SCREENING OF SECONDARY METABOLITES, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY FROM THE PETALS OF MORINGA OLEIFERA

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ABSTRACT

Moringa oleifera is a small, fast-growing evergreen or deciduous tree and known for its various medicinal properties. The in vitro antimicrobial activity of aqueous methanol extract of Moringa oleifera petals was investigated against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Proteus vulgaris using the disc diffusion method. The methanol extract inhibited the growth of all the presently investigated bacteria with zone of inhibition between 12.4 – 23.4 mm at 20 µl/ml whereas the minimum inhibitory effect of the methanol extract of Moringa oleifera petals was effective at the highest concentration (20 µl/ml) against pathogenic microorganisms. Preliminary phytochemical analysis showed that the methanol extracts of Moringa oleifera petals contain flavonoids, tannins, saponins, alkaloids and glycosides. Studies have shown that the petals of Moringa oleifera may be a great natural source for the development of new drugs and might be useful for treating the bacterial diseases.

Keywords- Moringa oleifera, Antimicrobial activity, Phytochemical screening, Minimum inhibitory concentration (MIC),

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INTRODUCTION

Medicinal plants have been used for centuries before the advent of orthodox medicine. Secondary metabolites produced by plants constitute a major source of bioactive substances. These secondary metabolites have recently been referred to as phytochemicals. The medicinal values of the plants lie in their component phytochemicals, which produce definite physiological actions and pathological behavior infective disease on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids, phenolic compounds and around 40% of modern medicine are derived from medicinal plants [1] which possess antibacterial properties [2]. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free radicals [3].

Traditional medicine using plant methanol extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world (WHO, 2002). Infectious diseases continue to be the major concern in all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics [4, 5]. The search for newer sources of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs [6]. It is therefore very necessary that the search for newer antibiotic sources be a continued process. Plants are inexpensive and safer alternative sources of antimicrobials [7, 8, and 9].

*Moringa oleifera* is one of the 14 species of family Moringaceae and is commonly known as “Drumstick”. *Moringa oleifera* 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* leaves contains phytochemical having potent anticancer and hypotensive activity and are considered full of medicinal properties and used in siddha medicine [10]. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases and fever. 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 activity [11]. The flowers and roots contain an antibiotic that is highly effective in the treatment of cholera. The juice of the leaves is believed to stabilize
blood pressure, flowers are used to cure inflammations, pods are used for joint pain, and the roots are used to treat rheumatism [12]

In view of the importance of *Moringa oleifera* in ethanobotany as health remedy and the antimicrobial property of crude extracts of the petals of *Moringa oleifera* has been studied as part of the exploration for new and novel bio-active compounds

**MATERIALS AND METHODS**

**Collection of plant material**

The petals of *Moringa oleifera* were collected from the Sri Sairam Siddha Medical College and Research Centre, Herbal garden, Tamilnadu, India. It was ensured that the plant was healthy and uninfected. The plant was identified with the help of available literature and authenticated by Dr. S. Sankaranarayanan, Head of the Department, Department of Medicinal Botany, Sri Sairam Siddha Medical College, West Tambaram, Chennai. The petals were washed under running tap water followed by distilled water to eliminate dust and other foreign particles and shade dried for 5 days.

**Preparation of extract**

The dried petals were then blended using a household electrical blender. 20g of petal powder was extracted in 70% methanol. The extracts were then filtered using Whatman No. 1 filter paper. The methanol filtrate was concentrated to dryness in using a rotary evaporator at 40º C. The residue was partitioned with Petroleum ether, chloroform and ethyl acetate. The ethyl acetate extract was used for further studies.

**Determination of phytochemical constituents**

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, glycosides, saponins and terpenoids as described by Jigna et al & Harborne et al [13,14].

**Determination of total phenol content**

The amount of total phenol content was determined by Folin-Ciocalteu’s reagent method [15]. 0.5 ml of extract (1 mg/ml) and 0.1 ml Folin-Ciocalteu’s reagent (0.5 N) was mixed and the mixture was incubated at room temperature for 15 min. Then 2.5 ml saturated sodium carbonate solution was added and further incubated for 30 min at room temperature and the
absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extracted compound).

