Antioxidant and Antimicrobial Activities of Moringa Oleifera Leaves

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ABSTRACT

M. oleifera plant have high nutritional and medicinal values. Different parts of this plant such as flowers, leaves, immature pods and fruit have been found to be very useful and used in many countries as a highly nutritious vegetable. The plant belongs to Moringaceae family that is a monogenic family and consists of about thirty three species. It is cultivated in many aeras mainly in India, Sri Lanka, Africa, Pakistan, South America and Mexico in recent time. The importance of Moringa species is gained because of their numerous uses. It is used traditionally to treat inflammation, diarrhea, skin infection, cough, diabetes, headache and fever. The most used part in the plant is leaves as these are enriched with protein, carotenoids, ascorbic acid, antioxidant and phenolic. In this research crude protein, crude fat, total ash and carbohydrates were determined in the dry leaves powder beside mineral content which also was determined. Total polyphenols and flavonoids content of the plant leaves under investigation were estimated spectrophotometry. Phenolic and flavonoid compounds were fractionated using HPLC technique.

Keywords: Antioxidant activities, antimicrobial activities and moringa oleifera.

INTRODUCTION

Several compounds in Moringa leaves methanolic extracts were found using HPLC. Twenty phenolic acids and ten flavonoid were also detected. Total flavonoids and phenols in Moringa methanolic extract have average values of (91.37, mg GAE/ g). Total flavonoid content was also determined with the amounts of (65.7 mg CE/g) in the same extract Saleem et al., (2020). Another study was made by Braham et al., (2020) on Moringa leaves extract. They used HPLC analysis for phenolic compounds and detected many flavonoids compounds as quercetin, rutin, and kaempferol as well as phenolic acids such as ferulic, ellagic, chlorogenic and gallic acid. Thus, the M. oleifera phenolic compounds fractions have strong antioxidant properties and possesses high phenolic content, which may be done through direct trapping of free radicals and metal chelation. The aerial part of morgina are a good source of essential amino acid methionine and are also rich in minerals such as potassium (259-20616), iron (70-300), phosphorus (0.85-126) and calcium (440-3650) mg/100g of the plant (Singh et al., 2020). The abundance of phenolic content in Moringa species may be the cause of high antioxidant activity. These compounds stabilise the free radical generated in a cell by donating or accepting electrons, therefore acts as antioxidant compounds.

MATERIALS AND METHODS

Plant material

The present study was carried out using green aerial parts (leaves) of ornamental and medical plant, Moringa oleifera.
oleifera lam (Moringa). These plant samples were obtained from faculty of agriculture, Mansoura University, Egypt plantation in August 2019. For two weeks the plant parts were air dried in shade. The air-dried plant was crushed into fine powder then stored in plastic bags till use.

Plant methanolic extracts:
Moringa air dried samples were macerated separately in methanol for 2 days at room temperature, filtered and the residues were re-extracted with methanol 4 times. The collective filtrated extracts were evaporated at 45°C under pressure using rotary evaporator to gain crude methanol extracts which stored at 4°C in refrigerator until further analysis.

Chemical composition:
Moisture, crude protein, ash, crude lipid and fibre content of all samples were determined according to (AOAC, 2016). Total content of carbohydrates was estimated using the method of phenol-sulfuric acid as reported by odouro et al., (2008).

Mineral content:
Mineral content was determined by digestion of each air dried sample by Conc. H2SO4 and HClO4 acid as reported by Makkar and Becker, (1996).Nine element as : Cadmium (Cd++), lead (Pb++), cobalt (Co+++), copper (Cu++), chrome (Cr+++), zinc (Zn++), nickel (Ni++), iron (Fe+++), and manganese (Mn+++) were estimated by atomic absorption (VARIAN specter. AA.20) according to Ferreira et al., (2008) while, sodium (Na+) and potassium (K+) were determined using flame photometer. The determination was carried out in Delta Company for Fertilizer and Chemical Industries, Talkha, Dakahlia, Egypt.

