Characterization of Egyptian *Moringa peregrine* seed oil and its bioactivities

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Abstract

The seed oil of Egyptian Moringa peregrina (Forssk) was extracted with a mixture of dichloromethane/methanol (1:1, v/v). The oil was examined with respect to physicochemical properties, unsaponifiable (UnSap) and fatty acids profiles, tochopherols and phenolic contents, anticancer and antioxidant activities. Moringa oil (MO) showed a better overall quality, its acid, peroxide, iodine, saponification values were 0.02 mg KOH/g oil, 0.01 meq O_2/kg oil, 67 I_2 g/100 g oil and 177 mg KOH/g. The Un-Sap of MO was found to contain high amounts of hydrocarbon fraction C12 to C32 and phytoesterol fractions were found rich in campesteol, clerosterol and β sitosterol compounds. The major fatty acids were identified as oleic (C18:1 ω9, 65.36%) and linoleic (C18:3 ω6, 15.32%). Tocopherols and phenolic in oil accounted for 20.35 and 48.31 mg/100 g. The Moringa oil showed high growth inhibition against three human cancer cell lines, breast adenocarcinoma (MCF-7), hepatocellular carcinoma (Hep-G2), and colon carcinoma (HCT-116), with IC_{50} values of 2.92, 9.40 and 9.48 µg/ml, respectively. The MO showed remarkable antioxidant activity, compared with that of commonly used antioxidants (α-tocopherol, BHT and BHA) as determined by five antioxidant assays (includes, free radical scavenging of DPPH, ABTS, ·OH, anion-scavenging capability and reducing power. These results strongly suggested its potential use MO as non-conventional seed crop for high quality oil and as candidate in the area of natural anticancer and antioxidant compounds.

Key words: *Moringa peregrin*a, seed oil, anticancer, fatty acid, antioxidant activity.

Introduction

Moringa species are one of the most useful trees in the tropics and subtropics of Asia and Africa, with a multiple of uses. These species are the most widely cultivated of the *Moringaceae* family and was utilized by the ancient Egyptians, Romans and Greeks. Traditionally, almost all parts of *Moringa* flower, fruits and roots are edible, and have long been consumed as vegetable and used to treat many diseases such as abdominal tumors, hysteria, scurvy, paralytic attacks, helmintic bladder, prostate troubles, sores and skin infections (Rahman, et al. 2009). The leaves are highly nutritious, which contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and more protein than milk and eggs (Sreelatha, et al. 2011). Moreover, leaves of *Moringa* species are rich in various phytochemicals like carotenoids, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phytoestrogens caffeoylquinic acids and phenolics (Anwar, et al. 2007). Fruits and seeds have been reported as a rich source of protein, essential elements (Ca, Mg, K and Fe) and vitamins (vitamin A, C and E). The seed oil contains all fatty acids contained in olive oil, except linolieic and was used as its acceptable substitute (Lalas and Tsakinis, 2002). In addition, *Moringa* oil has been used in salads, in industry as fine machine lubrication and in the manufacture of perfume and hair care products. Moreover, seeds contain a dimeric cationic proteins (13 kDa) having coagulant and antimicrobial properties, and traditionally is utilized for purification and treatment of high turbid water (Anwar, et al. 2007). More recent, *Moringa* gums, roots and seeds are widely used in traditional medicine and folk remedies for treated many diseases such as inflammation, cardiovascular and liver diseases, immune boosting agent, blood sugar and cholesterol regulator (Rao and Misra, 1998). Thus, *Moringa* trees are an important food commodity as all plant parts can be used in the indigenous systems of human medicine for the treatment of a variety of ailments and as high nutritive vegetable in many countries of the tropics and sub-tropics (Anhwange, *et al.* 2004 and). In Ghana and some parts of Asia (particularly India and Bangladesh) *Moringa oleifera* was used as a nutritional supplement and remained popular among the lower socio-economic class (Anhwange *et al.*, 2004). In Egypt, the oil obtained from seeds of moringa trees were used as edible oil sources in south cost of Red Sea region. However, the physiochemical properties, fatty acid composition, phenolic and tecopherols contents, anticancer antimicrobial and antioxidant activities of the Egyptian *Moringa* oil has not well established yet. Therefore, the objectives of this study were to determine physicochemical, antioxidant contents (tochopherols and tocopherols) and unsaponifiable and fatty acid profiles of oil extracted from the seeds of Egyptian *Moringa pergringa* trees. Also, antioxidant, antibacterial and antiprolifiration activities of oil (against three cancer cell lines) were evaluated.