**Reducing power assay**
The reducing power was determined by the Fe3+ - Fe2+ transformation in the presence of the extracts as described in the literature [16]. The Fe2+ can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. The different concentration of methanol extract (5-20µl) was mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. Supernatant (2.5 ml) was diluted with 2.5 ml of water and shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated greater reducing power. Vitamin C was used as a positive control.

**Metal chelating assay**
To determine metal chelating ability the protocol according to Eric Decker and Welch was used [17]. The different concentration of extract (5-20 µl) was added to a solution of 0.1 ml of 2 mM FeCl2. This was followed by the addition of 0.2 ml of 5 mM ferrozine solution, which was left to react at room temperature for 10 min under shaking conditions before determining the absorbance of the solution at 562 nm. The percentage inhibition of Ferrozine–Fe2+ complex formation was calculated using the formula

\[ I = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100 \]

EDTA was used as positive control

**Hydrogen peroxide scavenging activity**
The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch et al. (1989)[18]. A solution of H2O2 (40 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). The different concentration of Crude extract (5-20 µl) was added to 0.6 ml of H2O2 solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contains sodium phosphate buffer without H2O2. The percentage of H2O2 scavenging of crude extract and standard compounds were calculated using the following equation

\[ \text{H}_{2}\text{O}_2\text{ scavenging effect (}%) = (1 - \frac{\text{As}}{\text{Ac}}) \times 100 \]
where $A_C$ is the absorbance of the control and $A_S$ is the absorbance in the presence of the sample extract or standards.

**Antioxidant activity in a hemoglobin induced linoleic acid**

The antioxidant activity of Methanol extracts was determined by the method of Kuo et al (1999) [19]. Methanol extracts (5-20 µl/ml) was mixed with 1 ml of 1 mmol/l of Potassium phosphate buffer (pH-6.5) followed by the addition of 20 µl of 0.0016% hemoglobin was shaken vigorously. The mixed solution was incubated at 37°C for 45 minutes. After incubation, 2.5 ml of 0.6% Hc1 in ethanol was added and mixed thoroughly to stop the lipid peroxidation. Then, 100 µl of 0.02 mol/l FeCl3 and 100 µl of ammonium thiocyanate (15g/50ml) was added and vortexed thoroughly. The total antioxidant activity determination was performed in triplicate using the thiocyanate method by reading the absorbance at 480 nm.

**Test organisms**

The bacterial cultures were *Staphylococcus aureus* MTCC 29213, *Bacillus subtilis* MTCC441, Gram negative; *Escherichia coli* MTCC 25922, *Pseudomonas aeruginosa* MTCC 2488, *Proteus vulgaris* MTCC 1771 used for the antibacterial activity. They were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial cultures were maintained at 4°C on nutrient agar.

**Antimicrobial assay**

Antibacterial activity was carried out using disc diffusion method of Sathyabama et al [20]. Petriplates were prepared with 20 ml of sterile nutrient agar (HIMEDIA). The tested cultures were swabbed on top of the solidified media and allowed to dry for 10 minutes. The crude extract impregnated discs (Whatman No.1 filter paper was used to prepare discs) were prepared and air dried well. The test was conducted at four different concentrations of the crude extract (5, 10, 15 & 20 µl/ml) with 3 replicates. The loaded discs were placed on the surface of the medium and incubated at room temperature for 24 hrs. The relative susceptibility of the organisms to the crude extract indicated by the clear zone of inhibition around the discs, were observed, measured and recorded in millimeters.

**Determination of Minimum inhibitory concentration (MIC)**

The minimum inhibitory concentration was determined according to method of Velickovic Ana et al [21]. The different concentration of methanol extracts (5-20µl/ml) was mixed with
0.5 ml bacterial cultures were incubated at 37°C for 18 hrs and OD was measured spectrophotometrically at 580 nm.

