www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 513-521

www.thepharmajournal.com
Received: 17-11-2018
Accepted: 23-12-2018

Dr. C Sivaraj
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Chennai, Tamil Nadu, India

V Aswitha
Department of Biotechnology, Rajalakshmi Engineering College, Rajalakshmi Nagar, Thandalam, Chennai, Tamil Nadu, India

M Srinidhi
Department of Biotechnology, Rajalakshmi Engineering College, Rajalakshmi Nagar, Thandalam, Chennai, Tamil Nadu, India

K Saraswathi
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Chennai, Tamil Nadu, India

P Arumugam
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Chennai, Tamil Nadu, India

Correspondence
Dr. C Sivaraj
Phytochemistry and Natural Products Laboratory, ARMATS Biotek Training and Research Institute, Chennai, Tamil Nadu, India

Antibacterial, antioxidant activities and GC-MS analysis of leaves extract of *Millingtonia hortensis* L

Dr. C Sivaraj, V Aswitha, M Srinidhi, K Saraswathi and P Arumugam

Abstract
Millingtonia hortensis L. (Indian cork tree) belongs to the family of Bignoniaceae. The leaves used as sinusitis, cholagogue, antipyretic and tonic in folklore medicine. The aim of present research study was to evaluate the antibacterial, antioxidant activities of methanol leaf extract of *M. hortensis* and to identify the bioactive compounds by GC-MS analysis. Different types of antioxidant assays were carried out for the evaluation of antioxidant activity. Antibacterial activity was carried out by well diffusion method, in which the leaf extract showed maximum zone of inhibition of 22 mm for *Micrococcus luteus* at 500 µg/mL concentration. The maximum DPPH˙ radical scavenging activity was 92.49±0.41 at 300 µg/mL concentration and the IC_{50} was 140.97 µg/mL concentration. The maximum superoxide (O_2·-) radical scavenging activity was 54.30±0.13 at 60 µg/mL concentration and the IC_{50} was 51.53 µg/mL concentration. The maximum Fe^{3+} reduction and Mo^{6+} reduction were 72.76±0.22 and 88.85±0.28 at 120 µg/mL concentration. The RC_{50} of Fe^{3+} and Mo^{6+} reduction were 21.41 µg/mL and 21.45 µg/mL concentration respectively. The GCMS analysis of methanol extract of leaves of *M. hortensis* revealed the presence of bio-active compounds such as Isoquinoline,1-ethyl, Flavone, Oleic acid, Coumarine,8-methoxy-3-(4-nitrophenyl) and Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl] exhibiting various therapeutic applications such as antioxidant, antimicrobial, anti-proliferative and immuno-modulatory activities.

Keywords: *Millingtonia hortensis*, DPPH˙ radical, phenols, flavonoids, antibacterial, GC-MS

1. Introduction
Millingtonia hortensis L. (Indian cork tree) belonging to the family of Bignoniaceae. It is an important medicinal plant in southern Asia ranging from India, Burma, Thailand and South China. It is also called as akash neem, neem chameli, tree jasmine or Indian cork tree, native to South-East Asia [1]. It is tall deciduous tree and grows up to 25 m. It has corky bark and straight trunk, leaves are large, very ornamental and pinnately compound. Long leaves bear two or three widely spaced pinnae, and its imparipinnate and it resembles that of neem with 5-7 smooth leaflets, oval, pointed and slightly round-toothed, 1-3 inched long. Fruit is a 2-valued senticidal capsule, smooth flat capsule and it's partitioned. Fruit is acute at both ends, oblongoid, seeds discoid and winged (board-winged seeds). The flowers are in corymbose, long tubular, trumpet shaped, white and delightfully fragrant, mostly this tree flowers twice a year. They are bisexual and zygomorphic [2, 3]. The bell-shaped sepals of flower have five lobes. The flowers have four stamens with parallel anthers [4]. The whole plant is used to treat antimutagenic, anticancer, antifungal, antimicrobial and other medicinal uses. Flowers are used in treatment of asthma, sinusitis, cholagogue and tonic [5].

1.1 Plant Taxonomy
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Lamiales
Family	:	Bignoniaceae
Genus	:	*Millingtonia*
Species	:	*hortensis*
Common name	:	Neem chameli

Fig 1: Habitat of *Millingtonia hortensis*

2. Materials and Methods

2.1 Collection of leaves and preparation of extract

The leaves of *M. hortensis* were collected from Thiruvanmiyur, Chennai. The leaves were washed with tap water, shade dried for 10 d and course powdered by mechanical blender [6]. Twenty gram of leaves powder was soaked in methanol for 72 h and the supernatant was filtered by filter paper and condensed in a hot plate at 50°C, which yields gummy extract.

2.2 Qualitative phytochemical analysis

The methanol leaves extract of *M. hortensis* was subjected to preliminary phytochemical analysis for different classes of phytoconstituents using specific reagents [6] according to standard methods [7].

2.3 Determination of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds [8] with slight modifications. One hundred µL of methanol leaves extract (1mg/mL) of *M. hortensis* was mixed with 900 µL methanol and 1 mL of Folin-Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of aqueous solution of Na_2CO_3 (20% w/v) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured by UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference standard.

