Pacific Journal of Medical and Health Sciences
ISSN: 2456-7450 | Vol.9, No. 2, 2024, pp - 94 - 103

PJMHS
PACIFIC JOURNAL OF MEDICAL AND HEALTH SCIENCES

Review

Ethnomedicinal Uses, Phytochemistry and Pharmacology of *Millingtonia hortensis L.f.*

Anju Kumari[1] and Vartika Jain[2]*

[1]Assistant Professor, Department of Botany
Shri Kalyan Rajkiya Kanya Mahavidyalya, Sikar, Rajasthan, Bharat

[2]Assistant Professor, Department of Botany
Government Meera Girls' College, Udaipur, Rajasthan, Bharat

***Corresponding Author Email:** vartikabotany@gmail.com

ABSTRACT

Millingtonia hortensis L.f.; a member of family Bignoniaceae; is a large ornamental tree. It is native to Burma but widely cultivated in gardens of India and referred to as Neem Chameli, Akash Neem, and Indian Cork Tree. It not only possess ecological benefits but also useful for medicinal purpose. The tree is traditionally used for the treatment of abdominal pain, body ache, headache, cough, diabetes, diarrhea, fever, indigestion, asthma and vomiting by ethnic communities of India. Phytochemical study of various plant parts has exposed the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, saponins, betacyanins, coumarins, terpenoids, phenolic and cardiac glycosides. Moreover, many therapeutic bioactive compounds such as hispidulin, scutellarein-5-galactoside, scutellarein, cornoside, hortensin, A-B, rengyol, rengyoside isorengyol, β-carotene, β-sitosterol and millingtonine have been isolated from this plant. It has also demonstrated a range of pharmacological properties, including anti-inflammatory, antibacterial, antioxidant, anthelmintic, hepatoprotective, antimutagenic, cytotoxic, larvicidal, anti-convulsant, anti-proliferative and anti-aging in several studies. These pharmacological properties have established a strong scientific foundation for various medicinal uses of M. hortensis as reported in traditional medicine. However, further in vivo as well as large scale clinical studies are required to establish its therapeutic potential.

KEYWORDS: Ethnic Communities, Tree Jasmine, Millingtonine, Hispidulin, Anti-inflammatory

INTRODUCTION

Millingtonia hortensis L.f. syn. *Bignonia azedarachta* Kon. & Sims and *Bignonia suberosa* Roxb. is a member of botanical family- Bignoniaceae. It is commonly known as Indian cork tree, Tree jasmine, *Akas Nim, Mini-Chambeli, Neem Chameli, Cork-Gach, Akash Mallige, Akas-Nimb, Mara-Malli, avala-Nimb, Kavuki, Berate, Beratu, Reali, Katesam Bakeni, Mach-Mach, Kat-Malli, Buch, Sitahara,* and *Bakeni* etc. in various languages[1].

It is a fast-growing, tall, deciduous, perennial tree with a straight trunk, few branches and rich green leaves. It has a height of twenty-four meters [Figure 1] and usually cultivated in gardens as an ornamental tree. Its bark is tough, corky, and yellowish-grey. Its leaves are 2-3 pinnate, with oval to ovate-lanceolate leaflets. It blooms at night and sheds its flowers before dawn. The flowers are grouped in terminal panicles or corymbose clusters and are long, tubular, silvery-white or pinkish, and highly fragrant

[Figure 2]. The compact, thin capsule fruit bears numerous flat, winged seeds[1,2].

The plant is also reported for its ecological benefits. For example, its high air pollution tolerance property could be useful for urban landscaping[3]. A recent study from parks of Bangkok in Thailand has revealed the carbon dioxide absorption potential of *M. hortensis* which has shown significant increase with a deficit in vapour pressure until it reached its maximum and reduced with decrease in vapour pressure deficit without showing any seasonal difference. This shows that its plantation in urban green spaces could be helpful for allowing adaptability to unfavourable environmental consequences, such as urban heat island effects and droughts due to climate change[4]. Dried leaves of *M. hortensis* have also reported to produce silver nanoparticles which have revealed a potent cardioprotective effect in male wistar rats against isoproterenol-induced cardiotoxicity[5].

It is a valuable therapeutic plant found throughout Southern Asia, including Southern China, Vietnam, Thailand, Burma, Myanmar, and Cambodia; widely cultivated in Malaysia, India and Indonesia and sometimes naturalized[2,6]. The plant grows best in tropical forests under direct sunlight. It grows in a variety of soil types. Its flower petals have been used in rituals and bark is utilised for producing yellow dye[2,7]. Though, some review articles have been written on the plant species[2,7], but due to its multiple benefits as evidenced in the present technology era; an attempt has been made in this paper to provide a recent updated review on its ethnomedicinal uses, phytochemistry and pharmacological activities.

METHODOLOGY

For preparing a comprehensive review, online literature search was carried out using the keywords for example, *Millingtonia hortensis, Akas Nim,* Indian Cork Tree, Pharmacology, Chemical composition, Ethnobotany, Traditional knowledge, Animal study, *In vitro, In vivo* etc. using the popular databases namely, Pubmed, Springer Link, Scopus, Science Direct, Google Scholar, and Research gate. Moreover, certain useful books were also consulted. After this, the gist of all the important findings is presented in following sections under the three main headings; Ethnomedicinal uses, Phytochemical profile and Pharmacological profile.

ETHNOMEDICINAL USES

M. hortensis is a significant medicinal plant, used to treat numerous ailments in traditional medicinal system by ethnic communities. It is reported as an antipyretic in Indonesia[1]. Its

Figure 1: Tall tree of *Millingtonia hortensis*

Figure 2: Floral buds of *Millingtonia hortensis*

desiccated flower is beneficial for the lungs. It is also used for cough diseases[2]. The plant is employed to treat abdominal pain, headache, body ache, cough, diabetes, diarrhea, fever, indigestion, vomit, asthma, sinusitis and liver diseases etc. by various ethnic communities of India[8]. Its flowers and leaves are used for the treatment of jaundice and chest pain by Khampti tribe in Arunachal Pradesh located in North-East India[9].

In Myanmar, leaves are used in the treatment of hypertension and menstruation. Hypertension and heart palpitations can be treated by consuming a soup prepared with flowers or by consuming shoot. A paste of the root with sugar or salt is used to treat dizziness and heart palpitation, a paste of bark and root can cure sore eyes, a paste of root is used to treat gas disorders, and rubbing a paste made from bark or root on the tongue can cure alcoholic toxicity. The fresh root boiled with jaggery is used to treat vitiligo[6].