RESULTS AND DISCUSSION

Phytochemical Screening of *Moringa oleifera* Petals

Investigations on the phytochemical screening of *Moringa oleifera* aqueous methanol extracts revealed the presence of saponins, tannins, glycosides, alkaloids and flavonoids (Table 1). These compounds are known to be biologically active and therefore aid the antimicrobial activities of *Moringa oleifera*. These secondary metabolites exert antimicrobial activity through different mechanisms.

Tannins have been found to form irreversible complexes with proline rich protein [22] resulting in the inhibition of cell protein synthesis. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes [23]. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [24]. These observations therefore support the use of *Moringa oleifera* in herbal cure remedies.

Another secondary metabolite compound observed in the petals of *Moringa oleifera* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines [25]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer drugs [26]. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects [27].

Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [28,29]. Saponins prevent the excessive intestinal absorption of this cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension[30]. Recent studies have shown that, many polyphenol compounds contribute significantly to the total antioxidant activity of many plants. The total phenols contain good antioxidant, antimutagenic and anticancer properties [31].
Flavonoids, another constituent of *Moringa oleifera* methanol extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties [32]. Flavonoids, are potent water-soluble antioxidants and free radical scavengers [33], which prevent oxidative cell damage, have strong anticancer activity. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity.

**Table 1: Phytochemical constituents of crude extracts of *Moringa oleifera* petals.**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Test</th>
<th>Methanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
<td>--</td>
</tr>
</tbody>
</table>

++ Presence of constituent  -- Absence of constituent

**Total phenolic content**

Plant phenolics and flavonoids are a major group of compounds which have the following effects; choleretic and diuretic functions, decreasing blood pressure, reducing the viscosity of the blood and stimulating intestinal peristalsis [34], as well as primary antioxidation or free radicals scavenging activities [35].

In the present study the phenolics content of petals of *M. Oleifera* was found to be 62.75% in terms of gallic acid equivalents.

**Reducing capacity assessment**

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antio-xidant activity [36]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxi-dation processes, so that they can act as primary and secondary antioxidants. The maximum reducing property was found at 20µl/ml of *M. oleifera* petals. (Graph 1). The reducing power of crude extract increased gradually in concentration dependent manner.
Metal chelating activity

The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in human cardiovascular disease [37]. Because Fe2+ also has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe2+ concentration in Fenton reactions affords protection against oxidative damage. The absorbance of Fe2+-ferrozine complex was decreased dosedependently, i.e. the activity was increased on increasing concentration from 5 to 20 µl/ml. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (38).

Table 1: Metal chelating activity of methanol extract of M.oleifera petals

<table>
<thead>
<tr>
<th>Methanol extract (µl/ml)</th>
<th>aFe2+ chelating activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>52.93 ± 12.61</td>
</tr>
<tr>
<td>10</td>
<td>66.22 ± 13.42</td>
</tr>
<tr>
<td>15</td>
<td>81.96 ± 16.12</td>
</tr>
<tr>
<td>20</td>
<td>102.72 ± 20.32</td>
</tr>
</tbody>
</table>

a Results are expressed as % inhibition of Fe2+ chelating with respect to control. Each value represents Mean±SD

Hydrogen peroxide scavenging activity

Hydrogen peroxide has strong oxidizing properties. It can be formed in vivo by many oxidizing enzymes, such as superoxide dismutase and can cross cellular membranes and may
slowly oxidize a number of intracellular compounds. The methanol extract of *M. oleifera* petals was capable of scavenging H$_2$O$_2$ in a concentration dependent manner. Hydrogen peroxide scavenging activity of the methanol extract was found to be 53.06 ± 3.88 at 20µl/ml. These results showed that the extract had an effective hydrogen peroxide scavenging activity.