2.4 Determination of total flavonoids

The total flavonoid content of methanol leaves extract of *M. hortensis* was determined using aluminum chloride reagent method with slight modification as described by Liu *et al* [9]. Five hundred µL of extract (1mg/mL) was mixed with 0.5 mL of methanol and 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL of 10% (w/v) aluminium chloride solution was added and followed by 0.1 mL of 1 M NaOH solution was added. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using UV-Vis spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent.

2.5 *In vitro* antioxidant activities

2.5.1 DPPH˙ radical scavenging activity

The antioxidant activity of extract of *M. hortensis* leaves was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical [10]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (50-300 µg/mL) of plant extracts. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as standard reference. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample} \times 100}{\text{Control}}$$

2.5.2 Screening of antioxidant activity by dot-blot DPPH staining method.

Drops of DPPH (0.4 mM) were loaded onto a 5 cm x 5 cm TLC plate (silica gel 60 F254; Merck) in each column and allowed to dry for 3 min. The first row of TLC plate was considered as control, containing only DPPH. In second row, various concentrations of methanolic extract of leaves of *M. hortensis* were carefully loaded onto the DPPH spot. The third row of TLC plate was considered as standard reference, where ascorbic acid was carefully loaded onto the DPPH spot. The staining of the silica plate was based on the procedure of Soler- Rivas. Stained silica gel layer revealed a purple background with yellow to white spots at the location where radical scavenging capacity observed. The intensity of disappearance of purple color depends upon the amount of antioxidant molecules present in the extract of leaves of *M. hortensis* [11].

2.5.3 Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide radical scavenging activity was carried out for the methanol extract and the reaction mixture contains different concentrations (10-60 µg/mL) leaves extract of *M. hortensis* 50 mM of phosphate buffer (pH 7.8), 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT, added in that sequence [12]. The reaction was started by illuminating the reaction mixture for 150 s. After complete illumination, the absorbance was measured at 590 nm and the IC_{50} was calculated. Ascorbic acid was used as standard reference.

$$\% \text{ of superoxide } (O_2^{\cdot-}) \text{ radical inhibition} = \frac{\text{Control} - \text{Sample} \times 100}{\text{Control}}$$

2.5.4 Ferric (Fe^{3+}) reducing power activity

The reducing power of leaves extract of *M. hortensis* leaves determined by slightly modified method of Yen and Chen [13]. One mL of plant extract of different concentrations (20-120 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH-

6.6) and potassium ferricyanide [K_3 Fe $(CN)_6$] (1 mL, 1 % w/v). The mixtures were then incubated at 50°C for 20 min. One mL of trichloroacetic acid (10 % w/v) was added to each mixture. Then to the mixture 1 mL of $FeCl_3$ (0.1% w/v) was added and the absorbance was measured at 700 nm using Spectrophotometer. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \frac{\text{Sample} - \text{Control} \times 100}{\text{Sample}}$$

2.5.5 Phosphomolybdenum reduction activity

The antioxidant capacity of the extract of *M. hortensis* leaves assessed as described by Prieto *et al* [14]. The plant extract with concentrations ranging from 20 to 120 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), Sodium phosphate (28 mM) and Sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 90^0C for 90 min. The absorbance of the colored complex was measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum reduction} = \frac{\text{Sample} - \text{Control} \times 100}{\text{Sample}}$$

2.6 Antibacterial activity by well diffusion method

2.6.1 Bacterial strains

The microorganisms of Gram positive strains such as (*Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*) and Gram-negative strains *(Escherichia coli, Proteus vulgaris, Shigella flexneri*) were used for the evaluation of antibacterial activity.

2.6.2 Reference and control

Tetracycline was chosen as the standard reference for bacteria. The controls consist of solidifying agar onto which was solvent, and the test compounds were soluble in it.

2.6.3 Aseptic conditions

The aseptic chamber which consist of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

2.6.4 Nutrient broth agar medium

Nutrient broth agar medium was prepared (peptone-5 g; yeast extract-3 g; NaCl-5 g; distilled water-100 mL; pH-7±0.2; agar-20 g) according to the standard methods and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 15 lbs and at 121ºC for 15 minutes. The hot medium was poured in sterile Petri plates which were kept in the aseptic laminar chamber. The medium was allowed to solidify for 15 min [15]. Determination of antibacterial potential of the extracts was carried out using the agar well diffusion method. The solidified nutrient agar in the Petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The control, extract and standard were then poured into each well of desirable concentrations. Tetracycline was used as the standard with the concentration of 25 µg. All the plates containing sample loaded wells were incubated for 24 h at 37°C. After the incubation period, zone of inhibition in each plate, for each concentration of extract and standard were measured by calculating the diameter of zone of inhibition.

2.7 Thin layer chromatography

Thin layer chromatography (TLC) was carried out for the methanolic extract of *M. hortensis* leaves in Merck TLC aluminium sheets, silica gel 60 F254 (20 x 20 cm), preloaded plates. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 254 nm. The R_f values of the colored spots were recorded [16]. The ratio in which distinct bands appeared was optimized and R_f values were calculated.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

2.8 Gas chromatography–Mass Spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250^0C; interface temperature of 250°C; mass range of 50-600 mass units [17].

