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Physicochemical properties and chemical composition of Seinat (*Cucumis melo* var. *tibish*) seed oil and its antioxidant activity

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SUMMARY: Seinat (*Cucumis melo* var. *tibish*) seeds were analyzed for their physiochemical properties and chemical composition of the oil in addition to antioxidant activity. The crude oil content was 31.1%, while the moisture, fiber, protein, ash and total sugar contents were 4.2%, 24.7%, 28.5%, 4.3%, and 6.9%, respectively. The main fatty acids were linoleic, oleic, palmitic, and stearic acids (61.10%, 18.75%, 10.37% and 9.18%, respectively). The total phenolic content was 28.17 mg·g^{-1} oil. Seinat seed oil also contained a good level of tocopherols; of which δ-tocopherol (63.43%) showed the highest content. β-sitosterol was found at 289 mg·100g^{-1} oil of total sterols (302 mg·100g^{-1}) in the oil. The crude oil showed a good antioxidant activity in four assays including reducing power, β-carotene bleaching inhibition activity, ABTS and DPPH radical scavenging activities.

KEYWORDS: *Antioxidant activity; Fatty acids; Seinat (Cucumis melo var. tibish) seed oil; Sterols; Tocopherols*

RESUMEN: ***Propiedades físico-químicas y composisción química de aceites de semillas de Seinat (Cucumis melo var. Tibish) y su actividad antioxidante***. Se analizó las propiedades fisicoquímicas y la composición de aceites de semillas de seinat (Cucumis melo var. Tibish) además de su actividad antioxidante. El contenido de aceite crudo fue de 31,1%, mientras que la humedad, fibra, proteínas, cenizas y contenido total de azúcares fue de 4,2%, 24,7%, 28,5%, 4,3%, y 6,9%, respectivamente. Los principales ácidos grasos fueron: linoleico, oleico, palmítico, y esteárico (61,10%, 18,75%, 10,37% y 9,18%, respectivamente). El contenido de fenoles totales fue de 28,17 mg.g^{-1} de aceite. El aceite de semilla de Seinat también contiene un buen nivel de tocoferoles, de los cuales el mayoritario es δ-tocoferol (63,43%). β-sitosterol es el esterol mayoritario con 289 mg·100 g^{-1} de aceite y los esteroles totales 302 mg·100 g^{-1} de aceite. El aceite crudo mostró una buena actividad antioxidante en cuatro ensayos incluyendo la reducción de potencia, actividad de inhibición de blanqueo del β-caroteno, actividad captadora de radicales ABTS y DPPH.

PALABRAS CLAVE: *Aceite de semillas de Seinat (Cucumis melo var. Tibish); Ácidos grasos; Actividad antioxidante; Esteroles; Tocoferoles*

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1. INTRODUCTION

Seinat (*Cucumis melo* var. *tibish*) is a type of melon that belongs to the cucurbitaceae family. The cucurbitaceae family, also commonly referred to as the cucumber, gourd, melon, or pumpkin family is a medium-sized plant family, primarily found in the warmer regions of the world. It is a family of economically important species, of which the fruits are used for nutrition and medicinal purposes (Jeffrey, 1990). Most species of the cucurbitaceae family are oilseed and their kernels have tremendous food value in Africa (Badifu, 2001). Seinat is grown mostly in Sudan but is not well known in its neighboring countries. Seinat is cultivated for its edible seed. There are mainly five types of melons that are grown in Sudan. The melons have different morphological characteristics and they also differ in their use. Each of these types has a specific local name (Mohamed and Pitrat, 1999). The types of melons which are grown in Sudan include; sweet melon, snake melon, a salad melon known locally as *tibish*, and a melon locally known as *seinat*. *Tibish* and *seinat* cultivars are of local landraces and seem to belong to a different melon group, which is grown only in Sudan (Ali, 2009). The seinat type could be considered as a type of (*Cucumis melo*). Seinat seeds are roasted and eaten. Other well-known and more investigated oilseed proteins such as pumpkin and melon seeds are utilized directly as snacks after salting and roasting mostly in Arabian countries (El-Adawy and Taha, 2001). Determination of the chemical composition and antioxidant activity would significantly contribute to the valorization of cucurbit oil potential in food, cosmetic and pharmaceutical industries. (Rezig *et al*., 2012). No study on the physiochemical properties, chemical composition or antioxidant activity of the oil extracted from the seinat (*Cucumis melo* var. *tibish*) seed has been reported so far. Thus, in the current research, the physiochemical properties, chemical composition and antioxidant activity of the oil isolated from seinat seeds were evaluated. This may lead to the investigation of seinat seed oil as an option for food and industrial applications.

