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Natural antioxidants of the Jaffna variety of *Moringa Oleifera* seed oil of Indian origin as compared to other vegetable oils

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RESUMEN

Antioxidantes naturales de aceites de semillas de *Moringa oleifera* variedad Jaffna de origen indio en comparación con otros aceites vegetales

Las cosechas de *Moringa oleifera* se cultivan en toda la India por sus nutritivas vainas, sus hojas y sus semillas. Las semillas de *M. oleifera* están dotadas de un buen contenido de aceite (~39%) y se asemejan al aceite de oliva debido a su alto contenido de ácido oleico (~65-75%). El objetivo del estudio fue evaluar los antioxidantes naturales de aceites crudos de semillas de *M. oleifera* variedad de Jaffna (MSO). Los antioxidantes naturales como tocoferoles y fenoles así como esteroles, carotenoides presentes en MSO y su actividad de captación de radicales se midió en comparación con los de aceite crudos de coco desecado (copra) (CNO), de sésamo (SESO), de semilla de niger (NSO), de palma (CPO), de salvado de arroz (RBO) y de cacahuete refinado comercial (GNO). MSO contenía 88 ppm de tocoferoles totales, siendo α-tocoferol el mayoritario (56,2 ppm). MSO también contenía 117,9 ppm de fenoles totales siendo el ácido gálico (48,5 ppm) el mayoritario. MSO contenía también cantidades importantes de esteroles (1700,8 ppm) y carotenoides (17,9 ppm). MSO mostró un valor de CI50 de 35,5 mg mL^{-1}. El estudio indica que MSO tiene características antioxidantes mejores que CNO y comparable con las de GNO.

PALABRAS CLAVE: Aceites de semillas de la India – Actividad captadora de radicales – Carotenoides totales – Esteroles totales – Fenoles – IC50 valor – Moringa oleifera variedad Jaffna – Tocoferoles.

SUMMARY

Natural antioxidants of the Jaffna variety of *Moringa oleifera* seed oil of Indian origin as compared to other vegetable oils

The *Moringa oleifera* crop is grown all over India for its nutritious pods, leaves and seeds. *M. oleifera* seeds are endowed with a high oil content (~39%) which resembles olive oil because of its high oleic acid content (~65-75%). The objective of the present study was to evaluate the natural antioxidants of crude seed kernel oil from the Jaffna variety of *M. oleifera* (MSO). Natural antioxidants like tocopherols and phenolics as well as minor compounds such as sterols and carotenoids present in MSO and its radical scavenging activity were measured in comparison to dried coconut kernel (copra) crude oil (CNO), crude sesame oil (SESO), crude niger seed oil (NSO), crude palm oil (CPO), crude rice bran oil (RBO) and commercially refined groundnut oil (GNO). MSO contained 88 ppm of total tocopherols with α-tocopherol (56.2 ppm) being the major tocopherol. MSO also contained 117.9 ppm of total phenolics with gallic acid (48.5 ppm) being the major phenolic. MSO contained fair amounts of sterols (1700.8 ppm) and carotenoids (17.9 ppm). MSO showed an IC_{50} value of 35.5 mg mL^{-1}. This study indicates that MSO has antioxidant characteristics which are better than CNO and comparable to those of GNO.

KEY-WORDS: IC_{50} value – Indian seed oil – Moringa oleifera Jaffna variety – Phenolics – Radical scavenging activity – Tocopherols – Total carotenoids – Total sterols.

1. INTRODUCTION

Moringa oleifera is a pan-tropical species and the most widely cultivated species of a monogeneric family, Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. The *M. oleifera* tree is also known as the horseradish tree, drumstick tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree (Sengupta and Gupta, 1970; Ramachandran *et al*., 1980). *M. oleifera* is an important vegetable crop and minor oil seed crop in India, however, since there is no organized cultivation of moringa crops, its production quantities are not documented. Other countries which cultivate and use moringa crops are Pakistan, Bangladesh, Malaysia, Indonesia, Ethiopia, Philippines, Sudan, West, East and South Africa, tropical Asia, Latin America, the Caribbean, Florida and the Pacific Islands (Sengupta and Gupta, 1970; Ramachandran *et al.,* 1980). The common varieties available in India like Jaffna, Chauakacheri Murunga, Palmurungai and Periyakulam 1 are all used as vegetables. The pods, leaves and seeds of these moringa varieties are used for various culinary preparations.

Moringa seeds are endowed with high oil contents (35-40%) and are rich source of omega-9 fatty acids i.e. oleic acid; however, they are underutilized as an oil source (Anwar *et al*., 2007; Fahey, 2005). There are some reports on the composition and characteristics of *M. oleifera* seed oil varieties from different countries of origin, e.g., India (Lalas and Tsaknis, 2002; Ogunsina *et*

al., 2011), Kenya (Tsaknis *et al*., 1999), Malaysia (Abdulkarim *et al*., 2005), Pakistan (Anwar *et al.,* 2006; Manzoor *et al*., 2007; Latif and Anwar, 2008), and Bangladesh (Rahman *et al*., 2009) which examine its prospect as an alternative vegetable oil source.

