

Original Research Article

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A Study on Biochemical Constituents and Anti-Bacterial Activity in the Bark Extract of *Millingtonia hortensis*

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ABSTRACT

Millingtonia hortensis Linn. (Bignoniaceae) commonly known as Cork tree, Akash neem, Neem chameli. It is an important medicinal plant in Southern Asia ranging from India, Burma, Thailand and Southern China, cultivated in most parts of India in gardens and avenues. It can grow up to 25 meter tall. Flowers have very rich and pleasant scent, used in the treatment of asthma, sinusitis, cholagogue, tonic and in rituals. The stem bark is used traditionally as mainly lung tonic, anti-asthmatic and antimicrobial properties. Leaves and roots of cork tree used as antiasthmatic and antimicrobial activity. Fruit is very long and narrow, pointed at both ends and contains thin, flat seeds. Trees do not seed very easily in India. The plant has antifungal, antibacterial, larvicidal, antioxidant, antiproliferative, antimutagenic, anthelmintic and hepatoprotective activities. The study was carried out to find the biochemical constituents and anti-bacterial activities of the bark extract of *Millingtonia hortensis*. The objectives of the study were to analyse the chemical constituents of the bark and to analyze the anti-bacterial activity of the bark of *Millingtonia hortensis*. In this study, an experiment was conducted to study the biochemical constituents of the bark, the extract was give for GCMS test to SITRA (South Indian Textile Research Association). The results reveals that the bark have four compounds which are all mainly used in medicinal propose. The anti-bacterial activity of the bark of *Millingtonia hortensis* is studied through laboratory *Bacillus subtilis*.

Keywords

Millingtonia hortensis, Bio-chemical, GC-MS

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Introduction

Millingtonia hortensis Linn. F is an important medicinal plant in Southern Asia, ranging from India, Burma, Thailand and Southern China. A very tall tree, Flowers have very rich & pleasant scent. It is a drought resistant tree. The Biological name of *Millingtonia hortensis* belonging to the family of

Bignoniaceae. Propagation by Seeds, suckers. Longevity is Perennial. It is a tall deciduous tree. It grows up to 25 meter.

The leaves are pinnately compound. Long leaves bear two or three widely spaced pinnae, each with 5-7 smooth leaflets, oval, pointed and slightly round-toothed, 1-3 inches long.

Sometimes the lower pinnae are again divided and bear one pair of three leaved pinnae, 1-2 pairs of leaflets and one leaflet at the end. Flowers in corymbose panicles, long tubular, silvery-white and delightfully fragrant. It requires full sunlight for its growth. Stem and roots of the Cork tree have great medicinal value. Its dried flower is a good lung tonic. It is also used in the cough diseases. Its flowers are used in the rituals. Its bark is used to produce yellow dye.

Extract of the leaves of *Millingtonia hortensis* has good antimicrobial activity. Trees 8-25 m tall. Leaves 40-100 cm; Inflorescences cymose-paniculate. It is a drought resistant, tall deciduous tree, grows at an altitude of 500-1200m. Largely seen in tropical forest with an average altitude of 0-922m. The tree grows well in all types of soil with variable climate. The propagation of the tree is carried through seed and suckers.

***Millingtonia hortensis* and its pharmacological activity**

Antifungal activity

Sharma *et al.*, (2007) was described the Antifungal activities of different extracts of *Millingtonia hortensis* were investigated against various fungal pathogens. Methanol extract was found to have stronger activity than fluconazole against yeast like fungi: 4 fold against *Candida krusei* with 4 µg/ml minimal inhibitory concentration and 2 fold (MIC- 2 µg/ml) against *Sacharomyces cerevisiae*, though it showed the same activity as fluconazole against *Candida glabrata*.

Aqueous extract also exhibited 4 fold stronger activity against *Candida krusei* (MIC- 4 µg/ml) and 4 fold (MIC; 2 µg/ml) against *Sacharomyces cerevisiae*. Chloroform and ethyl acetate extract showed lower activities against all fungal pathogens except for

Candida krusei, compared with the standard. Against the filamentous fungus, *Trichosporon cutaneum*, all extracts showed less activity than the standard.

