Phytochemical analysis and antioxidant activity of aqueous fraction of Moringa oleifera Leaves

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INTRODUCTION

The World Health Organization (WHO) estimates that 4 billion people, which is 80% of the world’s population, presently use herbal medicines for some aspect of primary health care in conjunction with conventional medicines. They have been tested and proven to be safe and efficacious with wide cultural acceptability and lesser side effects than modern pharmaceutical medicines. The chemical constituents present in them are part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. The importance of antioxidants in preventing tissue damage or cellular function cannot be overemphasized, and this has resulted in increased production of antioxidants worldwide. Plants are cheap source of varieties of antioxidants in addition to their safety and efficacy. Naturally occurring antioxidants play a major role in the prevention and development of chronic diseases such as cancer, coronary heart diseases, diabetes, obesity and hypertension and these disorders are highly linked to oxidative stress. The antioxidants compounds have the ability to neutralize free radicals, therefore play an important role in the prevention of these diseases. Large body of researches has investigated the potential role of antioxidants in the prevention of these chronic diseases. The interaction between ulcers and bacteria can be stratified into four levels: contamination, colonization, critical colonization and infection. While contamination and colonization by microbes are not believed to inhibit healing, the line between colonization and infection can be difficult to define.

The use of traditional medicine is widespread. Plants still present large source of natural antioxidants that might serve as leads for the development of novel drugs. Moringa oleifera commonly known as horse radish tree or drumstick from the family Moringaceae is a cosmopolitan plant that grows almost everywhere even in poor soils. It is found almost everywhere especially around the tropics. Almost all the parts of this plant: root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders. Moringa oleifera Lam is a fast growing, aesthetically pleasing small tree adapted to arid, sandy conditions. The species is characterized by its long, drumstick shaped pods that contain its seeds. It is the most widely cultivated species of the monogeneric family Moringaceae. It is native to India, Pakistan, Bangladesh and Afghanistan and was utilized by the ancient Romans, Greeks and Egyptians. Now it is available all over the tropics. It was reported to possess antimicrobial action and anti-inflammatory, anti-diabetic, antioxidant and anticancer properties. In addition, it has been confirmed that it has significant hepatoprotective action against hepatotoxic induced liver injury.

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Keywords: Moringa oleifera, phytochemical compounds, free radicals, antioxidants,
In recent years, considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption. It is in view of this that we investigated the in vitro antioxidant activity of aqueous fraction from the leaves of methanolic extract of Moringa oleifera leaves.

**MATERIALS AND METHOD**

**Plant collection, extraction and fractionation**

The leaves of *M. oleifera* Lam (MO) were procured from Kara Market in the city of Sokoto, Sokoto state, Nigeria, in October, 2020. They were authenticated by a staff of Pharmacognosy department, Usmanu Dانfodiyo University Sokoto. The air-dried powdered leaves of *M. oleifera* (500g) were extracted successively with methanol, ethanol and water (three times for each solvent to ensure complete extraction) with the help of a shaker (Lab Tech shaker; model: LSI- 3016R, Korea set at 25°C). The extracts were then filtered and the solvent evaporated with a rotary evaporator (Buchi, Switzerland) at room temperature to dryness under reduced pressure and further freeze dried with a freeze drier (Virtis Bench Top K, United States). The yield of each dried extracts was calculated and stored at –20°C until required for further use.

**Differential fractionation of the active crude methanolic extract**

The methanolic crude extract was subjected further to differential fractionation using n-hexane, dichloromethane, ethyl acetate, n-butanol and water. The methanolic crude extract was dissolved in methanol: water (50:150) and partitioned against equivalent volumes each of n-hexane, dichloromethane, ethyl acetate and n-butanol. Five fractions were obtained with these respective solvents and subjected to in vitro screening to obtain the most active aqueous fraction. The aqueous fraction was freeze dried and stored at -20°C until required for further study.

**Phytochemical screening of methanolic crude extract and aqueous fractions**

Phytochemical examinations were carried out qualitatively according to the established methods. Test for flavonoids (alkaline reagent test). 3ml aliquot of the fraction plus 10% NaOH produced a yellow color which indicated the presence of flavonoid compounds. Test for tannins. 5% ferric chloride solution was added drop by drop to each of the 2-3ml the fraction and a dark green color was observed which indicated the presence of tannins. Test for Saponins. 5ml of each of the fraction was shaken strongly with 5ml of water for about 15mins; formation of a layer of foam that lasted for several minutes indicated the presence of saponins. Test for Glycosides. 2.5ml of 50% H$_2$SO$_4$ was added to 5ml of the fraction and heated in boiling water with NaOH. 5ml of Fehling’s solution was then added and the mixture was boiled. A brick red precipitate formed indicated the presence of glycosides. Test for alkaloids. 2ml of each of the fractions was stirred in 2ml of 10% HCl. 1 ml was stirred with few drops of Wagner’s reagent and another 1ml with a few drops of Meyer’s reagent. Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids. Test for cardiac glycosides (Keller-Killiani’s test). 3.5% ferric chloride solution was added to the fraction and allowed to stand for a minute. 1ml of conc. H$_2$SO$_4$ was carefully poured on the walls of the tube to form a lower layer. A reddish brown ring at the interface indicated the presence of glycosides. Test for steroids (Salkowski). 0.5ml of the fraction was dissolved in 5ml of chloroform. 2ml of conc. H$_2$SO$_4$ carefully added to form a lower layer. A reddish brown color at the interface indicated the presence of a steroidal ring. Test for saponin glycosides. 2.5ml of Fehling’s solution A and B was added to each extract and fraction. A bluish green precipitate showed the presence of saponin glycosides. Test for volatile oils. 1ml of the fraction was mixed with dil. HCl. A white precipitate was observed which indicated the presence of volatile oils. Test for anthraquinones. 0.5ml of the fraction was shaken with 10ml benzene and 5ml of 10% ammonia solution. There was no presence of any color change which indicated the absence of Anthraquinones.