Material and Methods

Plant material

The *M. peregrina* seeds were collected from one tree in the Orman Botanical Garden, ministry of Agriculture, Giza, Egypt. After the removal of seed coat, the seeds (500 g) were air dried and crushed into a coarse powder using a laboratory blender.

Reagents

All the reagents (analytical and HPLC grade) were obtained from Sigma-Aldrach Co. (St Louis, MO, U.S.A) or Merck Ltd (Darmstadt, Germany). The standard fatty acids (>98%, GC) and tecopherols (> 98% by HPLC) were purchase from Sigma-Aldrich. All cell culture material was purchased from Cambrex BioScience (Copenhagen, Denmark).

Oil extraction

Total lipids content from seeds was extracted with 2 liters of dichloromethane/methanol (1:1, v/v) mixture in Soxhlet apparatus.

Determination of the physical chemical characteristics

The extracted *Moringa* oil was analyzed immediately for acid value (AV), iodine value, saponification matter (SM), TBA, unsaponifiable matter (USM) and peroxide value (PV) and the absorbance of diene (A 232 nm) and triene (A at 270 nm) -conjugated fatty acids as described in AOCS methods (1998).

Identification of un-saponifiable matter

The un-saponifiable matters in *Moringa* oil was analyzed by an HP 5890 gas chromatograph equipped with FID detector and DB-5 capillary column (30 m, 0.25mm (5%-phenyl)–95%-methylpolysiloxane, 0.25 um film thickness, 280 °C temperature injector and 300°C temperature transfer line. The oven temperature was programmed as follows: initial temperature; 100°C for 2 min, increase 10°C/min up to 300°C, and then hold for 20 min. The carrier gas was N_2 (2 ml/min). The identification of the different compounds was performed by comparing of its relative retention times with those of authentic reference compounds (Aldrich-Sigma, > 98%).

Identification of fatty acids

The fatty acids of *Moringa* oil was analyzed by an HP 6890 series as chromatograph system with an HP 5973 Network mass selective detector. The system was equipped with a TR-FAME (MS, 30 m x 0.25 mm i.d., (70%-cyanopropyl-polysil phenylene), 0.25 μm film thickness) capillary column, 250 °C temperature injector and 240 °C temperature transfer line. The oven temperature was programmed as follows: initial temperature; 80°C for 2 min, increase 3°C/min up to 220°C, and then hold at 220°C for 20 min. The carrier gas was He_2 (1.5 ml/min). The amount of sample injected was l μl (about 2 mg/ ml) and the ionization energy was 70 eV. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds or by comparison of their retention indices and mass spectra with those shown in the NIST (2007) MS spectra. The relative amounts (RA) of individual components of the fatty acids oil were expressed as percentages of the peak area relative to the total peak areas as described by Abd El Baky et al. (2012).

Determination of total phenolic content

Total phenolic compounds (TPC) of MO were spectrophotometrically determined using Folin-Ciocalteu reagent as described by Singleton, et al. (1999). A standard calibration curve was prepared using gallic acid.

Determination of total tocopherols content

Total tocopherols of MO were spectrophotometrically determined as described by Wong, et al. (1988).

Antioxidant activity

The antioxidant activity of *Moringa* oil (MO) was measured by the scavenging ability of hydroxyl, ABTS and DPPH radical and reducing power methods. All tests were performed in triplicates and averaged.

1- DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay

The ability of the MO to scavenge DPPH radical was estimated based on the method of Tagashira and Ohtake (1998). In brief, 3 ml of 0.15 mM DPPH (in methanol) was added to the different dilutions of the extract (amounting to 1.0 ml). The reaction mixture was incubated for 30 min after which its absorbance was measured at 517 nm. The methanol was used as both a blank and negative control. BHT, BHA, α-tochopherol and ascorbic acid (5–50 μg/ml) were used as a reference antioxidants standard. The radical-scavenging activity of MO was calculated from a calibration curve. The concentration providing 50% inhibition (IC_{50}) was calculated from a graph representing the inhibition percentage against MSO concentration.

2- ABTS scavenging radical assay

TBAS radical scavenging activity of the MO was determined according to the Re, et al. (1999)

method. The reduction percentage of absorbance at 15 min compared to the initial absorbance value was determined.

