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Original Article

# PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *MILLINGTONIA HORTENSIS* (L)

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**ABSTRACT**

**Objective:** *Millingtonia hortensis* Linn (Bignoniaceae) is commonly known as cork tree and Akash neem. Aim of studies to determine the antioxidant activity and antibacterial activity.

**Methods:** The antioxidant activity of different solvent extracts were measured by chemical analyses involving the assay of 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging activity and super oxide radical scavenging activity.

**Results:** Phytochemicals (secondary metabolites) screening of methanol, chloroform, ethanol, petroleum ether, aqueous leaf extracts revealed the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, betacyanins, phenols and coumarins.

**Conclusion:** The presence of these phytochemicals and antioxidant capacity support the use of this plant as an antibacterial agent against the group of micro organisms tested.

**Keywords**: *Millingtonia hortensis*, Phyto compounds, Antioxidant activity, GC-MS analysis, Anti bacterial activity


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## INTRODUCTION

Medicinal plants play a key role in healthcare and about 80 % of the world's populations rely on the use of traditional medicine [1, 2]. Phytochemicals are primary and secondary metabolites of plants which fight to protect our health against diseases. Phytocompounds commonly found in plants have been reported to have multiple biological effects including antioxidant activity. In recent years, secondary plant metabolites (phytochemical) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents [3]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers and thus act as antioxidants [4].

Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases including cancer, cardiovascular diseases and inflammatory diseases. It is believed that high intake of antioxidant-rich food is associated with decreased risk of degenerative diseases, particularly cardio vascular diseases and cancer [5]. The synthetic antioxidants (e. g) butylated hydroxyl anisole (BHA) butylated hydroxyl toluene (BHT) or vitamin E [6-8] are commonly used. These synthetic antioxidants such as butylated hydroxyl anisole (BHA) butylated hydroxyl toluene (BHT) etc. may have carcinogenic and other harmful effects on the lungs and liver [9-11] of human beings. Scientists have continuously engaged in the search for naturally occurring potential and non-toxic antioxidants which could prevent free radical related disorders in human beings and also can replace the harmful synthetic antioxidants [9-11].

Plant derived antioxidants have advantages as they are less toxic and more effective and economical and hence there is a growing interest in identifying natural antioxidants of plant origin [12]. The search for cheap and abundant sources of natural antioxidants is attracting worldwide interest.

The drugs already in use to treat the infectious disease are of concern because drug safety remains an enormous global issue. Most of the synthetic drugs cause side effects and also most of the microbes have developed resistance against the synthetic drugs [13]. To alleviate this problem, antimicrobial compounds from potential plants should be explored. These drugs from plants are less toxic, side effects are scanty and also cost effective. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [14].

Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials is the need of the hour. Antimicrobials of plant origin have enormous therapeutic potential. Plant-derived antimicrobials have a long history of providing the much-needed novel therapeutics [15]. Plants constantly interact with the rapidly changing and potentially damaging external environmental factors. Being organisms devoid of mobility, plants have evolved elaborate alternative defence strategies, which involve an enormous variety of chemical metabolites as tools to overcome stress conditions.

*Millingtonia hortensis* Linn is an important medicinal plant, found throughout South Asia and is well known for its fragrant flowers. The leaves of *M. hortensis* are used as antipyretic, sinusitis, cholagogue and tonic in folklore medicine [16] and is a rich source of essential oil, flavonoids, tannin and alkaloids [17].

## MATERIALS AND METHODS

### Preparation of plant extract

*Millingtonia hortensis* leaves were collected and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre (Voucher No: PARC/2015/3058), West Tamabaram, Chennai-45. Leaves dried under shade at room temperature for about 20 d. The dried leaves were powdered and sieved to get fine powder using an electric blender. 70 g of the powder was filled in the thimble and extracted successively with methanol, petroleum ether, chloroform, ethanol, using soxhlet extractor for 10 h. All the extracts were concentrated using rotary flash evaporator and preserved at 4 °C in an airtight bottle until further use. (fig. 1)

### Phytochemicals screening

The phytochemical screening was carried out using standard procedures. [18] By this analysis, the presence of several phytochemicals listed in (table 1) were tested.