Earlier, in our laboratory, we carried out the work on the quality and stability characteristics of crude seed oil from the Indian *M. oleifera* Jaffna variety (Ogunsina *et al.,* 2011). We have reported the physico-chemical characteristics of cold pressed and hexane extracted crude seed oil from the Indian *M. oleifera* Jaffna variety such as color in 1’ cell (36.0 Lovibond units), refractive index (1.47), density at 25 °C (0.92 g mL^{-1}), specific gravity (0.90), viscosity (43.6 mPa.s), iodine value (68.5 g I_2 100 g^{-1}), saponification value (191.2 mg KOH g^{-1}), unsaponifiable matter (0.65 g 100 g^{-1}) and total tocopherols (90.2 ppm). We also reported its fatty acid composition which showed oleic acid to the extent of 79% and polyunsaturated fatty acids (PUFA) to the extent of 2%. However, apart from physico-chemical characteristics, the quality of oil also depends on its inherent natural antioxidants such as tocopherols, phenolics, sterols and carotenoids which were not reported in the earlier study (Ogunsina *et al*., 2011). Hence, in continuation with the previous work, the inherent natural antioxidants of crude seed oil from the Indian *M. oleifera* Jaffna variety are quantified and its radical scavenging ability is reported in this study. Tocopherols, phenolics, sterols and carotenoids are inherent natural antioxidants and bioactive molecules of oil which help in preventing free radical damage to the tissues apart from performing various physiological functions in the body. The natural antioxidants of crude seed oil from the Indian *M. oleifera* Jaffna variety and its radical scavenging activity have not been reported so far. The objective of this study was to evaluate the crude seed oil from *M. oleifera* Jaffna variety for its natural antioxidants and radical scavenging activity in comparison to other common vegetable oils used in India like coconut oil, palm oil, rice bran oil, niger seed oil, sesame oil and groundnut oil.

2. MATERIALS AND METHODS

2.1. Materials

Dry seeds of the *M. oleifera* Jaffna variety (5 kg) were purchased from the local market of Mysore, Karnataka, India. After dehulling, seed coats (1.73 kg) and kernels (3.27 kg) were obtained. The kernels and seed coats comprised 65.4% and 34.6%, respectively, of the dry seeds of the *M. oleifera* Jaffna variety. Dried coconut kernels (copra), sesame seeds, niger seeds and commercially refined groundnut oil –Goldwinner brand (GNO) were purchased from a local supermarket in Mysore. Rice bran was purchased from a local rice mill in Mysore. Crude palm oil was also purchased from a local palm oil industry in Mysore. Standard α-tocopherol, γ-tocopherol, δ-tocopherol, β-carotene, cholesterol, gallic acid, caffeic acid, ferulic acid, vanillic acid, cinnamic acid, vanillin, sesamol, sesamin and 2,2-diphenyl-1-picryl hydrazyl free radicals (DPPH) were obtained from the Sigma Chemical Co., St. Louis, USA. Tert-Butyl hydro quinone (TBHQ) was obtained from Loba Chemie, Mumbai, India. The other chemicals and reagents used for analysis were of analytical reagent grade.

2.2. Oil extraction

The oil extraction from ground moringa kernels was performed according to the Soxhlet extraction method with commercial hexane as the extraction solvent at a ratio of 1:10. Briefly, moringa kernels (1 kg) were ground into a fine powder and packed in cellulose extraction thimbles (Whatman). The thimble was placed in the extractor and warm hexane (35-40 °C) was percolated continuously for 8 h for complete oil extraction. After 8 h of extraction, miscella was desolventized by flash evaporation and the oil content was determined gravimetrically and expressed as g kg^{-1}. Similarly, dried coconut kernels (copra), sesame seeds, niger seeds and rice bran, 100 g each, were ground and packed in cellulose extraction thimbles and the oil was extracted using the Soxhlet extraction method with commercial hexane as the extraction solvent with a material to solvent ratio of 1:10.

2.3. Unsaponifiable matter of oils

Unsaponifiable matter of the oil samples was determined according to the AOCS Method No. Ca 6a-40 (Firestone, 1998).

2.4. Tocopherol determination

2.4.1. Total tocopherol determination by spectrophotometry

The total tocopherol content of the oils was determined according to the IUPAC Method No. 2.301 (Paquot and Havtfenne, 1987).

2.4.2. Tocopherol analysis by HPLC

The tocopherol content of the oil was determined according to the AOCS Method No: Ce 8-89 (Firestone, 1998) by normal phase HPLC separation on a silica column (Lichrosorb Si60 pore size 6 nm, pore volume 0.75 mL/g, particle size 7 µm, LiChrosorb®, Merck Millipore, Merck KGaA, Frankfurter, Darmstadt, Germany) employing Shimadzu HPLC system consisting of an LC-10A pump, injector fitted with 20 µL loop and flourescence detector. The mobile phase was hexane: isopropyl alcohol (99.5:0.5, v/v) at the flow rate of 1 mL min^{-1}. An excitation wave length

of 290 nm and emission wavelength of 330 nm were maintained for the fluorescence detection of all the peaks. Standard α, γ and δ- tocopherols were used as external and internal standards for the identification and quantitation of tocopherols in the samples by comparing the peak retention times and peak areas of the standard compounds with those of the samples. The tocopherol content in the oil was expressed as ppm.

