A REVIEW ON INDIAN CORK TREE - MILLINGTONIA HORTENSIS LINN.F

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ABSTRACT

Millingtonia hortensis Linn.F (Bignoniaceae) is a traditional medicinal plant widely used in south - East Asia. It is commonly known as Cork tree, Akash neem and Neem chameli. The tree is cultivated in various parts of India for its ornamental purposes. It can grow up to 25m, with woody stem and thick cork. Flowers have very rich and pleasant scent, used in the treatment of asthma, sinusitis, and cholagogue and in rituals. Leaves and roots of cork tree are used as antiasthmatic and antimicrobial. The stem bark is used traditionally as lung tonic, antiasthmatic and antimicrobial. The tree has antifungal, antioxidant, antibacterial, larvicidal, antimutagenic, antiproliferative, anthelmintic and hepatoprotective properties. This paper highlights about the tree on its, pharmacognosy & phytochemistry and medicinal aspects.

KEYWORDS: Millingtonia hortensis, Cork tree, Bignoniaceae, Pharmacognosy, phytochemistry and pharmacological activities.

1. INTRODUCTION

Millingtonia hortensis Linn.F (Bignoniaceae) a large ornamental tree of Southern Asia is cultivated in various part of India and is largely seen in central India, Myanmar(Burma) and Thailand. The name Millingtonia comes from Thomas Millington, an English Botanist, while hortensis means grown in gardens. The tree is commonly known as Cork tree, Akash neem
and Neem chameli; Hindi: Neem chameli; Kannada: Akash mallige, beratu, birate mara; Konkani: akasnimb; Malayalam: Katesam; Marathi: akash chameli, buch, kaval nimb; Oriya: bakeni, mach-mach, sitahara; Tamil: Kat-malli; Telugu: Kavuki.\textsuperscript{[2]}

2.0 PLANT PROFILE

Scientific classification

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<td>\emph{Bignonia suberosa} Roxb, \emph{Millingtonia hortensis} L.\textsuperscript{[3]}</td>
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![Fig. 1 Millingtonia hortensis L.F Tree](image1)

![Fig. 2 Millingtonia hortensis L.F stem bark](image2)
2.0 DISTRIBUTION

The tree is native to Burma and the Malay Archipelago, but grows largely in all parts of India. It is cultivated in India, Burma, Thailand and South China.\textsuperscript{3,4}

3.0 HABIT AND HABITAT

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.\textsuperscript{5} The propagation of the tree is carried through seed and suckers.\textsuperscript{3}

4.0 DESCRIPTION

\textit{Millingtonia hortensis} L.F is a tall deciduous tree grows to a height of about 18 to 25m and can reach a maximum of about 80m, spread to an area of 7 to 11m.\textsuperscript{3} The tree bears a straight trunk with corky bark and lesser branches. It flowers at the night and shed flowers early in the morning.\textsuperscript{4}
4.1 Leaves
The leaves are large, ornamental, imparpinnate, opposite, tripinnately compound, exstipulate, petiolated. The upper tertiary leaflets are sessile, exstipellate and are ovate-lanceolate with a rounded or cuneate base, serrate margins with acuminated tips and 1-3 inches long.

4.2 Inflorescence
Paniculate cymes, terminal or axillary, the cymules 3-flowered and bracts are minute.

4.2.1 Flowers
Flowers are white, waxy, trumpet shaped and somewhat 2 lipped with 5 sub equal lobes. The tree flowers twice a year and the white flowers come as large panicles which emit a pleasant fragrance. They are bisexual, zygomorphic. The bell-shaped sepals of the flower have five small lobes with four stamens and paralleled anthers. The corolla is a long tube with 5 lobes. The lobes are valvate, ovate-lanceolate, densely pubescent adaxially margin. Ovary sessile, ovoid. Style long; stigma lingulate, compressed, 2-lobed, slightly exerted from corolla tube. Capsule dehiscing septicidally long linear, compressed. Flowering usually takes place from April until the rains and again in October to December.

4.3 Fruits and Seeds
Fruits are smooth, flat, 2 valved septicidal capsule, oblongoid, acute at both ends, woody. The seeds are discoid, compressed, winged, except the base, the wing is narrow at the apex and non endospermic. The fruiting period is November-February.

4.4 Bark
Evergreen tree with an elongated pyramidal stem. Yellowish white wood, soft, brittle. Corky bark, Dark brown coloured, characteristic odour. It has a straight trunk and has a fewer branches. The inferior cork is processed from its corky bark that’s why it is called cork tree, externally rough with irregular ridges and fissures.

