Antioxidant activity of *moringa oleifera* aqueous extract from leaves and seeds

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*Moringa oleifera* is rich with many compounds that widely used in natural medicine. The aim of this present study were to compare the antioxidant activity and total phenolic content of aqueous extracts from *M. oleifera* leaves and seeds and to evaluate the biological activities (anticoagulation and fibrinolytic) of aqueous and sulfated extracts from *M. oleifera* leaves and seeds. Both aqueous extracts from *M. oleifera* leaves and seeds exhibited good DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity (76.61% and 72.88%) of seeds and leaves, respectively. In the present study it was clear that aqueous extract from seeds of *M. oleifera* showed higher contents of phenolic compounds than the contents of phenolic compounds in leaves extract. Our results indicated that leaves and seeds aqueous extracts have anticoagulant activity. Additionally, adding sulfate group to leaves and seeds aqueous extracts improved the anticoagulation activity. Moreover, leaves extract exhibited fibrinolytic activity.

**Keywords:** *Moringa oleifera*, aqueous extracts, antioxidant activity (DPPH) biological activity.

INTRODUCTION

*Moringa oleifera* is known as ‘a miracle tree’ because all plant parts are having nutraceutical and functional properties (Singh et al., 2012). It is the most widely cultivated species of the genus *Moringa* of Moringaceae family. Moringa is a good source of nutrition for the people of all ages which can save lives (Fuglie, 2001). Studies by various researchers have proved that plants are one of the major sources of drug discovery and development (Gordon and David, 2005; Rebecca et al., 2006). Plant parts including leaves, stem, roots, seeds and flowers have been reported as source of different biochemical compounds with anti-inflammatory, antidiabetic, anti-carcinogenic, antioxidant, and antimicrobial effects (Goyal et al., 2007; Chumark et al., 2008; Peixoto et al., 2011). The ethanolic extract of *Moringa oleifera* leaves contain various phytochemicals and exhibited antibacterial activity against various organisms (Amabye and Tadesse, 2016).

*Moringa oleifera* oil is highly resistant to oxidation and also, leaves and seeds are a good source of antioxidant (Anwar et al., 2006). The previous studies referred to the ethanolic extract of *Moringa oleifera* seeds possess antioxidant activity (Kumar et al., 2013). *M. oleifera* saline and ethanolic extracts had antioxidant activity that supported the use of the plant tissues as food sources (Santos et al., 2012). Kumar et al., (2012) indicated that the methanolic extract of *Moringa oleifera* leaves considered as a source of antioxidants and natural antibacterial. The phenolic content extracted from dried leaves of *Moringa oleifera* exhibited the yield of methanolic extract was 7.6% (Anwar et al., 2006). *Moringa oleifera* might be regarded as a promising candidate as a natural plant rich in phenolic
compounds. Antioxidant activity of *M. oleifera* leaves extracts, which was attributed to phenolic compounds and flavonoids might be responsible for human body protection against free radicals causing the damage to the body (El Sohaimy et al., 2015). Heparin and warfarin have been used as anticoagulant drugs for treating the clotting from about fifty years (Dobesh et al., 2004). Many of previous studies have been trying to find anticoagulant from natural sources (Félix-Siliva et al., 2014; Edemeka and ogwa, 2000; Weirong et al., 2013). Therefore, the present study was planned to compare the antioxidant activity and total phenolic content presented in *Moringa oleifera* leaves and seeds. The fibrinolytic and anticoagulation activities were determined for aqueous and sulfated extracts from leaves and seeds.

**MATERIALS AND METHODS**

This study was carried out at National Research Center, in Botany Department and Natural and Microbial Products Chemistry Department. *Moringa oleifera* leaves and seeds were purchased from Egyptian Scientific Society of Moringa (ESSM), National Research Center, Dokki, Cairo, Egypt. Reagent and standards were obtained from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGa A, Darmstadt, Germany).

**Preparation of aqueous extracts**

Extraction with hot water: Using the method of (Whistler and Saarnio, 1957) with some modifications 5 g of the ground plant sample were extracted successively with distilled water (200 ml x 3 times) at 85°C under reflux for 3 h. After cooling and filtration, the combined extracts were dried and weighed.