2.8.1 Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. Results and Discussion

3.1 Phytochemical analysis

The phytochemical analysis of methanolic leaves extract of *M. hortensis* showed (Table 1) the presence of alkaloids, terpenoids, phenolic compounds, flavonoids, glycosides and saponins.

Table 1: Qualitative analysis for methanol leaves extract of *M. hortensis*

S. No	Phytochemicals	Tests	Results
1.	Alkaloids	a. Mayer's reagent	+
		b. Hager's reagent	+
2.	Terpenoids	$CHCl_3$ + conc. H_2SO_4	+
3.	Flavonoids	NaOH solution	+
4.	Phenols	$FeCl_3$ solution	+
5.	Glycosides	Sodium nitroprusside solution+Con.H_2SO_4	+
6.	Saponins	Foam test	+
7.	Tannins	$FeCl_3$ solution	+

3.2 Total phenols and flavonoids

Total phenols and flavonoids were quantified in the methanol

extract leaves of *M. hortensis* showed to be responsible for the antioxidant activity. The total phenol content of leaves was 190.51±0.38 µg/mg and of GAE and total flavonoid content of leaves (Table 2) was 7.71±0.14 µg/mg of QE in the extract. These results provide a comprehensive profile of the antioxidant activity of leaves *M. hortensis* with respect to their phenols and flavonoids content.

Table 2: Quantitative phytochemical estimation of phenols and flavonoids of methanol leaves extract of *M. hortensis*

Phytochemicals	Amount (µg/mg)
Phenol content	190.51±0.38 GAE
Flavonoid content	7.71±0.14 QE

3.3 DPPH˙ radical scavenging activity

The ability of methanol extract of *M. hortensis* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). In leaves, the maximum DPPH˙ radical scavenging activity was 92.49±0.41 at 300 µg/mL concentration. Methanol extract of *M. hortensis* demonstrated high capacity for scavenging free radicals (Table 3) by reducing the stable DPPH (1,1- diphenyl-2-picrylhydrazyl) radical to the yellow colour 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract (Fig 2) [18]. The IC_{50} value in leaves was found to be 140.97 µg/mL concentration and was compared with standard (IC_{50} = 11.98 µg/mL concentration) ascorbic acid.

Table 3: DPPH˙ radical scavenging activity of methanol leaves extract of *M. hortensis*

S. No	Concentration (µg/mL)	% of inhibition* DPPH˙ radical
1	50	21.85±0.22
2	100	33.33±0.36
3	150	53.20±0.12
4	200	76.82±0.37
5	250	88.96±0.29
6	300	92.49±0.41

(*Average value of 3 replicates)

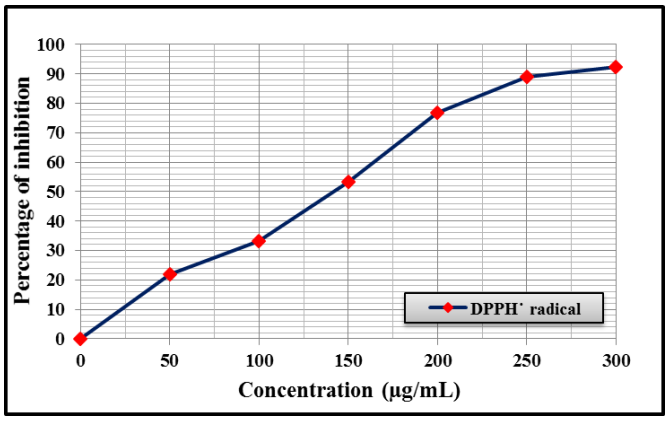


Fig 2: DPPH˙ radical scavenging activity of methanol leaves extract of *M. hortensis*

3.4 Dot-blot assay for rapid radical scavenging activity

The results of dot-blot assay showed colored spots where the aliquots of methanolic extract of *M. hortensis* leaves were placed in row. The purple zone on the plate indicates no (free radical scavenging) antioxidant activity and the yellow zone indicates antioxidant activity. The more intense the yellow color, the greater the antioxidant activity (Fig 3). The result indicates that the methanolic extract of *M. hortensis* leaves have significant antioxidant activity when compared to standard ascorbic acid.