5.0 MICROSCOPICAL STUDIES
5.1 Bark
Transverse section of bark shows a wide cork consisting of 32 - 40 layers of large, thin walled lignified cells. They are radially elongated except in the innermost region where they are tangentially elongated and are arranged in radial rows. The cork cells are measuring 33 –
58 – 86 μm x 13-38-66 μm. The cambium is composed of 8-20 layers of tangentially elongated cells arranged in regular radial rows. It is followed by a wide zone of parenchyma 12-30 layers thick. They are circular – oval or polyhedral showing intercellular spaces. Strands of sclerenchymatous pericycle are small, each strand consisting of 8 - 16 fibres. The remainder of the bark is a very wide zone of secondary phloem, which is traversed by medullary rays. The phloem consists of sieve-tubes, companion cells, phloem parenchyma and fibres. The phloem which presents a stratified appearance consists of 14-20 tangentially elongated strands of fibres alternating with soft tissues. In the inner phloem region fibre strands usually extend from one medullary ray to other, whereas in the middle and outer region a few strands alternate with parenchyma between two medullary rays. The medullary rays are 2-4 cells wide, radially elongated, the biseriate and triseriate rays being more common. Medullary ray cells are measuring 32-50-62 μm x 14-22-30 μm.\[9\]

6.0 COMMON USES

It is an ornamental tree with pleasant fragrance of flowers, which make them suitable as a garden tree. The wood is used as timber, whereas the bark is used as cheap substitute for cork. The leaves are used a substitute for tobacco in cigarettes.\[^{3,10}\]

7.0 TRADITIONAL USES

7.1 Leaves
Leaves are used as antiasthmatic, antimicrobial and antipyretic in folklore medicine.\[^{3,4}\]

7.2 Flowers
Flower buds are used in the treatment of atma, sinusitis, cholagogue and tonic. The flowers are added to tobacco for smoking as treatment of throat ailments.\[^{3,4}\]

7.3 Stem
Stem is used as lung tonic and as a cough suppressant.\[^{3,4}\]

7.4 Bark
It is used as for the production of a yellow dye.\[^{3,4}\]

7.5 Root
Roots are used as antiasthmatic and antimicrobial.\[^{3}\]

7.6 Whole Plant: The whole tree is used as antipyretic, antitubercular, antimicrobial,
larvicidal, antimutagenic, anticancer and antifungal.\textsuperscript{[1,8]}

8.0 Phytochemical Studies

8.1 Leaves
The leaves are said to contain hispidulin,\textsuperscript{[11]} rutinoside,\textsuperscript{[12]} \(\beta\)-carotene,\textsuperscript{[13]} and Dinatin.\textsuperscript{[4,12]}

8.2 Flowers
The flowers are reported to contain Glycosides: Scutellerin Scutellarein-5-galactoside,\textsuperscript{[14]} Salidroside, 2-Phenyl rutinoside, 2-(3,4-dihydroxy phenyl)-ethyl glucoside, acetoside, phenyl propanoid glucosides, p-coumaryl alcohol glucoside, isoeugenol glucoside, cornoside, rengyolone, rengyoside B, rengyol, rengyoside A, isorengyol,\textsuperscript{[3,15]} Millingtonine.\textsuperscript{[16]}

Flavanoids: Scutellarein-5-glucuronide, Hispidulin, Scutellarein,\textsuperscript{[11,17]} Hortensin,\textsuperscript{[18-20]} 3,4-dihydroxy-6,7-dimethoxyflavone. \textsuperscript{[18]}

8.3 Fruits
The fruits contain Acetyl oleanolic acid.\textsuperscript{[11]}

8.4 Bark
The bark is reported with \(\beta\)-sitosterol,\textsuperscript{[21,22]} bitter substances and tannins.\textsuperscript{[12]}

8.5 Roots
Lapachol, \(\beta\)-sitosterol and poulownin were reported from roots.\textsuperscript{[23]}

9.0 PHARMACOLOGICAL STUDIES

9.1 Antimicrobial activity
Twenty bacterial strains and two yeast cultures were tested against the polar extracts of leaves of \textit{Millingtonia hortensis}. The hydroalcoholic extract exhibit good antimicrobial activity against all strains. However, the extract was found to be significantly effective against \textit{Escherichia coli} and \textit{Salmonella typhimurium} with MIC values of 6.25 \(\mu\)g/ml as compared to the standard antibiotic such as gentamycin and nystatin.\textsuperscript{[24]}

