, chloroform and methanol (8:2 and 1:1) extract alone were selected for elution of compounds using column chromatography and its antibacterial efficacy was determined (Tables 2 and 3).

Chloroform and methanol in the ratio 8:2 were prepared for chromatographic fractions. All the fractions exhibited excellent activity against the tested organisms namely *E. coli*, (19, 20, 25 and 28 mm) followed by *Bacillus subtilis* (18, 18, 20 and 22 mm) at concentrations 0.625, 1.25, 2.5 and 5 mg/ml, respectively, whereas the other organisms such as *K. pneumoniae* (23 and 24 mm), *P. aeruginosa* (12 and 16 mm) and *Salmonella typhi* (13 and 17 mm) generated inhibition halos only at the higher concentrations (Table 2) of the eluted extracts (2.5 and 5 mg/ml, respectively). Elution with the same solvent in the ratio 1:1 was tested for its antibacterial efficacy, with different results obtained (Table 3).

Based on this investigations methanol extract and its elution fractions with chloroform were found to have significant antibacterial activity. Daud et al. (2005) found that ethanol extract of flowers of *Phrygilanthus acutifolius* and this extract was bactericidal against *Staphylococcus aureus* and bacteriostatic against *P. aeruginosa*. Test results would tend to corroborate the folk belief that the flowers of this plant are efficacious against respiratory infections and would justify its further investigation. This study support why crude extracts of *M. philippinensis* may be used for treatment of several infectious diseases.

Table 2. Antibacterial activity of column elution with chloroform : methanol (8:2) of bark of *Mallotus Philippinensis* var. Tomentosus.

Microorganism	Concentration (mg/ml)				Standard (10 µg/ml)
	5	2.5	1.25	0.625	
Escherichia coli	28	25	20	19	28 (Norfloxacin)
Klebsiella pneumoniae	24	23	-	-	22 (Ciprofloxacin)
Pseudomonas aeruginosa	16	12	-	-	20 (Tobramycin)
Salmonella typhi	17	13	-	-	30 (Ciprofloxacin)
Bacillus subtilis	22	20	18	18	24 (Ampicillin)

Table 3. Antibacterial activity of column elution with chloroform : methanol (1:1) of bark of *Mallotus Philippinensis* var. Tomentosus.

Microorganisms	Concentration (mg/ml)				Standard (10 mg/ml)
	5	2.5	1.25	0.625	
Escherichia coli	-	-	-	-	28 (Norfloxacin)
Klebsiella pneumoniae	15	13	-	-	22 (Ciprofloxacin)
Pseudomonas aeruginosa	25	24	-	-	20 (Tobramycin)
Salmonella typhi	26	23	-	-	30 (Ciprofloxacin)
Bacillus subtilis	14	14	13	13	24 (Ampicillin)

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