Sulfation of aqueous extracts (sulfated extract): Adding sulfated group to aqueous extract achieved adopting on the method reported by (Yang et al., 2005) with some modification.

**Antioxidant activity (DPPH assay)**

DPPH' scavenging capacity: DPPH' assay was carried out following Martínez-Avila et al., (2012). The antioxidant activity of the extracts and standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. 10 μL of each extract or standard (from 0.0 1 μg/ml) was added to 90 μL of a 100 μM methanolic solution of DPPH in a 96-well microtiter plate (sigma-Aldrich Co., St. Louis, MO, US). After incubation in the dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using an ELISA micro plate reader. The percentage of scavenging inhibition was determined and compared with ascorbic acid, which was used as the standard. The radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH' percent of inhibition:

\[
\text{Inhibition} \% = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

Where Abs control is the absorption of the blank; Abs sample is the absorption of the extract sample.

**Determination of total phenolic**

The total phenolic content in plant extracts was determined by using Folin-Ciocalteu colourimetric method based on oxidation-reduction reaction (Waterhouse, 2002). Various concentrations of gallic acid solutions in methanol were prepared. In a 20 ml test tube, 1 ml gallic acid of each concentration was added to 5 mL of Folin-Ciocalteu reagent (10%) and 4 mL of 7% Na2CO3 were added to get a total volume of 10 mL. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 °C in a water bath. Then the absorbance was measured at 725 nm against blank. All experiments were carried out in triplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.

**Anticoagulation activity**

The activity of *M. oleifera* aqueous and sulfated extracts were determined according to the time for clot formation of fresh plasma of human blood (colman et al., 1994) and compared with heparin sodium preparation as a standard.

**Fibrinolytic Activity**

This was performed by exposing a plasma clot to *M. oleifera* aqueous and sulfated extracts. The results were compared with the effect of hemoclar on plasma clot that used as standard. Preparation of the plasma clot was done according to the method reported by Pharmacopeia (1960).

**Statistical analysis**

The results of all experiments were carried out in three replicates (n=3). The data were expressed as mean and standard deviation (±SD) was calculated.
RESULTS AND DISCUSSION

Antioxidant activity (DPPH)

The antioxidants converted the deep violet colour of free radical DPPH into colorless solution of 1, 1-diphenyl-2-picryl hydrazine during the reaction. An aqueous extract of M. oleifera (leaves and seeds) and ascorbic acid as the standard showed the antioxidant activity.

In this result, DPPH free radical was carried out in leaves and seeds aqueous extracts of M. oleifera to determine the richer extracts in antioxidant activity. In our results revealed that both extracts from leaves and seeds have antioxidant activity in (Figure1 and Table 1) the antioxidant activity of seeds 76.61% and leaves 72.88% compared to ascorbic acid, that considered as a source of natural antioxidants.

Chumark et al., (2008) studied the in vitro and ex vivo antioxidant properties of water extract of M. oleifera leaves by DPPH assay. The scavenging DPPH free radicals of the extract had of 78.15±0.92. Measurement of antioxidant activity is the most widely accepted analysis to attribute the several benefits of phenolic content (Yemis et al., 2008).

Lako et al., (2007) who reported Moringa oleifera had high antioxidant activity. Moringa oleifera leaves are potential source of natural antioxidants due to their marked antioxidant activity. Pakade et al., (2013) found that M. oleifera leaves had greater antioxidant activity than other vegetables (cabbage, spinach, broccoli, cauliflower and peas). These results due to the total phenolic compounds and total flavonoid compounds of dry moringa leaves more than those of the selected vegetables.