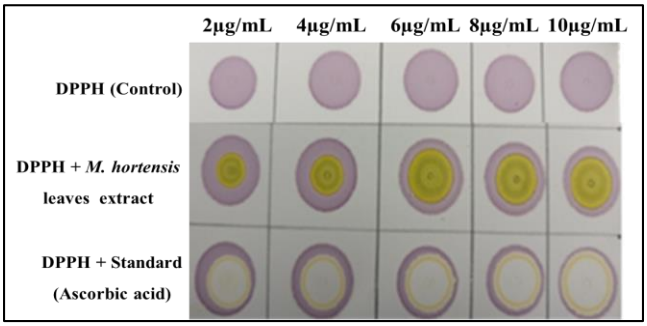


Fig 3: DPPH dot -blot assay of methanol extract of leaves of *M. hortensis*

3.5 Superoxide (O_2˙-) radical scavenging activity

Superoxide radical scavenging activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Superoxide is an oxygen-centered radical with selective reactivity. Although it is a relatively weak oxidant, superoxide exhibits limited chemical reactivity but can generate more dangerous species, including singlet oxygen and hydroxyl radicals, which cause the peroxidation of lipids. These species are produced by a number of enzyme systems. Superoxide can also reduce certain iron complexes such as cytochrome C. Superoxide anions are thus precursors to active free radicals that have potential for reacting with biological macromolecules and thereby inducing tissue damage. Also, superoxide has been observed to directly initiate lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical [19]. Superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents [20]. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the formazan, which is measured spectrophotometrically at 590 nm. Antioxidants are able to inhibit the blue NBT formation. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide reduction for leaves extract of *M. hortensis* was 54.30±0.13 at 60 µg/mL concentration (Table 4 and Fig 4). The IC_{50} value of leaves extract was 51.53 µg/mL concentration. It was considered to the standard of ascorbic acid (18.65 µg/mL concentration).

Superoxide radical, known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributes to tissue damage and various diseases. In a biological system, its toxic role can be eliminated by superoxide dismutase. The radicals may also play an important role during the peroxidation of unsaturated fatty acids and other potential susceptible substances. Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. It is an oxygen-centered radical with selective reactivity.

It also produced by a number of enzyme systems in auto oxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen. The biological toxicity of superoxide is due to its capacity to inactivate iron-

sulfur cluster containing enzymes, which are critical in a wide variety of metabolic pathways, thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. It can also reduce certain iron complex such as cytochrome c. Superoxide anions are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage. It has been implicated in several pathophysiological processes due to its transformation into more reactive species such as hydroxyl radical that initiate lipid peroxidation [21]. Also, superoxide has been observed to directly initiate lipid peroxidation. In addition, it has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical. Superoxide anion plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA. Superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Superoxide anion derived from dissolved oxygen by riboflavin methionine illuminate system and reduces NBT in this system [22]. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the blue formazan which is measured spectrophotometrically at 560 nm.

Table 4: Superoxide ($O_2^{\cdot-}$) radical scavenging activity of methanol leaves extract of *M. hortensis*

S. No	Concentration (µg/mL)	% of inhibition*
		Superoxide ($O_2^{\cdot-}$) radical
1	10	19.56±0.25
2	20	29.74±0.36
3	30	32.26±0.30
4	40	43.24±0.18
5	50	48.51±0.29
6	60	54.30±0.13

(*Average value of 3 replicates)

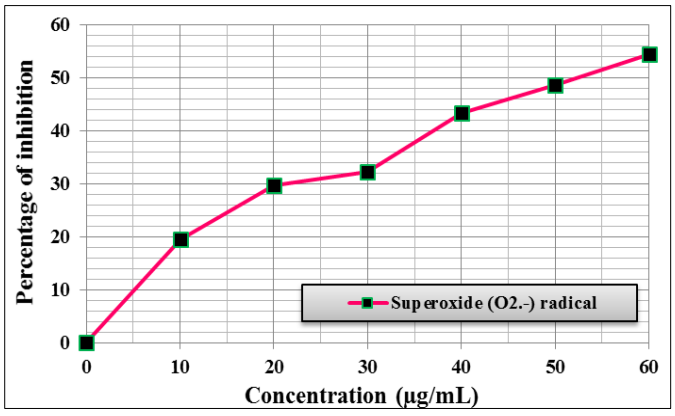


Fig 4: Superoxide ($O_2^{\cdot-}$) radical scavenging activity of methanol leaves extract of *M. hortensis*

3.6 Ferric (Fe^{3+}) reducing power activity

Reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the methanol extract of leaves of *M. hortensis* and the subsequent formation of ferro-ferric complex. The reduction ability increases with increase in concentration of the extract [23]. The maximum Fe^{3+} reduction of leaves was 72.76±0.22 at 120 µg/mL concentration (Table 5 and Fig 5) and the RC_{50} of leaves extract was 21.41 µg/mL concentration. It was compared with standard ascorbic acid (13.63 µg/mL concentration).

3.7 Phosphomolybdenum reduction activity

Phosphomolybdenum assay revealed the reduction of Mo (VI) to Mo (V) by the methanol extract of leaves of *M. hortensis* and formation of a Mo (V) complex at acidic pH. Increase in absorbance of the reaction mixture indicates increase in reducing power. The significant increase of methanol extract was found to be 88.85±0.28 at 60 µg/mL concentration.The result obtained was confirmed by the high potency of the methanol extract of leaves of *M. hortensis* towards the reduction of transition metal ions. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [24]. The experiment demonstrated higher antioxidant activity the RC_{50} of 21.45 µg/mL concentration for methanol extract of leaves of *M. hortensis* (Table 5 and Fig 5) and was compared with standard Ascorbic acid (RC_{50} value as 14.28 µg/mL concentration).

