

Original Research Article

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Potency of Two Commonly Available Plants *Pisonia alba* and *Mukia maderaspatana* in the Health Industry

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ABSTRACT

Pisonia alba commonly known as Leechikottaikerai in tamil is a widely used plant. In the alternative system of medicine *Pisonia alba* leaves are used as anti-inflammatory, anti-diuretic and antifungal. *Mukia maderaspatana* is an important traditional medicinal plant used generally in Western districts of Tamilnadu. It has properties like anti-rheumatic, antifatulent and anticancer also. The present study was carried out to investigate the phytochemical screening, antioxidant and antimicrobial activity of aqueous and ethanolic extracts of the leaves of both the plants. The phytochemical analysis of the leaf extracts of *Mukia maderaspatana* and *Pisonia alba* revealed the presence of tannins, saponins, quinines, flavanoids, cardiac glycosides, phenolic acids, terpenoids, coumarins, steroids and betacyanins. The results showed that the phytochemical properties of the leaves have the potency of being used in the health industry. The strongest radical scavenging activity(76.4%) was exhibited by the ethanolic extract of *Pisonia alba*, moderate activity(59.8%) was recorded in ethanolic extract of *Mukia maderaspatana* and weakest activity(51.2%) was exhibited by the aqueous extract of *Pisonia alba* followed by aqueous extract of *Mukia maderaspatana*(46.4%). The zone of inhibition by disc diffusion method were measured for determining antimicrobial action of ethanolic extracts of *Pisonia alba* and *Mukia maderaspatana* leaves against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aureginosa* and *Staphylococcus aureus*. Among the various extracts, maximum antimicrobial activity was exhibited by ethanolic extract of *Pisonia alba* followed by ethanolic extract of *Mukia maderaspatana*.

Keywords

Pisonia alba,
Mukia maderaspatana,
Phytochemical
screening,
Antioxidant,
Antimicrobial activity,
Zone of inhibition.

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Introduction

Plants, our gifts of nature which are the sources of bioactive constituents have been used traditionally to cure various ailments in Ayurvedha, Unani and Siddha. During last few years, synthetic drugs occupy the highest position for curing various disease due to their side effects, scientists are now focusing highest on exploring the potentiality of traditional medicines (Gopalakrishnan sasi priya *et al.*, 2012).

As we move into the 21st century, we observe a big change in the attitude of physicians, researchers and the general public towards prophylaxis and therapeutics originating from plant drugs. Nearly all-major pharmaceutical houses are back into research on plant product (Shah *et al.*, 1986).

The plant *Pisonia alba*, belonging to the family Nyctaginaceae, is an evergreen

glabrous garden tree with young shoots are minutely puberulous. In the alternative system of medicine *Pisonia alba* leaves are used as analgesic, anti-inflammatory, diuretic (Radha, *et al.*, 2008), hypoglycemic agent (Sunil *et al.*, 2009) and antifungal (Shubashini *et al.*, 2010). It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing, rheumatism and arthritis (Prabhu *et al.*, 2008).

Mukia maderaspatana (L.) M.Roem. of the family, Cucurbitaceae is an important traditional medicinal plant generally practiced in western districts of Tamil Nadu. The species is mainly distributed in tropical regions of India, especially in the lower hills of the Western Ghats (Singh and Panda, 2005). The plant is bitter, sweet, refrigerant, carminative, vulnerary, expectorant and tonic and it is useful in vitiated conditions of pitta, burning sensation, dyspepsia, flatulence, colic, constipation, ulcers, cough, asthma and vertigo (Sowndhararajan *et al.*, 2010). Further it is reported to have the properties viz., anti-rheumatic, anti-flatulent, anti-inflammatory, anticancer, anti-diabetic, diuretic and stomachic also. Squeezed plant is applied to treat scabies of animals (Mallikadevi *et al.*, 2012).