PHYTOCHEMICAL PROFILE

Various phytochemicals have been isolated from different parts of *M. hortensis* which have revealed biological activities and have the ability to protect human health against various ailments[10].

The bark of *M. hortensis* contains a bitter substance and some tannin[1]. Hispidulin, a flavonoid was reported from the dried flowers of *M. hortensis*[11]. Several phyto-constituents from different parts of *M. hortensis* such as hispidulin from flowers and hispidulin, hispidin, β-carotene, dinatin, rutinoside, hortensin from leaves, bitter substances and tannins from bark and hentriacontane, paulownin, rapachol, beta-sitosterol from roots have been reported[12]. Scutellarein, hispidulin and hortensin have been reported by Nair and Sivakumar[13], recimic rengyolone, cornoside, rengyoside B, rengyoside A rengyol, and isorengyol by Takeshi *et al*.[14] and millingtonine by Takeshi *et al*.[15].

Maneemegalai and Monika[16] have reported presence of flavonoids, alkaloids, saponin, tannins, phlobatannins, cardiac glycosides and terpenoids from its leaves. A preliminary qualitative screening of phytochemicals indicated the presence of tannins, steroids and flavonoids in various extracts of *M. hortensis* stem bark[17]. The ethanolic flower extract of *M. hortensis* was found to contain amino acids, proteins, flavonoids, and phenolics. The total phenolic content was measured as 241.2 ± 3.0 mg tannic acid equivalent per g, and the total flavonoid content was found to be 58.5 ± 2.06 mg equivalent of catechin per gram[18]. Qualitative phytochemical analysis methanolic extract of of *M. hortensis* leaves was carried out by Jadhav *et al*.[19] which revealed the presence of triterpenoids and flavonoids. A new phytoconstituent- Rutin was also revealed by GC-MS analysis.

Janani and Ananthi[20] evaluated *M. hortensis* flower extract for qualitative phytochemical screening and identification of bioactive phytocompounds by GC-MS analysis. Preliminary phytochemical screening of the ethanolic extract of *M. hortensis* flower showed the presence of carbohydrates, glycosides, flavonoids, phenolic compounds, and reducing sugar. Major identified compounds with retention time (RT) were Aspidoseprmidin 17-ol, 1- acety-19, 21- epoxy-15, 16-dimethoxy (27.90); 2- methyltetracosane (28.24); cis-13, 16-Docasdienoic acid (30.86); Alfaxalone (29.17); Olean-12-ene-3, 15, 16, 21, 22, 28-hexol (33.14); Stigmasta-5, 22- dien-3- ol, acetate, (3β) (34.20); β-Sitosterol (35.72); Stigmasta-5,24(28)-dien-3-ol,(3β) (36.10); Betulin (37.86).

Chumbhale *et al*.[21] screened the phytochemicals in different extracts of *M. hortensis* stem which revealed the presence of tannins, carbohydrates, flavonoids, glycosides, and phenolic compounds in the methanolic, ethyl acetate insoluble fraction of methanolic extract and ethyl acetate soluble fraction of methanolic extract, while aqueous and petroleum ether extracts revealed the presence of proteins and phytosterol, respectively. The total phenolic content was observed as 16% in petroleum ether, 24% in ethyl acetate soluble, 28% in ethyl acetate insoluble, 32% in aqueous and 38% in methanol extracts, respectively.

Kaleena *et al*.[10] prepared different extracts namely, aqueous, methanol, ethanol, chloroform and petroleum ether extracts of *M. hortensis* leaves and shown the presence of carbohydrates, alkaloids, phenols, flavonoids, saponins, coumarins, tannins, and betacyanins. They also identified phytoconstituents in ethanol extract of leaves using GC-MS analysis. GC–MS was performed employing a database of National Institute Standards and technology containing over 62,000 patterns. Various bioactive compounds were reported with their retention time namely, Z-7-Pentadecenol (22.58), Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester (19.75), Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester (23.21), Aspidofractinine-3-methanol (2a,3a,5a) (19.52), 4-Piperidineacetic acid,1-acetyl-5-Ethyl-2[3-[2-hydroxyethyl]-1H-Indol-2-yl]-a-methyl-methyl ester (19.12), Heptadecanoic acid, 15-methyl-ethyl ester (18.9), Oleic Acid (17.83), 8-Octadecenoic acid, methyl ester (17.13), Pentadecanoic acid,14methyl-methyl ester (15.73), Estra-1,3,5(10)-trien-17a-ol (14.05), 9-Oxabicyclo[3.3.1]nonane-2,6-diol (13.67), 7-Octadecenal (12.7), 4,6, Heptadien-3-one,1,7,-diphenyl (11.6).

Sivaraj *et al*.[22] described the presence of various phytoconstituents namely, Isoquinoline,1-ethyl, flavone, oleic acid, coumarin, 8-methoxy-3-(4-nitrophenyl), and phenol,2,6-bis (1,1-dimethyl ethyl)-4- [(4-hydroxy-3,5- dimethyl phenyl) methyl] from methanolic extract of *M. hortensis* leaves by GC-MS technique. Aqueous extract of flowers exhibited the presence of flavonoids, terpenoids, phenolic, cardiac glycosides, and carbohydrates with remarkably high phenolic content[23]. Recently, Kumari[24] identified 37 compounds from the methanolic extract of *M. hortensis* root through GC-MS technique. The maximum peak area was covered by Guanosine (47.99%) followed by Linoleic acid (8.33%), Glyceraldehyde (6.87%), 5-Hydroxymethylfurfural (5.07%), 3-Diazo-2,4-Pentanedione (4.51%), n-Hexadecanoic acid (4.06%), 1,2,3-Propanetriol, monoacetate (2.07%), etc.

Deethae *et al*.[25] identified eighteen bioactive compounds namely, Eucalyptol, Bicyclo[2.2.1]heptan-2-one, 1,7,7-

trimethyl-, (1S)-, endo-Borneol, Cyclohexanol, 1-methyl-4-(1-methylethyl)-, Linoleyl methyl ketone, 9-Octadecene, 1,1-dimethoxy-, (Z)-, trans-13-Octadecenoic acid, Oleic acid, Isopropyl palmitate, Hexadecanoic acid, ethyl ester, Ethyl 14-methyl-hexadecanoate, 19,19-Dimethyl-eicosa-8,11-dienoic acid, 9,12-Octadecadienoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, Octadecanoic acid, ethyl ester, 9,12-Octadecadienoic acid (Z,Z)-, 2,3- dihydroxypropyl ester, 5à-Pregn-16-en-20-one, 6-Hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9, 10,10a-octahydro-1-phenanthrenemethanol (1à, 4aá, 10a.alpha) from the ethanol extract of *M. hortensis* stem bark using GC-MS technique.