**Antioxidant assay**

**DPPH method**

The 1, 1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay was first described by [19] and was later modified slightly by numerous researchers. It is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is then measured by the decrease in absorption at 515 nm. In this method, a 0.1 mmol solution of DPPH in methanol is prepared, and 4 ml of this solution are added to 1 ml of the sample solution in methanol at varying concentrations. Thirty minutes later, the absorbance was measured at 517 nm. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound.

**Superoxide radical scavenging assay**

The superoxide anion radicals are produced in 2 ml of phosphate buffer (100 mmol, pH 7.4) with 78 µM-nicotinamide adenine dinucleotide (NADH), 50 µM nitro blue tetrazolium chloride (NBT) and test samples at different concentrations. The reaction mixture is kept for incubation at room temperature for 5 min. It is then added with 5-methylphenazinium methosulphate (PMS) (10 µM) to initiate the reaction and incubated for 5 min at room temperature. The colour reaction between superoxide anion radical and NBT is read at 560 nm. Gallic acid is used as a positive control agent for comparative analysis. The reaction mixture without test sample is used as a control and without PMS is used as blank [20]. The scavenging activity is calculated as follows,

A) % Scavenging activity = [(Control OD–Sample OD)/Control OD] × 100

**GC–MS analysis**

GC–MS analysis were carried out an SHIMADZU QP 2010T which comprised of an auto sampler and gas chromatography interfaced to a mass spectrometer (GC–MS) instrument employing the following condition: capillary column –624 ms (30 m × 0.32 mm × 1.8 m) operating in an electron mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1.491 ml/min and injection volume of 1.0 ml, injector temperature was 140 °C; ion source temperature of 200 °C. The oven temperature was programmed from 45° C. Mass spectra were taken at 70 eV.

**GC-MS identification of compounds**

Interpretation of mass spectrum GC–MS was conducted using a database of National Institute Standard and technology having more than 62,000 patterns. The spectrum of the unknown compounds stored in the NIST library. The compound prediction was based on Dr. Duke's Phytochemical and ethnobotanical database by Dr. Jim Duke of the agricultural research service/USDA. The names of the components of the test material were ascertained.

**Antimicrobial assay**

**Micro organisms**

All microbial strains were provided from the microbiology department, Presidency College, Chennai-05. Bacterial strains were maintained by subculture on nutrient agar favourable to their growth for 24 h in the dark at 37 °C. The antibacterial activity was evaluated using *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, *Salmonella typhi*, *Citrobacter braakii*.

**Antibacterial assay**

The antimicrobial activity of the extracts was determined by disc diffusion method with minor modifications which is based on the spread of antimicrobial compound in the solid medium [21, 22]. The Mueller–Hinton agar was poured in sterile Petri dishes (90 mm diameter). The paper discs (6 mm diameter) that were impregnated with ethanolic extract 10, 15, 20, 25, 30µg/µl and antibiotic tested standard discs were placed on the inoculated agar surface. Petri dishes were allowed to stand for 30 min at room temperature before incubation at 37 °C for 24 h. The effect of the ethanolic extract was reflected by the appearance around the disc with a transparent circular zone corresponding to the absence of growth. The diameter of inhibition zone was measured in mm. The larger the diameter of the area the more susceptible the strain [22, 23].

**Statistical analysis**

Three replicates of each sample concentration were used for statistical analysis by the Graphpad Prism version 5. Results with $P<0.05$ were considered to be significant.

**RESULTS**

**Phytochemical screening**

The preliminary phytochemical screening is a means of evaluating the potential phyto compounds in the leaf extract of *Millingtonia hortensis*. Phytochemical characterizations of solvent extracts of *M. hortensis* are presented in (table 1). The phytochemical screening revealed the strong presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, betacyanins, phenols and coumarins.

**DPPH radical scavenging activity**

DPPH radical scavenging activity of methanol, petroleum ether, chloroform, ethanol, aqueous extracts of *M. hortensis* leaf are shown in (fig. 3). Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity. At 70µg/ml concentration of ethanol leaf extract showed nearly 80% scavenging activity of DPPH.

**Superoxide radical scavenging activity**

In this assays, the liberated radicals are scavenged and the inhibition was estimated for different solvent extracts of *M. hortensis* leaf extracts (fig. 4). In the presence of an antioxidant, 70% inhibition was observed in ethanol extract at 100 µg/ml concentration followed by methanol extract.