2. MATERIALS AND METHODS

Dried seinat fruits were brought from a local farm in Wad Medani City, Gezira State, Sudan, and transported to the Food Processing and Ingredients laboratory in Jiangnan University, People's Republic of China.

2.1. Proximate composition analysis of seinat seeds

The protein, fat, moisture, fiber and ash contents of seinat seed flours were determined using the standard AOAC methods 932.06, 925.09 and 923.03, respectively (AOAC, 1990) and carbohydrate content was determined by difference. Iron content was estimated using a UV-Visible spectrophotometer (TECHCOM Co., Shanghai, China) at 480 nm (AOAC, 1995). Magnesium was analyzed by the Ranganna (1986) method. The blue color that developed was read at 650 nm in a UV-Visible spectrophotometer and expressed as magnesium mg·100 g^{-1} meal. Other minerals were estimated by atomic absorption spectroscopy (Shimadzu AA 6701F, Atomic Absorption Flame Emission Spectrophotometer equipped with a hollow cathode lamp).

2.2. Oil extraction

Two hundred g of seeds were milled and mixed with 1 L of n-hexane using a shaker (IKA® RW 20 digital, UK-plug) at a rate of 440 rpm for 4 h. The mixture was then centrifuged for 20 min at a temperature of (4 °C). The supernatant was then filtered. The extraction process was repeated. The oil was then recovered by evaporating off the solvent using a rotary evaporator (Model N-1, Eyela; Tokyo Rikakikal, Japan), and the solvent was removed under a laboratory fume hood for 30 min at (37 °C). The extracted seinat oil was drained under a nitrogen stream and was then stored in a refrigerator at (4 °C) for analysis.

2.3. Color parameters

A Hunter Lab digital colorimeter (TC-PIIG system, Beijing Optical Instrument Co. Ltd., Beijing, China) was used to measure the seinat oil color and color scales as L*a*b* values were recorded. A cylindrical plastic dish (58 mm in diameter and 15 mm in depth) containing the same amount of sample was placed at the light port (50 mm in diameter).

2.4. Oxidative stability

The oxidative stability of seinat seed oil was measured using the Rancimat method (743 Rancimat, Metrohm Co., Basel, Switzerland). Briefly, 3.6 g of oil were weighed into the reaction vessel, which was placed into the heating block kept at 120 °C. Air flow was set at 20 $L \cdot h^{-1}$. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL distilled water. The conductivity of this solution was measured and recorded.

2.5. Chemical analysis of oil

American Oil Chemist's Society (AOCS, 1997) methods were used for the determination of free fatty acids (method Ca 5a-4), peroxide value (method Cd 8-53), saponification value (method Cd 3-25), unsaponifiable matter (method Ca 6a-40), and specific gravity (using a 10 ml pycnometer

at 25 °C). The refractive index was determined using an Abbe refractometer at 25 °C. Iodine value was calculated following the procedures of Kyriakidis and Katsiloulis (2000). Specific absorptive values k232 and k270 were determined using a UV spectrophotometer by measuring the absorbance.

2.6. Determination of fatty acid composition

Fatty acids were converted to their methyl esters (FAME) following the method of He and Xia (2007) with a slight modification. In brief, 1μL of FAME sample was injected into the gas chromatograph (Series PEG30 M) equipped with a flame ionization detector. GC separation was conducted on a capillary column (PEG30 M; 30m × 0.32mm × 0.50 μm). The carrier gas was nitrogen and the column flow rate was 0.8 mL·min^{-1}. Initially, the oven temperature was calibrated at 190 °C for 1 min, increased from 190 to 230 °C at a rate of 3 °C·min^{-1} and then maintained at 230 °C for 10 min. The temperatures of the injection port and detector were 240 and 250 °C, respectively. The peaks were identified on the chromatogram according to retention data from analyzed standard samples. Finally, fatty acid contents were calculated as percentages (%).

