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Isolation, Chemical Analysis, and Free Radical Scavenging Activity of the Volatile Components of two *Moringa* species

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ABSTRACT

Background: *An increase of oxidative species in our body is implicated in the expansion and progression of chronic diseases. Identification of new natural antioxidants resources may provide hopeful expectancies for the upgrading of human healthcare concerning the prevention of the expansion of the chronic diseases.* ***Objective:*** *The objective of this study was to perform the isolation, chemical analysis, and antioxidative activity of the volatile oils of Moringa peregrina and Moringa oleifera from Saudi Arabia.* ***Method:*** *The volatile oils were isolated by hydrodistillation method. The physicochemical characteristics were also analyzed by the reported methods. The DPPH method was used to assess the antioxidant potential of the isolated oils.* ***Result:*** *The hydrodistillation method provided the volatile oil from M. peregrina and M. oleifera in 0.06% and 0.05% yield, respectively. The color, odour, solubility in water, ethanol, methanol, n-hexane, dichloromethane, ethyl acetate, chloroform, and petroleum ether, refractive index, and the acid value of these isolated oils were similar. The isolated volatile oils from M. peregrina and M. oleifera provided good* IC_{50} *values, which were 85.48% and 85.57%, respectively, with respect to ascorbic acid (100%).* ***Conclusion:*** *It is expected that the chemical composition of the volatile oils of these two species of Moringa might be similar, which can be identified by their GC-MS analysis. Accordingly, further investigations are suggested.*

Keywords: *Moringa peregrina, Moringa oleifera*, Volatile oil, Antioxidant, DPPH

INTRODUCTION

Free radicals are produced throughout standard cellular purpose, and they are a portion of the normal biological progression of almost all live organisms [1]. They perform dual functions and are beneficial as well as harmful to our body [2]. An excess of the free radicals causes "oxidative damage", which is also known as "oxidative stress". Oxidative stress is implicated in the undiscriminating harm to an extensive collection of biomolecules [3]. It is usually considered the preliminary signal for the beginning of numerous chronic illnesses and also plays main part in the expansion of chronic and deteriorating disorders [4-7]. Therefore, consumption of the exogenous natural antioxidant supplements is effective and encouraging method to prevent the expansion of chronic diseases. Accordingly, identification of new antioxidants, preferably of the natural origin, may provide new, hopeful expectancies for the upgrading of human healthcare concerning the prevention of the expansion of the chronic diseases [8]. The *Moringa* genus (Family: Moringaceae), comprises of 13 species [9]. *Moringa peregrina* (Forssk.) Fiori and *Moringa oleifera* Lam. are two main species of the *Moringa* genus. *Moringa* species are famous for their various traditional uses or folk medicine, for example, paralysis and skin rashes [10], diabetes [11], disinfectant [12], and wound healing [13]. Research has also been performed on the different parts of *Moringa* tree, wherein the extracts of different solvents are

shown to possess antioxidant, antimicrobial, antispasmodic and hepatoprotective properties, including lipid-lowering, anti-inflammatory and anticancer activities [14-16]. There are a negligible number of reports [14] on the antioxidant activity evaluation of the phytochemicals of the *M. peregrina* and *M. oleifera* found in Saudi Arabia. Because of the above facts, it has been decided to perform the isolation, chemical analysis, and antioxidative activity of the volatile oils of *M. peregrina* and *M. oleifera*.

MATERIAL AND METHODS

Collection of the Plant Material

The leaves of *M. peregrina* and *M. oleifera* (2 kg, semi-dried) were acquired from Al Oula region in January 2019, the *Moringa* leaves were validated by Prof. Abdulhakim Bawadekji and specimens were conserved in the herbarium with the reference Oul. 1 and Oul. 2 respectively. The leaves material was cleaned and air dry for eight days at 25 to 30°C. The dried leaves of the *M. peregrina* and *M. oleifera* were used for the isolation of the volatile oil. These leaves were grounded in powder form employing a grinder. The powder was sieved and kept in polyethylene bags for the solvent extract purpose.

Isolation of the Volatile Oil

The dried leave powder (100 g) was hydrodistilled for 3 hours as per the method provided in the European Pharmacopoeia [17,18]. The resulting oil was mixed in n-hexane. The mixture was dried with anhydrous Na_2SO_4 and kept in the dark place (+4°C).

Physicochemical Analysis

The physicochemical properties of the isolated oils were performed as per the literature [19,20].

Preparation of the Ethanolic Extracts

The powdered leaves of *M. peregrina* (50 g) were taken in a 1000 ml flask. Ethanol (500 ml) was added to the flask. The mix was stirred at 25°C to 30°C for half-hour and kept in the dark for four days with infrequent shaking. The mixture was filtered, and the filtrate was concentrated to acquire a semisolid residue. The semisolid ethanolic extract of *M. oleifera* was also obtained in the same manner.

Phytochemical Studies of the Extracts

The phytochemical studies of the extracts were carried out as per the standard procedures [21].

Antioxidant Activity Evaluation

It was performed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test [18,22]. The test was carried out in microplates by mixing the appropriate dilution of the volatile oil and methanolic DPPH solution. The absorbance (517 nm) was measured spectrophotometrically. The methanolic DPPH solution served as control. The scavenging activity has been reported as IC_{50} values.

RESULTS AND DISCUSSION

The volatile oil from *M. peregrina* and *M. oleifera* were obtained in 0.06% and 0.05% yield. The physicochemical characteristics were analyzed using reported methods [19,20]. The color, odour, solubility in different solvents, refractive index, and the acid value of these isolated oils were similar [14], and are mentioned in Table 1. According to the data of Table 1, the two oils differ slightly with respect to their refractive index, and acid value. This slight variation may due to the difference at the level of species which are related to the same genus.