Photochemical are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. It is believed that photochemical may be effective in combating or preventing disease due to their antioxidant effect (Halliwell and Gutteridge, 1992). Photochemical are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. They are non-nutritive compounds. These photochemical are the secondary metabolites present in smaller quantities in higher plants and they include the alkaloids, Steroids, flavonoids,

terpenoids, tannins and many others (Peteros, 2010). Antioxidants are substances that may protect our body cells against the effects of free radicals. Free radicals are molecules produced when our body breaks down food (Padamanabhan vasanthi *et al.*, 2014). Increasing the anti-oxidant intake can prevent diseases and lower health problems. Research is increasingly showing that antioxidant rich foods and herbs have health benefits. Medicinal herbs are the richest sources of antioxidants compounds (Sies *et al.*, 1992). Natural antioxidants tend to be safer and also possess anti-viral, anti-inflammatory, anti-cancer, anti-mutagenic, antitumor and hepatoprotective properties (Baravalia *et al.*, 2009).

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-Bari *et al.*, 2006). Plant materials remain an important resource to combat serious diseases in the world. The pharmacological investigations of plants were carried out to find novel drugs or templates for the development of new therapeutic agents (Iwu *et al.*, 1999). These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents (Natarajan *et al.*, 2010). The present study is aimed to evaluate the efficacy of the two plants namely *Pisonia alba* and *Mukia maderaspatana* as potent antioxidants and antimicrobial agents and also to investigate the phytochemical compounds.

Materials and Methods

Collection and preparation of plant materials

The leaves of *Mukia maderaspatana* and

Pisonia alba were collected in local areas of Chennai city. The leaves of the plants *Mukia maderaspatana* and *Pisonia alba* were cleaned and shade dried. The dried leaves were crushed and sieved through mesh cloth to get fine powder.

Preparation of the extracts was done according to a combination of the methods used by (Janarthanam & Sumathi, 2010). About 1g of dried leaf powder of *Mukia maderaspatana* and *Pisonia alba* plant materials were extracted with 20 ml ethanol (75%) and aqueous for 1 minute using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40°C to a constant weight and then dissolved in ethanol and water. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18°C until use.

Phytochemical Screening of the Leaf Extracts

The phytochemical screening of leaf extracts were assessed by standard method as described by (Savithramma *et al.*, 2011; Selvaraj *et al.*, 2014).

Phytochemical screening was carried out on the leaf extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins, quinines, steroids and beta cyanin. General reactions in these analyses revealed the presence or absence of these compounds in the leaf extracts tested.

Test for Tannins

For identification of tannins, 1ml of the plant

extract, 1ml of ferric chloride (5% FeCl₃) was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for Saponins

For identification of saponins, 2ml Plant extract, 2ml of distilled water was added and shaken in graduated cylinder for 15min lengthwise, formation of 1cm layer of foam indicates the presence of saponins.

Test for Quinones

For identification of quinones, 1ml Plant extract, 1ml of concentrated sulphuric acid (H₂SO₄) was added. Formation of red colour indicates the presence of Quinones.

Test for Flavonoids

For identification of flavonoids, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Alkaloids

For identification of alkaloids, 2ml Plant extract, 2ml of concentrated Hydrochloric acid (HCl) was added. Then few drops Mayer's reagent was added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Glycosides

For identification of glycosides, 2ml of the plant extract, 3ml of chloroform and 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

Test for Cardiac Glycosides

For identification of Cardiac glycosides, 0.5

ml of the plant extract, 2 ml of glacial acetic acid and few drops of 5 % ferric chloride were added. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

Test for Terpenoids

For identification of terpenoids, 0.5 ml of the plant extract, 2ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Phenols

For identification of phenols, 1ml of the plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue / green colour indicates the presence of phenols.

Test for Steroids

For identification of steroids, 0.5ml of the plant extract, 2ml of chloroform and 1ml of Sulphuric acid (H₂ SO₄) were added. Formation of reddish brown ring at interface indicates the presence of steroids.

Test for Coumarins

For identification of coumarins, 1ml of plant extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Betacyanin

For identification of betacyanin, 2ml of the plant extract, one ml of 2N sodium hydroxide (NaOH) was added and heated for 5min at

100°C. Formation of yellow colour indicates the presence of betacyanin.

