



ISSN 2320-3862

JMPS 2016; 4(3): 104-106

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Received: 09-03-2016

Accepted: 05-04-2016

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## Antibacterial Activity of *Malotus philippensis* Fruit Extract

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### Abstract

The antibacterial activity of plant *M. philippensis* belonging to family, Euphorbiaceae was evaluated *in-vitro* against some selected human pathogenic microorganisms (*Escherichia coli*, *Yersinia pestis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) following agar-well diffusion method using different concentrations (30%, 50%, 70% and 100%). Two solvents methanol and acetone were used for extraction of different bioactive constituents from fresh fruits. It was concluded from the results that methanolic as well as acetone fruit extracts of *M. philippensis* were quite effective in inhibiting the growth of *Staphylococcus aureus* which is a serious human pathogen causing infections in wounds. Therefore, the fruit extracts of this plant can be selected for further investigation to determine their therapeutic potential.

**Keywords:** *M. philippensis*, Leaf extracts, Antibacterial activity, Agar-well diffusion

### Introduction

Medicinal plants have long been used for treatment of many ailments and such traditional knowledge is the foundation of discovery of many drugs and novel molecules. Nature has been a source of medicinal agents for thousands of years and numerous drugs have been isolated from plants, many based on their use in traditional healing properties [1]. Medicinal plants have always had an important place in the therapeutic world of human beings. According to WHO [2] approximately 80% of world population depend 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 their medicinal properties and about less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential worldwide [3, 4]. Nowadays in India interest of people has been diverted to traditional medicines (Ayurveda) from allopathy. At least 25% of the medicines prescribed issued in the USA and Canada contain bioactive constituents that are derived from plant natural products [5]. Many pharmaceutical industries are showing interest in chemicals isolated from plants for therapy of many pathological diseases [6]. Number of plants have been screened for their medicinal properties and their products are playing major role in healing various diseases [7] *Mallotus philippensis* (family: Euphorbiaceae) locally known as Kamla is a large genus of trees and shrubs distributed mainly in the tropical and subtropical regions of the World with around 20 species in India [8]. *Mallotus philippinensis* (commonly called Kamala, Kampillaka, and Kapila, Shendri) is a common perennial shrub or small tree found in outer Himalayas ascending to 1500 meters. Fruits of this plant have glandular hairs collected as reddish brown powder which is collected in cloth by shaking and rubbing the fruits by hand. Kamla has long been used to produce dye colouring silk. Beside being used as dye, powder from this plant claim to have many medicinal properties. It is used in Ayurvedic medicine to relieve cough, constipation, wounds, ulcers etc. This plant is also applied externally for skin disorders such scabies and cutaneous troubles and other parasitic infections. In India powder of leaves and bark is used as poultice skin disorders. In literature various medicinal properties of this plant has been reported such as antifilarial [9], antibacterial, anti-inflammatory, and immune-regulatory activity [10] and also used as purgative, antihelminthic, vulnerary, detergent, carminative, and is useful in treatment of bronchitis, abdominal diseases, spleen enlargement, antimicrobial, antiparasitic [11]. Medicinal plants are one of the best source of obtaining antimicrobial drugs [12]. Therefore such plants should be investigated further to understand better about their properties, safety and efficacy [13].

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## Materials and Methods

**Collection of Plant Material:** Fruits of *M. philippensis* were collected from village Kaloha, District Kangra, Himachal Pradesh, India. The collected plant material was brought to the laboratory for further analysis.

**Processing Of Plant Material:** The collected *M. philippensis*'s fruit were plucked from the plant and washed thoroughly under tap water and then with 2% Mercuric chloride. The fruits were cut into smaller pieces for quick drying. Cleaned fruit were shade dried for 15-20 days. The dried plant material was crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in air tight container at room temperature.

**Preparation of Methanolic and Acetone Fruit Extracts of *M. philippensis*:** The dried fruit material (50 g) was pulverized in a blender to get a coarse powder and soaked separately in 300 ml of methanol and acetone separately in Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and stock solution of conc. 50 mg/ml was prepared.

**Procurement Of Bacteria:** Bacterial strains used for determining antimicrobial activity of fruit extracts of *M. philippensis* procured from Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla, India. Pathogens used for the study were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia pestis*.

**Revival of Pathogen:** The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

**Screening The Antibacterial Activity of Methanolic and Acetone Extracts of *M. philippensis*:** Screening of fruit extract (methanol and acetone) of *M. philippensis* was done using agar-well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptones 5g,

Agar 20g, Distilled Water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours. A 100µl of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each petriplate. The wells in each plate were loaded with 30%, 50%, 70% and 100% concentration of prepared extracts of *M. philippensis*. The petriplate kept as control contained pure solvent in the well. The plates were incubated at 37±2°C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction for all the three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard [14].