9.1.1 Antibacterial studies
The essential oils distilled by vapor distillation from the flowers of \textit{Millingtonia hortensis} were tested against both gram positive (4 strains) and gram negative (2 strains) bacteria’s and
found to be a broad spectrum antibacterial at low concentration. The strains under study were *S. aureus, S. epidermi, B. subtilis, L. plantarum, E. coli* and *P. vulgaris.*\(^{[25]}\)

The methanol, ethanol and aqueous extracts of flowers and leaves of *Millingtonia hortensis* were tested against primary and opportunistic pathogens using disc diffusion method. Primary pathogens such as such as *Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi A, Vibrio cholerae, Shigella dysentrae* and *Bacillus subtilis* and opportunistic pathogens - *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis* were used for the study. The methanolic and ethanolic extracts of leaves were potent against all bacteria’s except *Salmonella typhi, Pseudomonas aeruginosa* and *Proteus mirabilis*, whereas the flower extract shows less activity against *Proteus mirabilis.* The leaves aqueous extract was found to exhibit antibacterial activity as compared to the flower aqueous extract. The MIC ranges between 25mg/ml-50mg/ml depending upon the type of extract and microorganisms.\(^{[26]}\)

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 rug. The MIC for *S. aureus* was found to be 50, 100, 50, 50 and 25μg/ml for petroleum ether, benzene, chloroform, methanol and aqueous extracts respectively, where as for *B.subtilis* it was 25, 100, 50, 50 and 25μg/ml of the same order. The *P. aeruginosa* exhibited MIC of 10, 50, 50, 50 and 50μg/ml in the same order of solvent extracts confirming the antibacterial potential of the stem bark of *Millingtonia hortensis* Linn.\(^{[27]}\)

### 9.1.2 Antifungal activity

Aqueous, methanol, chloroform and ethyl acetate extracts of leaves of *Millingtonia hortensis* were tested against yeast like fungi such as *Candida krusei, Saccharomyces cerevisiae, Candida glabrata* and *Trichosporon cutaneum.* 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 *Trichosporon cutaneum*, all extracts showed less activity than the standard.[28]

The aqueous extract of leaves of *Millingtonia hortensis* was tested against 8 fungal pathogens of maize at 10, 20, 30, 40 and 50% concentration. At 50% concentration the aqueous extract exhibited maximum activity against *A. flavus* followed by *F. oxysporum*, *F. solani*, *F. moniliforme*, *A. candidus*, *A. niger*, *A. flavipes* and *F. graminearum*. Moderate activity was observed for 20, 30 and 40% concentrations, whereas 10% exhibits least activity as compared to bavistin and thiram of synthetic class with 100% inhibition.[29]

### 9.2 Antimicrobial and Cytotoxic activity

The study aims at exploring the cytotoxic potential and antimicrobial capacity of the aqueous extract of stem bark of *Millingtonia hortensis* Linn. Aqueous extract of stem bark of *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.[30]

### 9.3 Mutagenecity and antimutagenecity activity

The flavanoids hisidulin and hortensin isolated from *Millingtonia hortensis* was studied for its mutagenic and antimutagenic activity using liquid pre incubation method of the Salmonella/micro some test. At the highest dose tested, 100 µg/plate, both compounds showed no mutagenecity and no cytotoxicity towards *S.typhimurium* strains TA98 and TA100 either in the presence or absence of S9 mix. However, these substances were antimutagens towards 2-aminoanthracene, aflatoxin B1(in TA98) and dimethylnitrosamine (in TA100); but neither substances inhibited the direct mutagenic activity of (2-furyl)-3-(5-nitro-2-furyl) acrylamide nor that of sodium azide ion strains TA98 and TA100, respectively.[31]
9.4 Larvicidal activity: *Millingtonia hortensis* leaf extract has been screened against three species of mosquito vectors such as *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The acetone extract of the tree leaves were evaluated at 25, 50, 100, 200, 300 and 500ppm dilutions against these different larval instars. The study revealed that the plant has a potent mosquito larvicidal property.\[32\]

Acetone extracts of eight plant species collected in the state of Andhra Pradesh, India, were tested for their larvicidal activity against the yellow fever mosquito, *Aedes aegypti* L. The buds of Tail Pepper, *Piper cubeba* L, Capers *Capparis spinosa* L and Indian Black Berry, *Syzygium cumini* L. the florals of Indian Oleander, *Nerium indicum* (Mill.), Indian Cork tree, *Millingtonia hortensis* L. and Royal Poinciana, *Delonix regia* L., leaves of Wood Apple, *Limonia acidissima* L. and Physic Nut, *Jatropha curcas* L were collected locally, shade dried and extracted in the soxhelet apparatus. Six of the 8 plants studied exhibited toxicity against the 3rd instar larvae. *Millingtonia hortensis* extract was found to be very less effective.\[33\]