Antioxidant Activity

Qualitative Analysis of Antioxidant Activity

The antioxidant activity of leaf extracts of *Mukia maderaspatana* and *Pisonia alba* was determined by following the method as described by (George *et al.*, 1996; Samundeeswari & Chittibabu, 2013). 50µL of leaf extracts of the two plants were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative Analysis of Free Radical Scavenging Activity

The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2003). Subsequently, at every 5min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm.

Free radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample}) \times 100}{(\text{Absorbance of control})}$$

The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate.

Antibacterial Activity of Leaf Extracts

The leaf extracts of *Mukia maderaspatana* and *Pisonia alba* plants were used for antibacterial study (Janarthanam & Sumathi, 2010; Ozkan *et al.*, 2004). Different concentration (50, 100 and 150 mg/ml) of the concentrated leaf extracts was tested for its antibacterial activity against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The bacterial cultures were grown in Muller Hinton Agar and Muller Hinton broth (Hi media) (Lopez *et al.*, 2001).

Antibacterial activity was measured using the standard method of diffusion disc plates on agar (Ertuk *et al.*, 2006). Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assay, all bacterial strains were grown in Muller Hinton Broth Medium (Hi media) for 24 hours at 37°C and plated on Muller Hinton Agar (Hi media) for agar diffusion experiments. Paper disc (6mm in diameter) were placed on the agar medium to load 10µl, 20µl and 30µl of different concentrations of leaf extracts of *Mukia maderaspatana* and *Pisonia alba* were tested. Zone of Inhibition diameters were measured after incubation for 24 - 48 hours at 37°C.

Results and Discussion

Phytochemical screening

Phytochemical screening of the leaf extracts of *Pisonia alba* & *Mukia maderaspatana* revealed the presence of different kind of chemical groups that are summarized in table. 1. Aqueous and ethanolic extracts of leaves of *Pisonia alba* contain tannins, saponins,

flavanoids, phenolic acids, terpenoids, coumarins, steroids, betacyanin. Aqueous and ethanolic extract of leaves of *Mukia maderaspatana* contain tannins, saponins, quinones, flavanoids, cardiac glycosides, phenolic acids, terpenoids, coumarins, steroids, betacyanins. All the tested extracts failed to detect alkaloids and glycosides.

The Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, steroids and related active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems etc (Balakumar *et al.*, 2011; Paulraj *et al.*, 2011; Kala *et al.*, 2011).

Saponins are generally regarded as antinutrients but are also believed to be useful in human diet for controlling cholesterol. Its presence in this plant therefore could suggest that the plant is of medicinal value. There is evidence of the presence of saponins in the traditional medicine preparations (Asl & Hosseinzade, 2008; Xu *et al.*, 1996).

Among the observed phytoconstituents saponins and phenolic acids are abundantly present in all the tested extracts of leaves when compared to other chemicals. These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study it also reveal that the plant contains bioactive compounds which are connected with antimicrobial activity of plants.

Antioxidant assay

Among the leaf extracts of *Pisonia alba* and *Mukia maderaspatana*, Ethanolic extracts was found to have positive antioxidant activity,

whereas their Aqueous extract had semi-positive antioxidant activity. The results of the free radical scavenging activity of the 1,1-diphenyl - 2 picryl - hydrazyl (DPPH) assay showed percentage antioxidant activity (%AA) is 76.4% in ethanolic leaf extract of *Pisonia alba* followed by its Aqueous extract with 51.2% of Antioxidant activity. In *Mukia maderaspatana*, the free radical scavenging activity was highest in ethanolic extract with 59.8%, followed by its aqueous extract with 46.4% which is summarized in table. 2 & fig. 1. Ethanol extract of *Pisonia alba* and Ethanol extract of *Mukia maderaspatana* was found to have highest antioxidant activity both qualitatively and quantitatively.

The Antioxidant shows an important Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) is widely used in pathogenesis of many diseases. The usage of synthetic antioxidant components may shows many side effects like toxicity and mutagenic effects, it made an alternative search of naturally occurring antioxidants (Saritha *et al.*, 2014).