Determination of total phenolic content

The total phenolic content (TPC) of aqueous extracts of leaves and seeds were determined by using the Folin-Ciocalteu assay is expressed in terms of gallic acid equivalent (The standard curve equation: $y = 0.1293x + 0.0256$, $R^2 = 0.9995$) (Figure 2). The total phenolic contents of are presented in Table (1). The results indicated that the total phenolic content of seeds extract were higher (65.5±1.13) than which presented in leaves extract (51.1±2.26) expressed as gallic acid equivalents. The phenolic compounds concentration in M. oleifera plant differed according to plant part extracted. Lallas and Tsakins (2002) characterized the antioxidants in seeds of Moringa oleifera grown in Malawi and reported that seeds contained appreciable amount of phenolic antioxidants that could be used to prolong the shelf life of edible oils without affecting on characteristics. The high concentrations of phenolic compounds indicated that these compounds contribute to the strong antioxidant activity (Stankovic, 2011).

Anticoagulant and fibrinolytic activities

In this study evaluated the anticoagulant activity of aqueous and sulfated extracts of –M. oleifera leaves and seeds at 2000µg/ml concentration on fresh plasma of human blood. Both of aqueous and sulfated extracts from seeds exhibited anticoagulant activity on coagulation process than leaves extracts as comparing with heparin by increasing in time required for clot formation (Figure 3).

The anticoagulant activity of M. oleifera varied with their part tested and extracts without or with adding sulfated group. Both of sulfated extracts from leaves and seeds prolonged the clotting time than aqueous extracts, also sulfated extract of seeds exhibited higher clotting time than other extracts. The anticoagulant activity of sulfated extracts is related to the presence of sulfated groups. Shanmugam and Mody (2000) referred to the variation in anticoagulation activity may be due to the active polysaccharides and sulfated groups attached to them. The extracts of M. oleifera plant present a potent anticoagulant activity varying according to the amounts of different compounds in each extract. According to pawlaczzyk et al., (2013) anticoagulant effect of polyphenol and flavonoid has been attributed to a large quantities of them presented in Fragaria vesca L. Fibrinolytic activities for aqueous extract of M. oleifera leaves, seeds and their sulfated extracts. –M. oleifera leaves extracts have activity at (+5) clotting lysis for aqueous extract and sulfated extract exhibited (+6) lysis for clots than heomclor (+4) as a control (Figure 4).

![Table 1. DPPH scavenging activity and Total phenolic content of M. oleifera leaves and seeds.](https://example.com/table1.png)

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH scavenging %</th>
<th>Total phenolic (mg of GA/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>72.88±0.035</td>
<td>51.1±2.26</td>
</tr>
<tr>
<td>Seeds</td>
<td>76.61±0.028</td>
<td>65.5±1.13</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Standard deviation. GA, gallic acid
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Figure 1. Total antioxidant activity of *M. oleifera* leaves and seeds.

![Figure 1](image)

Figure 2. Total phenolic content for standard Gallic acid

![Figure 2](image)

Figure 3. The effect of different extracts of *M. oleifera* on clotting time of normal human plasma.


![Figure 3](image)
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**Figure 4. In vitro fibrinolytic activity of aqueous and sulfated extracts of *M. oleifera* leaves and seeds.**

- **a.** Effect of aqueous extract of leaves.
- **b.** Effect of sulfated extract of leaves.
- **c.** Effect of aqueous extract of seeds.
- **d.** Effect of sulfated extract of seeds.

The aqueous and sulfated extracts from seeds haven't activities comparing with control. Satish et al., (2012) suggested that the extracts of *M. oleifera* root and leaves have fibrinogenolytic and fibrinolytic activity.

**CONCLUSION**

In the present study, the results indicated a strong correlation between the results of total phenolic content and DPPH radical scavenging activity. So, it was clear that the extracts from seeds of *M. oleifera* showing higher contents of phenolic constituents also exhibited good DPPH radical activity. *M. oleifera* seed have anticoagulant activity and leaves extracts exhibited Fibrinolytic activity.

**CONFLICT OF INTEREST**

No potential conflict of interest was reported by the authors.

**ACKNOWLEDGEMENT**

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**AUTHOR CONTRIBUTIONS**

WAH proposed the research idea, designed the experiments and reviewed the manuscript. ARA performed the experiments, wrote and sent the manuscript. AAH designed and performed some experiments. ARA AAH preparing the materials and tools and analyzed the data. All authors read and approved the final version.

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