9.5 Induction of Apoptosis on RKO Colon Cancer cell line

*Millingtonia hortensis* aqueous and ethanolic extracts were evaluated for the induction of apoptosis in an RKO human colon cancer cell lines. The assessment was carried out using MTT reduction assay. The aqueous extract of tree inhibited cell growth and proliferation in a dose and time dependent manner, however ethanolic extract fails. Apoptotic cells were determined by flow of cytometry and DNA fragmentation assay. Apoptic cell numbers increased in a dose-dependent manner after treatment with aqueous extract. DNA ladders were clearly observed in RKO cells treated with 200, 300 and 400μg/ml of the aqueous extract of *M. hortensis* suggesting that it inhibited cell proloiferation in an RKO colon cancer cell line via the apoptosis pathway.\[34\]

An aqueous crude extract of this plant has been shown the apoptosis induction on RKO colon cancer cells. However, its mechanism remains unknown. Further, the partially purified crude extract using Sephadex LH-20 and three aqueous fractions were collected. Each fraction was investigated for cytotoxicity using MTT assay. Fraction 1 showed antiproliferative effect on RKO cells with dose-dependent manner, while fraction 2 and 3 had no effect. Induction of apoptosis was determined using flow cytometry and DNA fragmentation method.\[35\]

9.6 Antioxidant activity: The antioxidant activity of aqueous extract of *Millingtonia hortensis* Linn (Bignoniaceae), stem bark by various methods. Both the extract and standard
drug quercetin was evaluated for its antioxidant potential at 10, 20, 30, 40 and 50mg/ml. In addition, the amount of total phenol (241 mg/gm) and total flavanoid (172mg/gm) were determined. The extract showed its antioxidant potential: DPPH radical scavenging activity (IC50 29.05mg/ml), FRAP radical scavenging activity, DCF/AAPH assay (TRAP) (IC50 41.10mg/ml), ABTS radical scavenging activity (IC50 24.0mg/ml), Superoxide anion scavenging activity assay (IC50 26.0mg/ml) and Nitric oxide assay (IC50 31.0mg/ml). The present study depicts that Millingtonia hortensis Linn bark has a potent natural antioxidant that can be used as a supplementary drug for various ailments.[36]

Successive extracts of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of M.hortensis was evaluated for in vitro antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and reducing power determination method. Total phenolic and flavonoids content in each extracts were also determined. Gallic acid and ascorbic acid were used as reference standards. The extracts exhibited strong antioxidant DPPH radical scavenging activity with IC50 value of 0.4358, 80.75, 54.07, 49.98, 26.22 and 39.07 μg/ml for gallic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of M.hortensis respectively. The absorbance for reducing power was found to be 0.504,0.064, 0.057, 0.076, 0.190 and 0.226 for ascorbic acid, petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of M.hortensis respectively. Total phenol content was found to be 16, 7.42, 28, 144 and 32 mg equivalent to gallic acid per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of M.hortensis respectively. Total flavonoids content was found to be 4.98, 21.56, 50.79, 64.92 and 19.67 mg equivalent to rutin per gram of petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of M.hortensis respectively. From the above data, it is clear that among all the extracts used, the methanolic extract had strong antioxidant activity which could be due to the presence of flavonoids and phenols.[37]

9.7 Antioxidant and hepatoprotective activity

Millingtonia hortensis flower ethanolic extract was evaluated for the hepatoprotective and antioxidant potential. The extract was evaluated against carbon tetrachloride induced hepatotoxicity. Phytochemical studies were carried out to determine the total phenolic and flavanoid contents. Lipid peroxides, glutathione, superoxide dismutase, catalase and total protein levels were assessed along with histopathological studies of the liver. Pretreatment
with the flower extract of *Millingtonia hortensis* significantly enhanced the level of endogenous antioxidant and reduced the level of endogenous antioxidants and reduced the levels of hepatic marker enzymes in relation to the CCl$_4$ treated group (p<0.05).\[^{[38]}\]