3- Hydroxyl radical assay

Hydroxyl radical scavenging activity of the MO was carried out according to the method of Muller (1997). BHT, BHA and α-tecopherol were used as positive controls.

4- Reducing power assay

Total reducing power was spectrophotometrically determined at 700 nm according to the method described by Zhu, et al. (2003).

5- Superoxide anion radical scavenging assay

The superoxide anion scavenging activity was spectrophotometrically determined at 560 nm according to Nishikimi, et al. (1972). BHT, BHA and α-tecopherol were used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percent inhibition (%) of superoxide anion generation was calculated as follows: %Inhibition = [(A_0, control – A, sample) / A_0, control] A_0, control X 100. The scavenging of superoxide anion as IC_{50} value (50% of inhibitory concentration in mg/ml) was calculated from inhibition curve.

Anticancer activity

The cytotoxic activity of MSO oil on various cancer cells was measured by MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay. The assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form dark blue insoluble formazan crystals which is greatly impermeable to cell membranes, resulting in its accumulation within healthy cells (Hansen, et al. 1989).

1. Cell Culture

Human cancer cells MCF-7 (breast adenocarcinoma cells), Hep-G2 (hepatocellular carcinoma cells) and HCT-116 (colon carcinoma) were used to evaluate the cytotoxic activity of MSO oil and etoposide (positive control, 25 µg/ml). The cells were cultured in DMEM (Dulbeco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/l), penicillin G sodium (100 U/ml), streptomycin sulphate (100 units/ml), amphotericin B (250 ng/ml) and maintained at 37ºC with 5% CO_2 in a humidified atmosphere. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested oil were dissolved in dimethylsulphoxide (DMSO), and then diluted thousand times in the assay. All experiments were performed in triplicate.

2- **MTT bioassay**

Cells ($0.5X10^5$ cells/ well), in serum-free media, were plated in 96-wells microplates, and treated with 20 µl of MSO solution at concentration ranging from 1.0 – 20 µg, subsequently the cells were incubated for 48 h. Negative control was treated with DMSO alone. After incubation, media were removed and 40 µl MTT (5mg/ml of MTT in 0.9 %NaCl) solution / well were added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol (0.04 N HCl in absolute isopropanol) / well and plate was shacked at room temperature, followed by photometric determination at 570 nm. The data were obtained from three independent assays, using five well for each assay, and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by 100% relative viability

Statistical analysis

All measurements were carried out in triplicate. Statistical analyses were performed using one-way analysis of variance ANOVA, and the significance of the difference between means was determined by Duncan's multiple range tests. Differences at $P < 0.05$ were considered statistically significant. The results were presented as mean values ± SD (standard deviations).

Results and Dissection

Characteristics of Moringa oil

The total oil yield and physical-chemical property of *M. peregrina* oil are shown in Table I. The data revealed that the oil were yellow in color and had acceptable odor. The oil yield was 42.23%, and not so different from those reported for *M. Peregrine* (44.90%) seeds from Saudi Arabia (Manzoor, et al., 2007). However, oil content in the present analysis was considerably quite higher than that reported for some common seed oil crops: cotton (15.0–24.0%) and soybean (17.0–21.0 %) and some what comparable with safflower (25.0-40.0%) (Pritchard, 1991). The acidity, iodine value (IV), peroxide value (PV), saponification number (SpN) and unsaponification matter (USM) of *Moringa* oil, the major

characterization parameters for oil quality are depicted in Table 1. The total acidity expressed as the acid value (AV) was found to be 0.02 mg KOH/g oil, these value was almost comparable to those of other *Moringa* oils investigated in several countries. A low AV for oil in the present analysis is indicated of a good resistance of this oil to hydrolysis (Manzoor, et al. 2007).

Table (1): Physical amd chemical characteristics of *Moranga peregrina* oil

Properties	Moranga peregrina oil
Refractive index (40 C)	1.43± 0.14
Density (g/cm3) 24°C	0.82 ± 0.11
Saponification value (mg of KOH/g of oil)	179± 1.52
Unsaponifaiable mater (%)	0.34± 0.001
Acid value (mg KOH/ g oil)	0.01± 0.001
Iodine value (g of I_2/100 g of oil)	67.9± 0.70
Peroxide value (meq kg-1 of oil)	0.01± 0.001
Conjugated diene (λ232)	
Conjugated triene (λ270)	

Tthe data are mean ± SD for three separate experiments.