**Antioxidant assay of bioactive aqueous fraction**

**Determination of total antioxidant capacity of the aqueous fraction**

For sample preparation, 1mg of the freeze dried aqueous fraction sample was measured and dissolved completely in 1 ml of methanol and a 1mg/ml of sample solution was obtained. Serial dilutions of 50, 100, 150, 200, 250 and 300µg/ml were made from the stock solution of the aqueous fraction sample. For the preparation of the standard solutions, ascorbic acid was used as a standard. 1mg of the ascorbic acid was measured and dissolved completely in 1ml of methanol and a 1mg/ml standard solution was obtained. Serial dilutions 50, 100, 150, 200, 250 and 300µg/ml were made.

**Phosphomolybdenum Assay**

The method described by with slight modification was adopted to measure the total antioxidant capacity of the aqueous fraction of *M. oleifera*. Briefly, to a known aliquot of the aqueous fraction sample, 0.4ml was placed in a vial; 4ml of the reagent solution (containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and incubated in a water bath at 95°C for 90 mins. The samples were cooled down to room temperature and absorbance was measured at 630nm. The blank solution contained 4 ml of reagent solution and 0.4 ml of methanol; the control solution consisted of 4ml of reagent and 0.4 ml of various concentration of ascorbic acid (50 – 300µg/ml). The control and blank solutions were treated in the same procedure as that of sample solution. A calibration curve was prepared by using the standard solution of ascorbic acid and the antioxidant activity was expressed as µg of ascorbic acid equivalent antioxidant capacity (AAEAC) per gram of extract.

**Ferric reducing antioxidant power (FRAP Assay)**

The typical FRAP assay method was employed using 96-well microplates. Briefly, the FRAP reagent was prepared immediately by mixing 10 ml of acetate buffer (pH 3.6) with 1 ml of ferric chloride hexahydrate (20 mM in distilled water) and 1 ml of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM in HCl 0.04 N) and placed in a water bath at 37°C. Then, 25µL of each sample were pipetted into the wells in triplicates and 175µL of FRAP reagent was added in each well. Microplates were incubated in darkness at 37°C for 30 min and the absorbance was measured using microplate reader at 595 nm. The blank solution contained 175µL of FRAP reagent and 25µl of methanol; the control solution consisted of 175µl of reagent and 25µl of various concentrations of ascorbic acid (50 –
RESULTS AND DISCUSSION

Phytochemical analysis

The results of qualitative phytochemical analysis revealed the presence of tannins, flavonoids, saponins, glycosides, alkaloids, cardiac glycosides, saponin glycosides, steroids and volatile oils while anthraquinones were absent. The presence or absence of phytochemicals is presented in Table 1.

Table 1: Phytochemical composition of methanolic crude extract and aqueous fraction

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Aqueous fraction</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponin glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: (−): Absent. (+): Present.

Antioxidant activity

Phosphomolybdenum and Ferric reducing potential of aqueous fraction.

The results of Phosphomolybdenum assay and ferric reducing capacity of aqueous fraction of M. oleifera is presented in Table 4.4. The results indicated that, aqueous fraction demonstrated a good antioxidant activity by reducing phosphate molybdenum (VI) to phosphate molybdenum (V) at concentration of 56.35 ± 0.29 mg ascorbic acid equivalent/g of aqueous fraction of M. oleifera and reduction of ferric ion at concentration of 124.16 ± 0.6 mg ascorbic acid equivalent/g of aqueous fraction of M. oleifera. This is represented in table 2.

Table 2: Ferric reducing antioxidant capacity (FRAP) and Total antioxidant capacity of aqueous fraction of M. oleifera by Phosphomolybdenum assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>(mg) of ascorbic acid equivalent by phosphomolybdenum assay</th>
<th>mg ascorbic acid equivalent by FRAP assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous fraction</td>
<td>56.35 ± 0.29</td>
<td>124.16 ± 0.6</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD of triplicate values (mg of ascorbic acid /g of aqueous fraction of M. oleifera).
DPPH radical scavenging capacity
The radical scavenging activity of aqueous fraction of *M. oleifera* following DPPH assay was observed to increase with increasing concentration. The minimum scavenging activity of 10% was obtained at lower concentration of 10 µg/mL, and a maximum scavenging activity of about 70% was obtained at concentration of 500 µg/mL. Similarly, maximum activity of 70% was also obtained from standard ascorbic acid. The IC\(_{50}\) of aqueous fraction was 179µg/ml as shown in figure 1.