PHARMACOLOGICAL PROFILE

Several pharmacological properties for example, antimicrobial, antioxidant, anti-proliferative, larvicidal, cytotoxic, anti-inflammatory, hepatoprotective, anthelminitic, anticonvulsant etc. have been reported in scientific investigations from various parts of *M. hortensis* and a number of bioactive constituents identified from *M. hortensis* are accountable for such activities[2,7].

Antimicrobial Activity

The antibacterial efficacy of *M. hortensis* leaf aqueous alcohol extract has been shown by Jetty and Iyengar[26] against twenty distinct bacterial strains and two yeast cultures. With minimum inhibitory concentration (MIC) values of 12.5 µg/mL, *Bacillus subtilis* and *Bacillus polymixa* were the most susceptible Gram-positive bacteria, whereas *Bacillus pustulis, Bacillus licheniformis,* and *Bacillus cereus* had MIC values of 25 µg/mL. *Escherichia coli, Salmonella*, and *Pseudomonas aeruginosa*; the Gram-negative bacteria had the lowest minimum inhibitory concentrations of 6.25, 12.5, and 12.5 µg/mL, respectively. *Sarcina lutea* and the other two species of *Pseudomonas* had MIC values of 25 µg/mL, while *Clostridium* sp. is inhibited at 100 µg/mL. *Candida albicans* and *Saccharomyces cerevisiae* exhibited MIC values of 50 and 100 µg/mL, while nystatin had MIC values of 1.56 and 3.13 µg/mL, respectively.

Sharma *et al*.[27] have investigated *M. hortensis* extracts against various pathogenic fungi. The results showed that the methanol extract was more effective than fluconazole against yeast-like fungi: 4-fold against *Candida krusei* with a minimal inhibitory concentration of 4 µg/mL and 2-fold (MIC-2 µg/mL) against *S.*

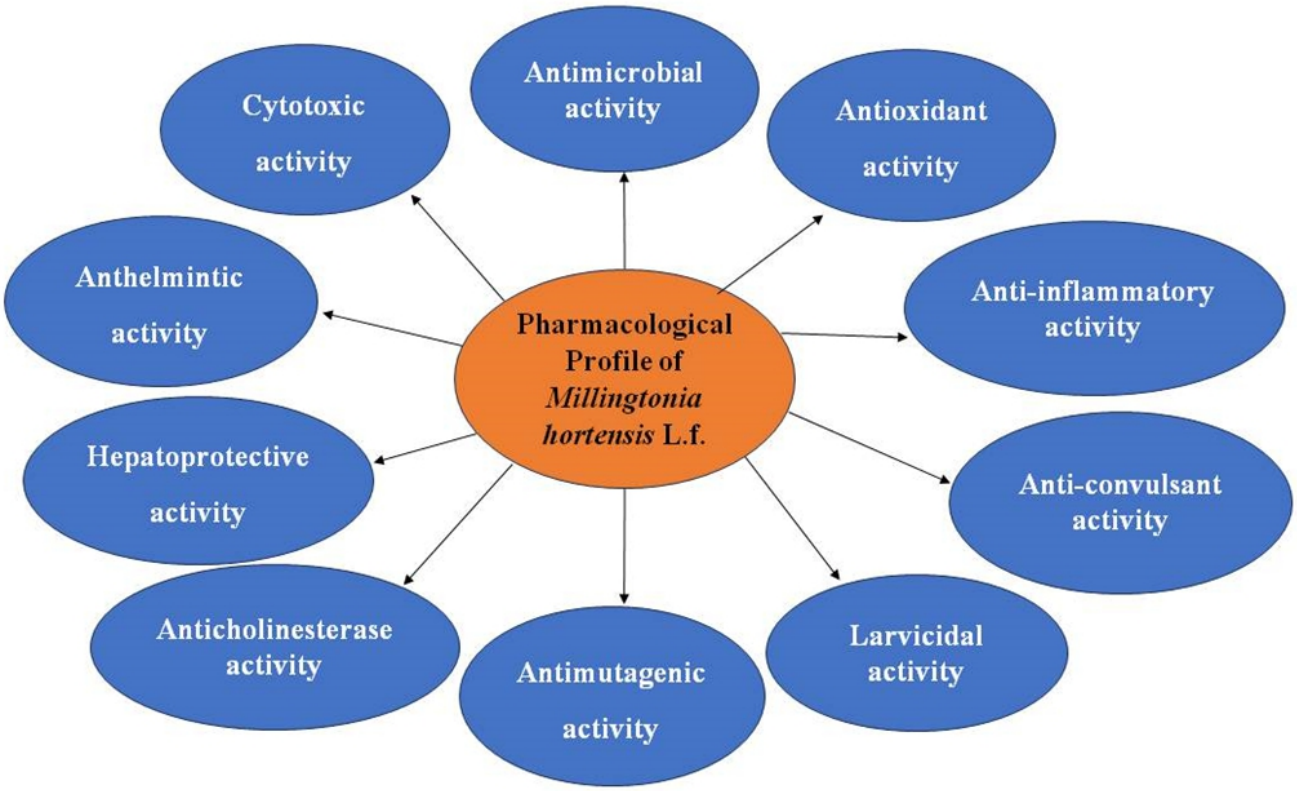

Figure 3: Pharmacological Profile of *Millingtonia hortensis*

cerevisiae, whereas it was as effective as fluconazole against *Candida glabrata*. The aqueous extract was also found to be four times more effective against *C. krusei* (MIC: 4 µg/mL) and *S. cerevisiae* (MIC: 2 µg/mL). As compared to the standard, chloroform and ethyl acetate extracts were less effective against all fungal pathogens except *C. krusei*. All extracts demonstrated less activity against *Trichosporon cutaneum*; filamentous fungus than the standard.

The essential oil of *M. hortensis* L. flower was evaluated against several bacterial species by Sittiwet[28] and reported broad antimicrobial spectrum activity at low concentrations. Flowers were vapor-distilled for their essential oil yielding between 0.5 and 2%. The agar diffusion susceptibility test revealed that six out of ten tested microbes, four gram-positive bacteria, namely *Staphylococcus epidermidis*, *B. subtilis*, *S. aureus*, and *Lactiplantibacillus plantarum*, and two gram-negative bacteria, namely *Proteus vulgaris* and *E. coli* were inhibited. The MICs and MBCs (minimum bactericidal concentration) of essential oil of *M. hortensis* flower were determined using the agar dilution and broth macro dilution methods, with results ranging from 0.5- 2 ml/L and 1-4 ml/L, respectively.