Peroxide value (PV) is one of the most important assays used for measuring oxidative rancidity in oils and fats, which is the measure of levels of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Generally, the PV should be less than 10 meq O_2 /kg oil in the fresh oils as any increase in this value (>10) are unstable and easily become rancid (having a disagreeable odor) (Pearson, 1976). The PV of *M. peregrina* oil in the present study was 0.1 meq peroxide/kg, this value was much lower than that reported for *M. peregrine* oil (0.4 meq/kg of oil) from Saudi Arabia (Manzoor, et al. 2007). However, the lower PV of *Moringa* oil was good indices for the absence of specific products from oxidative rancidity. Moreover, as shown in Table 1, *M. pergrena* oil had very low values of conjugated-diene (λ 232 nm, 0.01) and -triene (λ 270 nm, 0.01), which revealed the process of initiation oxidative deterioration and purity of the oil. Iodine value is used as indicator for un-saturation degree of oils and measured the stability of oil in industrial applications (Xu and Josiah, 2007). The IV of *M. peregrina* oil was 67 mg I_2/100 g oil, thus it could be classified as non-drying oil. Also, the low IV of oil reflected its characteristics such as the higher resistance to oxidation, longer shelf life and higher quality. Saponification number (SpN) and unsaponifiable matter (USM) matters of *M. peregrina* oil were 177 mg KOH g^{-1} and 0.78%, respectively. The lower SN of *Moringa* oil indicates a very high content of low molecular weight triacylglycerols. However, the physical-chemical property of *M. peregrina* are close agreement with those of figures previously reported in the similar *Moringa* seed oil from different countries (Manzoor, et al. 2007). For example, the levels of AV, IV, PV, SN and SM (Table 1) in Egyptian *M. peregrina* oil were considerably similar with those reported in *M. peregrine* oil (0.34%, 69.6 mg I/100 g oil, 0.40 meq kg^{-1} of oil, 179 mg of KOH/g oil and 0.78%, respectively) from Pakistan, India and Saudi Arabia (Lalas and Tsaknis, 2002). On contrary, some physico-chemical properties of the oil were considerably varied from those reported in oil of other *Moringa* species. For instant, the values of AV, IV, PV, USM and SM in the present analysis for Egyptian of *M. peregrine* was lower than those reported for *M. peregrine* oil native to Saudi Arabia (0.04 mg KOH/g, 69.5 mg I/100g, 2.3 meq/kg, 0.3 % and 182.9 mg KOH/g oil) (Somali, et al. 1984). Generally, the variation in oil yield and physical chemical characteristics between Egyptian *M. peregrina* and same *M. pergrina* grown in other countries might be attributable to the differences in genotypes, growing conditions, geological conditions of the regions, ripening stage, the harvesting time of seeds and extraction methods used (Rahaman, et al. 2009).

Un-saponifiable matter

The un-saponiifable matter of *M. pergrina* seed oil consisted of hydrocarbon and steroid compounds (Table 2). The major saturated hydrocarbon in MO was C20, C22 and C 28. β-sitosterol (56.76%), campsterol (23.24%) and sigmasterol (8.11%) ware identified as the main phytosterols in steroids fraction. The results in agreement with the study of Lalas and Tsaknis (2002) which found that *M. oleifera* oil showed

high content of β-sitosterol and stigmasterol and campesterol.

Fatty acids composition

As shown in Table 3 and Fig. 1, *M. pergrina* seed oil was found to contain a high level of oleic acid (C18:1 n-9), which accounted for 65.36% of the total fatty acid. Thus, *M. pergrina* oil is belongs to the oleic acid oil category (Sonntag, 1982). Palmitic (C16:0, 12.44%) and linoleic (C18:2, 15.32%) acids were found in high quantity. Whereas, palmitoleic (C16:1, 1.54%) and stearic (C18:0, 4.35%) were identified as minor (<10%) compounds. The high percentage of mono-unsaturated fatty acids (MUFAs, 67.44%) in *Moinga* oil was not so varied from those of *M. peregrine* oil

Table (2): Unsaponified matter composition of *Morenga peregrina* seeds oil[a]