Larvicidal activity

R. Kaushik *et al.*, (2008) was studied *Millingtonia hortensis* L. (Family: Bignoniaceae) a plant commonly known as 'Akas neem' and also as the "Indian cork tree" was first reported which reveals the mosquito larvicidal property of *M.hortensis*. R. Kaushik *et al.* (2009) was Screened 11 plant species of local flora against the IV instar larvae of *Aedes aegypti* (Diptera: Culicidae).

Antimicrobial activity

A.Jetty; *et al.*, (2000) was described the Polar extracts of the leaves of *Millingtonia hortensis* showed good antimicrobial activity. Twenty different bacterial strains and two yeast cultures were used. The aqueous alcohol extract showed good activity against all microbes tested, particularly *Escherichia coli* and *Salmonella typhimurium*, both Gram-negative bacteria, with MIC values of 6.25 µg/ml. The activity is compared with known antibiotics such as gentamycin and nystatin.

Mutagenicity and antimutagenicity

Malyn Chulasiri *et al.*, (2006) was studied the mutagenicity and antimutagenicity of hispidulin and hortensin, the flavonoids from *Millingtonia hortensis* L. (Bignoniaceae), were performed using the liquid pre-incubation method of the Salmonella/microsome test.

At the highest dose tested, 100 g/plate, both compounds showed no mutagenicity and no cytotoxicity toward *S. typhimurium* strains TA98 and TA100 either in the presence or absence of S9 mix.

However, these substances were antimutagens toward 2-aminoanthracene, aflatoxin B1 (in TA98), and dimethylnitrosamine (in TA100); but neither substance inhibited the direct mutagenic activity of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide nor that of sodium azide in strains TA98 and TA100, respectively.

Antiproliferation activity

Siwapong Tansuwanwong *et al.*, (2009) was described the *Millingtonia hortensis* is a medicinal plant widely used in many Asian countries. An aqueous crude extract of this plant has been shown the apoptosis induction on RKO colon cancer cells.

However, its mechanism remains unknown. To learn more about this plant extract, we partially purified the crude extract using Sephadex LH-20 and three aqueous fractions were collected. Each fraction was investigated for cytotoxicity using MTT assay.

The crude petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *Millingtonia hortensis* were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* using agar disc diffusion method using ampicillin as the standard drug.

Petroleum ether extract exhibits significant Zone of Inhibition (ZI) against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, whereas the benzene, chloroform, methanol and aqueous extracts were completely effective against all the four strains under study as compared to the standard drug.

Millingtonia hortensis Linn is tested against the Human cervical cancer cell line by MTT Assay. Antimicrobial capacity of the extract is evaluated by means of agar diffusion method using 12 strains of microbes.

The extract is found to be a poor cytotoxic and antibacterial agent, however it is found to be an effective antifungal agent.

Materials and Methods

The experimental material to be present study consisted of bark collection from at Forest college and research institute, Mettupalayam. From this species, the bark was collected from *Millingtonia hortensis* bark of (approximately five year old tree). The collected bark was chopped, shade dried and coarsely powdered by using electrical blender. The Bark powder was used for experiments.

Solvent extraction

5g of the coarsely powdered bark was used for the solvent extraction process. The 5g powdered bark was filled in the thimble of Soxhlet apparatus using methanol as a solvent. The process of extraction continued for an hour at 55°C. The solvent extracts collected in airtight containers were preserved in refrigerated condition at 5°C for further use.

The extracted solvent was analysed for its bio chemical constituents through Gas Chromatography Mass Spectrometer (GC-MS). The Instrument used was THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. The Column in the equipment was DB 35 - MS CAPILLARY STANDARD NON - POLAR COLUMN and the carrier gas used was Helium (He) and its flow was 1.0 ML/Min.