Table 5: Ferric (Fe^{3+}) reducing power and Phosphomolybdenum reduction activity of methanol leaves extract of *M. hortensis*

S. No	Concentration (µg/mL)	% of reduction*	
		Fe^{3+} reduction	Phosphomolybdenum reduction
1	20	46.70±0.20	46.62±0.26
2	40	55.61±0.11	51.55±0.39
3	60	62.79±0.38	75.27±0.46
4	80	64.36±0.17	83.89±0.43
5	100	67.03±0.33	87.51±0.12
6	120	72.76±0.22	88.85±0.28

(*Average value of 3 replicates)

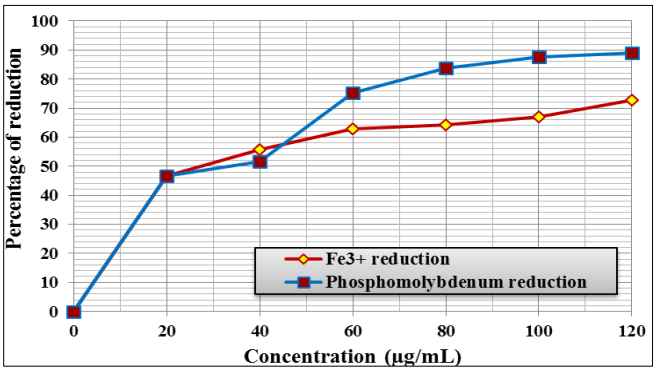


Fig 5: Ferric (Fe^{3+}) reducing power and Phosphomolybdenum reduction activity of methanol leaves extract of *M. hortensis*

3.8 Antibacterial Activity by well diffusion method

The methanol extract of leaves of *M. hortensis* was investigated for antibacterial activity against microorganism including Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus*) and Gram-negative bacteria *(Escherichia coli, Proteus vulgaris, Shigella flexneri*). The antibacterial sensitivity of the crude extract and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petri plates (Table 6 and Fig 6).

The antibacterial activity of these extracts could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth and metabolism of microbes. The comparatively poor antimicrobial profile indicated by the leaves extract which contain lesser levels of alkaloids and terpenoids, as evident from the quantitative analysis supports this correlation.

Table 6: Antimicrobial activity of methanolic leaves extract of *M. hortensis* against bacterial pathogens

S. No	Test Bacterial pathogens	Zone of inhibition (mm)				Standard (Tetracycline)
		125 µg	250 µg	375 µg	500 µg	
1	*Bacillus subtilis*	10	14	15	18	24
2	*Staphylococcus aureus*	12	14	16	20	23
3	*Micrococcus luteus*	14	16	18	22	18
4	*Escherichia coli*	10	12	14	17	22
5	*Proteus vulgaris*	-	14	15	16	22
6	*Shigella flexneri*	14	16	18	21	18

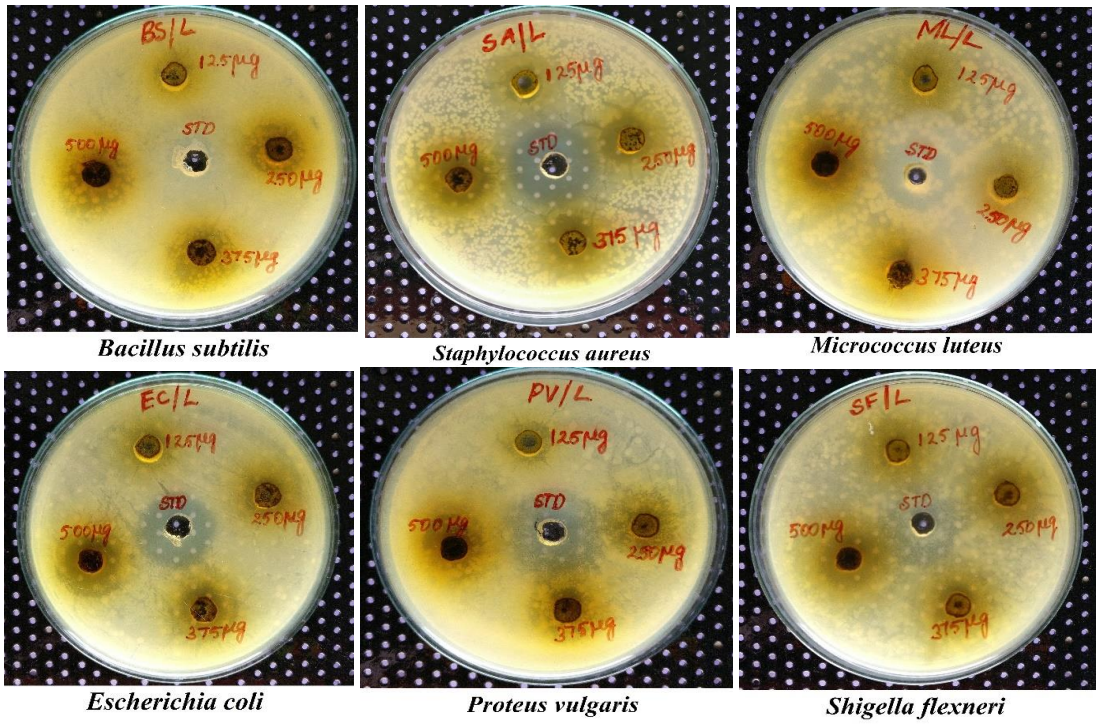


Fig 6: Antimicrobial activity of methanolic leaves extract of *M. hortensis* against bacterial pathogens

3.9 Thin Layer Chromatography

Thin layer chromatography analysis was carried out in the solvent system of Toluene: Ethyl acetate with the ratio of 1:1. The separated active compounds were visualized in UV light and iodine stain. The R_f values of the separated compounds were measured and tabulated (Table 7).