Different accessions of *Pisonia alba* and *Mukia maderaspatana* leaf samples were used for antioxidant studies. Analysis on different extraction of ethanol (75%) and aqueous extract showed the presence of antioxidants. Among the two different solvent extracts of *Pisonia alba* and *Mukia maderaspatana* the ethanolic leaf extracts of both the plants recorded the most effective DPPH radical scavenging activity (76.4% & 59.8%) In each case, ethanolic leaf extracts recorded higher percentage of free radical scavenging activity than aqueous extract. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers.

The Ethanolic extracts of *Pisonia alba* and *Mukia maderaspatana* showed good results, and was used further in testing their antimicrobial activity.

Antimicrobial activity

The zone of inhibition diameter of Ethanolic extract of *Pisonia alba* and Ethanolic extract of *Mukia maderaspatana* for laboratory strains *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, is shown in table. 3 & 4.

In the Ethanolic extract of *Pisonia alba* at the lower concentrations of 10 μ l the extract showed no antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, where as for *Bacillus cereus* and *Pseudomonas aeruginosa* showed antibacterial activity with 9mm and 8mm inhibition. At the concentration of 20 μ l the extract showed antibacterial activity for each bacterial strain of *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (10,10,10,8mm) except *Escherichia coli*. When the concentration was increased to 30 μ l, the plant extract showed higher antibacterial activity with significant inhibition of bacteria. Significant zone of inhibition was observed in bacteria including *Staphylococcus aureus* and *Bacillus cereus* with maximum zone of inhibition being 13mm and 19mm respectively at the concentration of 30 μ l in *Pisonia alba* ethanol extract. Rest of the tested bacteria also had zone of inhibition though lesser than the other bacteria (11,10,9mm).

Mukia maderaspatana ethanol extract was found to be not effective against bacterial strains *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at lower concentration of 10 μ l, whereas for *Bacillus cereus* and *Pseudomonas aeruginosa* it showed antibacterial activity to some extent about 7mm and 11mm. At the concentration of 20 μ l the plant extract showed antibacterial activity for all bacterial strains though with lesser zone of inhibition. When concentration

was increased to 30µl, the plant extract showed Significant zone of inhibition in bacteria *Pseudomonas aeruginosa* with maximum of 18mm respectively. Rest of the bacteria *Bacillus cereus* (10mm), *Bacillus*

subtilis(11mm), *Escherichia coli*(9mm), *Staphylococcus aureus*(11mm) also had zone of inhibition though lesser than *Pseudomonas aeruginosa*.

Table.1 Phytochemical Analysis of *Pisonia alba* and *Mukia maderaspatana*

Constituent tests	Aqueous extract of <i>Pisonia alba</i>	Ethanollic extract of <i>Pisonia alba</i>	Aqueous extract of <i>Mukia maderaspatana</i>	Ethanollic extract of <i>Mukia maderaspatana</i>
Tannins	+	+	+	+
Saponins	++	++	+	++
Quinones	-	-	+	+
Flavanoids	++	++	+	-
Alkaloids	-	-	-	-
Glycosides	-	-	-	-
Cardiac glycosides	-	-	-	+
Phenolic acids	++	++	++	+
Terpenoids	-	+	+	+
Coumarins	++	++	+	+
Steroids	+	+	+	+
Beta cyanins	+	+	+	+

++ : Double Positive

+ : Positive

- : Negative

Table.2 DPPH free radical scavenging activity of *Pisonia alba* and *Mukia maderaspatana*

Minutes	0	5	10	15	20	25	30
Pisonia-Aqueous(%)	37.0	43.3	45.7	47.2	49.6	49.6	51.2
Pisonia-Ethanol(%)	72.4	76.4	76.4	76.4	76.4	76.4	76.4
Mukia-Aqueous(%)	37.0	40.9	44.1	44.9	45.7	45.7	46.4
Mukia-Ethanol(%)	41.7	51.9	52.7	59.8	59.8	59.8	59.8
BHT(standard)%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
DPPH(control)	1.27						

Table.3 Antibacterial activity in ethanolic extract of *Pisonia alba* against bacterial pathogens

Name of the Pathogenic Bacteria	Inhibition Zone (mm)		
	10µl	20µl	30µl
<i>Bacillus cereus</i>	9	10	19
<i>Bacillus subtilis</i>	-	10	9
<i>Escherichia coli</i>	-	-	10
<i>Pseudomonas aeruginosa</i>	8	10	11
<i>Staphylococcus aureus</i>	-	8	13