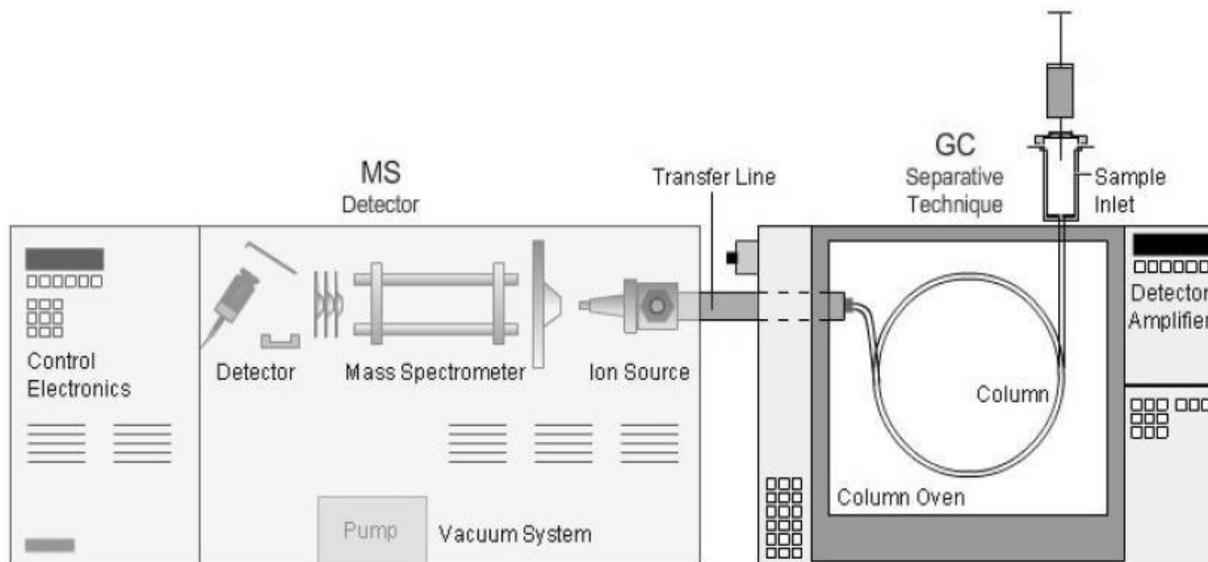
GC-MS studies: principle of gas chromatography –mass spectrometry

Gas Chromatography is used to separate volatile compounds in a mixture. The extracted solvent was analysed for its bio chemical constituents through Gas

Chromatography Mass Spectrometer (GC-MS). The GC-MS analysis was carried out using a Thermo GC - Trace Ultra ver: 5.0, Thermo MS DSQ II with 30m×0.25mm×0.25µm of capillary standard non-popular column. The instrument was set to an initial temperature of 70°C, and maintained at this temperature for 3 min. At the end of this period the oven temperature was rose up to 260°C, at the rate of an increase of 6°C/min, and maintained for 2 min. Injection port temperature was ensured at 250°C and Helium flow rate at 1.0ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1.

Mass spectral scan range was set at 40-700 (m/z). The ion source temperature was maintained at 220°C and Interface temperature was at 240°C. The MS start time was 0.00 min, and end time was 40.51 min. Interpretation on mass spectrum of GC-MS was done using the database of the South India Textile Research Association (SITRA). The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the SITRA library. The name, molecular weight and structure of the components of the test materials were confirmed (Fig. 1).

Fig.1 GC-MS studies: principle of gas chromatography –mass spectrometry



Results and Discussion

The GC-MS analysis led to the identification of 4 compounds from the Gas Chromatography fractions of the methanol extract of *Millingtonia hortensis*. The active principles with their retention time (RT), molecular formula and molecular weight (MW) in the methanol extract are presented in Table 1.

The Figure 2 to 4 depicts the peak of the

chemical constituents present in the methanol extract.

Anti-bacterial activity

Antibacterial evaluation studies revealed that the extracts under investigation were found to be completely effective against bacteria employed, the gram positive bacteria, *Bacillus subtilis*. It showed the presence of zone of inhibition for about 0.2 mm against *Bacillus subtilis*.