Unsaponified matter	Relative content %[b]	Relative %[c]
Hydrocarbon		
C8	4.93	
C9	5.36	
C10	7.65	
C11	1.32	
C12	2.87	
C13	0.61	
C14	2.34	
C15	1.02	
C16	0.53	
C17	4.87	
C18	5.71	
C19	1.47	
C20	5.06	
C21	0.69	
C22	3.67	
C23	2.38	
C24	1.22	
C25	1.42	
C26	11.71	
C27	1.57	
C28	3.52	
C29	2.01	
C30	1.63	
C32	1.26	
Steroids		
Campesterol	3.16	23.24
Stigmasterol	1.01	8.11
Beta-Sitosterol	7.72	56.76
Δ^7-Avenasterol	1.62	11.90

[a]: Unsaponified matter was identified based on the total known hydrocarbon and steroids (Retention time)
[b]: The relative % of the unsaponified matter was evaluated through the peak area.
[c]: The relative % of the steroids compounds were s evaluated through the peak area.

reported from Saudi Arabia (70.52%). The presence of polyunsaturated (PUFAs, 15.32%) and saturated fatty acids (SFs, 17.88%) were very low in comparison with the MUFAs. Thus, *Moringa* oil presented relatively low contents of saturated fatty acids (SFAs), high contents of unsaturated fatty acids (UNFAs) compared with other common vegetable/fruit seed oils, such as corn, olive and sesame and soybean (Manzoor, et al. 2007). Generally, the fatty acid composition of the Egyptian *M. peregrina* was quiet varied from those of *Moringa* oils species reported in other countries (Rahman, et al. 2009) and was also not so varied from those of other *Moringa* oil reported from India, Bangladesh, Saudi Arabia and Kenya (Lalas and Tsaknis 2002 and Manzoor, et al. 2007). On the other hand, the Egyptian *Moringa* oil had approximate amounts of C16:0 and C18:2 that reported in olive oil, and could not be compared with other conventional vegetable oils (Manzoor, et al. 2007). Thus, the levels of C16:0 and C18:2 were in good agrees with the determined iodine value (67 g I/100g) that was also low. High oleic acid in moringa oil makes it desirable in the term of nutrition and high stability cooking and frying oil (Abulkarim, et al. 2005). The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rate of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, *Moringa* oils with high proportion of oleic acid are more stable than the others. Also, oleic acid is less susceptible to oxidation than polyunsaturated fatty acid from the n-6 series (linoleic acid). Another interesting fact is that considerable content of linoleic acid (C18:2) as an essential fatty acid in the *Moringa* oil may be provide high nutritional remuneration and render beneficial healthy effect on blood lipid, blood pressure and cholesterol contents (Cheikh-Rouhou, et al. 2008) and it is preferred by industries when oil hydrogenation is required.

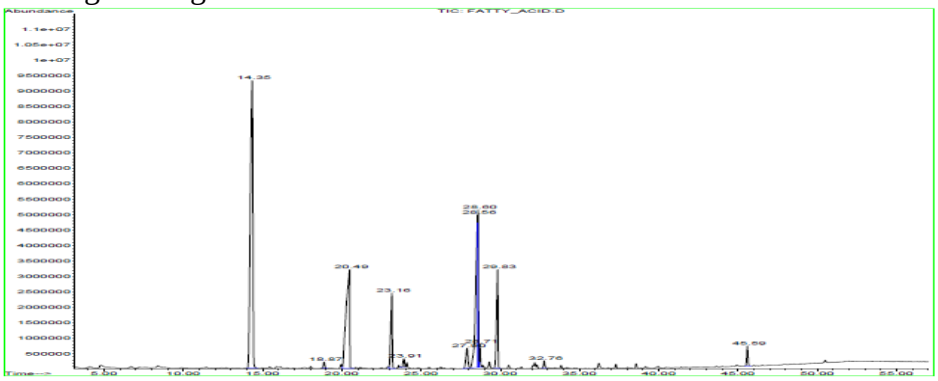


Figure (1): Fatty acid profile of *M. peregrina* oil

Total phenolic (TP) and Tocopherol (TOC) content of *M. peregrina* oils

Phenolic and tocopherols compounds have been proved to be responsible for antioxidant activity on many vegetable seeds oils, its is mainly due to their redox properties, which can play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Thus, determination of total TP and TCO contents represents a good estimative of antioxidant potential of food products. The TPC and TOC in the *M. peregrina* seed oil extract are presented in Table 4. The *Moringa* oil was found to be rich in TP and TOC contents, with values of 48.31 and 20.35 mg/ 100 g oil, respectively.