2.7. Determination of tocopherol composition

To determine tocopherol composition, the NY/T 1598–2008 method was used. A solution of 250 mg of seinat oil, 25 mL of ethanol, 5 mL of 10% ascorbic acid and 10 mL of a 50% KOH solution were saponified in a capped flask in a water bath at 90 °C for 1 h. After cooling, 100 mL of distilled water were added and mixed. The solution was extracted with 50 mL of diethyl ether. The upper layer was collected and washed with distilled water until a neutral pH was reached. The organic layer was then separated and dried over Na_2SO_4. After filtration of this solution, the solvent was evaporated to dryness under a vacuum at 40 °C. The dry matter was dissolved in 2 mL of ethanol, and then centrifuged at 5.000 rpm for 5 min. The upper layer (10 μL) was injected for the HPLC analysis. The HPLC system (Waters, USA) included a Waters 2996 PAD detector and a Spherisorb Silica column (250 mm × 4.6 mm, 5 μm), operating at 30 °C. The separation of tocopherols was based on a mobile phase of methanol/ water (98:2, v/v) at a flow rate of 1.0 mL·min^{-1} with detection at 290 nm. Tocopherols were calculated as mg/g oil.

2.8. Sterols analysis

The sterol composition of seinat oil was determined by derivatization with N-methyl-N-trimethylsilyl-heptafluor butyramid as silylation agent. The assignments were made using the retention times of the individual sterols and calculation of the relative retention times in relation to betulin as internal standard (ISO/FIDS, 1999). Briefly, 250 mg of oil were saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was extracted by solid-phase extraction on an aluminum oxide column on which fatty acid anions were retained and sterols passed through. The sterol fraction from the unsaponifiable substance was separated by thin-layer chromatography (TLC) on 20×20 cm silica gel, 0.25 mm layer thickness using hexane/diethyl ether as developing solvent was extracted again from the TLC substrate. The sterol fraction was detected by gas liquid chromatography using an internal standard (betulin). Unclear cases were detected by GC mass only. Finally, other parameters were hydrogen which was the carrier gas; split ratio, 1:20; injection and detection temperature calibrated to 320 °C; temperature was programmed between 245–260 °C at 5 °C·min^{-1}.

2.9. Determination of total phenolic contents (TPC)

TPC was determined with the Folin-Ciocalteu method. In brief, 100 mg oil equivalents·mL^{-1} were mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of methanol. The mixture was shaken for 1 min, and then 1.5 mL of 15% Na_2CO_3 was added and the mixture was shaken again. Finally, the solution was brought up to 10 mL by adding distilled water. The mixture was incubated at 50 °C for 20 min and centrifuged at 3,000 rpm for 10 min. The absorbance was recorded at 750 nm. TPC of the oil was calculated using Gallic acid as a standard (Liu and Yao, 2007).

2.10. Reducing power assay

The reducing power of the oil was determined using the method of Zheng *et al*. (2012) with slight modification. Oil concentrations of 5, 10, 15, 20 and 25 mg oil equivalents·mL^{-1} were mixed with a sodium phosphate buffer (200 μL, 0.2 M, pH 6.6) and potassium ferricyanide (200 μL, 10 mg·mL^{-1}) and incubated at 50 °C for 30 min. Trichloroacetic acid (200 μL, 100 mg·mL^{-1}) was added and the mixtures were again incubated for 5 min to stop the reaction. Then 680 μL of the reaction mixture were mixed with 680 μL of distilled water and 68 μL of ferric chloride (10 mg·mL^{-1}). 0.5 mM of Ascorbic acid was used as the reference compound. The absorbance of solution was recorded at 700 nm.

2.11. ABTS radical scavenging assay

To measure the ABTS radical scavenging activity of seinat oil, an aliquot of oil 5, 10, 15, 20 and 25 mg oil equivalents·mL^{-1} concentrations were mixed with 900 μL of 100 mM Tris-HCl buffer (pH 7.4), 40 μL of methanol and 50 μL of 0.5% (w/w)

Tween 20 solution (Bruni *et al.*, 2004). An ABTS radical was generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate via incubation at 23 °C in the dark for 12 h. Then, 0.1 mL of the sample solution was mixed with 2.6 mL of a diluted ABTS radical solution. The absorbance of the solution was recorded at 734 nm after incubation at 23 °C for 6 min. ABTS radical scavenging assay was calculated using the formula: scavenging activity % = $(A_B–A_S /A_B) \times 100$, where A_B: absorption of blank sample and A_S: absorption of the sample. BHT was used as the reference compound.