### 9.8 Anti-phlorgistic activity

Hispidulin, a bioactive flavanoid isolated from the flowers of *Millingtonia hortensis* Linn. F., was tested for anti-phlorgistic effect by observing the inhibitory activity in 5-lipoxygenase pathway. The test was performed by incubating the hispidulin with 1-$^{14}$C-arachidonic acid and porcine leukocyte suspension containing 1–lipoxygenase. After the incubation, the 1-$^{14}$C-arachidonic acid and its metabolite were separated and quantified by RP-HPLC. Hispidulin showed inhibition of 65% at 64μM.\[^{[39]}\]

### 9.9 Anticonvulsant activity

The functional characterization of hispidulin (4’, 5, 7 – trihydroxy-6-methoxyflavone), a potent benzodiazepine(BZD) receptor ligand, was initiated to determine its potential as a modulator of central nervous system activity. After chemical synthesis, hispidulin was investigated at recombinant GABAA/BZD receptors expressed by *Xenopus laevis* oocytes. Concentrations of 50 nm and higher stimulated the GABA-induced chloride currents at tested receptor subtypes (α1-3, 5,6β2γ2S) indicating postive allosteric properties. Maximal stimulation at α1β2γ2S was observed with 10μM hispidulin. In contrast to diazepam, hispidulin modulated the α6β2γ2S-GABAA receptor subtype. When fed to seizure-prone Mongolian Gerbils (*Meriones unguiculatus*) in a model of epilepsy, hispidulin (10mg kg bw/day) and diazepam (2mg, kg bw/day) markedly reduced the number of animals suffering from seizures after 7 days of treatment (30 and 25% of animals in the respective treatment groups, vs 80% in the vehicle group). Permeability across the blood-brain barrier for the chemically synthesized, 14cs-labelled hispidulin was confirmed by a rat in situ perfusion model. With an uptake rate (Kin) of 1.14ml min$^{-1}$ g$^{-1}$, measurements approached the values obtained with highly penetrating compounds such as diazepam. Experiments with Caco-2 cells predict that orally administered hispidulin enters circulation in its intact form. At a concentration of 30 μM, the flavone crossed the monolayer without degradation as verified by the absence of glucuronidated metabolites.\[^{[40]}\]

### 9.10 Antiasthmatic activity

The methanolic extract of flowers of *Millingtonia hortensis* was found to possess significant bronchodilating effect on the isolated rat trachea. This
extract was further fractionated into petroleum ether, chloroform, n-butanol and aqueous fractions. Chloroform fraction possess prominent pharmacological effect. On further fractionation of the chloroform fraction by column chromatography enabled hispidulin, the branchodilating agent, to be isolated. Detection by TLC indicated that hispidulin is one of the compounds present in the smoke of the dried flowers. It is therefore likely that the antiasthmatic activity of the dried flowers of *M. hortensis* Linn. is due to hispidulin. Hispidulin is more potent than aminophylline on a molar basis. It was interesting to observe that the aqueous extract of these flowers exhibits a bronchoconstricting action which gradually diminishes upon storage.\[^{[17]}\]

9.11  Antihelmintic activity
Petroleum ether, benzene, chloroform, methanol and aqueous extracts of stem bark of *Millingtonia hortensis* (Bignoniaceae) was evaluated against adult earthworm *Pheretima posthuma*. Piperazine citrate was used as the standard drug. The methanolic extract showed dose dependent activity and a better activity in comparison to reference standard. Chloroform and benzene extracts 20mg/ml concentration also showed similar activity in comparison to piperazine citrate at dose of 60mg/ml. Aqueous extract was found to be inactive. Preliminary phytochemical screening revealed the presence of steroids, flavanoids and tannins in different extracts.\[^{[41]}\]

9.12  Antiinflammatory activity
Aqueous extract of *Millingtonia hortensis* Linn.F (Bignoniaceae) stem bark was evaluated for its antiinflammatory property by carrageenan induced paw edema. The potency of the extract was evaluated at about two doses of 200mg/kg and 400mg/kg and compared with the standard drug indomethacin 10mg/kg. The results showed that the drug possess significant antiinflammatory potential at both the doses as compared to the control groups and the results were comparable with the standard drugs.\[^{[42]}\]

10.0 CONCLUSIONS
The updated report on *Millingtoniahortensis* Linn.F(Bignoniaceae)- Commonly known as cork tree is a perennial herb, cultivated in various parts of India for its ornamental purpose. The tree is used for various indigenous treatments such as asthma, rheumatism, cancer, sinusitis, tuberculosis and tonic. The current review on the tree highlights about the botanical, Pharmacognostical, phytochemical and pharmacological aspects of the tree. This updated
review on the tree will be much more helpful for all those researchers who are all carrying out their investigations and research on this tree.

11.0 REFERENCES