Table (4): Oil content of *Moranga peregrina* seed and total tecopherols and phenolic content of *Moranga peregrina* oil

Parameter	*Moranga peregrina oils*
Oil content (g oil /100 seed)	42.23 ± 1.14
Total tecopherol (mg/100g oil)	20.35± 0.84
Total phenolic (mg/100g oil)	48.31± 1.22

The data are mean ± SD for three separate experiments.

These values were well comparable to those reported in other *Moringa* species native to Kenya, Saudi Arabia, Bangladesh and Pakistan, and also was in close with that values reported in conventional edible oils (cottonseed, peanut and olive oils) (Manzoor, et al. 2007). Thus, *Moringa* oil is expecting to offer some auto-oxidation protection during storage and processing (Manzoor, et al. 2007). Many reports in the literature demonstrated a high oxidative stability of seed oils of different of *Moringa* species have been positive associated with levels antioxidant compounds includes TOC and TP (Lalas and Tsaknis, 2002).

Antioxidant activity

Antioxidant activity of oil obtained from Egyptian *M. pergrina* (MO) were assessed with scavenging of DPPH, ABTS, superoxide ($^{\cdot-}O_2$), $^{\cdot}OH$ radicals and reducing power techniques.

Radical.$^{\cdot-}O_2$ and $^{\cdot}OH$ scavenging activity

Superoxide anion and hydroxyl radicals are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage. Table 5 shown the scavenging activity of MO against superoxide anion ($^{\cdot-}O_2$) and $^{\cdot}OH$ radicals generated from non-enzymatic systems. The MO showed a comparable high scavenging activity against $^{\cdot-}O_2$ and $^{\cdot}OH$ radicals to that of standard antioxidants (BHT, BHA and TOC). However, these effects were dose-dependent, with IC_{50} values of 56.36 and 45.62 µg /ml, respectively.

Table (5): In vitro superoxide and OH radical Scavenging activity of *M. peregrina* oil,

BHA, BHT and α -Tocopherol

Assay	IC_{50} µg ml^{-1}				LSD at level (P< 0.01)
	BHA	BHT	α -Tocopherol	*M. peregrina* oil	
Superoxide (%scavenging $O^{\cdot-}_2$ radical)	8.64	16.38	23.34	56.36	1.23
OH$^{\cdot}$ (% Scavenging OH radical)	6.39	14.34	21.24	45.62	1.33

The target oxidative substances values represent the % of scavenging activity.
Each value represent the mean of three
(n=3)

ABTS and DPPH scavenging activity

As giving in Fig. 2 and Table (6), MO exhibited good scavenging abilities against DPPH and ABTS radicals with concentration dependent manner. The IC_{50} values were 25.65 and 95 µg/mL, respectively. Thus, the MO exhibited a high ABTS radical scavenging efficiency than the DPPH radicals, at the same concentration, which could be attributed to a different mechanism. In the DPPH assay, the scavenging action may be due to the hydrogen donating ability, whereas scavenging of ABTS radical is due to scavenging of proton radicals induced through donation of electrons (Chu, et al. 2010).

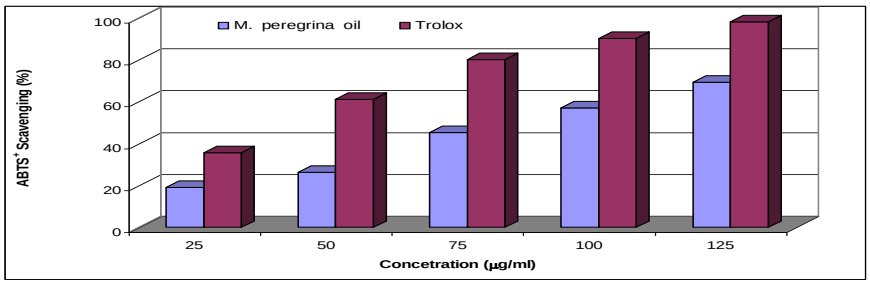


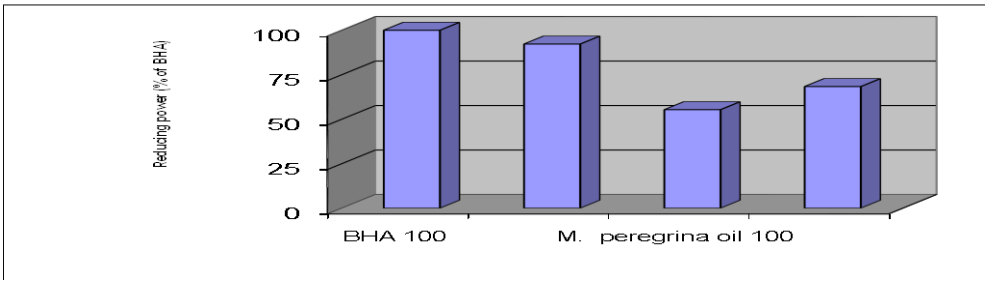
Figure. (2): ABTS^{+} radical scavenging effects of *M. peregrina* oil .