2.12. DPPH radical scavenging assay

The DPPH radical scavenging assay of seinat oil was conducted using a method described by Brand *et al.* (1995) with a slight modification. Oil concentrations of 5, 10, 15, 20, 25 and 30 mg oil equivalents/ mL were mixed with 3.5 mL of DPPH solution with absorbance at 517 nm. The mixtures were incubated for 30 min at 25 °C. Then, the absorbance was recorded at 517 nm. The DPPH radical scavenging activity was calculated by the formula: scavenging activity % = $(A_C–A_S /A_C) \times 100$, where $A_{S:}$ absorbance of oil sample and A_C: absorbance of control. Ascorbic acid was used as the reference.

2.13. Antioxidant assay using the β-carotene bleaching method

An amount of one mL of β-carotene solution in chloroform (3.34 mg·mL^{-1}) was pipetted into a flask containing 50 mg linoleic acid and 500 mg Tween 20. The chloroform was removed by rotary evaporation at 40 °C for 5 min and 100 mL of distilled water were slowly added to the solution with vigorous agitation to form an emulsion. A 5 ml aliquot of the emulsion was added to a tube containing 0.2 mL of the antioxidant solution at 200 mg·L^{-1} and the absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 40 °C and the absorbance measurements were made again at 15 min intervals (Lu and Foo, 2000). BHT was used for comparison.

2.14. Differential scanning calorimeter (DSC)

The thermal parameter of seinat oil was measured by using a DSC (DSC-Q2000, V24.9, Instruments, Newcastle, USA). The seinat oil sample (4.8 mg) was weighed directly into a DSC-pan. The oil was rapidly cooled to −60 °C with a speed of 15 °C·min^{-1}, maintained for 15 min and heated to 120 °C with a heating speed of 15 °C·min^{-1}. The heating process was repeated and the DSC thermographs were recorded during the second melting. An empty DSC pan was used as reference.

3. RESULTS AND DISCUSSION

3.1. Proximate analysis of seed

As shown in Table 1, fat, protein, fiber, total sugars, and ash were 31.13%, 196 28.58%, 24.75%, 6.94% and 4.33% on dry weight, respectively, and the moisture content was 4.27%. The high level of oil makes seinat seeds convenient for oil industry application. Despite high percentages of oil in the recorded sample seeds, there are variations in these levels that have been reported. The variations in the oil content can be related to differences in plant species, cultivation climate, ripening level and the isolation method used (Nyam *et al.*, 2009). Since seinat (*Cucumis melo* var. *tibish*) seeds also contain a good level of crude fiber, they could be a source of dietary fiber for animals. Seinat seeds were also found to contain high levels of minerals (Table 1). Potassium was the most abundant element (9,548.33 mg·100 g^{-1}), while calcium was found at the lowest level.

3.2. Chemical analysis of oil

As shown in Table 2, the chemical composition of seinat seed oil was studied. The Relative density value (0.91) was similar to that found by El-Adawy and Taha (2001). The iodine value (110.99) was similar to that reported by Mariod *et al.*, (2009), for *Cucumis. melo* var. *flexuosus*. The high iodine value is due to its high content of unsaturated fatty acids (80.18 mg·100 g^{-1} oil); this indicates that the

Table 1. Macronutrient and micronutrient of seinat (*Cucumis melo* var. *tibish*) seed

Components (%)	
Moisture	4.27±0.12
Crude protein	28.58±0.50
Carbohydrates[a]	6.94±0.55
Crude fat	31.13±0.90
Crude Fiber	24.75±0.34
Ash	4.33±0.14
Minerals (mg·100 g^{-1}) of dry weight flour	
Zinc (Zn)	44.03±1.53
Iron (Fe)	81.17±1.52
Copper (Cu)	9.30±0.72
Manganese (Mn)	15.20±0.72
Potassium (K)	9,548.33±1.52
Sodium (Na)	386.13±0.81
Magnesium (Mg)	3,299.27±0.64
Calcium (Ca)	8.34±0.12

All determinations were carried out in triplicate and mean value ± standard deviation.
[a]Carbohydrate obtained by difference.