Maneemegalai and Monika[16] evaluated aqueous, methanol and ethanol extracts of *M. hortensis* leaves and flowers against various opportunistic and primary pathogens using the agar disc diffusion technique. The primary pathogens used were *Salmonella typhi, Salmonella paratyphi* A, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Vibrio cholerae*, and *B. subtilis*, and the opportunistic pathogens were *E. coli, Enterococcus faecalis, S. aureus, Proteus mirabilis*, and *P. aeruginosa*. The ethanolic and methanolic extracts of leaves were the most active against all organisms except *P. aeruginosa, P. mirabilis* and *S. typhi* whereas the flower extract was less effective against *P. mirabilis*. The leaf aqueous extract demonstrated greater antimicrobial activity than the aqueous extract of flowers. The MIC varied between 25 mg/mL and 50 mg/mL based on the microorganisms and extracts used.

Kumar and Ravichandran[29] reported the antimicrobial potential for aqueous extract of stem bark of *M. hortensis* against various strains of microorganisms by agar disc diffusion method. Evaluation was carried out against different bacteria such as *S. aureus*, *B. subtilis*, *S. paratype, K. pneumoniae, Micrococcus luteus, Staphylococcus albus, V. cholerae, Corynebacterium diphtheriae, P. aureginosa* and fungi such as *Monococcus purpurea* and *Aspergillus fumigatus*. The extract showed poor antibacterial activity but good antifungal activity. Nagaraja and Paarakh[30] evaluated *in vitro* antibacterial activity of crude petroleum ether, chloroform, benzene, methanol, and aqueous extracts of *M. hortensis* stem bark against *E. coli, S. aureus*, *P. aeruginosa,* and *B. subtilis* using the agar disc diffusion method. In comparison to ampicillin, a standard drug, petroleum ether extract was effective against *P. aeruginosa, B. subtilis, and S. aureus*, whereas benzene, chloroform, methanol, and aqueous extracts were effective against all four test organisms. *S. aureus* had MICs of 50, 100, 50, 50, and 50 µg/mL; *B. subtilis* had MICs of 25, 100, 50, 50, and 25 µg/mL; *E. coli* had MICs of 200, 100, 50, 50, and 25 µg/mL; and *P. aeruginosa* had MICs of 10, 50, 50, 50, and 50 µg/mL for petroleum ether, benzene, chloroform, methanol, and aqueous extracts, respectively.

The antimicrobial effect of ethanolic extract of *M. hortensis* leaf was evaluated against five microorganisms using disc diffusion method. The results demonstrated a maximum diameter zone of 13 mm in *P. aeroginosa* and a minimum diameter zone of 6.1 mm in *S. typhi*. Other microorganisms, such as *K. pneumoniae, Citrobactor brakii,* and *E. coli*, exhibited a minimum inhibition zone concentration diameter varying from 10µg/µL to 25µg/µL at a concentration of 30 µg/µL. However, at the concentration of 30 µg/µL, almost all the microorganisms revealed a higher inhibition zone which is significantly comparable to standard diameter zones[10].

The well diffusion method was used by Sivaraj *et al*.[22] to evaluate the antibacterial activity of the methanolic leaf extract of *M. hortensis*. At a concentration of 500 µg/mL, the extract exhibited a maximal zone of inhibition of 22 mm against *Micrococcus luteus*. Sasidharan *et al*.[31] have investigated the antibacterial potential of methanolic extract of *M. hortensis* bark against gram-positive bacteria, *B. subtilis*. The study was found effective with 0.2 mm zone of inhibition.

The antibacterial activity of aqueous and ethanol extracts of leaf and stem bark of *M. hortensis* was evaluated by Deethae *et al*.[25] against nine bacterial strains namely, *E. faecalis, Enterococcus faecium, S. aureus, B, cereus, E. coli, P. aeruginosa, K. pneumoniae, Acinetobacter baumannii, P. mirabilis*. The aqueous extract of leaves showed zone of inhibition (6.5 ± 1.0 mm) against only *B. cereus*, while aqueous extract of stem bark revealed zone of inhibition against two strains namely, *B. cereus* (7.3 ± 0.9) and *S. aureus* (7.2 ± 1.3) at concentration of 500 mg/mL. The ethanol extract of leaves showed inhibition zone against *E. faecium* (8.4 ± 0.7), *S. aureus* (7.4 ± 0.5), *B. cereus* (7.8 ± 0.8), while ethanol extract of stem bark exhibited zone of inhibition against *E. faecalis* (14.4 ± 0.8), *E. faecium* (11.5 ± 1.1), *S. aureus* (10.7 ± 1.0), B. cereus (11.8 ± 0.9), *P. aeruginosa* (8.8 ± 0.5) and *A. baumannii* (8.6 ± 1.2) at 500 mg/mL concentration. The results revealed the broad spectrum antibacterial activity of ethanol extract of stem bark.

Anti-arboviral Activity

Hispidulin was isolated from ethanolic extract of *M. hortensis* leaves after evaluation of the anti-arboviral activity. With EC_{50} ranging from 37.8 to 134.1 µg/mL for *M. hortensis* leaves extract, anti-arboviral activity against the three viruses, namely, Chikungunya (CHIKV), Zika and Mayaro virus (MAYV) was evident. Viral cytopathic impact of MAYV and CHIKV was suppressed by hispidulin with EC_{50} values of 32.2 µM and 78.8 µM, respectively[32].

Antioxidant Activity

Babitha *et al*.[18] demonstrated *in vivo* antioxidant potential of the ethanolic extract of *M. hortensis* flower in rats exposed to carbon tetrachloride (CCl_4). The application of extract mitigates the oxidative changes caused by CCl_4. Compared to the control group, CCl4 significantly ($p<0.05$) increased the levels of malondialdehyde (MDA) and decreased the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) in rat liver tissue. Pretreatment of rats with 200 and 400 mg/kg of flower extract prior to CCl_4 significantly ($p< 0.05$) increased the levels of enzyme and non-enzymatic antioxidants in comparison to the CCl_4-intoxicated group. Following treatment with the extract, MDA levels decreased considerably compared to the CCl_4 control group ($p< 0.05$). Curcumin as a standard increased the antioxidant levels and decreased the MDA levels significantly ($p< 0.05$) compared to the CCl_4-treated group.