2.5. Phenolic compounds

2.5.1. Phenolic compounds extraction

The phenolic compounds were extracted from the oil samples using liquid–liquid extraction according to Taga *et al*., 1984 with minor modifications. Twenty grams of oil were dissolved in 50 mL hexane and extracted three times with 30 mL of methanol/water (80:20, v/v). The extracts were pooled and the volume made up to 100 ml with methanol/water (80:20, v/v) and then used for spectrophotometric and HPLC analyses.

2.5.2. Total phenolic compound analysis by spectrophotometry

The concentration of total phenols in the methanolic extract of the oils was estimated with the Folin-Ciocalteau reagent. A sample aliquot of 100 μL was added to 900 μL of distilled water and 0.5 mL of Folin- Ciocalteau reagent were added, mixed and kept for 3 min. After 3 min, 1 mL of 15% Na_2CO_3 solution was added; the contents were mixed and the volume made up to 10 ml with distilled water. After 45 min of incubation at room temperature, the absorbance was measured at 750 nm against the reagent blank. Gallic acid was used as the standard for preparing the calibration curve (0.05-0.4 mg mL^{-1}). The sample absorbance was interpolated on the standard graph and the total phenolic compounds (such as gallic acid) were calculated and expressed as ppm.

2.5.3. Phenolic compounds analysis by HPLC

HPLC analysis of the phenolic compounds in oil samples was done according to Siger *et al*., 2008. Individual phenolic compounds were separated on a RP-C18 column (250 mm × 4.5 mm) connected to a high-performance liquid chromatograph (LC 10 A, Shimadzu, Kyoto, Japan) fitted with a 20 μL injector loop and UV detector. The gradient mobile phase consisted of solvent A – methanol and solvent B – 2.5% acetic acid in water (v/v) with the flow rate at 1 mL min^{-1}. The dried samples were diluted with methanol/water (60:40, v/v) to a concentration of 1 mg mL^{-1} and 20 μL were injected into the injector loop. The phenolic compounds in the samples and the standards were detected at 280 nm (for hydroxybenzoic acid derivatives i.e. gallic acid, vanillic acid, vanillin) and 320 nm (for hydroxycinnamic acid derivatives i.e. caffeic, cinnamic, ferulic acids) at 25 °C. The identification was carried out by comparing the peak retention times of oil samples with those of standard compounds. The amount of individual phenolic compounds in the oils was determined using external and internal standards of the individual phenolic compounds and expressed as ppm.

2.6. Total carotenoid determination by spectrophotometry

The total carotenoid contents of the oils were determined according to the method of Dauqan *et al*., 2011 with minor modifications. Oil samples (100 mg) were weighed and diluted with 10 mL acetone, mixed well by vortexing and read at 446 nm (UV-1601, UV visible spectrophotometer, Shimadzu Corporation, Koyoto, Japan) against a blank of pure acetone. Standard β-carotene in acetone (1 mg mL^{-1}) solution was prepared and its five aliquots (0.5-2.5 mL) were read at 446 nm and a standard β-carotene curve was generated by plotting the absorbance of β-carotene against the amount of β-carotene (μg). The sample absorbance was interpolated on the standard graph and the total carotenoid content (as β-carotene) were calculated and expressed as ppm.

2.7. Total sterol determination by spectrophotometry

The total sterol contents of the oils were determined following the Liberman-Burchard method according to Sabir *et al.* 2003. The oil samples (1 g) were weighed and diluted with 10 mL chloroform and mixed well by vortexing. Aliquots of 3 mL were taken and 2 mL of the Liberman-Burchard reagent (0.5 mL of sulphuric acid dissolved in 10 mL of acetic anhydride) were added to them and the final volume was made up to 7 mL with chloroform. The Liberman-Burchard reagent reacted with the sterol to produce a characteristic green color whose absorbance was read at 640 nm. A standard cholesterol solution (1 mg mL^{-1}) was prepared and its five aliquots of 0.5-2.5 mL were taken. 2 mL of Liberman-Burchard reagent were then added and the final volume was made up to 7 mL with chloroform. This mixture was incubated in the dark for 15 minutes and read at 640 nm. A standard cholesterol curve was generated by plotting the absorbance of cholesterol against the amount of cholesterol (μg). The sample absorbance was interpolated on the standard graph and the total sterol contents (as cholesterol) was calculated and expressed as ppm.

2.8. Oryzanol determination in rice bran oil

The oryzanol content in extracted crude rice bran oil was determined using the spectrophotometric method (Gopala Krishna *et al.,* 2001) by dissolving

0.01 g of the sample in 10 ml of hexane and reading the absorbance at 314 nm in a 1cm cell (double beam uv-visible recording spectrophotometer model UV-1601, Shimadzu corporation, Kyoto, Japan). Oryzanol content (mg 100 g^{-1}) was calculated using the formula: [(A / W) × (100 / 358.9)]; Where, A = absorbance of the sample, W = weight of the sample in gram per 100 mL, 358.9 = $E^{1\%}_{1cm}$ for oryzanol.