**GC–MS analysis**

The composition and identification of the compounds present in the ethanol leaf extracts of *M. hortensis* by GC-MS analysis shown in (table 2). The active principles with their retention time (RT), molecular formula, molecular weight (MW) are presented in (fig. 4). The main constituents were Heptadecanoic acid, 15-methyl-methyl ester, Oleic Acid and Estra-1,3,5(10)-trien-17a-ol.

**Antibacterial assay**

The antimicrobial activity of the investigated extracts against five bacterial used by disc diffusion method was shown in (table 3). The obtained results of *M. hortensis* ethanolic leaf extract revealed the maximum diameter zone of 13 mm in *Pseudomona aeroginosa* and minimum diameter zone of 6.1 mm in *Salmonella typhi*, other microbes like *Klebsiella pneumonia*, *Citrobactor brakii* and *Escherichia coli* showed minimum inhibition zone concentration diameter ranging from 10µg/µl to 25 µg/µl. But almost all the microbes exhibited higher diameter zone at the concentration of 30 µg/µl nearer to standard inhibition zones. Antibacterial activity of *M. hortensis* leaf ethanol extract was assayed *in vitro* by disc diffusion method against microbes of *E. coli* and *P. aeroginosa, K. pneumonia*, *S. typhi*, *C. braakii*. The given (table 3). Shows the microbial growth inhibition of ethanolic leaf extracts of *M. hortensis*. Among the varying concentration of leaf extracts, higher concentration exhibited maximum antibacterial activity against the two clinical isolates. table 3 shows the zone of inhibition formed by the extracts against the bacterial strains on Muller Hinton agar. The sequence of antibacterial activity of leaf extract against *E. coli* exhibited no activity at 10µl but produced 8.1 mm and 13 mm zones of inhibition observed at 30 µl, whereas the plant extracts showed no activity against *K. pneumonia* at 10 µl but produced 8.2 mm inhibition zone in 30µl concentration (table 3). Thus antibacterial activity was observed at varying degrees with the difference in concentration. Higher concentration of the leaf extract shows highest antibacterial activity. The results obtained might be considered sufficient for further studies for isolation and identification of active principle and for the evaluation of possible antimicrobial activity

## DISCUSSION

The phytochemical constituents of the different solvent extracts of *M. hortensis* were exhibited high antioxidant and antibacterial activity.

The result of DPPH scavenging activity indicates that the plant has bioactivity. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron which is responsible for free radical's reactivity [24]. Among four extracts, ethanol extract showed maximum scavenging activity followed by the aqueous extract. The DPPH radical scavenging activity may be due to the presence of various phytochemicals including polyphenols and flavonoids in *M. hortensis* leaf extracts.

Superoxide anions are the most common free radicals *in vitro* and are generated in a variety of biological systems either by auto-oxidation processes or by enzymes. The concentration of superoxide increases under conditions of oxidative stress and related situations [25]. Moreover, superoxide anions produce other kinds of cell damaging free radicals and oxidizing agents [26]. Therefore *M. hortensis* leaf was undertaken to test whether it has a scavenging activity of superoxide anions. 70% inhibition was recorded in ethanolic leaf extract. The results clearly indicate that *M. hortensis* leaf extracts have noticeable effect as scavenging superoxide radical.

In the present study, phytoconstituents like 4,6,Heptadien-3-one,1,7,-diphenyl, 7-Octadecenal, 9-Oxabicyclo[3.3.1]nonane-2,6-diol, Estra-1,3,5(10)-trien-17a-ol, Pentadecanoic acid,14methyl-methyl ester, 8-Octadecenoic acid,methyl ester, Oleic Acid, Heptadecanoic acid, 15-methyl-ethyl ester, 4-Piperidineacetic acid,1-acetyl-5-Ethyl-2[3-[2-hydroxyethyl]-1H-Indol-2-yl]-a-methyl-methyl ester, Aspidofractinine-3-methanol (2a,3a,5a), Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester, Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester, Z-7-Pentadecenol were identified from ethanol extract of *M. hortensis* by using a gas chromatography-mass spectrography. The basic structural features of phenolic compounds is an aromatic ring bearing methyl esters, hexadecanoic acid, eicosanoic acid, ethyl ester with more hydroxyl groups [27]. Phenolic compounds are known as powerful chain breaking antioxidants and they are very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups [28]. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, antihepatotoxic, anti ulcer, anti-allergic, antiviral and anticancer activities [29] Phytochemical components are known to produce therapeutic activity like antibacterial, antifungal and antioxidant etc [30].