Chumbhale *et al*.[21] observed significant antioxidant potential in various extracts of *M. hortensis* stem as compared to the standard drug ascorbic acid using different radical scavenging assays. In comparison to other extracts, the methanolic extract has greater efficacy and potent activity, such as 0.3563 ± 0.010 for nitric oxide radical scavenging activity, 0.3992 ± 0.015 for superoxide anion radical scavenging activity, 0.2618 ± 0.012 for hydrogen peroxide radical scavenging, and 0.3989 ± 0.011 for anti-lipid peroxidation. Kaleena *et al*.[10] performed DPPH radical scavenging activity and superoxide radical scavenging activity of methanol, ethanol, aqueous, chloroform, and petroleum ether extracts of *M. hortensis* leaf. Among the solvents examined, ethanol extract showed the highest DPPH radical scavenging potential. The 80% inhibition was observed at 70µg/mL concentration of ethanol extract of leaf. In superoxide radical scavenging assay, 70% radical scavenging activity was measured at 100 µg/mL concentration of ethanolic extract.

Sivaraj *et al*.[22] demonstrated the antioxidant potential of the methanolic extract of *M. hortensis* stem which showed DPPH scavenging activity (92.49±0.41) at 300 µg/mL with IC_{50} value of 140.97 µg/mL and superoxide radical scavenging activity (54.30±0.13) at 60 µg/mL with IC_{50} value of 1.53 µg/mL.

Antioxidant potential of leaves of *M. hortensis* was evaluated using DPPH, FRAP, ABTS, and ORAC assays. The dichloromethane fraction of the extract exhibited the highest free radical scavenging activity when DPPH, ORAC and ABTS assays were applied, while, in the FRAP assay, n-hexane extract had the highest effect. Twenty-eight phytocompounds were identified in the ethanolic leaf extract through LC-MS/MS analysis. The total phenolic content of the ethanolic extract was 34.137 ± 0.509 µg Gallic acid Equivalent/mg, while the total flavonoid content was 10.256 ± 0.579 µg Rutin Equivalent/mg demonstrating the possible reasons behind strong antioxidant potential of *M. hortensis*[33].

Anti-inflammatory Activity

Kumar *et al*.[34] demonstrated the anti-inflammatory activity of an aqueous extract of *M. hortensis* stem bark in rats with carrageenan-induced paw edoema. The potency of extract was evaluated at two concentrations, 200mg/kg and 400mg/kg, as compared to 10mg/kg of indomethacin, the standard drug. Compared to the control groups, the results showed that both doses of the drug possess significant anti-inflammatory potential, and the results were comparable to the standard drugs.

In vitro and *in vivo* anti-inflammatory effects and molecular mechanisms of ethanol extracts (Mh-EE) of *M. hortensis* were determined. The Griess reagent was used to assess the generation of nitric oxide (NO), and the MTT test was used to assess the viability of RAW264.7 and HEK293T cells. PCR and RT-PCR were used to assess the mRNA expression of inflammatory cytokines, and constituent analysis of Mh-EE was conducted using GC/MS-MS and HPLC. Western blotting was used to assess protein levels and CESTA was conducted for evaluation of Mh-EE's thermal stability. Lastly, western blotting was used to assess the levels of protein expression in an *in vivo* model of gastritis caused by HCl/EtOH. In LPS-induced RAW264.7 cells, Mh-EE decreased NO generation without significantly compromising cell viability. Furthermore, Mh-EE inhibited the production of pro-inflammatory factors, including COX2, IL-1β, and iNOS. Mh-EE also decreased phosphorylation of Syk kinase, IKKα, IκBα, and AKT and downregulated expression of TLR4 and recruitment of MyD88. In mice, stimulated with HCl/EtOH, Mh-EE concurrently reduced NF-κB signalling. As the role of Syk kinase in disease onsets such as inflammatory disorders and cancers is evident, the effect of Mh-EE thus keeps significance because the ethanolic extract has clearly exhibited Syk inhibitory and anti-inflammatory effects in the *in vivo* acute gastritis mouse model[35].

Larvicidal Activity

Kaushik and Saini[36] looked into the larvicidal efficacy of *M. hortensis* acetone leaf extract against larvae of *Culex quinquefasciatus, Anopheles stephensi*, and *Aedes aegypti*. Significant results were obtained regarding the extract's efficacy, with LC_{50} values of 83.18 ppm, 104.70 ppm, and 138 ppm, respectively. Kaushik and Saini[37] evaluated 11 plants of semi-arid region for larvicidal potential against IV instar larvae of *Aedes aegypti* mosquitoes. Among them, acetone extract of *M. hortensis* leaves revealed the highest larvicidal efficacy with an LC_{50} value of 123 ppm and LC_{90} value of 323.6 ppm.

The larvicidal activity of essential oils extracted from the flowers of *M. hortensis* was evaluated against *A. aegypti* larvae by Thongpoon and Poolprasert[38]. The larvae third to fourth-stage were treated with different concentrations (control, 25, 50, 100, 250, and 500 ppm) of flower extract. These larvae showed an average lethal concentration of approximately

208.5 ppm to kill 50% of treated larvae within 24 hours. On the basis of the mortality rate, the dose of extract at 500 ppm revealed the highest efficacy in regulating the larvae, with 98% mortality after 24 hours of exposure.

Hepatoprotective Activity

Rats with CCl4-induced hepatotoxicity were used to test the ethanolic flower extract of *M. hortensis* for its *in vivo* hepatoprotective properties. The activities of serum alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), direct bilirubin, and total bilirubin increased significantly ($p<0.05$) in the CCl_4 inducer control, although total protein levels dramatically reduced. Compared to the inducer control, pre-treatment with the ethanolic extract of flower decreased the levels of the above-mentioned markers and increased the levels of tissue total protein significantly ($p < 0.05$). Maximum protection was observed at the highest dose of the extract. In addition, the histopathological investigations of the liver of CCl_4-exposed rodents revealed fatty alterations, swelling, necrosis, and inflammatory infiltration, as well as a loss of hepatocytes. Liver sections from rodents treated with a higher dose of the extract demonstrated regeneration of hepatocytes, normalization of lipid changes, and a reduction in liver necrosis. In the curcumin-treated group (control), lipid accumulation and necrosis returned to normal levels[18].