2.9. Lignans determination in sesame oil

The lignan content in extracted crude sesame oil was determined according to Kamal Eldin and Appelqvist, 1994. The analysis of lignans was performed with HPLC (model LC-10A VP Shimadzu corporation, Kyoto, Japan) equipped with uv-detector set at 290 nm and a C18 phenomenex column (250 mm length × 4.6 mm i.d.) using 70% methanol as the mobile phase at a flow rate of 1 mL min^{-1}. Standard sesamol and sesamin were used for the quantification of lignans in the sesame oil samples by comparing the retention times and peak area of the standard compounds with those of the oil samples. The total lignan content of sesame oil was also determined according to the spectrophotometry method as per the published procedure of Bhatnagar *et al*., 2013.

2.10. Radical Scavenging Activity (RSA) toward DPPH Radicals in oils and determination of IC_{50} value

RSA and the presence of hydrogen donors in the oils were examined by the reduction of DPPH radicals in toluene. A toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M according to Bhatnagar *et al*., 2009. The oil samples (50 ± 1 mg) were placed in test tubes and a 4-mL aliquot of DPPH toluenic solution was added and vortexed for 20s at ambient temperature. Against a blank of pure toluene without DPPH radicals, the decrease in the absorption at 515 nm was measured in a 1-cm quartz cell using a UV-visible spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto, Japan) after 1, 30 and 60 min of mixing. The RSA toward DPPH radicals was estimated from the differences in the absorbance of the toluenic DPPH solution with or without the sample (control). The inhibition percent was calculated using the following equation: Percentage Inhibition = [absorbance of control-absorbance of test sample)/absorbance of control] X 100. The IC_{50} value of the oils, expressed as mg mL^{-1}, denotes the concentration of oil in the DPPH toluenic solution required to cause a 50% inhibition of DPPH radicals. 5-50 mg of oil samples were taken and a 1-mL aliquot of DPPH toluenic solution was added. The reaction mixture was incubated in the dark for 15 minutes and the absorbance was measured at 515 nm in a 1-cm quartz cell. A graph was plotted for percent DPPH remaining against the concentration of oil in the DPPH toluenic solution (mg mL^{-1}) and the IC_{50} value was determined from the interpolation of values on the graph.

2.11. Statistical analysis

All oil extractions were performed in duplicate and estimations carried out in triplicate making six determinations (n = 6) and the mean ± standard deviation values are reported. The data were analyzed using the statistical program, GraphPad InStat Demo- [DATASET1.ISD] (Graphpad InStat, 2012). The two-tailed *p* value was determined to show the significant differences. A significant difference was considered only when the *p* value ≤ 0.05.

3. RESULTS AND DISCUSSION

3.1. Oil content, unsaponifiable matter and natural antioxidants

The oil content of *M. oleifera* Jaffna variety seed kernels was found to be 392.2 g kg^{-1} (Table 1). This agreed well with our previous literature report of 394 g kg^{-1} by Ogunsina *et al*., (2011) and 383 g kg^{-1} by Lalas and Tsaknis (2002) on Indian *M. Oleifera* varieties Jaffna and Periyakulam-1, respectively. The oil contents of dried coconut kernels (copra), rice bran, niger seeds and sesame seeds were found to be in the range of 177.2-647.4 g kg^{-1} (Table 1). The results indicate that copra is the richest source of oil followed by sesame, niger, moringa and rice bran. In comparison to *M. Oleifera*, copra and sesame were found to be 1.6 X and 1.4 X richer while niger seed was almost equal in oil content. However, *M. Oleifera* was richer in oil content than rice bran by 2.2 X. The refining of oil removes its inherent natural antioxidants thus bringing down the unsaponifiable matter of the oil to a large extent. *M. Oleifera* Jaffna variety seed kernel oil (MSO) studied in the present work was of crude (unrefined) nature; hence it has been compared with other crude (unrefined) vegetable oils. The unsaponifiable matter of MSO was found to be 6.5 g kg^{-1} (Table 1). The unsaponifiable matter of CNO, CPO, RBO, NSO and SESO was found to be in the range of 1.9-57.4 g kg^{-1} (Table 1). The results suggest that RBO contains the highest amount of unsaponifiable matter followed by SESO, NSO, MSO, CPO and CNO. The natural antioxidants of oil such as sterols, tocopherols, phenolics and carotenoids are present as the unsaponfiable matter of oil (Christie, 2012) hence oil with more abundant amounts of unsaponifiable matter would have higher quantities of these natural antioxidants. The total sterol contents of the oils were found to be in the range of 811.7-9787.5 ppm in the order of decreasing prevalence of RBO > SESO > MSO > NSO > CNO > CPO with RBO containing the highest and CPO the lowest amount of sterols while MSO had 1700.8 ppm of sterols (Table 1). The total tocopherol contents of the oils were found to be in the range of 32.5-

Table 1
Oil, unsaponifiable matter and natural antioxidant contents of different oil crops