Table 2. Physicochemical properties of seinat (*Cucumis melo* var. *tibish*) seed oil

Parameters	
Physical state at room temperature	Liquid
Color	28.31 Y+1.21 R
Refractive index (25 °C)	1.440±0.03
Relative density (25 °C)	0.91±0.02
Free fatty acids (oleic acid %)	1.51±0.06
Saponification value (mg KOH·g^{-1} oil)	186.20±0.11
Iodine value	110.99
Peroxide value (meq O_2·kg^{-1} oil)	2.50±0.20
Unsaponifiable matter (%)	1.20±0.11
k_{232}	3.06±0.13
k_{270}	1.54±0.07
Oil stability index (h)	4.28

R Value on the red slide, *Y* value on the yellow slide. Values are the means of three replicate samples (n=3), mean±standard deviation, Iodine value was calculated following Kyriakidis and Katsiloulis (2000).

seed oil has good edible and drying oil qualities (Eromosele *et al*., 1997). The peroxide value and free fatty acid composition was 2.50 meq O_2·kg^{-1} oil and 1.51%, respectively. The peroxide values indicate that the oil started to degrade since it was identified as highly unsaturated oil. The saponification value of seinat seed oil was recorded as 186.20 mg of KOH·g^{-1} of oil. This value was lower than that reported by El-Adawy and Taha, (2001) in their research on watermelon (*Citrullus sp*) seed kernel oil and higher than that of Rezig *et al*. (2012) on pumpkin (*Cucumis maxima*) seed oil. Seinat oil also showed unsaponifiable matter of 1.20%. K232 and k270 were calculated from the absorbance recorded at 232 and 270 nm as 3.06 and 1.54, respectively.

3.3. Color

The CIE Lab coordinate values (L*, a*, b*) of seinat seed oil were 64.60, 1.21 and 29.31. Seinat (*C. melo* var. *tibish*) seed oil showed a slightly lower L* value and lower a* and b* values than (*C. melo* var. *agrestis*) seed oil studied by Mariod *et al*., (2009), this means that seinat seed oil was not as light in color. The CIE-Lab (L*, a*, b*) values of other vegetable oils, such as palm, soybean, sunflower, olive, and corn ranged from 63.4 to 69.5, 3.8 to 4.4 and 9.2 to 10.4, respectively (Hsu and Yu, 2002). This indicates the presence of yellow pigments like carotenoid compounds.

3.4. Oxidative stability

The results from the Rancimat test are presented in Table 2. The stability of seinat seed oil expressed as the oxidation induction time was about 4.28 h. This value may be justified by the fact that the oil was crude. A linear regression based on the linoleic ratio and the contents of phenols and tocopherols in virgin olive oil showed a good correlation with the oxidative stability measured by Rancimat (Aparicio *et al*., 1999). Similarly, the *Cucumis. melo* var. *agrestis* seed oil obtained by Mariod and Matthäus, (2006) reported an oxidation stability index of 5.7 to 5.9 h under the same conditions.

3.5. Fatty acid composition

The fatty acid composition of seinat seed oil is shown in Table 3. Of the nine fatty acids determined, five were unsaturated. Linoleic acid was found in the highest content (61.10%), followed by oleic acid (18.75%). These percentages are near to those reported by El-Adawy and Taha, (2001), for watermelon seed oil. It has been suggested that its high level of linoleic acid makes seed oil specifically prone to oxidation. This fatty acid may have favorable nutritional implications and beneficial physiological effects in the prevention of diseases such as cancer and coronary heart disease (Oomah *et al*., 2000).

Table 3. Fatty acid (%) and Tocopherols (mg·100 g^{-1} oil) composition of seinat seed oil

Fatty acid Composition (%)	
ΣSFA	19.82
Myristic acid (C14:0)	0.05±0.01
Palmitic acid (C16:0)	10.37±0.02
Stearic acid (C18:0)	9.18±0.04
Arachidic acid (C20:0)	0.22±0.04
ΣUFA	80.18
Palmitoleic acid (C16:1)	0.08±0.02
Oleic acid (C18:1)	18.75±0.1
Linoleic acid (C18:2)	61.10±0.08
Linolenic acid (C18:3)	0.16±0.03
Eicosenoic acid (C20:1)	0.09±0.02
ΣMUFA	18.92
ΣPUFA	61.26
Ratio SFA/UFA	4.01
Tocopherols (mg·100 g^{-1} oil)	
Total	43.20
α-Tocopherol	2.70±0.17
β-Tocopherol	ND
δ-Tocopherol	27.40±0.53
γ-Tocopherol	13.10±0.41

All determinations were carried out in triplicate and mean value ± standard deviation reported. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; ND, not detected.

3.6. Tocopherols content

The tocopherol contents (α, δ and γ) of the crude oil are shown in Table 3. δ-tocopherol was the highest content and was about 63.43% of the total tocopherols, followed by γ-tocopherol and α-tocopherol (30.32% and 6.25%, respectively). Oils and derived compounds are a main source of tocopherols. Alpha-tocopherol is benefitial to human nutrition because it has a higher biological activity than other tocopherols (Fatnassi *et al*., 2009). The total tocopherol content in this crude oil was 43.20 mg·100g^{-1} oil, higher than that of *Cucumis. melo* var. *flexuosus* seed oil as reported by Mariod *et al*., (2009).

