INTRODUCTION

“Moringa oleifera” is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, South Asia, South America and the Pacific and Caribbean Islands. Because Moringa oleifera has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and “Mothers best friend”[1].

Moringa oleifera is commonly known as “Drumstick”. It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract[2]. Moringa oleifera is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark[3]. Moringa oleifera Lam is used as a highly nutritive vegetable in many countries. Its young leaves, flowers, seeds and tender pods are commonly consumed and they are having some medicinal properties.

Traditionally its roots are applied as plaster to reduce the swelling and rheumatism. The root, flower, fruit and leaf have analgesic and anti-inflammatory activity. Moringa grows more rapidly, reaching higher heights, when found in well-drained soils with ample water, but tolerates sandy soils, heavier clay soils and water limited conditions.

Moringa leaves contains phytochemical having potent antancer and hypotensive activity and are considered full of medicinal properties and used in siddha medicine[4]. The whole Moringa oleifera plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation, aqueous extracts of fruits have shown significant anti-inflammatory activity, methanolic extracts of leaves have shown anti-ulcer activity and ethanolic extracts of seeds exhibited anti-tumour activity[5]. Moringa oleifera is used as drug many ayurvedic practitioners for the treatment of asthma and evaluate the anthelmintic activity of methanolic extract of...
**Moringa oleifera** in adult Indian earthworms pheretima posthum.a at different doses[6]. The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer[7]. Other important medicinal properties of the plant include antispasmodic[8], diuretic[9], antihypertensive[10], cholesterol lowering[11], antioxidant, antidiabetic, hepatoprotective[12], antibacterial and antifungal activities[13].

Antibacterial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antibacterial Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistant[14] forms. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources[15]. In developing countries, due to the cost of efficient, Antibacterial a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases.

According to World Health Organization (WHO), more than 80% of the world’s population relies on traditional medicines for their primary health care needs[16]. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants[17].

Despite this array of uses to which parts of Moringa tree are put to, scanty literature is available on the uses of moringa oleifera as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitizing / preservative activity is to evaluate it is antibacterial activity against food – borne microorganisms[18].

**MATERIALS AND METHODS**

**Collection of plant materials:**

The experiment was conducted in the year 2011 in the college laboratory. Leaves were collected from the Moringa oleifera tree in the college campus. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves throughly and dried.

**Preparation of leaf extracts:**

20-30 grams of fresh leaves were boiled with 200 mL of solvent for 1 hour. The extract was filtered using Whatmann filter paper No. 1 and then concentrated in vacuum at 40°-50 °C using a rotary evaporator. Evaporation of solvent in the rotary evaporator affords a crude extract of the soluble components and these extracts were subjected to the qualitative phytochemical analysis and antibacterial studies.

**Phytochemical Analysis:**

The extracts were analyzed by the following procedures [19] to test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

**Saponins:**

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

**Tannins:**

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for gallic tannins and green color indicates for catecholic tannins.

**Reducing Sugars**

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling’s solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

**Glycosides:**

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

**Alkaloids:**

2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

**Flavonoids:**

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

**Volatile oils:**

2ml of extract was shaken with 0,1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

**Terpenoids:**

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid.

**Aqueous Extract**

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract[20]. The extract was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

**Ethanol Extract**

Moringa oleifera leaves (100 g) were ground into fine powder [20] using a stainless-steel grinder, and deep in100% ethanol (200 mL) for overnight. The ethanol
fraction was separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

**Chloroform Extract**

For preparation of chloroform extract ground plant sample (100 g) was added in chloroform respectively (200ml each case) and left for overnight at room temperature\(^{[21]}\). The extracts were separated using sterile muslin cloth and filter through sterile Whatman filter paper (no. 02).

**Source of microorganisms**

The organisms used were *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhii*. The organisms were obtained from the Microbial Lab of Department of Microbiology, A.V.C. College, Mayiladuthurai, Tamilnadu, India.

**Determination of Antibacterial Activity:**

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

**Table 1.** Antimicrobial activity of Chloroform, Ethanol, Water extract of medicinal plants against human pathogens.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Extracts</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Escherichia Coli</em></td>
</tr>
<tr>
<td><strong>Moringa oleifera</strong></td>
<td>Water</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>6</td>
</tr>
</tbody>
</table>

“C” – Chloroform, “E” – Ethanol “W” – Water

**Table 2:** Qualitative Phytochemical Analyses of the extracts of *Moringa oleifera* Leaf

<table>
<thead>
<tr>
<th>Solvents used for extraction</th>
<th>Alkaloid</th>
<th>Reducing sugar</th>
<th>Flavonoid</th>
<th>Saponin</th>
<th>Tannin</th>
<th>Volatile oil</th>
<th>Glycoside</th>
<th>Terpenoids</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>chloroform</td>
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<td>Ethanol</td>
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</table>

(+) indicates presence while (−) indicates the absence of the components

**RESULT**

The antibacterial activity of chloroform, ethanol and aqueous extracts was investigated using agar well diffusion method, against the selected human pathogens such as *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhii*. All the examined extract showed varying degrees of antibacterial activities against the pathogens. The Phytochemical test was done to find the presence of active chemical constituents such as glycosides, alkaloids, tannins, flavonoids, terpenoids, saponins.