Table 1 Physicochemical data of the volatile oils

S. No.	Physicochemical parameter	*M. peregrina*	*M. oleifera*
1	Yield	0.06%	0.05%
2	Colour	Faint Brown	Faint Brown
3	Odour	Pleasant	Pleasant

4	Solubility in	Water	Marginally miscible	Marginally miscible
		Ethyl alcohol	Marginally miscible	Marginally miscible
		Methyl alcohol	Marginally miscible	Marginally miscible
		Hexane	Miscible	Miscible
		Dichloromethane	Miscible	Miscible
		Ethyl acetate	Miscible	Miscible
		Chloroform	Miscible	Miscible
		Petroleum ether	Miscible	Miscible
5	Paper test		Oil dried without oily stain	Oil dried without oily stain
6	Refractive index at 20°C		1.220 ± 0.013*	1.241 ± 0.009*
7	Acid value		3.52 ± 0.54*	3.44 ± 0.14*

The phytochemical analysis of the ethanolic extracts of *M. peregrina* and *M. oleifera* was performed by the standard procedures [21]. These phytochemical tests revealed the presence of alkaloids, tannins, cardenolides, steroids, terpenoids, anthraquinone, flavonoids, carbohydrates, and glycosides. However, the absence saponins, proteins, and amino acid was observed in these extracts. The data of the Table 2 revealed a similarity in the phytoconstituents of these two *Moringa* species [14]. However, for the determination of the exact quantity of the phytoconstituents, further sophisticated analysis is advised, for example, gas chromatography, (GCMS).

Table 2 Phytochemical assessment data of the ethanolic extracts

S. No.	Phytochemical	Test	*M. peregrina*	*M. oleifera*
1	Alkaloids	Mayer's and Wagner's test	+	+
2	Tannins	$FeCl_3$ and Lead acetate test	+	+
3	Cardenolides	Baljet test and Kellar Killani test	+	+
4	Steroids	Liebermann-Burchard test	+	+
5	Terpenoids	Salkowski's test	+	+
6	Saponins	Foam test	-	-
7	Anthraquinone	Borntrager's test	+	+
8	Flavonoids	Ammonia test & alkaline reagent test	+	+
9	Proteins and amino acids	Ninhydrin test	-	-
10	Carbohydrates	Molisch's test	+	+
11	Glycosides	Nitroprusside test	+	+
(+): Detected; (-): Not Detected				

The DPPH method was used to assess the antioxidant potential of the isolated oils and the ethanolic extracts of *M. peregrina* and *M. oleifera*, and are expressed as IC_{50} values [18,22]. For comparison, the IC_{50} value of the standard ascorbic acid (15.19 µg/mL) was taken as 100%. The isolated volatile oils from *M. peregrina* and *M. oleifera* provided good IC_{50} values, which were 85.48% and 85.57%, respectively, with respect to ascorbic acid (100%). The IC_{50} values provided by the ethanolic extracts of *M. peregrina* and *M. oleifera* were 81.18% and 81.49%, respectively, with respect to ascorbic acid (100%) (Table 3).

Table 3 The antioxidant activity of the volatile oils and the extracts

Sample	% Antioxidant Activity (N=3)			IC_{50} (µg/mL) (% Activity relative to ascorbic acid)
	10 µg/mL	20 µg/mL	30 µg/mL	
M. peregrina oil	33.10 ± 0.11[a]	71.13 ± 0.22[a]	91.30 ± 0.45[a]	17.77 (85.48%)
M. peregrina extract	26.16 ± 0.40[a]	61.10 ± 0.21[a]	75.98 ± 0.50[a]	18.71 (81.18%)
M. oleifera oil	31.95 ± 0.16[a]	72.11 ± 0.22[a]	93.33 ± 0.19[a]	17.75 (85.57%)

M. oleifera extract	27.15 ± 0.40[a]	62.50 ± 0.50[a]	80.05 ± 0.20[a]	18.64 (81.49%)
Ascorbic Acid	46.33 ± 0.22[a]	85.12 ± 0.31[a]	99.65 ± 0.10[a]	15.19 (100%)
Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Values in mean ± standard deviation (SD); a: $p<0.05$ as compared to control				

It is expected that the antioxidative property of the ethanolic extracts were due to the presence of alkaloids, terpenoids, and flavonoids present in them. The chances of the presence of volatile oil in these extracts were low because of their volatile nature. It is also expected that the volatile oils had better antioxidative property because of their volatile compounds. The volatile components of other species of *Moringa* have reported good antioxidative properties. It is also observed that the antioxidative property provided by the *M. peregrina* and *M. oleifera* were almost equivalent. This suggests the presence of similar types of phytoconstituents in both plants. Our postulation is also supported by the phytochemical analysis data of Table 2, which provides the presence of the identical phytoconstituents in the ethanolic extracts of these two plants.

CONCLUSION

The isolated volatile oils from M. pregrina and *M. oleifera* collected Saudi Arabia from Al Oula region, exhibited similar preliminary physicochemical properties. The antioxidative potential of these isolated oils was also promising. Based on the similar phytochemical analysis results of the ethanolic extracts of these two plants, it is also expected that the chemical composition of the volatile oils of these two species of *Moringa* might be similar, which can be identified by their GC-MS analysis. Accordingly, further investigations are suggested to ensure the main antioxidative chemical compounds of the volatile oil and the ethanolic extracts of these plants.

DECLARATIONS

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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