Total polyphenol content (TPC):
The phenolic compounds amount in moringa extract was determined by using the colorimetric method of Folin–Ciocalteu’s which performed in microplates, as described by Brahma et al. (2020). The absorption was recorded at 755 nm. The analysis was carried out in triplicate and total polyphenol content was estimated using the following equation: (Y=0.0168 X + 0.861), (R²=0.9991). Gallic acid was used as a standard and the results were expressed as mg GAE/g D.W.

Total flavonoid content (TFC):
Total flavonoid content was determined as described by Brahma, et al., (2020) with some modification. The analysis was carried out in triplicate and the following equation : (Y = 0.0117 X + 0.0521), (R²= 0.9996), was used to calculate the total flavonoid content using Quercetin as a standard. Results were expressed as mg QE/g D.W.

HPLC determination of phenolic and flavonoid compounds:
Identification of phenolic compounds in investigated sample were carried out using the method described by Goupay et al., (1999), while identification of flavonoid compounds was done as described by Mattila et al., (2000). Separation of phenolic and flavonoid compounds was performed using an Agilent1260 infinity, HPLC Series (Agilent, USA), equipped with Quaternary pump, aKinex® 5μm EVO C18 100 mm x 4.6 mm, (Phenomenex, USA) and operated at 30°C. The separation is done using a ternary linear elution gradient with (A) HPLC grade water 0.2 % H3PO4 (v/v), (B) methanol and (C) acetonitrile. The injected volume was 20 μl. Detection: VWD detector set at 284 nm. All detected chromatograms were compared with those of external standards in Food Safety and Quality Control Laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt.

Antioxidant activity
In vitro antioxidant activity for plant sample methanolic extract was performed using three different methods:

DPPH radical scavenging assay
To evaluate antioxidant activity DPPH radical was applied depending on the rate of removing hydrogen atom by the free radical and reduction of colour from dark violet to yellow. DPPH method was carried out according to de Oliveira et al., (2020). The readings were performed at 517 nm using spectrophotometer. Each trial was performed in triplicate and BHT was used as a standard. Antioxidant capacity was expressed as percentage of DPPH radical-scavenging activity. Inhibition percentage (%I) was determined using the following equation:

\[
%I = \frac{(A0 - A)}{A0} \times 100
\]

Reducing power assay
Reducing power of methanolic extract sample was determined according to the method out lined by Da Rocha et al., (2019). Sample extract was reacted with potassium ferricyanide to form potassium ferrocyanide, then reacted with FeCl3 to form FeCl2 complex. The absorbance was measured at 700 nm. Reaction mixture with higher absorbance indicates greater reducing power. Ascorbic acid was used as reference antioxidant. All tests were carried out in triplicate. Determination of EC50 value was done using the corresponding regression equation.

Total antioxidant capacity assay
Determination of total antioxidant capacity was carried out using phosphomolybdate assay according to Tiliia et al., (2018) with slight modification. The absorbance was read at 695 nm. The total antioxidant capacity was expressed as mg of ascorbic acid equivalents per gram (AAE/g) of dry weight. Ascorbic acid is used as a standard and employed for evaluation as follows : ( Y=0.0031x-0.1614), (R²=0.9978).

Antimicrobial activity
Antimicrobial activity of moringa leaves methanolic extract was evaluated using disk diffusion technique, as detailed by (CLSI, 2015).

Bacterial strains
Two Gram negative and one Gram positive bacteria were used. The first strain was Gram negative, non-spore forming short rod bacteria namely Escherichia coli. The second Gram negative strain was Salmonella typhi (non-sporing short rod bacteria) while, gram positive bacteria were Staphylococcus aureus, it is potentially pathogenic, and coccoid shaped bacteria in clusters.

Cultivation media:
Cultivation of bacterial strains was done using nutrient agar (NA) and potato dextrose agar (PDA) media (Bagamboula et al., 2003).

Agar diffusion method:
Agar diffusion method was performed as described by Bagamboula et al., (2003). Moringa methanolic extract sample was used in amounts of 4mg/ml [4 mg of extract dissolved in a few drops of dimethyl sulfoxide (DMSO) and...
completed to 1 ml with distilled water. Tested sample was applied in wells in freshly agar plate inoculated with young culture (12 hours old). The plates of bacterial strains were incubated at 37°C for 24 hours. The inhibition zones were measured and recorded at the end of incubation periods.