Bicyclo [4.1.0] heptanes, 7-pentyl

From the GC-MS it was interpreted the peak around 166 belongs to Bicyclo [4.1.0] heptanes,7-pentyl which is found at retention time of 9.68. This compound is proved as versatile and also conservation material. It is to be used on wall paintings, plaster, ceramics, paper, textiles and metals.

DI-alpha-tocopherol

The peak around 430 belongs to DI-alpha-tocopherol which is found at retention time of 10.66. This compound is said to be a source of vitamin E for the production of tablets.

Octadecanoic acid, butyl ester

The peak around 340 belongs to Octadecanoic acid, butyl ester compound found at retention time of 10.66. This compound is said to be used in production of hormone that regulate a variety of functions including blood pressure, blood clotting and immune response.

Hentriacontane

The peak around 436 belongs to Hentriacontane found at retention time of 28.98. This compound is used for Slows down the growth of Mycobacterium tuberculosis, used in the treatment of tuberculosis.

Table.1 Components identified in the bark of *Millingtonia hortensis*

NO.	Compound Name	Molecular Formula	Molecular Weight	RT
1	Bicyclo[4.1.0]heptanes,7-pentyl	C ₁₂ H ₂₂	166	9.68
2	DI-alpha -tocopherol	C ₂₉ H ₅₀ O ₂	430	10.66
3	Octadecanoicacid, butyl ester	C ₂₂ H ₄₄ O ₂	340	10.66
4	Hentriacontane	C ₃₁ H ₆₄	436	28.98