The earlier study reveals that the ethanol leaf extracts of *A. squamosa* were more active against the clinical bacterial pathogens viz. *E. coli* and *P. aeroginosa* the antibacterial activity may be due to the presence of different phyto chemical compounds in the leaf extract including essential oils, flavonoids, terpenoids and other components which are classified as active antimicrobial compounds.

The results of the study supports to a certain degree, the use of traditional medicinal plants in human and animal disease therapy and reinforce the concept of ethno botanical approach in screening plants as potential sources of bioactive substances [31]. The aqueous extract generally exhibits a high degree of antibacterial activity which seems to confirm the traditional therapeutic claims of this plant [32].

These biologically important Phyto-compounds exerts antimicrobial activity by using a different mechanism for example flavonoids exhibits antimicrobial, anti-inflammatory, anti-angionic, analgesic, antiallergic and antioxidant properties [33]. Ability to scavenge free radicals, lipid peroxyl radicals makes them an essential phytoconstituent which is important for prevention of ailments associated with the oxidative damage [34]. Tannins are reported to have iron deprivation property, astringent in nature and exhibit antimicrobial activity [35]. Saponins have also been reported to possess antimicrobial activity due to their detergent like nature as they can cause leakage in the membrane by interacting with proteins and certain enzymes from the bacterial cell [36-38]. Alkaloids are reported to have anti-quorum sensing property [39].

## CONCLUSION

The present study suggests that the leaves of the *M. hortensis* contains a considerable amount of Phyto-compounds like saponins, alkaloids, flavonoids and phenols which are responsible for the antioxidant and antibacterial activities. Hence, this plant may be explored as a new source for potent antimicrobial drugs of natural origin. Further studies are being carried out to isolate and purify the active principle and to evaluate its mechanism of action.

**Fig. 1: *Millingtonia hortensis* leaves**

**Table 1: Phytochemical screening of leaf extracts of *Millingtonia hortensis***

| S. No. | Secondary metabolites | Aqueous | Ethanol | Methanol | Chloroform | Petroleum ether |
|---|---|---|---|---|---|---|
| 1 | Tannins | - | +++ | + | ++ | + |
| 2 | Saponin | ++ | +++ | ++ | + | - |
| 3 | Flavonoids | +++ | +++ | +++ | ++ | ++ |
| 4 | Alkaloids | +++ | +++ | +++ | + | ++ |
| 5 | cyanins | +++ | +++ | +++ | +++ | +++ |
| 6 | Quinones | + | - | - | - | ++ |
| 7 | Glycosides | - | - | - | ++ | + |
| 8 | Cardiac glycosides | ++ | +++ | ++ | - | - |
| 9 | Terpenoids | + | - | - | - | _ |
| 10 | Triterpenoids | _ | - | ++ | - | _ |
| 11 | Phenols | ++ | +++ | +++ | + | ++ |
| 12 | Coumarins | +++ | +++ | +++ | ++ | +++ |
| 13 | Acids | ++ | ++ | + | ++ | ++ |
| 14 | Proteins | _ | - | - | - | - |
| 15 | Steroids | + | +++ | ++ | ++ | - |