Table (6): Scavenging activity of *M. peregrina* oil on DPPH·

Treatments	Inhibition %[a]	IC_{50} µg/ml
M. peregrina oil	77.95± 1.51	25.65
BHA	93.00 ± 1.68	13.80
BHT	95.58 ± 1.87	16.00
α -Tocopherol	91.54 ± 1.95	18.30
LSD at level (P< 0.01)	1.52	1.11

a: Percentage of antioxidant inhibition was calculated from following equation:
%a = (A blank - A sample / A blank) x 100
where A blank = absorbance of methanolic DPPH.
A sample = absorbance of DPPH radical + samples.
$IC_{50:}$ concentration (µg/ml) for a 50% inhibition was calculated from the plot of inhibition (%) against M. peregrina oil concentration.
Tests were carried out in triplicate.

Reducing power (RP) The data in Figure (3) revealed that the RP activity was increased with increasing concentrations of Moringa oil, RP values were 0.1, 0.2 and 0.3 at concentration of 5, 20 and 50 µg/mL, respectively. Thus, the RP of MO was better than that of standard antioxidants (BHT= BHA). RP activity of MO may be attributed to its hydrogen-donating ability compared with that for both standard antioxidants. It is known that standard antioxidant BHT and BHA had a high RP values, due to ability donate a hydrogen atom to free radical, that terminating free radical reaction (Shahidi and Wanasundara, 1992).



General, a relationship was existed between concentration of MSO and its reducing power and scavenging activities against DPPH, ABTS, ·OH and $^{\cdot-}O_2$ radicals. The previous reports demonstrated that there was a positive correlation between antioxidant property of MSO in various oxidative systems and content of antioxidant compounds include: TOC and TPC compounds (Simirgiotis, et al. 2009). Manzoor, et al. (2007) reports that high concentration of tocopherols as the major lipid-soluble constitutes in different species of moringa displayed greater antioxidant activity compared with commercial antioxidant (BHT and BHA). It is well known that antioxidants are classified into two broad divisions, depending on whether they are soluble in lipids (hydrophobic) or in water (hydrophilic), lipid-soluble antioxidants protect cell membranes from lipid peroxidation. Finally, *Moringa* oil may play important roles in protecting of the body with antioxidants from oxidative damage induced by free radicals and reactive oxygen species by different mechanisms as follows: (i) suppressing their formation and (ii) acting as scavengers.

Antiproliferation activity

Antiproliferation activities (APF) of *M.* peregrine oil in the term of cell death of three human cancer cells MCF-7, Hep-G2, and HCT-116 are shown in Table 7and Fig.4. The results revealed that *M. pregrina* oil displayed a good APF effect on human cancer cells examined, with IC_{50} values of 2.92, 9.42 and 9.48 µg/ml, respectively. Among the 3 cell lines tested for cytotoxicity of *Moringa* oil, MCF7 was the most sensitive cell line followed by HeP-G2 and HCT-116. The anti-proliferation activity of *Moringa* oil (MO) could be attributed to cytotoxic effect of nature of saturated and unsaturated fatty acids contained in MO, which are shown to be very effective anti-proliferative action toward cancer cells could be

through apoptotic process (Singh, et al., 1996). However, the APF action of fatty acids could be related to their ability to increase free radical generation and lipid peroxidation process in cancer cells. Fernandes, et al. (1996) reported that free radicals and lipid peroxides can induce damage to a variety of enzymes, proteins and DNA as well as deplete ATP levels in cancer cells and induced apoptotic process.