Parameters	*M. Oleifera* Jaffna variety seed kernel	Dried Coconut kernel (copra)	Palm	Rice bran	Niger seeds	Sesame seeds
O C[1]	392.2 ± 1.9[a]	647.4 ± 3.5[b]	ND	177.2 ± 2.0[c]	392.6 ± 1.6[a]	541.5 ± 2.2[d]
USM[2]	6.5 ± 0.8[a]	1.9 ± 0.2[b]	5.7 ± 0.9[a]	57.4 ± 3.0[c]	13.3 ± 1.5[d]	18.4 ± 1.0[e]
T T[3]	88.0 ± 3.0[a]	32.5 ± 0.5[b]	796.8 ± 14.2[c]	1350.6 ± 20.5[d]	184.6 ± 4.7[e]	562.5 ± 7.3[f]
T S[4]	1700.8 ± 15.5[a]	896.4 ± 12.5[b]	811.7 ± 22.8[c]	9787.5 ± 52.6[d]	1351.4 ± 6.0[e]	7909.6 ± 11.4[f]
T P[5]	118.9 ± 3.9[a]	56.2 ± 4.5[b]	76.7 ± 2.8[c]	#309.3 ± 5.2[d]	264.8 ± 6.0[e]	$278.9 ± 4.3[f]
T C[6]	16.9 ± 0.4[a]	5.5 ± 0.5[b]	497.8 ± 3.5[c]	211.8 ± 4.1[d]	26.85 ± 1.4[e]	90.3 ± 5.2[f]
IC_{50}[7]	35.5 ± 0.3[a]	57.5 ± 2.2[b]	30.4 ± 1.8[c]	20.5 ± 1.1[d]	32.2 ± 0.9[e]	28.4 ± 0.6[f]

ND-not determined
[1] O C, Oil content (g kg^{-1}); [2] USM, Unsaponifiable matter of the oil (g kg-[1]); [3] T T, Total tocopherols in the oil (ppm); [4] T S, Total sterols in the oil (ppm); [5] T P, Total phenolics in the oil (ppm); [6] T C, Total carotenoids in the oil (ppm); [7] IC_{50}, IC_{50} value of the oil (mg mL^{-1})
Values are means ± SD (n = 6).
Values given in rows followed by different alphabetic superscripts are significantly different at $p \leq 0.05$.
Crude rice bran oil contained 19000 ± 150 ppm of oryzanol.
$ Crude sesame oil contained 10662 ± 80 ppm of lignans.

1350.6 ppm in the order of decreasing prevalence of RBO > CPO > SESO NSO > MSO > CNO with RBO containing the highest and CNO the lowest amount of tocopherols while MSO had 88.0 ppm of tocopherols (Table 1). The total phenolic contents of the oils were found to be in the range of 56.0-278.9 ppm in the order of decreasing prevalence of RBO > SESO > NSO > MSO > CPO > CNO with RBO containing the highest and CNO the lowest amount of phenolics while MSO had 118.9 ppm of phenolics (Table 1). The total carotenoid contents of the oils were found to be in the range of 5.5-497.8 ppm in the order of decreasing prevalence of CPO > RBO > SESO > NSO > MSO > CNO with CPO containing the highest and CNO the lowest amount of carotenoids while MSO had 16.9 ppm of carotenids (Table 1). Apart from tocopherols, sterols, phenolics and carotenoids, RBO and SESO also contained oryzanol (19000 ppm) and lignans (10200 ppm) respectively, both of which have strong antioxidant and bioactive functions (Gopala Krishna *et al.,* 2001; Namiki, 2007). The presence of natural antioxidants plays a vital role in free radical inhibition. The IC_{50} values of the oils were found to be in the range of 20.5-57.5 mg mL^{-1} in the order of increasing prevalence of RBO < SESO < CPO < NSO < MSO < CNO with RBO exhibiting lowest and CNO the highest IC_{50} value while MSO showed an IC_{50} value of 35.5 mg mL^{-1} (Table 1). The strong IC_{50} value of RBO and SESO could also be attributed to the radical scavenging activity of oryzanol and lignans present in them (Bhatnagar *et al.*, 2009).