Table.4 Antibacterial Activity In Ethanolic Extract of *Mukia maderaspatana* Against Bacterial Pathogens

Name of the Pathogenic Bacteria	Inhibition Zone (mm)		
	10µl	20µl	30µl
<i>Bacillus cereus</i>	7	10	10
<i>Bacillus subtilis</i>	-	9	11
<i>Escherichia coli</i>	-	9	9
<i>Pseudomonas aeruginosa</i>	11	15	18
<i>Staphylococcus aureus</i>	-	9	11

Fig.1 Free Radical Scavenging Activity of Different Extracts of *Pisonia alba* And *Mukia maderaspatana*

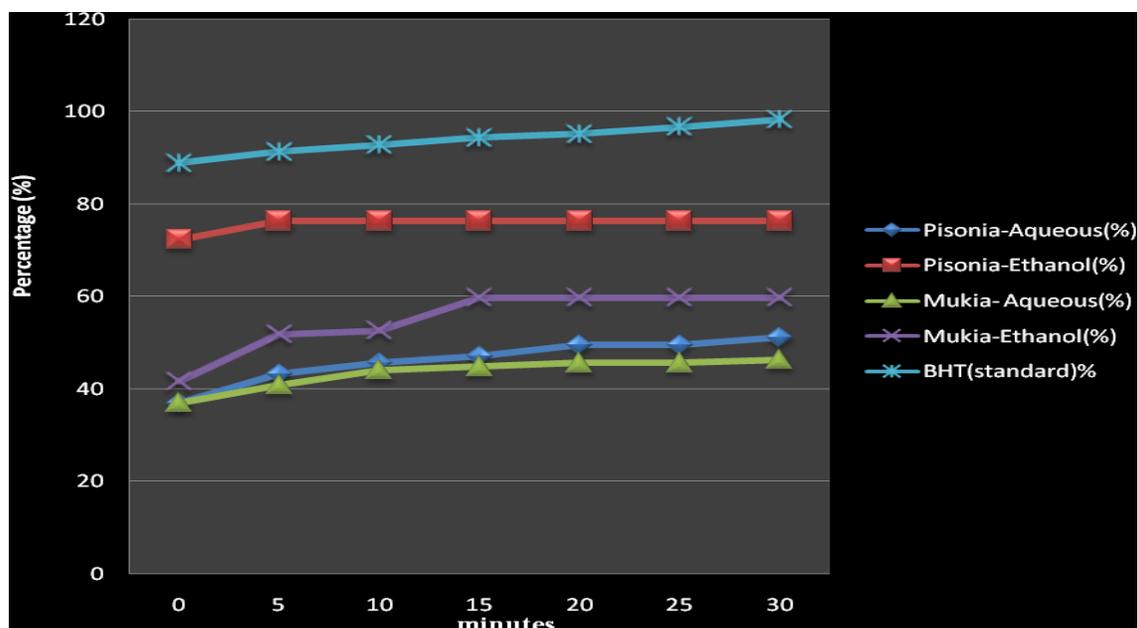


Fig.2 Comparative View of Inhibition of Zone Made by Ethanolic Extracts of *Pisonia alba* on Different Bacterial Strains