3.7. Sterols

The Sterols content of this crude oil is illustrated in Table 4. The main sterol in the seed oil was β-sitosterol (289 mg·100g^{-1} oil), followed by Δ5-Avenasterol (3.85 mg·100g^{-1} oil) from the total sterols (302.70 mg·100g^{-1} oil). The Δ5-avenasterol can act as an antioxidant and anti-polymerization agent in frying oils. Sterols with an ethylidene group in the side chain are most effective as antioxidants, and a synergistic effect of the sterols with other antioxidants could be occurring. The total sterols of seinat (*Cucumis melo* var. *tibish*) seed oil is higher than that reported by Rezig *et al*., 2012, for pumpkin (*Cucurbita maxima*) seed oil and close to the results obtained by Mariod and Matthäus, 2006, for (*Cucumis melo* var. *agrestis*) oil. Seinat seed oil compared with other oils could be a good source for human nutrition because it contains a high content of total sterols.

Table 4. Sterols and total phenolic compounds of seinat (*Cucumis melo* var. *tibish*) seed oil

Component	Composition
Sterols (mg·100g^{-1})	
Cholesterol	1.30±0.20
Campesterol	1.40±1.00
24-Methylenecholesterol	3.30±0.80
Stigmasterol	2.25±0.50
β-Sitosterol	289.00±1.00
Δ7-Stigmastadienol	1.60±0.50
Δ5-Avenasterol	3.85±0.50
Total sterols	302.70
Total phenolic compounds[a] *(mg·g^{-1} oil)*	28.17±0.25

All determinations were carried out in triplicate and mean value ± standard deviation.
[a]Gallic acid equivalent.

3.8. DSC thermal characteristics

As shown in Figure 1, the DSC of seinat seed oil was measured. The oil sample exhibited four transitions when heated from −60 °C to 120 °C. The first and the last transition respectively occurred at −53 °C and 0 °C, and showed approximately the same temperature range. Commonly, a crude oil sample with a high amount of saturated fatty acids

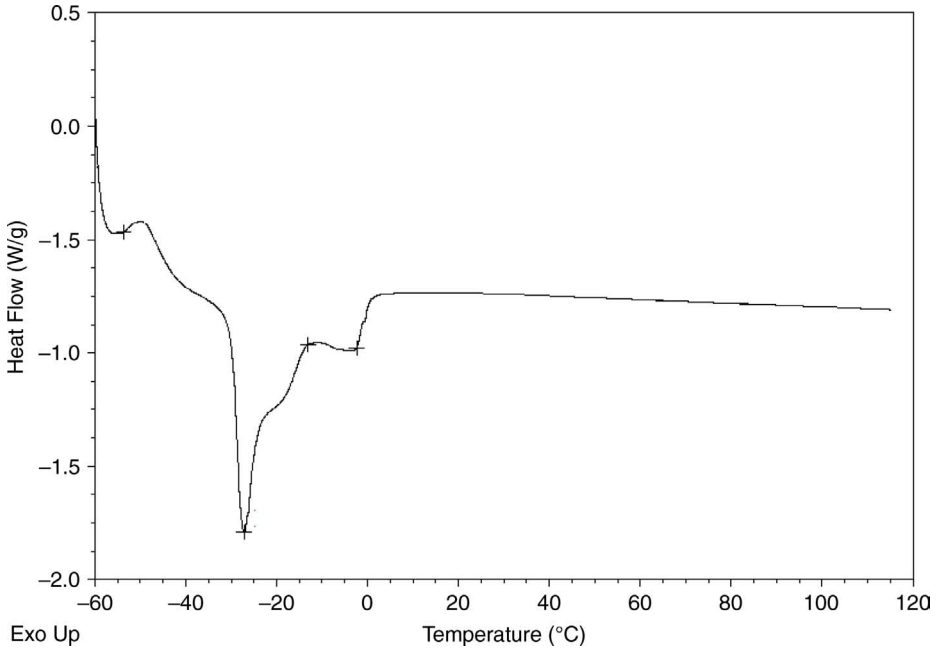

Figure 1. Melting thermogram curve of seinat (*Cucumis melo* var. *tibish*) seed oil.