![Figure 1: Graph of DPPH radical scavenging activity of aqueous fraction of *M. oleifera* by DPPH compared with standard ascorbic acid. Values are expressed as mean ± S.D of three independent experiments.](image1)

Nitric oxide scavenging activity
Following nitric oxide (NO) scavenging assay, the scavenging activity of NO by the aqueous fraction was seen to increase as concentration of aqueous fraction increases. At concentration of 10, 50, 100, 200, 300, 400 and 500 µg/mL, the NO scavenging activity was found to be; 35, 50, 52, 53, 54, 55 and 57% inhibition. However, the scavenging activity of standard ascorbic acid was found to be 60 % inhibition. The aqueous fraction with maximum activity of 57% inhibition and an IC\(_{50}\) of 46µg/ml. This is shown in figure 2.

![Figure 2: Graph of nitric oxide scavenging activity of aqueous fraction of *M. oleifera* compared with standard ascorbic acid. Values are expressed as mean ± S.D of independent experiments.](image2)

Hydrogen peroxide scavenging activity
Following hydrogen peroxide assay, the aqueous fraction of *M. oleifera* demonstrated a good scavenging activity. The fraction exhibited a concentration dependent scavenging activity with a minimum scavenging activity at 40% and a maximum scavenging of 86% inhibition while that of the standard ascorbic acid was 90% inhibition. The result of the hydrogen peroxide scavenging activity is illustrated in figure 3.

![Figure 3: Graph of hydrogen peroxide scavenging activity of aqueous fraction of *M. oleifera* compared with standard ascorbic acid. Values are expressed as mean ± S.D of three independent experiments.](image3)
DISCUSSION

In the present study, antioxidant properties of aqueous fraction of *Moringa oleifera* was evaluated for potential in wound healing applications. Preliminary qualitative phytochemical screening of the aqueous fraction revealed the presence of some useful phytochemical compounds that included tannins, flavonoids, saponins, glycosides, alkaloids, cardiac glycosides, saponin glycosides, steroids and volatile oil while antherquinones were absent. The antibacterial and antioxidant properties exhibited by aqueous fraction of *M. oleifera* may be linked to presence of some phytochemical compounds revealed in this study. These activities could be the result of an individual or additive effect of these compounds. This finding is in accordance with other previous studies that reported, constituents like alkaloids, triterpenoids and tannins found in *Vincia rosea* may play a major role in the process of wound healing in diabetic rats due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialization.

Another study also reported that, the wound healing effects of the chloroform, methanol, and aqueous extracts of *H. indicum* may be attributed to the presence of phytoconstituents like alkaloids, triterpenoids, tannins and flavonoids in the extracts which are known to promote the wound healing process mainly due to their antimicrobial property.

As widely reported, a strong relationship does exist between wound healing and oxidative stress. Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular.

The reactive oxygen species (ROS) generation by inflammatory cells within the dermis plays an integral role in mediating wound healing. Over production of ROS can affect proliferative and cell survival signaling to alter apoptotic pathways in the skin diseases and this contributes significantly to pathogenesis of impaired skin wound healing. In our antioxidant assays, aqueous fraction of *M. oleifera* was shown to possess significant antioxidant properties comparable to those of various standard ascorbic acid used. The antioxidant properties of our aqueous fraction could be linked to the presence of phenolic compounds which scavenger oxygen free radicals, thus help in prevention of tissue damage associated with delayed healing of wounds, thus, aqueous fraction of *Moringa oleifera* may help facilitate wound healing through its antioxidant capacity. The bioactive aqueous fraction of *M. oleifera* was obtained from crude methanolic extract which could be one of the reasons associated with its high antioxidant ability. This is in agreement with the previously reported study that reported methanolic extracts having higher antioxidant activity compared to other solvent extracts from plant derived food using various study models.

The high antioxidant nature of methanol solvent extracts may be linked to high chemical polarity of methanol when compared to other solvent system.

The phytochemical compounds such as flavonoids revealed aqueous fraction of *M. oleifera* which are poly phenolic which further justifies that, the major active compounds could be responsible for the antioxidant actions and the compounds are polar compounds that could be useful in promoting wound healing. The presence of some of these phytochemical compounds in the aqueous fraction of *M. oleifera* makes it a potential wound healing agent.

CONCLUSION

The ability to modulate production and quenching of free radicals may contribute significantly to the demonstrated property of bioactive aqueous fraction of *M. oleifera* to help in enhancing wound healing especially in chronic wounds. The results of this study are encouraging, proposing further investigation of the wound healing properties of bioactive aqueous fraction of *M. oleifera* that could have value clinically.

REFERENCES

1. WHO: Traditional Medicine. In Edited by Centre M, WHO, 2008;


25. Bios MS, Antioxidant determinations by the use of a stable free radical. Nature 1958; 181:1199-1200 https://doi.org/10.1038/1811199a0