Table 7: R_f values of methanolic leaves extract of *M. hortensis* active compounds separated by thin layer chromatography

Spots observed	R_f Value (UV light at 254nm)
1	0.98
2	0.88
3	0.84
4	0.69
5	0.65
6	0.60

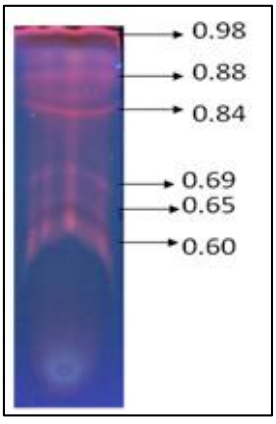
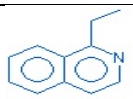
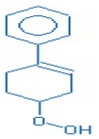
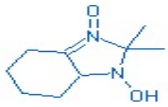
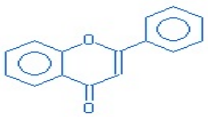
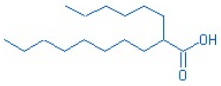
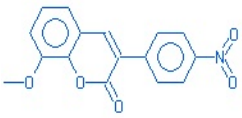
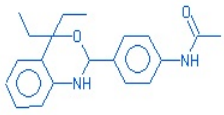
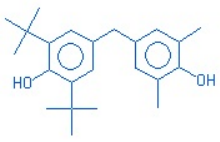
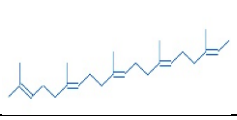


Fig 7: Active Compounds separated by Thin Layer Chromatography

3.10 GC-MS analysis

GC-MS analysis was carried out for the methanolic extract of *M. hortensis* leaves and the eluted compounds were showed in Table 8 and Fig 8. Oleic acid (9Z)-Octadec-9-enoic acid) ($C_{18}H_{34}O_2$) compound is fatty acid that occurs naturally in various animal and vegetable fats and oils. Oleic acid may be responsible for hypotensive (Table 9) (blood pressure reducing) effects of olive oil. Since adverse effects of both oleic acid and monosaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer, although consumption of oleate in olive oil has been associated with decrease in risk of breast cancer

Table 8: GC-MS analysis of methanol extract of leaves of *M. hortensis*

S. No	Retention Time	Compounds	Structure	Mol. Wt. (g/mol)	Mol. Formula
1	11.7	Isoquinoline,1-ethyl		157.21	$C_{11}H_{11}N$
2	13.73	1-cyclohexene,4-hydroperoxy-1-phenyl		190.23	$C_{12}H_{14}O_2$
3	16.02	1H-benzimidazol-1-ol,2,4,5,6,7,7a-hexahydro-2,2-dimethyl,3-oxide		158.20	$C_{10}H_{10}$
4	16.93	Flavone		222.24	$C_{15}H_{10}O_2$
5	17.65	2-Hexyldecanoic acid		256.43	$C_{16}H_{32}O_2$
6	18.58	11-octadecenoic acid, methyl ester		296.49	$C_{19}H_{36}O_2$
7	19.27	Oleic acid		282.46	$C_{18}H_{34}O_2$
8	19.42	Coumarine,8-methoxy-3-(4-nitrophenyl)		297.26	$C_{16}H_{11}O_5$
9	21.3	N-[4-[4,4-Diethyl-1,4-dihydro-2H-benzo[d][1,3] oxazin-2-yl]-phenyl]-acetamide		342.60	$C_{25}H_{42}$
10	24.63	Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]		340.50	$C_{23}H_{32}O_2$
11	26.95	2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene		342.60	$C_{25}H_{42}$