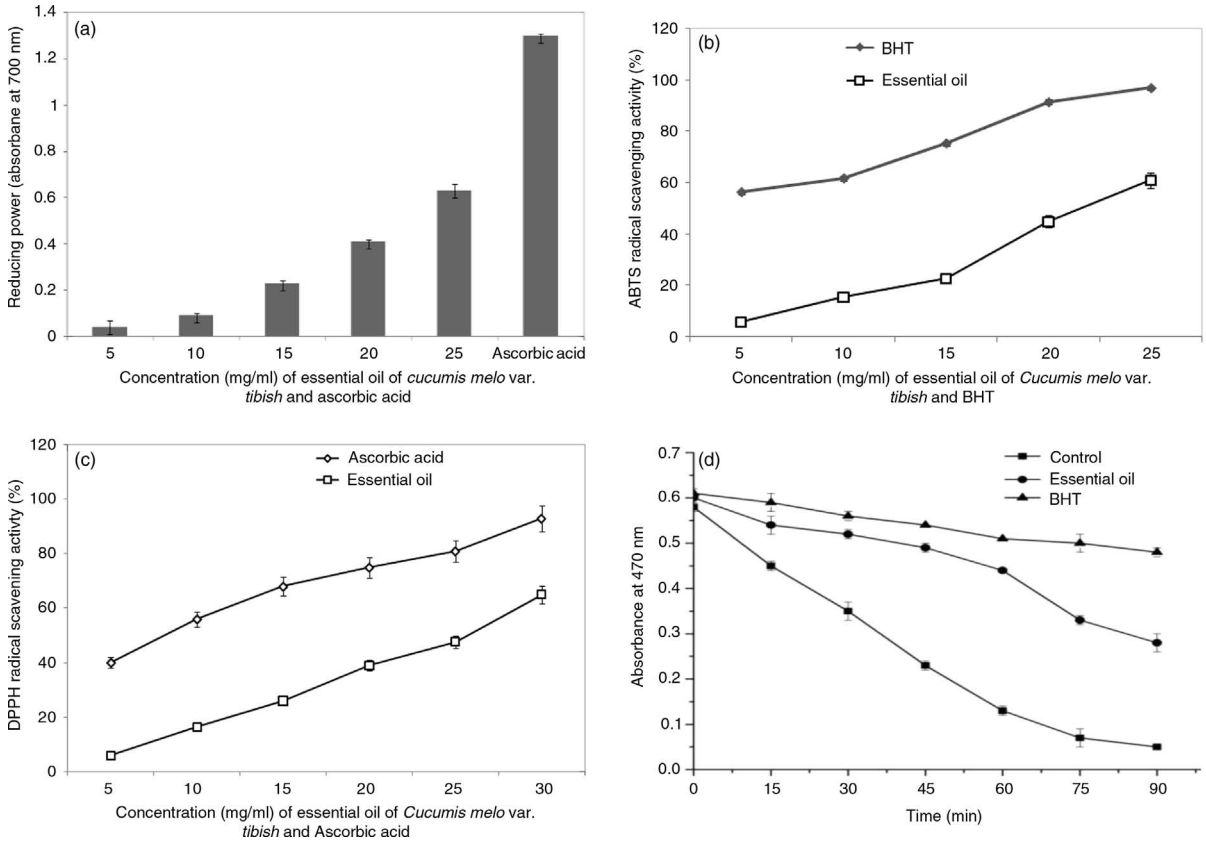

FIGURE 2. Antioxidant activity of seinat (*Cucumis melo* var. *tibish)* seed oil: (a) reducing power and the reference is ascorbic acid (5.0 mM); (b) ABTS radical scavenging activity; (c) DPPH radical scavenging activity; (d) β-carotene bleaching inhibition activity. Values are means of three determinations ± standard deviation.

illustrates a DSC melting profile at higher temperature zones when compared to an oil sample with a high amount of unsaturated fatty acids (Nyam *et al*., 2009). No endothermic phenomenon was shown beyond 0 °C. Such a feature may confirm the liquid state of the seed oil at room temperature (25 °C). It is worth noting that the asymmetry of the recorded peak at −27.17 °C can be regulated by two components of different weights. Actually, the thermogram seemed to correspond to a number of components higher than the visible ones, which suggested the presence of triglyceride fractions with melting points too near to be differentiated under the conditions used. (Herrera and Añón, 1991).