3.2. Tocopherols, sterols and carotenoid contents of MSO and GNO

Tocopherols are benzopyranols or methylated tocols consisting of 20-carbon phytyl tail (including the pyranol ring) attached to the benzene ring. Tocopherols are important minor constituents of vegetable oils which serve as antioxidants to retard oxidative rancidity and as a source of the essential nutrient vitamin-E. They are natural lipid soluble antioxidants and potent free radical scavengers present in the oil (Christie, 2012). The main biochemical function of tocopherols is believed to be the protection of consumed polyunsaturated fatty acids (PUFA) against peroxidation (Kamal-Eldin and Andersson, 1997). MSO contains no linoleic acid and only ~2% linolenic acid (Ogunsina *et al*., 2011). MSO contained 88 ppm of the total tocopherols. As shown in Table 2, α-tocopherol (56.2 ppm) was found to be the major tocopherol in MSO, while γ-tocopherol (12.6 ppm) and δ-tocopherol (19.2 ppm) were also present. These results agreed well with our previous literature report of 90.2 ppm by Ogunsina *et al.* (2011) on crude seed oil from the Indian *M. oleifera* Jaffna variety and 82.6 ppm by Tsaknis *et al.* (1999) on crude seed oil from the Kenyan *M. oleifera* Mbololo variety. The PUFA content of MSO is only ~2% which means that MSO contains a surplus of tocopherols for quenching free radicals and performing its other antioxidant functions in the human body. GNO contained significantly higher amounts ($p \leq 0.05$) of total tocopherols (102 ppm) with γ-tocopherol (70.5 ppm) being the major tocopherol followed by α-tocopherol (26.2 ppm) and δ-tocopherol (5.3 ppm) (Table 2). Sterols or steroid alcohols are a class of organic compounds that contain a common steroid nucleus plus an 8 to 10 carbon side chain and an alcohol group. Phytosterols are known to be effective in lowering low-density lipoprotein-cholesterol levels in humans (Christie, 2012). Sterols are generally present in higher amounts in crude oils than refined oils, as the deodorization step during the refining process removes most of the native sterols present in the oil. MSO, being of unrefined nature, contained significantly higher amounts ($p \leq 0.05$) of total

Table 2
Tocopherol, Phenolic, carotenoid, and sterol contents of MSO and GNO

Parameters	Indian *M. oleifera* Jaffna variety seed oil (MSO)	Commercially refined groundnut oil (GNO)	Indian *M. oleifera* Periyakulam 1 variety seed oil (Lalas and Tsaknis, 2002)	Kenya *M. oleifera* Mbololo variety seed oil (Tsaknis *et al.,* 1999)
α – T[1]	56.2 ± 1.6[a]	26.2 ± 1.0[b]	15.4	42.6
γ – T[2]	12.6 ± 0.8[a]	70.5 ± 1.2[b]	4.5	15.8
δ – T[3]	19.2 ± 0.6[a]	5.3 ± 0.3[b]	15.5	24.2
T T[4]	88.0 ± 3.0[a]	102.0 ± 2.5[b]	35.4	82.6
G A[5]	48.5 ± 1.2[a]	20.5 ± 1.5[b]	ND	ND
C A[6]	15.6 ± 0.7[a]	3.6 ± 0.2[b]	ND	ND
F A[7]	13.1 ± 0.5[a]	2.1 ± 0.3[b]	ND	ND
V A[8]	12.4 ± 0.3[a]	1.4 ± 0.2[b]	ND	ND
Cn A[9]	16.8 ± 0.8[a]	3.8 ± 0.4[b]	ND	ND
Vn[10]	11.5 ± 0.4	Nd	ND	ND
T P[11]	118.9 ± 3.9[a]	31.4 ± 2.6[b]	ND	ND
T C[12]	16.9 ± 0.4[a]	8.2 ± 0.2[b]	ND	ND
T S[13]	1700.8 ± 15.5[a]	1153.1 ± 9.4[b]	5600	ND

ND-not determined
Nd-not detected
[1] α – T, alpha tocopherol (ppm); [2] γ – T, gamma tocopherol (ppm); [3] δ – T, delta tocopherol (ppm); [4] T T, Total tocopherols (ppm);
[5] G A, Gallic acid (ppm); [6] C A, Caffeic acid (ppm); [7] F A, Ferulic acid (ppm); [8] V A, Vanillic acid (ppm); [9] Cn A, Cinnamic acid (ppm);
[10] Vn, Vanillin (ppm); [11] T P, Total phenolics (ppm); [12] T C, Total carotenoids (ppm); [13] T S, Total sterols (ppm)
Values are means ± SD (n = 6).
Values given in rows followed by different alphabetic superscripts are significantly different at $p \leq 0.05$.

sterols (1700.8 ppm) as compared to GNO which contained 1153.1 ppm of total sterols (Table 2). The total sterol content of MSO was found to be less than the total sterol content (5600 ppm) of crude seed oil from *M. oleifera* Periyakulam-1 variety as reported by Lalas and Tsaknis, 2002 (Table 2). Carotenoids are a class of hydrocarbons consisting of eight isoprenoid units. β-carotene, lutein, lycopene, zeaxanthin are some of the carotenoids present in vegetable oils (Christie, 2012). Carotenoids also contribute to the color of the oil and are generally present in higher amounts in crude oils than their refined counterparts, as the bleaching step during the refining process destructs major amounts of endogenous carotenoids present in the oil. MSO, being of unrefined nature, contained significantly higher amounts ($p \leq 0.05$) of total carotenoids (17.9 ppm) as compared to GNO which contained 10.2 ppm of total carotenoids (Table 2). The low carotenoid and sterol contents of GNO might be due to its refined form because refining includes bleaching and deodorization procedures which remove carotenoids and sterols respectively. Cold-pressed oils have better nutritive properties than solvent extracted and refined oils (Siger *et al.,* 2008). Cold pressed MSO, being clear oil with very low amounts of waxes and phospholipids (Ogunsina *et al.,* 2011) may be consumed in unrefined (virgin) form provided the oil is extracted from the seed kernels by cold pressing followed by filtration. This may also improve the bio-availability of natural antioxidants present in MSO to the human body upon consumption.