Statistical analysis:

All experiments were performed in triplicate. The statistical software program CoStat was used to calculate the least significant difference and the standard error at significance level (p) ≤ 0.05. The results are expressed as mean values ± standard error (SE).

RESULTS AND DISCUSSION

Chemical analyses:

Moisture, ash, crude protein, lipids, fibre and carbohydrates were determined in Moringa leaves sample. The results were recorded in Table (1). Moringa leaves contained high percentage values of 18.34, 10.0, 3.0 and 58.16 g/100g DW for crude protein, ash, lipid and total Carbohydrates respectively. The obtained results for Moringa leaves were agreement with those mentioned by ziani et al., (2019). They gave average values of 22.8, 6.5, 14.1 and 56.6 g/100g dw for crude protein, lipid, ash and carbohydrates respectively. Moringa leaves are used in the tropics as dietary supplements and a nutrient source especially in children and infants where malnutrition is a major concern (Brilhante et al., 2017).

Mineral content:

Proximate analyses for macro and micro elements were determined in Moringa leaves. In plants, minerals are involved in a lot of biological processes, they also play an important role in humane health. The results in Table (2) reveal that the main element in Moringa leaves was K followed by Ca, Mg, P and Na their average values are 9000, 7000, 3000, 2700 and 2500 mg/100g respectively. As well as Moringa leaves have high values for micro elements as Fe, Zn, and Cu average values of 1150, 170, 38 mg/100g respectively. These results are in agreement with those reported by Singh et al., (2020), they reported that Moringa leaves are a good source of some minerals such as phosphorus, potassium, calcium and iron with average values of 126, 2006, 3000 and 270 mg/100g respectively. In addition, Moringa dried leaves could be used to combat malnutrition because of presence of higher nutritive contents and that is an indication of the usefulness of the plant as a nutrition resource.

Table 1. Chemical analyses of Moringa leaves as g/ 100gdw.

<table>
<thead>
<tr>
<th>Components Sample</th>
<th>Moisture (g/100g)</th>
<th>Ash (g/100g)</th>
<th>Crude protein (g/100g)</th>
<th>Crude lipid (g/100g)</th>
<th>Fiber (g/100g)</th>
<th>Carbohydrates (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moringa leaves</td>
<td>9.0</td>
<td>10.0</td>
<td>18.34</td>
<td>3.0</td>
<td>1.5</td>
<td>58.16</td>
</tr>
</tbody>
</table>

Table 2. Mineral content of Moringa leaves as (mg/100g)

<table>
<thead>
<tr>
<th>Mineral Sample</th>
<th>Macro elements</th>
<th>Micro elements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>Moringa leaves (mg/100g)</td>
<td>2700</td>
<td>9000</td>
</tr>
</tbody>
</table>

Total polyphenol content (TPC):

Total polyphenols represent an important part of human and animal diets and they are considered as secondary plant metabolites. Data in Figure (1) present the total polyphenolic content of Moringa leaves extracts, the values are expressed as gallic acid equivalents per gram of dried Moringa leaves (mg GAE/g). The methanolic obtained extract exhibits high amount of phenolic compounds of 63.56±1.59 mg GAE/gdw. The obtained results clearly demonstrate that Moringa leaves are good source for polyphenols compounds may contribute significantly to the antioxidant and free radical scavenging activity. These results agreed with those mentioned by Saleem et al., (2020). They estimated total phenols in Moringa methanolic extract and showed almost equal value for total phenols (71.19 mg GAE/gdw).

Total flavonoid content (TFC):

Flavonoids are a large group of phenolic compounds consisting of flavonol, flavones and anthocyanin. Figure (1) summarizes the total flavonoid content (TFC) for moringa leaves extract, and shows that Moringa methanolic extract has high concentration of flavonoids 48.45±0.63 mg QE/g dw. These results are in harmony with those reported by Singh et al., (2020), they reported that Moringa leaves revealed lower level of flavonoids compound (40.23 mg QE/g dw) than the obtained results. Methanol extracts for moringa leaves are good source for flavonoids contents which had an important role in human health.