Percentage of growth inhibition =  $(\text{Control} - \text{Test} / \text{Control}) \times 100$   
Control = average diameter of bacterial colony in control.  
Test = average diameter of bacterial colony in treatment sets [15]

## Results and Discussion

The present study brings out that methanolic and acetone fruit extract of *M. philippensis* proved itself as good antibacterial agent. The methanolic extracts of *M. philippensis* showed considerable growth inhibition of test bacteria at different concentrations (30%, 50%, 70%, 100%) as compared to acetone fruit extract of the plant. The methanolic extract of *M. philippensis* was found to be most effective against *S. aureus* at (20mm at 100%) followed by (18mm at 70%), (15mm at 50%), (13mm at 30%), and it offered minimum inhibition in *P. aeruginosa* (14mm at 100%), (12mm at 70%), (10mm at 50%) and (9mm at 30%) as given in table 1. The acetone extract of *M. philippensis* was found to be most effective against *S. aureus* at (15mm at 100%) followed by (13mm at 70%), (14mm at 50%), (11mm at 30%), and it showed minimum inhibition towards *P. aeruginosa* (13mm at 100%), (10mm at 70%), (9mm at 50%) and (Nil at 30%) as shown in table 2.

**Table 1:** Percent inhibition of growth of human pathogenic bacterial *spp.* at different concentrations of methanolic extract of *M. philippensis*.

Concentration of methanolic extract of <i>M. philippensis</i> (In %)	Inhibition zone diameter (In mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>Y. pestis</i>	<i>P. aeruginosa</i>
Control	Nil	Nil	Nil	Nil
30	13	10	12	9
50	15	11	13	10
70	18	15	16	12
100	20	18	18	14

Each data represent mean of three replicates

**Table 2:** Percent inhibition of growth of human pathogenic bacterial *spp.* at different concentrations of acetone extract of *M. philippensis*

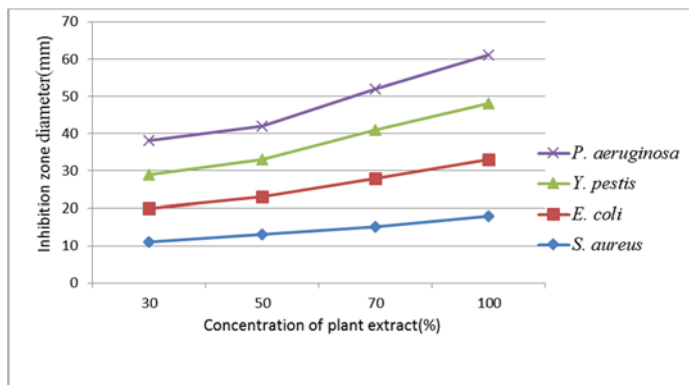
Concentration of methanolic extract of <i>M. philippensis</i> (In %)	Inhibition zone diameter (In mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>Y. pestis</i>	<i>P. aeruginosa</i>
Control	Nil	Nil	Nil	Nil
30	11	9	9	Nil
50	13	10	10	9
70	14	13	12	10
100	15	14	14	13

Each data represent mean of three replicates

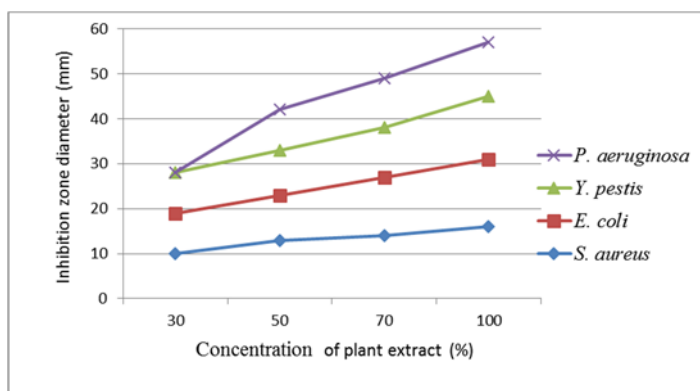
It was concluded from the results that methanolic as well as acetone fruit extract of *M. philippensis* were quite effective in inhibiting the growth of *Staphylococcus aureus* which is considered as a serious human pathogen causing infections in

wounds. Possible reason for this antibacterial activity of *M. philippensis* are presence of alkaloids, phenolics and flavanoids in its leaves [16]. Majority of phytochemical components are known to produce the therapeutic activity like

antibacterial, antifungal and antioxidant etc <sup>[17]</sup>. These findings are in accordance with the work carried out by <sup>[18, 19]</sup>. Our study was also found to correlate with the results of phytochemicals as well as antibacterial results obtained from the fruits of *M. philippensis* <sup>[20]</sup>. Thus it serves as an encouragement towards development of new drugs for the benefit of mankind.



**Fig 1:** Antibacterial activity of methanolic leaf extract of *M. philippensis* against various human pathogenic bacterial strains.



**Fig 2:** Antibacterial activity of acetone leaf extract of *M. philippensis* against various human pathogenic bacterial strains.

### Acknowledgements

We gratefully acknowledge the financial support provided for the present work by UGC Delhi in the form of BSR-Fellowship to the first author. The authors want to put on record their thanks to the chairperson, Department of Biosciences, Himachal Pradesh University, Summerhill Shimla for providing lab facilities.

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