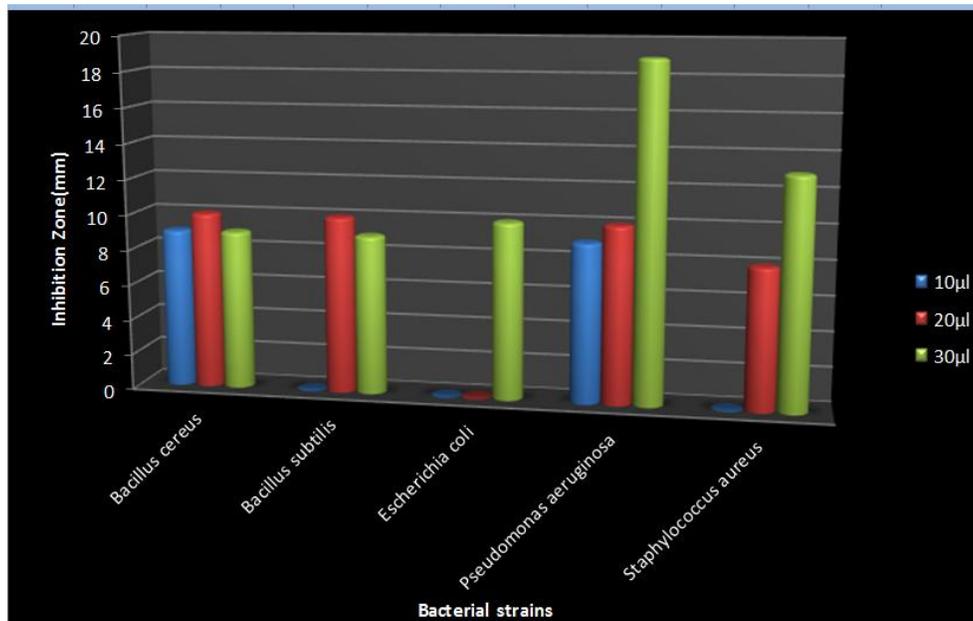
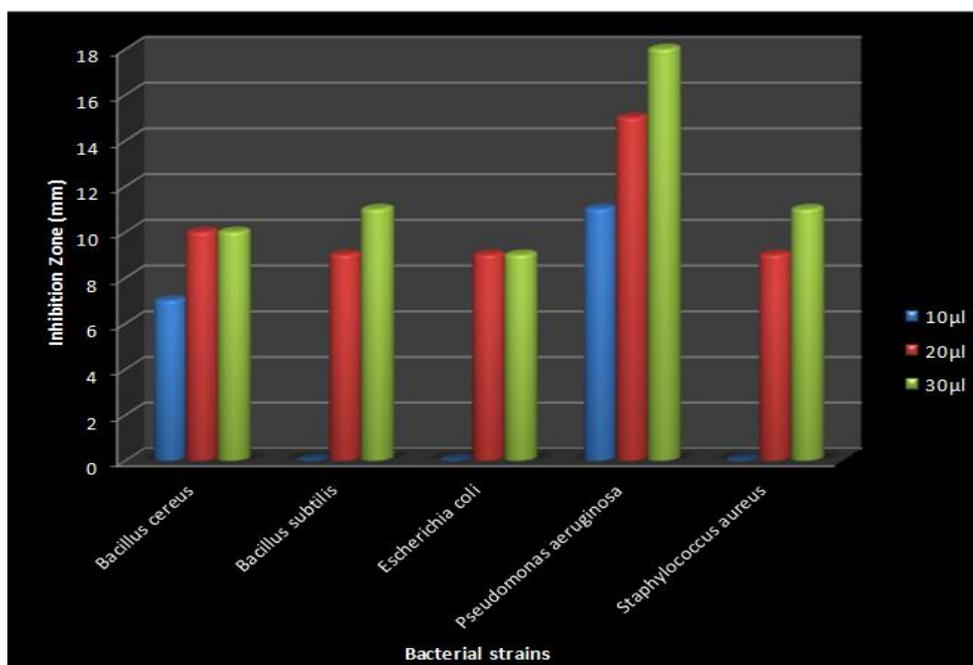


Fig.3 Comparative View of Inhibition of Zone Made by Ethanolic Extract of *Mukia maderaspatana* on Different Bacterial Strains



The *Mukia maderaspatana* leaves is most effective against *Pseudomonas aeruginosa*

and *Lachaikottei* leaves is most effective against *Bacillus cereus* hence these plant

extracts has great possess antimicrobial compounds. It can be used in the treatment of diseases caused by this pathogen by the discovery of drugs against this pathogen.

Earlier studies showed that the plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids (Tsuchiya *et al.*, 1996), phenolics and polyphenols (Mason & Wasserman, 1987), tannins (Ya *et al.*, 1988), terpenoids (Scortichini & Pia Rossi, 1991), sesquiterpenes (Goren *et al.*, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms. In this endeavour, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs.

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