HPLC determination of phenolic and flavonoid compounds:

Identification of phenolic and flavonoid compounds for Moringa methanolic extract sample was done using HPLC technique. The contents of phenolic and flavonoid compounds expressed as mg/g extract as shown in table (3). Fourteen phenolic compounds were detected. The major phenolic acid is Benzoic acid (303.43 mg/g) followed by Vanillic acid (118.7 mg/g), Resvertol (66.06 mg/g) and p-Hydroxy benzoic acid (62.02 mg/g). Catechol and Ferulic acid weren’t found in Moringa methanolic extract. While the major flavonoids are Kaempferol (78.71 mg/g) followed by Rutin (29.32 mg/g), Naringin (18.52 mg/g). Rosemarinic wasn’t found in Moringa methanolic extract. The recorded results were in harmony with those reported by Oguntibeju et al., (2019). They mentioned that the presence of coumaric acid (15.74 μg/ml), caffeic acid (8119 μg/ml) and chlorogenic acid (250 μg/ml) as phenolic acids, also rutin (9.55 μg/ml), myricetin (108.02 μg/ml) and quercetin (17.03 μg/ml) as flavonoids in Moringa methanolic extract. The difference in the qualitative and quantitative bioactive compounds and presence or absence of any compound may explained by several factors such as degree of ripeness, differences in cultivars, growing conditions, plant part type, extraction method and handling after harvest (Bennour et al., 2019).
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Fig. 1. Total polyphenols and flavonoids content of Moringa leaves methanolic extract.

Table 3. HPLC determination of phenolic and flavonoid compounds for Moringa methanolic extract sample as (mg/g)

<table>
<thead>
<tr>
<th>S.N</th>
<th>Compound name</th>
<th>Concentration (mg/g)</th>
<th>Ret. Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyrogallol</td>
<td>4.32</td>
<td>2.96</td>
</tr>
<tr>
<td>2</td>
<td>Quinol</td>
<td>7.27</td>
<td>3.12</td>
</tr>
<tr>
<td>3</td>
<td>Gallic acid</td>
<td>10.19</td>
<td>3.66</td>
</tr>
<tr>
<td>4</td>
<td>Catechol</td>
<td>n.d</td>
<td>5.40</td>
</tr>
<tr>
<td>5</td>
<td>p-Hydroxy benzoic acid</td>
<td>62.02</td>
<td>7.65</td>
</tr>
<tr>
<td>6</td>
<td>Chlorogenic</td>
<td>10.92</td>
<td>9.36</td>
</tr>
<tr>
<td>7</td>
<td>Vanillic acid</td>
<td>118.78</td>
<td>9.68</td>
</tr>
<tr>
<td>8</td>
<td>Caffeic acid</td>
<td>5.90</td>
<td>10.12</td>
</tr>
<tr>
<td>9</td>
<td>Syringic acid</td>
<td>3.36</td>
<td>10.40</td>
</tr>
<tr>
<td>10</td>
<td>p-Coumaric acid</td>
<td>22.13</td>
<td>12.86</td>
</tr>
<tr>
<td>11</td>
<td>Benzoic acid</td>
<td>303.43</td>
<td>14.39</td>
</tr>
<tr>
<td>12</td>
<td>Ferulic acid</td>
<td>n.d</td>
<td>15.40</td>
</tr>
<tr>
<td>13</td>
<td>O-Coumaric acid</td>
<td>30.62</td>
<td>17.54</td>
</tr>
<tr>
<td>14</td>
<td>Ellagic</td>
<td>4.63</td>
<td>17.01</td>
</tr>
<tr>
<td>15</td>
<td>Resveretol</td>
<td>66.06</td>
<td>19.83</td>
</tr>
<tr>
<td>16</td>
<td>Cinnamic acid</td>
<td>6.30</td>
<td>20.51</td>
</tr>
</tbody>
</table>