Anthelmintic Activity

Promising findings were seen in research that evaluated the anthelmintic activity of several extracts (petroleum ether, benzene, chloroform, methanol, and aqueous extracts) of *M. hortensis* stem bark against adult earthworm *Pheretima posthuma*. Piperazine citrate was used as the typical reference medication. Methanol had the highest level of activity and dose-dependent anti-helmintic properties among all the studied extracts when compared to the reference standard. When compared to piperazine citrate at a dose of 60 mg/ml, extracts of chloroform and benzene (20 mg/ml) also exhibited comparable efficacy. The aqueous extract lacked any activity[17].

Kumar and Kavimani[39] investigated the anthelmintic activity of an aqueous extract of *M. hortensis* stem bark against *Pheretima posthuma*. The experiment was conducted at the concentrations of 10, 20, 30, 40, and 50mg/mL, with piperazine citrate and piperazine hexahydrate serving as the standard. For each cohort, the duration between paralysis and death was calculated. The anthelmintic activity of the aqueous extract was found to be less than that of the standard drugs. They concluded that the aqueous extract *of M. hortensis* is not an effective anthelmintic drug.

Chumbhale *et al*.[40] examined the anthelmintic activity of different extracts of *M. hortensis* stem against *Pheritima posthuma* earthworms. Each extract was evaluated in a bioassay at a concentration of 20 mg/mL, which involved determining the time of worm paralysis and mortality. The extracts revealed significant anthelmintic activity with albendazole (20 mg/mL) and distilled water as a standard reference drug and control, respectively. The investigation revealed that among the various extracts, the soluble ethyl acetate fraction of methanolic extracts exhibited the most potent anthelmintic activity.

Anti-aging Activity

Jumnongprakhon *et al*.[23] assessed the impact of aqueous flower extract of *M. hortensis* on SK-N-SH cells exposed to hydrogen peroxide (H_2O_2)-induced senescence. Senescence-associated β-galactosidase (SA-β-gal) enzyme overactivation was the primary cause of the aging process. This enzyme mediated several negative reactions, including the creation of intracellular ROS, the control of cellular senescence, and synaptic loss. Utilising 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, flow cytometry, and ROS test, they showed that MH improved cell viability and lowered both apoptotic cells and ROS generation in a dose-dependent way when compared to the aging group ($P<0.01$). Along with the enhancement of Sirt-1 protein, there was a significant decrease ($P<0.01$) in the number of SA-β-gal-positive cells after administration of MH. Moreover, MH also enhanced synaptic plasticity in aging neurons by upregulating synaptophysin expression and reducing acetylcholinesterase activity ($P < 0.01$).

Cytotoxic Activity

The MTT assay was used by Deethae *et al*.[25] to determine the cytotoxic effect of ethanol and aqueous extracts of *M. hortensis* leaf and stem bark against macrophage AW264.7 cell line. Cell viability was found greater than 80% in ethanol extracts of *M. hortensis* leaf and stem bark at concentrations ≤0.625 mg/mL and in aqueous extracts of *M. hortensis* and stem bark at concentrations ≤5 mg/mL.

Hepatocellular carcinoma (HepG2) and colorectal cancer (LS-513) cell lines were used in an *in vitro* cytotoxic evaluation of *M. hortensis* leaf extract. Results showed that the ethanolic extract had the highest activity among all the fractions, with the least IC_{50} value[33].

Antimutagenic Activity

The liquid preincubation method of the *Salmonella*/microsome test was used to examine the mutagenicity and antimutagenicity of hispidulin and hortensin; the flavonoids reported from *M. hortensis* by Chulasiri *et al*.[41]. Both the compounds exhibited no mutagenicity or cytotoxicity against *S. typhimurium* strains TA98 and TA100 at the maximum dose tested, 100 µg per plate, in the presence or absence of S9 mix. However, these substances were antimutagenic against 2-aminoanthracene, aflatoxin B1 (in strain TA98), and

dimethylnitrosamine (in strain TA100). The direct mutagenic activity of 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide and sodium azide was not inhibited in strains TA98 and TA100, respectively.

Anti-proliferative Activity

A human colon cancer cell line RKO was used to test the effects of ethanol and aqueous extracts of M. hortensis stem bark on apoptotic induction. RKO cell viability was evaluated using the MTT (3-(4,5-di-methylthizol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In a dose- and time-dependent way, the aqueous extract of M. hortensis suppressed cell growth and proliferation, but not the ethanol extract. The techniques for DNA fragmentation and flow cytometry were used to identify the apoptotic cells. The amount of apoptotic cells increased in response to aqueous extract treatment in a dose-dependent manner. DNA ladders were evident in RKO cells treated with 200, 300, and 400 μg/ml of aqueous extract, indicating that it blocked the apoptotic pathway's ability to cause cell proliferation in an RKO colon cancer cell line[42].

RKO colon cancer cells have demonstrated the induction of apoptosis upon exposure to an aqueous crude extract of *M. hortensis*. Though, its mechanism is yet unknown. Three aqueous fractions and the partially purified crude extract using Sephadex LH-20 were also collected. The MTT test was used to examine the cytotoxicity of each fraction. While fractions 2 and 3 had no effect, fraction 1 exhibited a dose-dependent antiproliferative effect on RKO cells by decreasing the expression of anti-apoptotic protein, Bcl-xL and p-Bad and the expression of p-Akt whereas increasing the levels of total Akt[43].

Anti-asthmatic Activity

Methanol extract of *M. hortensis* flowers revealed a bronchodilating effect on isolated rat trachea. Methanol extract was further separated into aqueous, petroleum ether, chloroform, and n-butanol fractions. Pharmacological analyses revealed that the greatest noticeable impact was produced by the chloroform fraction. It was possible to isolate hispidulin, the bronchodilating agent, by further separating the chloroform fraction using short column chromatography. Hispidulin was found to be one of the components in the smoke of the dried flowers, according to TLC analysis. Thus, hispidulin is most likely responsible for the antiasthmatic properties of *M. hortensis* dried flowers showing more potency than aminophylline. It is important to note that the aqueous extract of the flowers had a broncho-constricting effect that becomes less pronounced with storage[11].

Anticholinesterase Activity

Ellman's approach was employed to detect the anticholinesterase activity of *M. hortensis* leaves. The results showed that the dichloromethane fraction had the highest activity against AChE, causing 80.6 ± 0.007% inhibition, while the ethyl acetate fraction had the lowest activity, causing 31.8 ± 0.02 % inhibition at a concentration of 0.1 mg/mL. Based on the molecular docking studies, apigenin was found to have stronger interactions with amino acid residues SerA:200, HisA:440, and those in the peripheral region, which were important for strong inhibitory effects against AChE, making it more effective than hispidulin[33].