3.9. Antioxidant activity of oil

As illustrated in Figure 2, the antioxidant activity of seinat seed oil was measured by four different assay systems: reducing power, ABTS radical scavenging activity, DPPH radical scavenging activity, and β-carotene bleaching inhibition activity. Edible vegetable oils contain many natural antioxidants such as phenolic compounds, phytosterols, tocopherols (Vitamin E) and carotenoid compounds that might prevent biological systems from reactive oxygen species, preventing chronic diseases such as cancer and cardiovascular diseases (Castelo and Torres, 2009).

3.9.1. Reducing power

The reducing powers of the crude oil of seinat and the reference compound (Gallic acid) are shown in Figure 2a. The reducing power increased with an increase in the concentration of the oil. The absorbance was recorded in the range of 0.04 for the concentration of 5 mg oil equivalents·mL^{-1} and 0.63 for the concentration of 25 mg oil equivalents·mL^{-1}. The results of the reducing power of seinat seed oil were weaker than the reference used; the absorbance was 1.3 at the concentration of 0.5 mM. The reducing power of a compound is used to assess its ability to donate electrons and may serve as an important indicator of its potential antioxidant activity (Meir *et al*., 1995).

3.9.2. ABTS radical assay

As shown in Figure 2b, the concentration response curves of the crude oil and the reference (BHT) to ABTS radical assay were not the same as the one recorded in the DPPH scavenging activity. The ABTS radical is also generally used to measure the in vitro antioxidant activity of different substances. IC_{50} value of the oil sample was 23.30 mg/mL compared with BHT of 10.52 mg·mL^{-1}. Decolorization of ABTS reflects the capacity of antioxidant species to donate electrons or hydrogen atoms to inactivate this radical action. ABTS discoloration assays have been applied to the evaluation of the total antioxidant activity of various substances and widly used in many recent studies for the detection of the total antioxidant activity of edible vegetable oils (Neffati *et al*., 2009).

3.9.3. DPPH radical scavenging

The DPPH radical scavenging potential of seinat seed oil is illustrated in Fig. 2c and the radical scavenging activity of the reference compound (Ascorbic acid) and oil are shown in the graph. According to the results recorded, the DPPH radical scavenging activity of the crude oil of seinat seed of the IC_{50} was 25.25 mg·mL^{-1},which is higher than the control (ascorbic acid) with an IC_{50} of 14.05 mg/mL, used at the same concentrations. The result of the recent investigation was comparable with previous study made by Tenore *et al*., (2011). De Oliveira *et al*., (2012) reported the DPPH radical scavenging activity of thymol rich essential oil from *Satureja montana*. The radical scavenging activity of its edible oil could be credited to the presence of its main total phenolic contents, especially thymol and their recognized impact on oil lipid oxidation (Guimaraes *et al*., 2010).

3.9.4. β-carotene bleaching inhibition

The β-carotene bleaching inhibition effect of the crude oil of seinat seed and the reference compound (BHT) are shown in Figure 2d. The anti-bleaching activity of β-carotene was determined by observation the color intensity of the emulsion recorded at 470 nm at every 15 min for 1.5 h. The concentration of the oil sample and the standard (BHT) was 0.95 mg·mL^{-1}. At the beginning absorbance of the oil sample was 0.61 nm. Then, after the next 15 min the sample showed an absorbance of 0.54 nm bleaching as compared to that of the standard at 0.59. After one hour of incubation, the absorbance decreased to 0.44 nm and 0.51 nm for the oil and the standard, respectively. Finally, at the last 90 min, the absorbance was 0.28 and 0.48 nm for the oil sample and reference, respectively. The assay of β-Carotene bleaching is dependent on the loss of the yellow color of β-carotene due to its interaction with radicals created by linoleic acid oxidation in an emulsion (Nanasombat and Wimuttigosol, 2011). In the β-carotene/linoleic acid assay, the oxidation of linoleic acid generates peroxyl free radicals which will then oxidize the highly unsaturated fatty acids, which means the presence of antioxidants will minimize the oxidation of β-carotene compounds.

4. CONCLUSIONS

In the present study, the analysis of a new source of edible oil belonging to the cucurbitaceae family is presented and the high percentage of oil in this seed is determined. The chemical composition of this crude oil showed promising results similar to other edible oils. Also the proportion of crude fiber in seinat seeds can be beneficial, even in animal feed. According to the tests carried out on the crude oil in order to assess the efficiency of the four methods of antioxidant evaluation, this oil can be a source for use in the food, cosmetics and pharmaceutical industries. This study is the first to take place on the seinat seed grown in Sudan, opening the way for further studies on these seeds.

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