3.3. Phenolic contents of MSO and GNO

Phenolics are a class of chemical compounds consisting of one or more hydroxyl functional groups (-OH) attached to an aromatic hydrocarbon ring (Christie, 2012). Phenolics are also natural lipid soluble antioxidants and potent free radical scavengers present in the oil. Phenolic compounds have great influence on the stability, sensory and nutritional characteristics of the oil and may prevent deterioration through the quenching of free radicals responsible for lipid oxidation. Olive oil is believed to be highly stable because of its large quantity of phenolics (Siger *et al.,* 2008). MSO contained 117.9 ppm of total phenolics with gallic acid (48.5 ppm) being the major phenolic, while caffeic acid (15.6 ppm), ferulic acid (13.1 ppm), vanillic acid (12.4 ppm), cinnamic acid (16.8 ppm), vanillin (11.5 ppm) were also present (Table 2). The present work is probably the first report on the qualitative and quantitative determination of the phenolics of crude seed oil from the Indian *M. oleifera* Jaffna variety. GNO showed significantly lower amounts ($p \leq 0.05$) of total phenolics (31.4 ppm) as compared to MSO. Gallic acid (20.5 ppm) was

found to be the major phenolic in GNO, while caffeic acid (3.6 ppm), ferulic acid (2.1 ppm), vanillic acid (1.4 ppm), cinnamic acid (3.8 ppm) were also present (Table 2). The low phenolic contents of GNO might be due to its refined form as refining includes alkali neutralization and water washing of the oil which removes phenolic acid compounds. As mentioned earlier, the consumption of cold pressed MSO may improve the bio-availability of its endogenous phenolics to the human body.

3.4. Radical scavenging activity of oils

Free radicals may cause reversible or irreversible damages to biological molecules such as DNA, proteins and/or lipids. These damages may cause cancer, heart diseases and arthritis, and could accelerate the aging of organisms (Siger *et al.,* 2008). Figure 1 shows the DPPH radical scavenging activity of MSO, GNO and GNO + 200 ppm TBHQ. At the first minute of of the incubation of MSO, GNO and GNO + 200 ppm, TBHQ showed 28, 19 and 63% inhibition respectively, which steadily increased with time and eventually showed 92, 84 and 99% inhibition, respectively after sixty minutes of incubation. MSO, being richer in bioactives like tocopherols, phenolics, sterols and carotenoids than GNO, showed significantly higher ($p \leq 0.05$) percent inhibition of DPPH radicals from the beginning to end of incubation. Since MSO is vastly rich in monounsaturated oleic acid (~79%) and very poor in PUFA (~2%) (Ogunsina *et al.*, 2011), it contains an excess of antiradical molecules like tocopherols, phenolics and carotenoids for preventing the peroxidation of its PUFA contents and quenching free radicals in the human body.

3.5. IC_{50} value of MSO and GNO

The IC_{50} value of an oil is its concentration which causes 50% reduction in the total amount of radicals (DPPH) in a solution. Figure 2 shows the IC_{50} value of MSO and GNO. MSO at a concentration of 35.5 mg mL^{-1} showed a 50% reduction in DPPH radicals while GNO showed 50% reduction in DPPH radicals at a concentration of 45.0 mg mL^{-1}. MSO, being rich in bioactives like tocopherols, phenolics, sterols and carotenoids, showed a significantly higher ($p \leq 0.05$) IC_{50} value as compared to GNO. GNO, being fairly rich in PUFA (~31%) (Bhatnagar *et al.*, 2009) may require most of its natural antioxidants for the prevention of peroxidation of its PUFA content. On the other hand, MSO, being poor in PUFA (~2%) (Ogunsina *et al.*, 2011) has higher amounts of natural antioxidants at its disposal for quenching free radicals. This could be the reason for a higher IC_{50} value of MSO than GNO despite being poorer in tocopherols.

4. CONCLUSIONS

The present study indicates that the Indian *M. oleifera* Jaffna variety seed contains edible oil rich in natural antioxidants. The consumption of this oil may provide health benefits in terms of hypocholesterolemic effects (due to the presence of phytosterols) and scavenging of free radicals in the body (due to the presence of tocopherols, phenolics and carotenoids). The crude seed oil from the Indian *M. oleifera* Jaffna variety, being rich in monounsaturated oleic acid and poor in PUFA contains surplus amounts of natural antioxidants and also exhibits better antiradical activity than