Anticonvulsant Activity

Hispidulin (4′, 5, 7-trihydroxy-6-methoxyflavone) is a strong benzodiazepine (BZD) receptor ligand. Its potential as a modulator of central nervous system activity led to the start of its functional characterisation. Following chemical production, hispidulin was examined at *Xenopus laevis* oocyte-expressed recombinant GABAA/BZD receptors. GABA-induced chloride currents at tested receptor subtypes (α1−3, 5,6β2γ2S) were activated at concentrations of 50 nM and higher, suggesting positive allosteric characteristics. With 10 μM hispidulin, maximum stimulation at α1β2η2S was seen. α6β2γ2S-GABAA receptor subtype was regulated by hispidulin, in contrast to diazepam. Hispidulin (10 mg Kg bw/day) and diazepam (2 mg Kg bw/day) were fed to seizure-prone Mongolian Gerbils (*Merionesun guiculatus*) in an epilepsy model, and after 7 days of treatment, the percentage of animals experiencing seizures was significantly lower (30% and 25% of animals in the respective treatment groups, vs. 80% in the vehicle group).

An *in situ* perfusion model in rats was used to validate the chemically synthesised, 14cs-labelled hispidulin's permeability across the blood–brain barrier. Measurements showed an absorption rate (Kin) of 1.14 ml/min/g, which was close to the values seen with more penetrating substances like diazepam. Hispidulin taken orally is expected to reach the bloodstream intact, according to studies conducted on CaCo-2 cells. The lack of glucuronidated metabolites at 30 μM indicated that the flavone had not broken down when it passed the monolayer[44].

Anti-phlorgistic Activity

An antiphlorgistic action was assessed by measuring the inhibitory activity in the 5-lipoxygenase pathway for hispidulin; a bioactive flavonoid, extracted from the flowers of *M. hortensis*. To conduct the test, hispidulin was incubated with a suspension of porcine leukocytes that included 5-lipoxygenase and 1–14C-arachidonic acid. 1-14C-arachidonic acid and its metabolite were isolated and measured using RP-HPLC following the incubation. Hispidulin revealed 65% inhibition at a concentration of 64 μM[45].

CONCLUSION

Millingtonia hortensis L.f. is a tall tree; usually cultivated in gardens. It has shown CO_2 absorption efficacy along with high

air pollution tolerance capacity. Various parts of the tree such as roots, leaves, stem, stem-bark and flowers are used for the treatment of several ailments by indigenous communities of India. Moreover, many therapeutic bioactive molecules namely, hispidulin, millingtonine, eucalyptol, betulin, β-Sitosterol etc. have been reported from its different parts. Pharmacologically, the plant has shown antioxidant, cytotoxic, antimicrobial, hepatoprotective, anti-inflammatory, antimutagenic, anti-proliferative, larvicidal, anthelmintic, anti-convulsant and anti-aging properties in several *in vitro* and *in vivo* studies. However, there is a need for conducting further *in vivo* and clinical studies for tapping its medicinal potential to the optimum levels.

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

REFERENCES

1. Sastri BN, *Millingtonia*: The Wealth of India. A Dictionary of Indian raw materials & industrial products. Raw Materials Vol. VI: L-M, Council of Scientific Industrial Research, New Delhi 1962; 380.
2. Ramasubramaniaraja R. *Millingtonia hortensis* Linn – An Overview. *Int J Pharm Sci Rev Res* 2010; 4(2): 123-25.
3. Yarnvudhi A, Leksungnoen N, Andriyas T, *et al*. Assessing the Cooling and Air Pollution Tolerance among Urban Tree Species in a Tropical Climate. *Plants (Basel)*. 2022; 11(22):3074. doi:10.3390/plants11223074.
4. Tor-Ngern P, Leksungnoen N. Investigating carbon dioxide absorption by urban trees in a new park of Bangkok, Thailand. *BMC Ecol*. 2020; 20(1):20. doi: 10.1186/s12898-020-00289-4.
5. Savitha R, Saraswathi U. A study on the preventive effect of silver nano particles synthesized from *Millingtonia hortensis* in isoproterenol induced cardio toxicity in male wistar rats. *World J Pharm Pharm Sci*. 2016; 5(8):1442–1450.
6. DeFilipps RA, Krupnick GA. The medicinal plants of Myanmar. *PhytoKeys* 2018; 102: 1–341.
7. Kumari A, Sharma RA. A review on *Millingtonia hortensis* Linn. *Int J Pharm Sci Rev Res* 2013; 19: 85-92.
8. Jain V, Jain SK. Compendium of Indian Folk Medicine and Ethnobotany (1991-2015). New Delhi, India: Deep Publications. 2016.
9. Bushi D, Bam K, Mahato R, *et al*. Ethnomedicinal plants used by the indigenous tribal communities of Arunachal Pradesh, India: a review. *Ethnobot Res Appl*. 2021; 22:34.
10. Kaleena PK, Janaki A, Elumalai D *et al*. Phytochemical screening, antioxidant and antibacterial activities of *Millingtonia hortensis* (L). *Int J Curr Pharm Res* 2017; 9(5):162-167.
11. Anulakanapakorn K, Nuntavan P, Jutamaad S. Phytochemical and Pharmacological studies of the flowers of *Millingtonia hortensis* Linn. *J Sci Soc* 1987; 13(2): 71-83.
12. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. PID, CSIR, 1995; Vol. 4: 478-479.
13. Nair AGR, Sivakumar R. Non-identity of hortensin from *Millingtonia hortensis* with 3,4′-dihydroxy-6,7-dimethoxyflavone. Phytochemistry 1992; 31*(*2): 671-673.
14. Takeshi H, Yuka K, Kazuhiro O. Cyclohexylethanoids and related glucosides from *Millingtonia hortensis*. *Phytochemistry* 1995; 39(1): 235-241.
15. Takeshi H, Kazuhiro O, Ryoji K *et al*. Millingtonine, an unusual glucosidal alkaloid from *Millingtonia hortensis*. *Phytochemistry* 1996; 41(1): 317-321.
16. Maneemegalai S, Monika P. Determination of activity of leaf and flower extracts of *Millingtonia hortensis* L. against primary and opportunistic pathogens. *J Herb Med Toxicol* 2010; 4(2): 65-70.
17. Nagaraja MS, Padmaa MP. *In vitro* anthelmintic activity of stem bark of *Millingtonia hortensis* Linn. *Int J Pharma Biol Sci* 2011; 2(2): 15-19.
18. Babitha S, Banji D, Banji OJ. (2012). Antioxidant and hepatoprotective effects of flower extract of *Millingtonia hortensis* Linn. on carbon tetrachloride induced hepatotoxicity. *J Pharm Bioallied Sci* 2012; 4(4): 307–312.
19. Jadhav SS, Yadav PJ, Mohite SK. Phytochemical screening and development and validation of HPLC method for isolated constituent in extract of *Millingtonia hortensis* Linn. *Int J Pharm Pharm Res* 2014; 1(2): 22-32.
20. Janani K, Ananthi T. GC-MS analysis of flowers of *Millingtonia hortensis* L. f. *Res J Pharm Technol* 2017; 10(7): 2003-2006.
21. Chumbhale DS, Chavan MJ Upasani CD. Phenolic content and *in vitro* antioxidant activity of *Millingtonia hortensis* Linn. *Int J Pharm Chem Biol Sci* 2017; 7(3): 224-29.
22. Sivaraj C, Aswitha V, Srinidhi M *et al*. Antibacterial, antioxidant activities and GC-MS analysis of leaves extract of *Millingtonia hortensis* L. *Pharma innov Int J* 2019; 8(1): 513-521.