+++Strongly positive++Positive, +Trace-Not detected

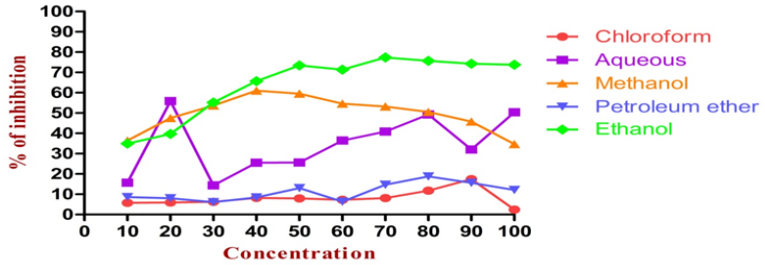


**Fig. 2: Antioxidant assay-DPPH radical scavenging activity of leaf extracts of *M. hortensis***

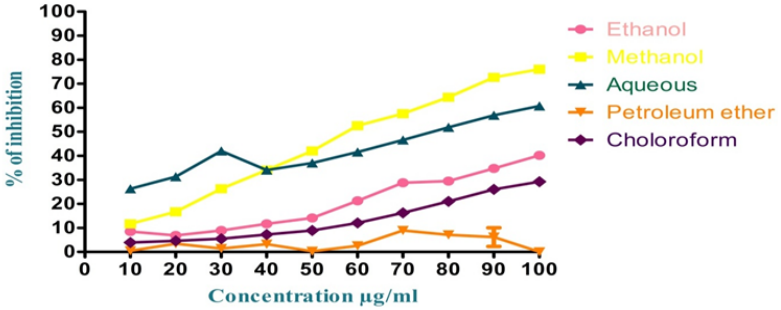


**Fig. 3: Antioxidant assay superoxide radical scavenging activity of leaf extracts of *M. hortensis***

**Table 2: Gas chromatography mass spectrometry of ethanol leaf extracts of *M. hortensis***

| S. No. | Retention time | Compound | Molecular formula | Molecular weight | Peak area % |
|---|---|---|---|---|---|
| 1 | 11.6 | 4,6,Heptadien-3-one,1,7,-diphenyl | $C_{19}H_{18}O$ | 262.34 | 6.75 |
| 2 | 12.7 | 7-Octadecenal | $C_{18}H_{34}O$ | 266.46 | 7.48 |
| 3 | 13.67 | 9-Oxabicyclo[3.3.1]nonane-2,6-diol | $C_8H_{14}O3$ | 158.19 | 7.26 |
| 4 | 14.05 | Estra-1,3,5(10)-trien-17a-ol | $C_{21}H_{26}O_2$ | 310.42 | 11.82 |
| 5 | 15.73 | Pentadecanoic acid,14methyl-methyl ester | $C_{17}H_{34}O_2$ | 270.45 | 4.8 |
| 6 | 17.13 | 8-Octadecenoic acid,methyl ester | $C_{19}H_{36}O_2$ | 296.48 | 5.8 |
| 7 | 17.83 | Oleic Acid | $C_{19}H_{38}O_2$ | 298.5 | 30.09 |
| 8 | 18.9 | Heptadecanoic acid, 15-methyl-ethyl ester | $C_{18}H_{34}O_2$ | 282.46 | 13.04 |
| 9 | 19.12 | 4-Piperidineacetic acid,1-acetyl-5-Ethyl-2[3-[2-hydroxyethyl]-1H-Indol-2-yl]-a-methyl-methyl ester | $C_{20}H_{40}O_2$ | 312.53 | 2.2 |
| 10 | 19.52 | Aspidofractinine-3-methanol (2a,3a,5a) | $C_{23}H_{32}N_2O_4$ | 400.51 | 3.9 |
| 11 | 19.75 | Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester | $C_{20}H_{26}N_2O_4$ | 310 | 3.7 |
| 12 | 23.21 | Octadecanoic acid,2[2-hydroxy ethoxy] ethyl ester | $C_{22}H_{44}O_4$ | 372.58 | 2.2 |
| 13 | 22.58 | Z-7-Pentadecenol | $C_{16}H_{34}$ | 224.44 | 1.2 |