**Table 2: Scavenging of hydrogen peroxide activity of methanol extract of *M. oleifera* petals**

<table>
<thead>
<tr>
<th>Methanol extract (µl/ml)</th>
<th>a Hydrogen peroxide scavenging activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>25.15 ± 5.70</td>
</tr>
<tr>
<td>10</td>
<td>36.62 ± 5.28</td>
</tr>
<tr>
<td>15</td>
<td>45.60 ± 4.64</td>
</tr>
<tr>
<td>20</td>
<td>53.06 ± 3.88</td>
</tr>
</tbody>
</table>

Antioxidant activity in a hemoglobin induced linoleic acid

The antioxidant activity of methanol extract of *M. oleifera* petals of medicinal plants was determined using hemoglobin induced linoleic acid system. The extract showed high inhibitory ability at 20µl/ml (0.178 ± 0.015). The extracts showed a rapid and concentration-dependent increase of antioxidant activity.

![Graph 2: Antioxidant activity of Hemoglobin induced linoleic acid](image)

**Antibacterial activity and Minimum Inhibitory Concentration of *Moringa oleifera* Petals**
The antibacterial activity of aqueous methanol extract of *Moringa oleifera petal* at different concentration was screened by disc diffusion technique and the zone of inhibition was measured mm diameter (Table 2). The methanol extract of *Moringa oleifera* was more effective against *B. subtilis* and *S. aureus* with a zone of inhibition percentage of 26 and 19.92 and was least effective against *P. aeruginosa*, *P. vulgaris* and *E. coli* with zone of inhibition percentage of 12.70, 13.77 and 17.14 respectively at the concentration of 20µl/ml (Graph-3).

The methanol extract of *Moringa oleifera petal* showed the maximum inhibitory activity at the highest concentration (20 µl/ml) than the lowest concentration (5 µl/ml) against gram negative bacteria such as *E. coli*, *P. vulgaris*, *P. aeruginosa* and gram positive *B. subtilis* and *S. aureus* (Table-3). The inhibitory effect of the methanol extract of *Moringa oleifera petals* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens. The presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study [39].

The results of this study showed that the methanol extract was more effective and may be due to the better solubility of the active components in organic solvents [40]. There are several reports published on antibacterial activity of different herbal extracts [41].It supports the earlier investigation that the phytoconstituents isolated from flower possess remarkable toxic activity against bacteria and may assume pharmacological importance [42].

**Table 3: Antibacterial activity of methanol extracts of *Moringa oleifera* petals.**

<table>
<thead>
<tr>
<th>Different Concentration of extract (µl/ml)</th>
<th>Zone of inhibition [in mm diameter] <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>E. coli</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td>5</td>
<td>11.36±0.77</td>
</tr>
<tr>
<td>10</td>
<td>13.43±0.73</td>
</tr>
<tr>
<td>15</td>
<td>15.3±0.75</td>
</tr>
<tr>
<td>20</td>
<td>17.93±0.60</td>
</tr>
</tbody>
</table>

*The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates*
Graph 3: Inhibition percentage of methanol extract of *Moringa oleifera* petal against pathogenic bacteria

Table 3: Minimum inhibitory concentration of methanol extracts of *Moringa oleifera* petals.

<table>
<thead>
<tr>
<th>Different Concentration of Flavanoid rich fraction (µl/ml)</th>
<th>Optical density at 580 nm (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S.aureus</em></td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
</tr>
<tr>
<td>0.642±0.010</td>
<td>0.666±0.012</td>
</tr>
<tr>
<td>5</td>
<td>0.56±0.006</td>
</tr>
<tr>
<td>10</td>
<td>0.523±0.010</td>
</tr>
<tr>
<td>15</td>
<td>0.472±0.010</td>
</tr>
<tr>
<td>20</td>
<td>0.312±0.010</td>
</tr>
</tbody>
</table>

\(^a\)The minimum inhibitory concentration was determined by optical density of inhibition that is the mean of triplicates ±SD of three replicates.

CONCLUSION

The petals of *Moringa oleifera* showed promising antibacterial activities and it can play a therapeutic role against number of epidemic and pathogen born diseases. On the basis of the results and promising activities of methanol extract of *Moringa oleifera* petals could be subjected to isolate most active components for drug development program and therapy of infection diseases.
ACKNOWLEDGEMENT
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