23. Jumnongprakhon P, Pinkaew D, Phuneerub P. The antiaging property of aqueous extract of *Millingtonia hortensis* flowers in aging neuron. *J Adv Pharm Technol Res* 2021; 12(1): 14-21.
24. Kumari A. GC-MS analysis of methanolic extract of root of *Millingtonia hortensis* L.f. *Int J Botany Stud* 2022; 7(12): 37-40.
25. Deethae A, Sritapunya T, Khetnaoanurak S. Evaluation of antibacterial, antiinflammatory activities and GC-MS profiling of *Millingtonia hortensis* Linn. leaf and stem bark extracts. *ASEAN J Sci Tech Report* 2023; 26: 72-80.
26. Jetty A, Iyengar DS. Antimicrobial activity of *Millingtonia hortensis* leaf extract. *Pharm Biol* 2000; 38(2): 157–60.
27. Sharma M, Puri S, Sharma PD. Antifungal activity of *Millingtonia hortensis*. *Indian J Pharm Sci* 2007; 69(4): 599-601.
28. Sittiwet C. Anti-microbial activities of *Millingtonia hortensis* Linn. Flowers essential Oil. *J Pharm Toxicol* 2009; 4(1): 41-44.
29. Kumar MS, Ravichandran N. Cytotoxicity and antimicrobial studies of stem bark of *Millingtonia hortensis* Linn. *Int J Pharm Pharm Sci* 2011; 3(5): 247-249.
30. Nagaraja MS, Paarakh PM. Antibacterial activity of *Millingtonia hortensis* Linn. stem bark. *Pharmacologyonline* 2011; 2: 841-844.
31. Sasidharan BR, Balaji P, Ashok KR *et al*. A study on biochemical constituents and anti-bacterial activity in the bark extract of *Millingtonia hortensis*. *J Curr Microbiol Appl Sci* 2020; 9(5): 2436-2442.
32. Cardoso Reis AC, Valente GM, Silva BM *et al*. Anti-arboviral activity and chemical characterization of hispidulin and ethanolic extracts from *Millingtonia hortensis* L.f. and *Oroxylum indicum* (L.) Kurz (Bignoniaceae). *Nat Prod Res* 2023; 37(4):613–617.
33. Elnaggar S, Zaghloul S, Kassem H. *et al*. (2024). Metabolic profiling of *Millingtonia hortensis* L.f. leaves using UHPLC-MS/MS and evaluation of its antioxidant, cytotoxic, anticholinesterase activities and molecular docking. *Egypt J Chem*. 2024; doi: 10.21608/ejchem.2024.302449.9974.
34. Kumar MS, Kumar SS, Astalakshmi N *et al*. Antiinflammatory effect of aqueous extract of *Millingtonia hortensis* L.f. stem bark on rats. *Int Res J Pharm* 2013; 4(4):104-105.
35. Liu Y, Kim JM, Lim HK *et al*. *Millingtonia hortensis* L.f. ethanol extract exerts *in vivo* and *in vitro* anti-inflammatory activities through inhibition of Syk kinase in NF-κB pathway. *J Ethnopharmacol* 2024; 332:118386.
36. Kaushik R, Saini P. Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. *J Vector Borne Dis* 2008; 45(1): 66–69.
37. Kaushik R, Saini P. Screening of some semi-arid region plants for larvicidal activity against *Aedes aegypti* mosquitoes. *J Vector Borne Dis* 2009; 46(3): 244–246.
38. Thongpoon C, Poolprasert P. Phytochemical screening and larvicidal activity of *Millingtonia hortensis* L.f. flower extract against *Aedes aegypti* Linn. *Witthayasan Kasetsat* 2015; 49: 597-605.
39. Kumar M, Kavimani S. Anthelmintic activity of *Millingtonia hortensis* Linn. F. stem bark on Indian adult earthworm. *Der Pharm Lett* 2014; 6(5): 89-91.
40. Chumbhale DS, Chaudhari S, Upasani C. Preliminary phytochemical analysis and *in vitro* anthelmintic activity of *Millingtonia hortensis* Linn. *Int J Pharm Chem Biol Sci* 2016; 6: 304-08.
41. Chulasiri M, Bunyapraphatsara N, Moongkarndi P. Mutagenicity and antimutagenicity of hispidulin and hortensin, the flavonoids from *Millingtonia hortensis* L. *Environ Mol Mutagen* 1992; 20: 307-312.
42. Tansuwanwong S, Hiroyuki Y, Kohzoh I *et al*. Induction of apoptosis in RKO cancer cells line by an aqueous extract of *Millingtonia hortensis*. *Asian Pac J Cancer Prev* 2006; 7: 641-644.
43. Tansuwanwong S. Hiroyuki Y, Imai K *et al*. Antiproliferation and apoptosis on RKO colon cancer by *Millingtonia hortensis*. *Plant Foods Hum Nutr* 2009; 64: 11-17.
44. Dominique K, Philipp S, Karesh AY *et al*. The flavone Hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, traverses the blood brain barrier and exhibits anticonvulsive effects. *Br J Pharmacol* 2004; 142(5): 811-820.
45. Moongkarndi P, Bunyapraphatsara N, Srisukh V *et al*. The inhibitory activity in 5-lipoxygenase pathway of hispidulin from *Millingtonia hortensis* Linn. F. *J Sci Soc Thail* 1991; 17: 51-56.