Table-1 showed the antibacterial activity of chloroform extract of *Moringa oleifera* showed maximum zone of inhibition (6 mm) against *Escherichia Coli*, *Salmonella typhii*. The antibacterial activity of chloroform extract of *Moringa oleifera* showed No zone of inhibition against *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The antibacterial activity of ethanol extract of *Moringa oleifera* showed maximum zone of inhibition (14 mm) against *Salmonella typhii* showed the minimum inhibitory zone (8 mm) against *Escherichia Coli*. The antibacterial activity of aqueous extract of maximum inhibitory zone (8 mm) against *Staphylococcus aureus*. The antibacterial activity of aqueous extract of No inhibitory zone against *Salmonella typhii*, *Pseudomonas aeruginosa*, *Escherichia Coli*.

The phytochemical analysis of plant extracts using chloroform, ethanol and aqueous was showed in Table - 2. From the phytochemical analysis catecholic tannins were found in *Moringa oleifera* in the solvents such as chloroform, ethanol and aqueous. The ethanol extract of *Moringa oleifera* showed the presence of flavanoids, tannins, glycosides and terpinoids were found in presence of ethanol and aqueous extract. Alkaloids were observed only in chloroform extract of *Moringa oleifera*. In all plant extracts found flavanoids except in chloroform extract of *Moringa oleifera*. Saponin were observed in the chloroform and aqueous extract of *Moringa oleifera*. Terpenoids were observed in the ethanol and water extract of *Moringa oleifera*. The ethanol, aqueous sand chloroform all extract of *Moringa oleifera* showed the absences of reducing sugar.

**DISCUSSION**

The findings of the preliminary Phytochemical investigations and the results of antibacterial activity were depicted in the respective Tables. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaloids, tannins, flavonoids, terpenoids, saponins Glycoside and compounds reducing were present in the extracts.

The results are recorded in Table 2. The ethanol, chloroform and aqueous extract showed considerable activity against *Salmonella typhii*. The ethanol extract was more active than the standard against *Salmonella typhii*. Previous study conducted by \(^{[22]}\) suggests that the essential...
oil of O. majorana posses antibacterial activity. The work conducted by [23] reveals that the leaves of marjoram have antimicrobial activity against Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus and Salmonella typhi. Similarly antimicrobial activity of ethanol, chloroform and water extract of Marrubium vulgare, was further assessed against, Salmonella typhi, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, were recorded [24].

Imran khan et al. [25], studied that phytochemical analysis of Azadiracta indica leaves by using different solvent such as Petroleum ether, chloroform, methanol show the presence of triterpenes, glycosides and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves. Antibacterial activity of Azadiracta indica was analyzed by previous workers showed that the chloroform extract of leaves possess significant activity, than petroleum ether and methanol extracts. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants. Hinal paudel chhetri et al., [26], reported that the ethanolic extract of Azadiracta indica whole plant shows presence of flavonoids and tannins only. Similarly the extract of Azadiracta indica is active against E.coli followed by Staphylococcus aureus. Earlier observation done by Srivivasan et al., [27] also showed the antifungal and antibacterial activity of A. indica.

S.K. Panda et al., [28], studied the antibacterial activity and phytochemical screening of ethanol; chloroform and extract of vites negundo were similar to our results. Antibacterial activity on vites negundo tested by [29] and [30] reported negative results. On the other hand, [31] reported positive results against B.subtilis, S. epidermis, E.coli & P. aeruginosa.

Bukar etal, [32] reported that Moringa oleifera leaf ethanolic extract had the broadest spectrum of activity on the test bacteria. The results showed that activity against four bacterial isolates Enterobacter sp (7 mm), Staphylococcus aureus (8 mm), Pseudomonas aeroginosa, (7 mm) and Escherichia Coli (7 mm) were sensitive at concentration of 200 mg/ml while shigella spp and Salmonella typhi were not sensitive at all concentrations used.

Napoleon et al., [33] also reported Enterobacter spp, S.aureus, P.aeruginosa, S.typhi and E.coli to be sensitive to ethanol, chloroform and aqueous extract of Moringa oleifera leaf at concentration of 200 mg/l. phytochemical analysis were similar report of our results. Maluventhan viji et al., [34] studied that ethanol, chloroform and aqueous extract of Cardiospermum halicacabum leaves shows the presence of flavonoids, tannins, steroids and glycosides, which were similar to our results. Antibacterial activity of Cardiospermum halicacabum was studied by same workers reported that ethanol extract was active against Steptococcus aureus followed by Salmonella typhi, E. coli & P. aeruginosa. It is also related to our results.

The ethanol, chloroform and aqueous extract of O. majorana and M. vulgar are because of its strong microbicidal property and superiority over commercial microbicides, may prove to be an effective herbal protectant against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic and ecofriendly.

CONCLUSION
Moringa oleifera leaves to treat common medical conditions but a few use it for preventing and treating malnutrition. Presence of phytochemicals indicates possible preventive and curative properties of M. oleifera leaves. There is need to carry out more pharmacological studies to support the use of M. oleifera as a medicinal plant.

REFERENCES

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