

ANTIBACTERIAL ACTIVITY OF *MILLINGTONIA HORTENSIS* LINN STEM BARK

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Summary

The antibacterial activity of crude petroleum ether, benzene, chloroform, methanol and aqueous extracts of *Millingtonia hortensis* stem bark were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The *in vitro* antibacterial activity was performed by agar disc diffusion method. The zone of inhibition was compared with the standard drug i.e. ampicillin. Petroleum ether extract was effective against *P. aeruginosa*, *B. subtilis* and *S. aureus*; benzene, chloroform, methanol and aqueous extracts were effective against the entire four test microorganism used respectively when compared to standard drug ampicillin. The minimum inhibitory concentration [MIC] for *S. aureus* was 50, 100, 50, 50 and 50 µg/ml; MIC for *B. subtilis* was 25, 100, 50, 50 and 25 µg/ml; MIC for *E. coli* was 200, 100, 50, 50 and 25 µg/ml and MIC for *P. aeruginosa* was 10, 50, 50, 50 and 50 µg/ml for petroleum ether, benzene, chloroform, methanol and aqueous extracts respectively suggesting the antibacterial activity of *Millingtonia hortensis*.

Key words: *Millingtonia hortensis*, antibacterial activity, zone of inhibition, minimum inhibitory concentration, stem bark.

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Introduction

Millingtonia hortensis Linn (Bignoniaceae) commonly known as Cork tree is important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. The stem bark is used traditionally as mainly lung tonic, anti asthmatic and antimicrobial¹. The scientific activities reported so far from the plants are antifungal², larvicidal^{3, 4}, antioxidant⁵, antiproliferative^{6, 7} and anthelmintic⁸ activities. However there is no report on antibacterial activity of this plant. In the light of the above information, the present investigation was under taken to evaluate the antibacterial potential of different extracts of stem bark of *Millingtonia hortensis* Linn.

Materials and Methods

Plant material

The fresh stem bark of *Millingtonia hortensis* Linn were collected from Bengaluru region, identified and authenticated by Dr Shiddamallayya N (SMPU/NADRI/BNG/ 2010-11/304) at National Ayurveda Dietetics Research Institute, Bengaluru, Karnataka. A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore.

Preparation of extracts

The dried stem bark powder were coarsely powdered and subjected to successive extraction by soxhlation. The extraction was done with different solvents in their increasing order of polarity such as petroleum ether, benzene, chloroform, methanol and distilled water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 1.44, 0.52, 0.61, 15.91 and 2.33 % w/w respectively with reference to the air dried plant material.

5 mg of the extract was weighed and dissolved in 5ml of DMSO which was labeled as stock 1. From stock 1 further dilution were made so as to get 10, 25, 50, 100, 200, 300, 400 and 500 µg/ml concentrations by using DMSO as solvent.

Microorganisms used:

All the microbial cultures, used for antimicrobial screening were procured from National centre for Industrial Microorganisms (NCIM), Pune, India and from The Oxford College of Science, Bangalore. The bacterial culture were maintained on Muller Hinton agar slants which were stored at 4°C

Antibacterial activity:

Determination of minimum inhibitory concentration (MIC)

The extract were screened for their antibacterial activity *in vitro* by disc diffusion method⁹ using *S.aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* as test organism. Agar cultures of the test microorganisms were prepared. Three to five similar colonies were selected and transferred to 5 ml broth with a loop and the broth cultures were incubated for 24 h at 37°C and suspension was checked to provide approximately 10¹⁰ colony forming units per ml. 0.1 ml of organism's suspension were spread evenly on the agar plates. For screening, sterile 3 mm diameter disc (Whatman filter paper No. 1) were impregnated with different concentration till saturation, dried and placed in inoculated plates of Muller Hinton agar medium. DMSO solvent was used as negative control. The plates were incubated at 37°C for 24 h. After incubation for 24 h, the results were recorded by measuring the zones of inhibition surrounding the disc and the lowest concentration of each extract which is showing inhibition of growth of bacteria was determined as MIC. Ampicillin (50 µg/ml) was used as standard for bacteria.

Results and Discussion:

The antibacterial activity of *Millingtonia hortensis* stem bark extracts was studied by employing disc diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results of minimum inhibitory concentration and zone of inhibition are given in Table 1 and Table 2.

It is clear from the Table 1 and 2, Petroleum ether extract was effective against *P. aeruginosa*, *B. subtilis* and *S. aureus*; benzene, chloroform, methanol and aqueous extracts were effective against the entire four test microorganism used respectively when compared to standard drug ampicillin. The minimum inhibitory concentration[MIC] for *S. aureus* was 50,100,50,50 and 50 µg/ml; MIC for *B. subtilis* was 25,100,50,50 and 25 µg/ml; MIC for *E. coli* was 200, 100,50,50 and 25 µg/ml and MIC for *P. aeruginosa* was 10,50, 50, 50 and 50 µg/ml for petroleum ether, benzene, chloroform, methanol and aqueous extracts respectively suggesting the antibacterial activity of *Millingtonia hortensis*. Work is under progress to reveal the chemical nature of the active constituents responsible for the antibacterial activity.

Table 1: MIC values of different extracts of stem bark of *Millingtonia hortensis*

Microorganism used	MIC with concentration of extract [µg/ml]				
	Petroleum ether	Benzene	Chloroform	Methanol	Aqueous
<i>Staphylococcus aureus</i>	50	100	50	50	50
<i>Bacillus subtilis</i>	25	100	50	50	25
<i>Escherichia coli</i>	200	100	50	50	25
<i>Pseudomonas aeruginosa</i>	10	50	50	50	50

Table 2: Zone of inhibition values (mm) in MIC of different extracts of *Millingtonia hortensis*

Microorganism used	Zone of inhibition (mm) of extracts and standard					
	Petroleum ether	Benzene	Chloroform	Methanol	Aqueous	Ampicillin
<i>Staphylococcus aureus</i>	8	8	8	8	8	12
<i>Bacillus subtilis</i>	7	7	7	7	7	11
<i>Escherichia coli</i>	7	7	8	8	7	7
<i>Pseudomonas aeruginosa</i>	7	7	8	7	7	10

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