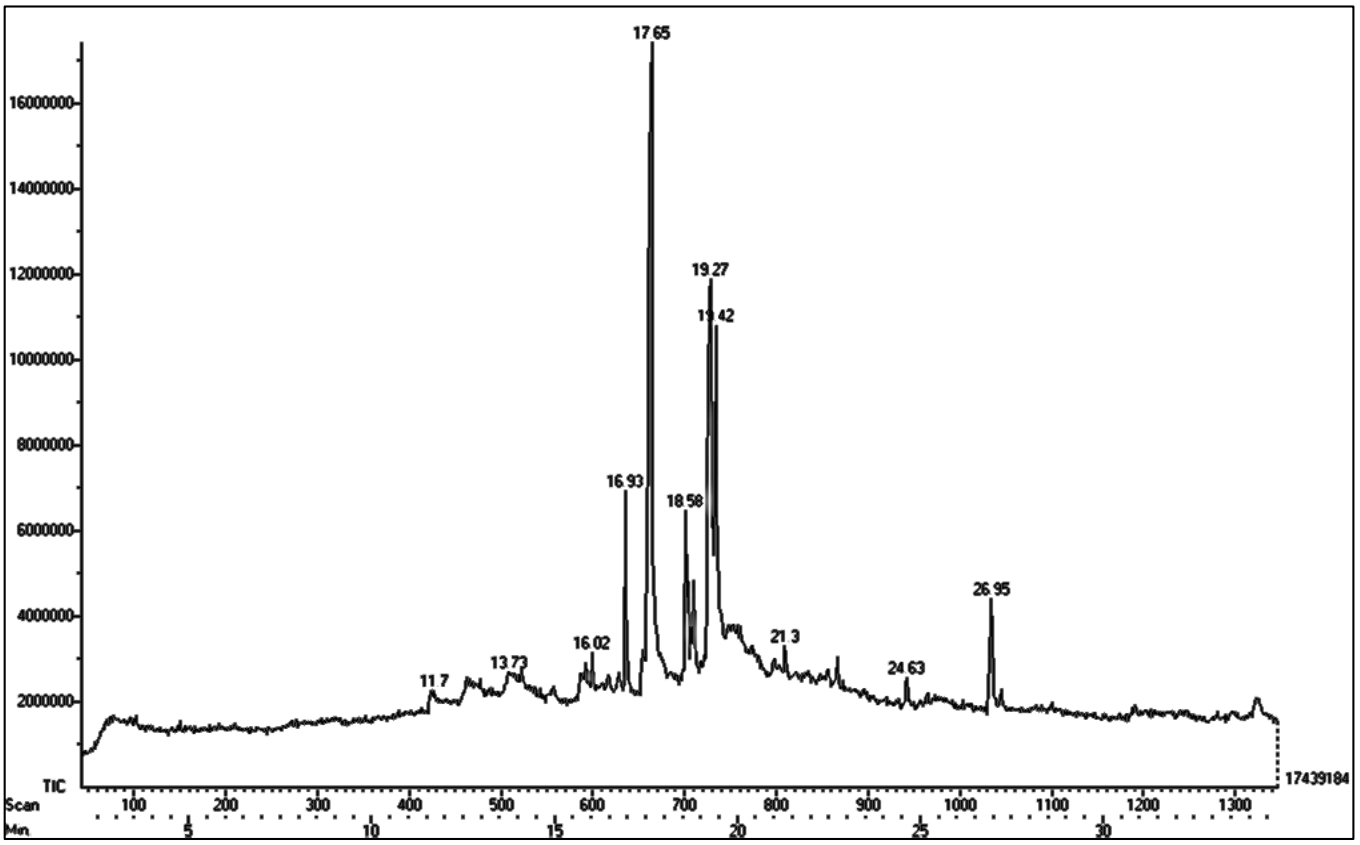


Fig 8: GC-MS chromatogram of methanol extract of leaves of *M. hortensis*

Table 9: Bio-activity of methanol extract of leaves of *M. hortensis* from GCMS analysis

S. No	Compound Name	Pharmacological Activity [25-29]
1	Isoquinoline,1-ethyl	Anticancer drug, Anti-angiogenic agent, Analgesic activity, Lipid peroxidation, Bone resorption, Antimalarial activity
2	Flavone	Reactive Oxygen Species (ROS) production can be reduced due to the presence of flavone. Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity.
3	Oleic acid	5 alpha reductase inhibitor, Hypocholestrolemic activity, Perfumery and flavor, Cancer preventing agent, Anti-inflammatory activity, Antibacterial activity
4	Coumarine,8-methoxy-3-(4-nitrophenyl)	Chronic infections, Cancer treatment, Blood coagulation, Anticoagulant activity, Edema (protent protective function)
5	Phenol,2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl) methyl]	Antimicrobial activity, Antioxidant activity, Antimalarial activity, Immuno-modulatory effect

4. Conclusion

The results of the present study indicate that extract of leaves *M. hortensis* has significant antioxidant activities to reduce harmful effect of radicals and anti-asthmatic activities to reduce asthma. Further molecular studies are required to find out the mechanism of action of bioactive compounds present in *M. hortensis* before it can be recommended for any practical widespread use of the plant. The results of various experiments conducted in the present study provide promising guideline regarding the potential uses of *M. hortensis* as an antioxidant agent. Medicinal plants are gaining much interest recently because their use in ethno medicine treating common disease such as cold, fever and other medicinal claims are now supported with sound scientific evidences. Any plant species showing promising bioactivity in extract is a valuable source for the wealth of resource poor communities. Most of the phytochemicals from plant sources such as phenolics and flavonoids have been reported to have positive impact on health and cancer prevention. High content of phenolic and flavonoids in medicinal plants have been associated with their antioxidant activities that play a role in the prevention of the development of age-related disease, particularly cause by oxidative stress. The important phytochemicals present in medicinal plants, shift towards natural products in pharmaceuticals and cosmoceuticals industry.

5. Acknowledgement

The authors are thankful to SAIF IIT MADRAS for providing facilities to carry out GC-MS analysis.