Flavonoids

<table>
<thead>
<tr>
<th>S.N</th>
<th>Compound name</th>
<th>Concentration (mg/g)</th>
<th>Ret. Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rutin</td>
<td>29.32</td>
<td>16.76</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin</td>
<td>5.90</td>
<td>21.55</td>
</tr>
<tr>
<td>3</td>
<td>Rosmarinic acid</td>
<td>n.d</td>
<td>22.00</td>
</tr>
<tr>
<td>4</td>
<td>Neringein</td>
<td>18.52</td>
<td>22.36</td>
</tr>
<tr>
<td>5</td>
<td>Myricetin</td>
<td>8.69</td>
<td>23.31</td>
</tr>
<tr>
<td>6</td>
<td>Kampherol</td>
<td>78.71</td>
<td>24.41</td>
</tr>
<tr>
<td>7</td>
<td>Catechin</td>
<td>1.98</td>
<td>8.99</td>
</tr>
</tbody>
</table>

n.d = not detected

**Antioxidant activity:**

To explain the potential antioxidant activity of the extract several methods with different mechanisms are frequently used. Mechanisms include reduction activity, metal chelation and radical scavenging. To clarify antioxidant activity of Moringa leaves methanolic extract three different methods were used in this study.

To evaluate antioxidant activity DPPH radical is applied depending on the rate of removing hydrogen atom by the free radical and reduction of colour from dark violet to yellow. The ability of investigated sample to scavenge DPPH radical was expressed as IC50 values (half maximal inhibitory concentration). A low IC50 values confirms high antioxidant activity.

Table 4 summarized results of Moringa leaves methanolic extract. It was clear that inhibition zones
increased gradually with increasing concentration of sample for all tested microorganisms. Results indicated that the effect of moringa extract showed variable inhibition zones ranging from 8 mm to 14 mm against all tested bacteria at different concentration. Moringa showed high inhibition zone (14 mm) against Candida albicans at concentration of 500 mg/ml compared with the other microorganisms, as well as its had the same inhibition zone (12 mm) against four bacteria at concentration of 500 mg/ml as Salmonella sp, Klebsiella, Staphylococcus aureus, Bacillus subtilis. While showed low inhibition zone against Bacillus subtilis 8 mm at concentration of 100 mg/ml. Furthermore, Moringa had no antibacterial activity against E.coli at concentration of 100 mg/ml. This results are agreement with those mentioned by Al-husnan and Alkahtani (2016). They reported that Moringa methanolic extract inhibited growth of bacteria which include Bacillus cereus, Staphylococcus aureus, Salmonella typhi, E.coli, Klebsiella and Candida albicans. An great effect was shown on the growth of bacteria by Moringa extracts with inhibition zone variable from 12.5 to 23.5 mm according to the type of tested bacteria.

![Figure 2. Antioxidant capacity of Moringa methanolic extract DPPH, TAC and Reducing power assay.](image)

### Table 4. Detected inhibition zone (mm) against Tested microorganisms as results of treatment with different Moringa methanolic extract concentrations.

<table>
<thead>
<tr>
<th>Samples</th>
<th>E.coli</th>
<th>Salmonella sp.</th>
<th>Klebsiella</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Diameter of inhibition zone (mm)</em></td>
<td>9 ± 0.87</td>
<td>10 ± 0.88</td>
<td>11 ± 0.92</td>
<td>12 ± 0.96</td>
<td>12 ± 1.05</td>
<td>14 ± 1.09</td>
</tr>
<tr>
<td><em>Tested microorganisms</em></td>
<td>100 mg/ml</td>
<td>300 mg/ml</td>
<td>500 mg/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### CONCLUSION

The present research summarises the photochemistry, traditional uses, nutritional value of Moringa leaves extract, indicating the versatility of the plant. *Moringa oleifera* is a rich source of carbohydrate, fat, vitamins, protein, and minerals. It also contains various secondary metabolites such as glycosides, phenolic acid, flavonoids, etc. which have shown efficacy as antioxidant and antimicrobial activities. It is recommended that moringa plant should be included in the daily diet as sources of nutrients and natural antioxidant to avoid danger of industrial antioxidant.

### REFERENCES


The antioxidant and anti-inflammatory potential of *Moringa oleifera* Lam. 