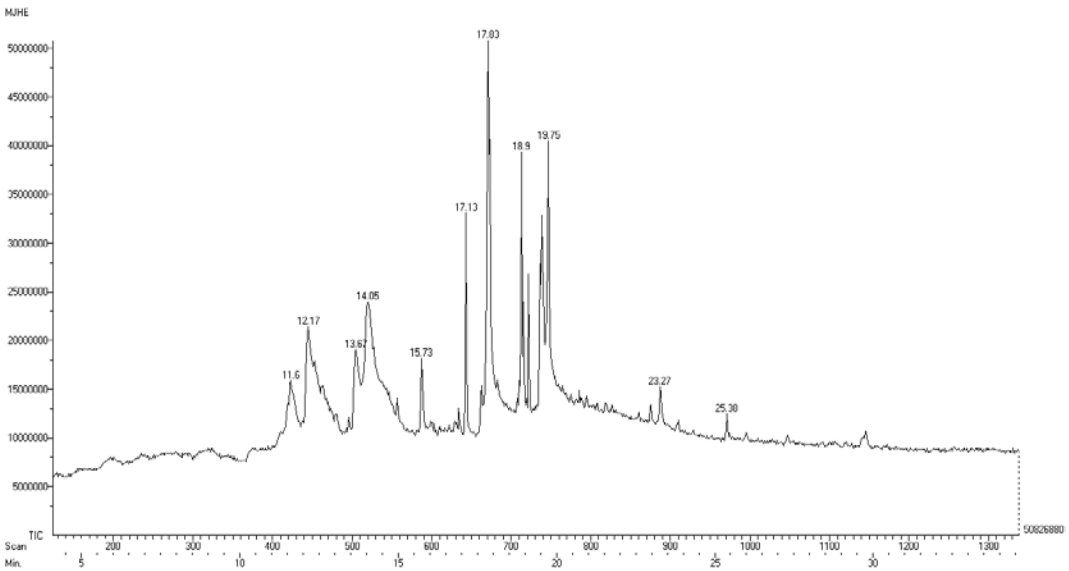


**Fig. 4: Gas chromatography mass spectrometry of ethanol leaf extracts of *M. hortensis***

**Table 3: Antibacterial activity of *Millingtonia hortensis* ethanolic extract**

| S. No. | Concentration of sample µg/µl | *E. coli* zone of inhibition mm | *Salmonella* zone of inhibition mm | *Citrobacter* zone of inhibition mm | *Klebsiela* zone of inhibition mm | *Pseudomonas* zone of inhibition mm |
|---|---|---|---|---|---|---|
| 1 | 10 | - | 6.1 | 6.2 | - | 6.3 |
| 2 | 15 | 6.8 | 6.3 | 6.3 | 6.5 | 6.7 |
| 3 | 20 | 7.2 | 6.4 | 6.5 | 6.7 | 7.3 |
| 4 | 25 | 7.6 | 6.5 | 9.4 | 6.8 | 10.6 |
| 5 | 30 | 8.1 | 9.3 | 9.8 | 8.2 | 13 |
| 6 | Std 30 µg/µl | 13 | 14 | 18 | 14 | 11 |

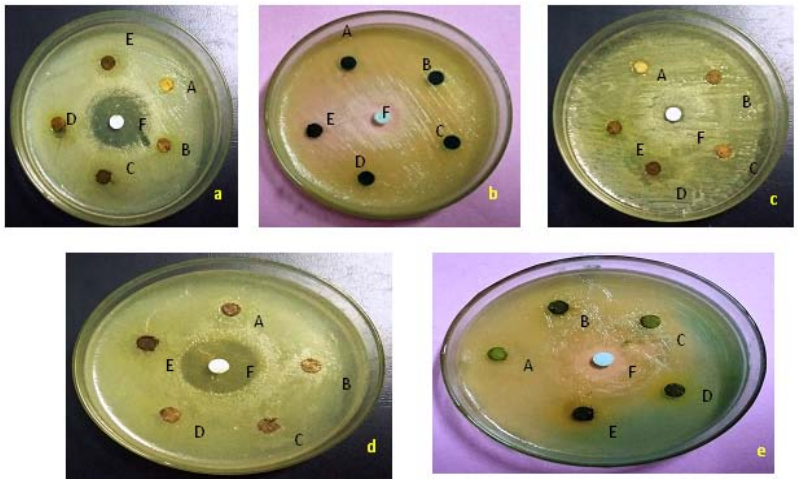


**Fig. 5: Antimicrobial activity of leaf extracts of *Millingtonia hortensis* by disc diffusion method, i.) *Escherichia coli*, ii) *Salmonella typhi,* iii) *Citrobacter braakii*, iv) *Klebsiella pneumonia*, v) *Pseudomonas aeroginosa*, A-10 µg/µl, B-15 µg/µl, C-20 µg/µl, D-25 µg/µl, E-25 µg/µl, F-Std 30 µg/µl**

**CONFLICT OF INTERESTS**

Declare none

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