Table (7): Antiproliferation activity of *M. peregrina* oil against three models human cancer cell lines breast MCF-7, HepG2, HCT-116 and Paclitaxel as anticancer drug

Human cancer cell lines	IC50 µg/ml	
	M. peregrina oil	Paclitaxel
MCF-7	2.92	0.45
HepG2)	9.40	0.52
HCT-116	9.48	0.35

The characterization of the oil from *Moringa* trees exhibited that the oil had fatty acids profile (high C18:1) and contain high considerable amount of tocopherols and phenolic compounds similar to that found in olive oil and could be utilized in diets as a source of vegetable oil for human consumption. However, despite the fact that

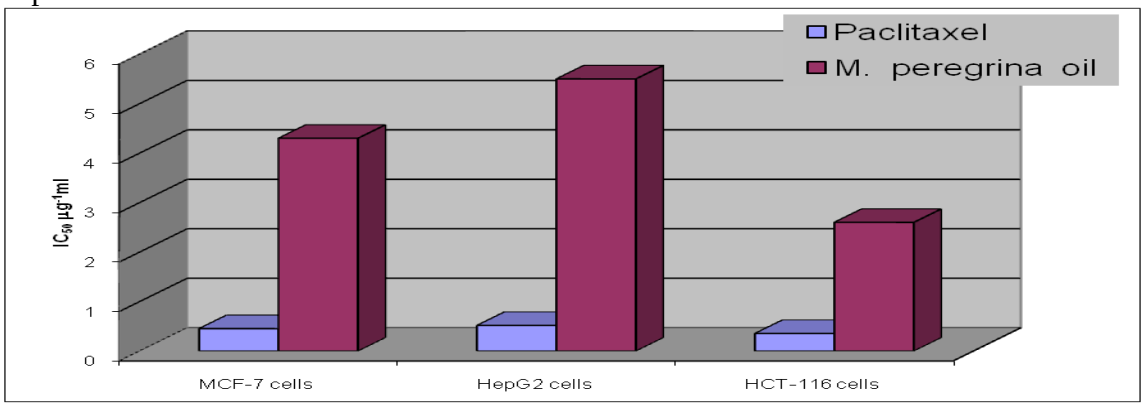
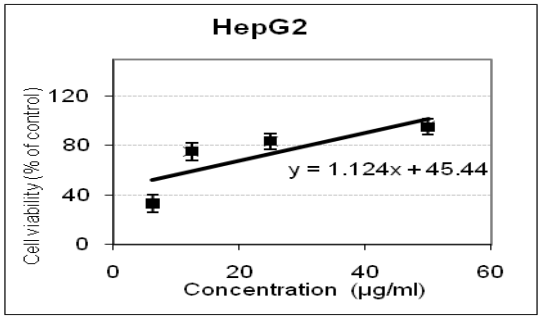
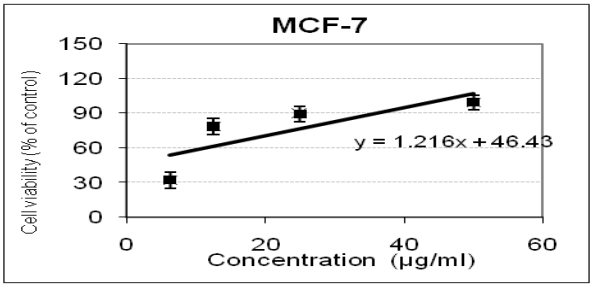
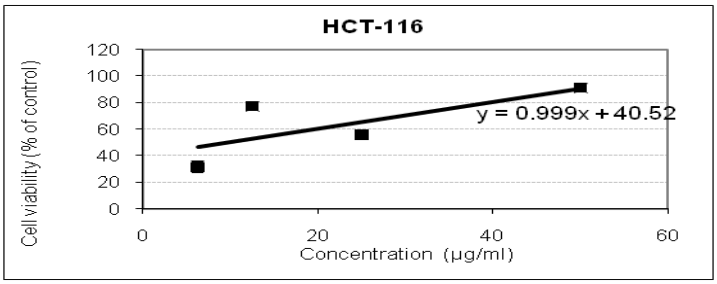


Fig.(4): Growth inhibitory effect (IC_{50} µγ/ml) of M. peregrina oil on three models human cancer cell lines breast Adencarcinoma cells (MCF-7), hepatocellular carcinoma cells (HepG2), and colon carcinoma (HCT-116) and Paclitaxel as anti-cancer drug







Egypt is an agrarian country it is not yet capable of producing oils for its domestic needs, which exported about 90% of the total demand. Therefore, the results revealed that *M. peregrina* contain significant amounts of high oleic oils, natural antioxidant and antiproliferation compounds could emerge as valuable crop, which could be cultivated in poor soil quality throughout the Egyptian deserts (about 95% of total area) for production of potentially yield useful oil for human consumption.

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