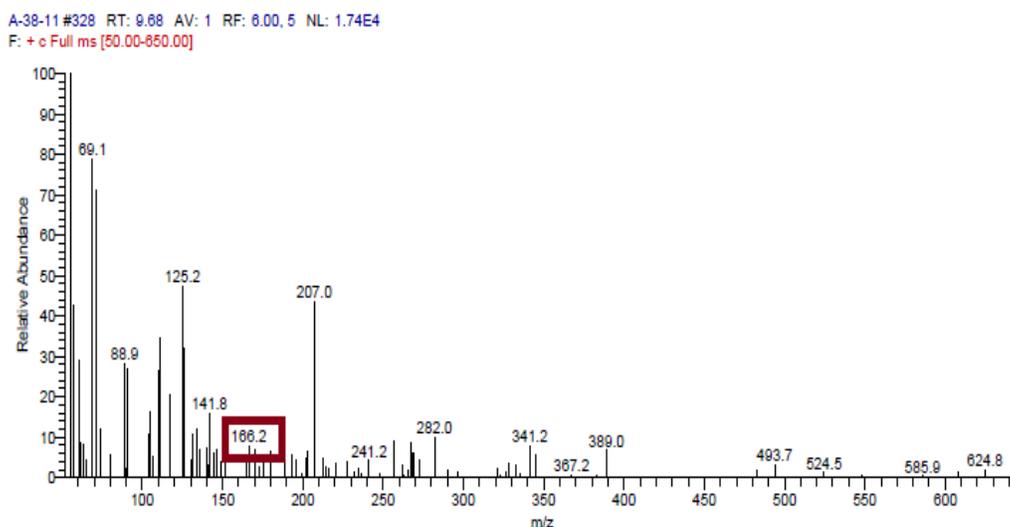


Figure.2 GC-MS Peak at which Bicyclo [4.1.0] heptanes,7-pentyl was detected

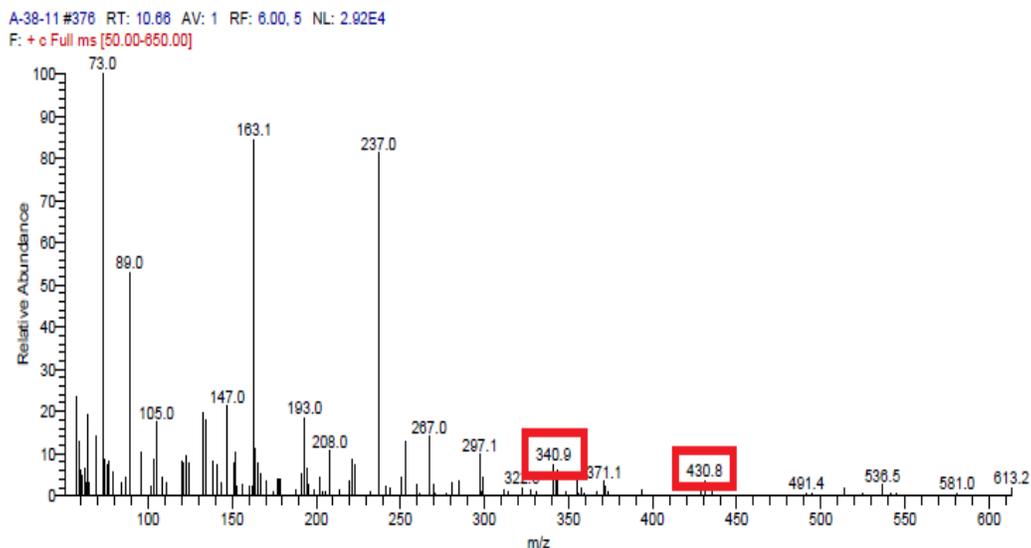


Figure.3 GC-MS Peak at which Octadecanoicacid, butylester, DI-alpha-tocopherol were detected

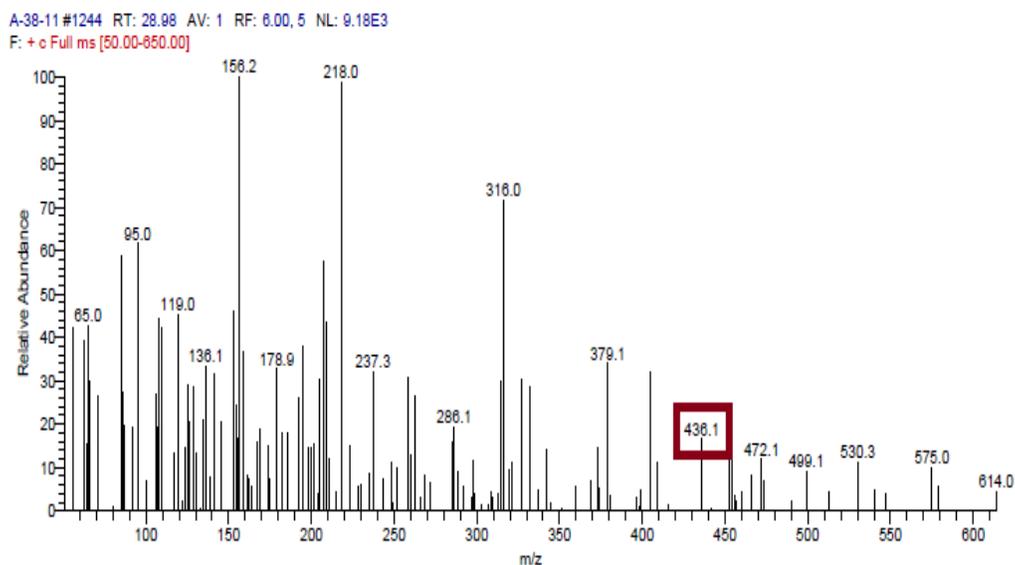


Figure.4 GC-MS Peak at which Hentriacontane was detected

The study on chemical constitution of *Millingtonia hortensis* bark showed that it have many phyto chemical properties and medicinal properties that are useful for Human being. The chemicals are used for conservation. *Millingtonia hortensis* L.is a potential Medicinal source with good medicinal components so it can be used in medicinal purpose. This study may give information to the possible production of

useful chemical components from *Millingtonia hortensis* in a large scale. The presents of Anti-bacterial activity showed that the bark can be used for bacterial infections.

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