6. References

1. Arunakumari, Sharma RA. A review on *Millingtonia hortensis* Linn. International journal of pharmaceutical sciences review and research 2013; 19(2).17, pp.85-92.
2. Surendra Kumar M, Astalakshmi N, Jeena Chandran N, Jesmi Jaison P, Sooraj P, Raihanath T *et al*. A review on

Indian cork tree -*Millingtonia hortensis* linn.F. World journal of pharmacy and pharmaceutical sciences 2014; 3(10).pp.256-271.
3. Ramasubramaniaraja R. *Millingtonia hortensis* Linn – Overview. International journal of pharmaceutical sciences review and research 2010; 4(2) pp.123-125.
4. Blatter E, Millard WS. Some beautiful Indian trees. The Bombay natural history society. Mumbai.1954, pp.6:106.
5. Nagaraja MS, Padmaa M Paarakh. *Millingtonia hortensis* Linn – a review. Pharmacology online 2011; Newsletter, 2, pp.567-602.
6. Trease GE, Evans WC. 1983. Textbook of Pharmacognosy.12th edn. Balliese Tindall and Company Publisher, London.1983, pp.343-383.
7. Rambabu M, Venkat Raji Reddy G, Vijay Kumar R, Krishna Reddy M. Phytochemical analysis of medicinal plant extracts. World journal of pharmaceutical research 2017; 6(3):585-590.
8. Spanos GA, Wrosltad RE. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry 1990; 38, 1565-1571.
9. Liu X, Dong M, Chen X, Jiang M, Lv X and Yan G. Antioxidant activity and phenolics of endophytic *Xylaria* sp. From *Ginkgo biloba*. Food Chemistry 2007; 105, pp. 548-554.
10. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 29: pp. 1199-1200.
11. Soler-Rivas C, Espin JC, Wichers HJ. An easy and fast test to compare total free radical scavenger capacity of foodstuffs. Phytochemical Analysis 2000; 11:1-9.
12. Lokesh Deb SK, Dubey, Avijeet Jain, Amit Kumar Jain, Pandian GS. Free radical scavenging activity of aqueous n- butanol fraction of *Prunus Persica* aqueous extract. Journal of Natural Remedies 2009; 9(2):152-158.
13. Yen GC and Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry 1995; 43: pp.27-32.
14. Prieto P, Pineda M and Anguilar M. Spectrophotometric quantisation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Analytical Biochemistry 1999; 269, pp.337-341.
15. Sheila John, Sarah Jane Monica, Priyadarshini S, Sivaraj C, Arumugam P. Antioxidant and Antimicrobial Efficacy of Lemon (*Citrus limonum* L.) Peel. International Journal of Pharmaceutical Sciences Review and Research 2017; 46(1), pp. 115-118.
16. Stahl E. Thin Layer Chromatography. 2nd ed. Springer Pvt. Ltd. New Delhi 2005; 85.
17. Vardhini SP, Sivaraj C, Himanshu Ranjan, Arumugam P. GCMS, Phytochemical analysis and antioxidant activities of fruit pulp of *Aegle marmelos* (l.) Correa. European Journal of Biomedical and Pharmaceutical Sciences 2018; 5(2) pp.569-574.
18. Awika M, Rooney LW, Wu X, Prior RL. Cisneros Zevallos L. Screening methods to measure antioxidant activity of Sorghum (*Sorghum ialmatei*) and Sorghum product. Journal of Agricultural and Food Chemistry 2003; 51:6657-62.
19. Wickens AP. Aging and the free radical theory, Respiratory Physiology, 2001; 128:379-391.
20. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochemical Pharmacology 1988; 37(5):837-41.
21. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Journal of Agricultural and Food Chemistry 1994; 42(3):629–632.
22. Liu F, Ooi VEC, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sciences 1997; 60(10):763–771.
23. Stadtman ER. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. Free Radical Biology and Medicine 1990; 9:315-325.
24. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem 2001; 49:4083-4089.
25. Gaspar Diaz, Izabel Luzia Miranda and Marisa Alves Nogueira Diaz. Quinolines, Isoquinolines, Angustureine, and Congeneric Alkaloids — Occurrence, Chemistry, and Biological Activity. Nogueira, Marisa & Diaz, Gaspar & Miranda, Izabel. Quinolines, Isoquinolines, Angustureine, and Congeneric Alkaloids — Occurrence, Chemistry, and Biological Activity 2015; 141-162.
26. Justyna Mierziak, Kamil Kostyn and Anna Kulma. Flavonoids as Important Molecules of Plant Interactions with the Environment. Molecules 2014; 19, 16240-16265.
27. Gomathi Rajashyamala L, Elango V. Identification of bioactive components and its biological activities of *Evolvulus alsinoides* linn. A GC-MS study. International Journal of Chemical Studies 2015; 3(1):41-44.
28. Jain PK and Himanshu Joshi. Coumarin: Chemical and Pharmacological Profile. Journal of Applied Pharmaceutical Science 2012; 02 (06):236-240.
29. Elaiyaraja A and Chandramohan G. Comparative phytochemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. Journal of Pharmacognosy and Phytochemistry 2016; 5(6):158-171.