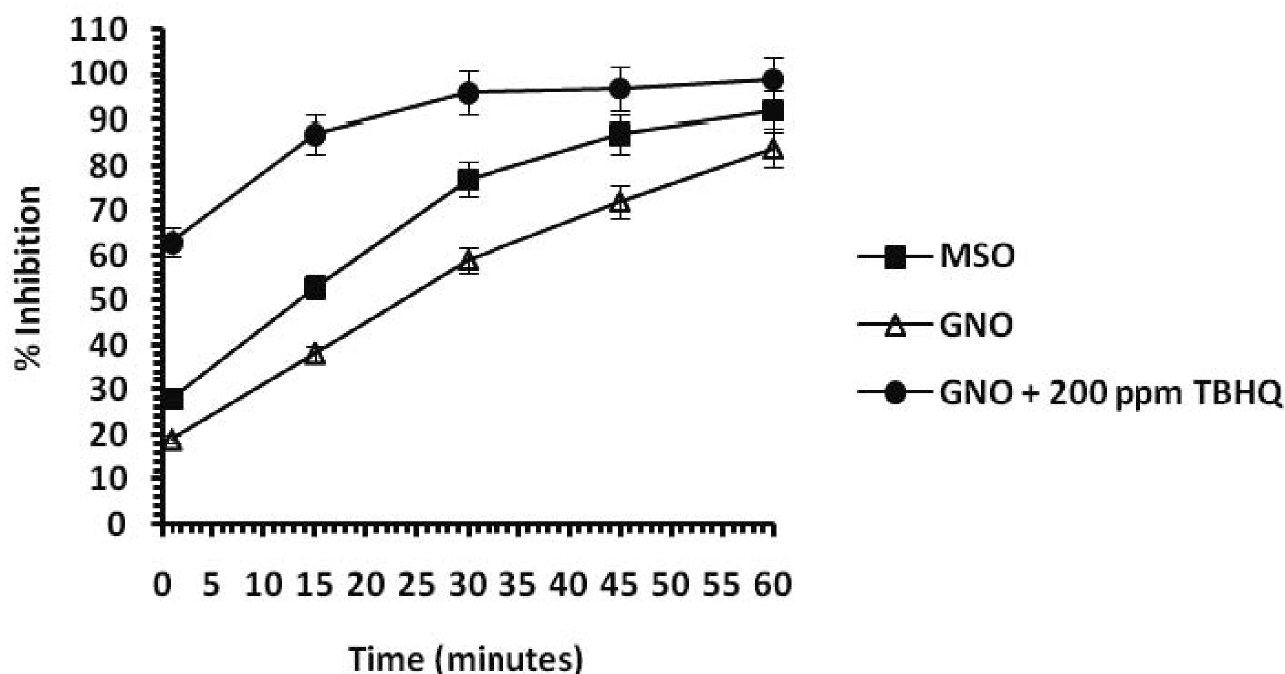

Figure 1
DPPH radical scavenging activity of oils.

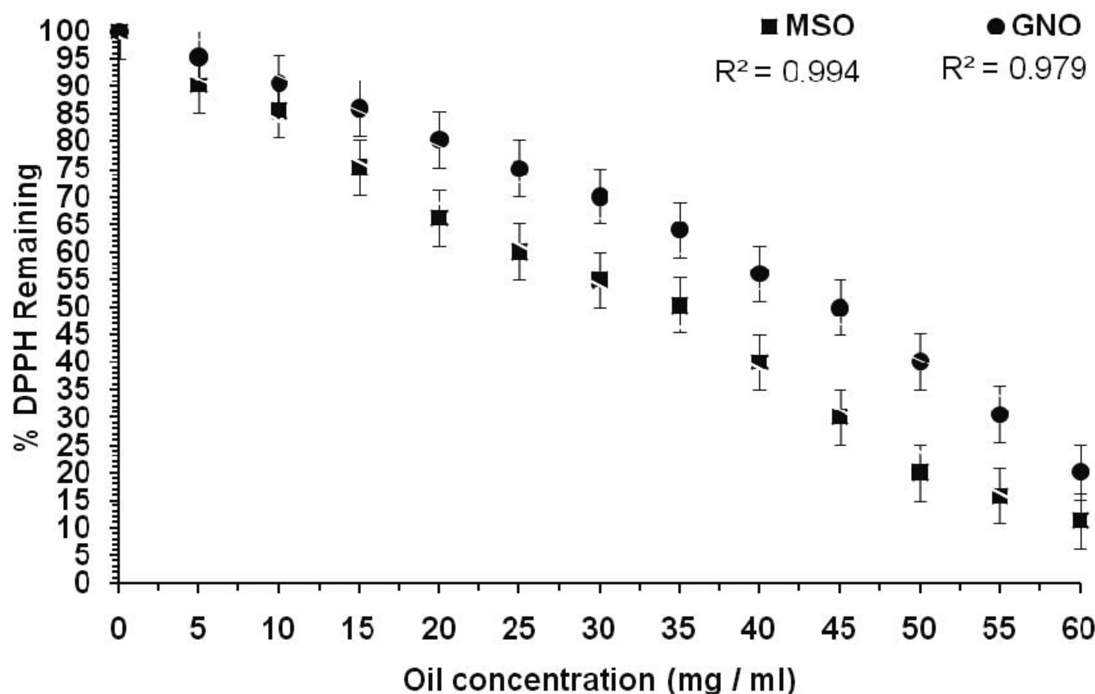

Figure 2
IC_{50} value of MSO and GNO.

commonly used copra oil and commercially refined groundnut oil. The crude seed oil from the Indian *M. oleifera* Jaffna variety, being rich in monounsaturated oleic acid and poor in PUFA, may also provide better bio-availability of its inherent natural antioxidants to the human body, which may be evaluated through in-vitro/in-vivo studies.

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