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INVESTIGACIÓN

Antioxidant activity of extracts from *Sclerocarya birrea* kernel oil cake

By **Abdalbasit A. Mariod*[1], Bertrand Matthäus[2], K. Eichner[3] and Ismail H. Hussein[4]**

[1] Food Sci & Technology Department, College of Agricultural Studies, Sudan University of Science & Technology, P.O Box 71 Khartoum North, Sudan. Phone +249 (0) 185 311082. Fax +249 (0) 185 311896. e-mail: elmariod@hotmail.com.
[2] Institute for Lipid Research, Federal Research Center for Nutrition and Food, Piusallee 68/76, D-48147 Münster, Germany.
[3] Institute for Food Chemistry, Westfälische-Wilhelms-Universität, Corrensstr. 45, D-48149 Münster, Germany
[4] National Oilseed Processing Research Institute (NOPRI), University of Gezira, P.O Box 20, Wad Madani Sudan.

RESUMEN

Actividad antioxidante de extractos de torta de aceite de semilla de *Sclerocarya birrea*

Se ha evaluado la actividad antioxidante de extractos metanólicos de torta de aceite de semilla de *Sclerocarya birrea* extraídos usando dos métodos diferentes. La extracción se llevó a cabo mediante agitación magnética del material en metanol/agua (80:20 v/v) durante toda la noche seguida de dos tratamientos con ultrasonidos durante 45 min. (extracto ONEXT) y solo tres tratamientos con ultrasonidos durante 45 min. (extracto USEXT), respectivamente. Se obtuvieron tres fracciones de cada extracto y el contenido total de compuestos fenólicos se determinó en cada fracción según el método de Folin-Ciocalteau como 34.6, 54.8 y 58.6 mg/g de producto seco en ONEXT y 29.6, 84.8 y 143.9 mg/g en USEXT, respectivamente. La actividad antioxidante de los extractos fue evaluada mediante el ensayo del β-caroteno-ácido linoleico donde los extractos y sus fracciones mostraron efectos significativos ($p<0.05$). Las propiedades antioxidantes de los extractos obtenidos mediante los dos métodos de extracción descritos fueron similares. El coeficiente de actividad antioxidante (AAC) de estos extractos y sus fracciones aumentó al aumentar la concentración del extracto.Se evaluó el efecto de ONEXT y USEXT, a niveles del 0,2 y 0,8 %, sobre la estabilidad oxidativa de aceite de girasol a 70°C y en la oscuridad y se comparó con el antioxidante sintético comúnmente utilizado BHA. Los índices de peróxidos (PVs) fueron significativamente ($p<0.05$) más bajos con la adición de extracto en comparación con un control En comparación con BHA (0.02%) el aumento de PVs después de la adición de ONEXT (0.2% y 0.8%) y USEXT (0.8%), respectivamente, se redujo. La oxidación del aceite de girasol tratado con 0.2%, 0.5%, y 1.0% de ONEXT y USEXT, respectivamente, fue probada usando el método Rancimat a 120 °C. En ambos extractos aumentó el periodo de inducción al compararlos con un control y BHA, y el factor de estabilización F aumentó con la concentración.

PALABRAS-CLAVE: Actividad antioxidante - Compuestos fenólicos - Ensayo ß-caroteno-ácido linoleico - Extractos metanólicos - Orujo de aceite de semilla - Sclerocarya birrea.

SUMMARY

Antioxidant activity of extracts from *Sclerocarya birrea* kernel oil cake

The antioxidant activity of methanolic extracts from *Sclerocarya birrea* kernel oil meal, extracted using two different methods was evaluated. The extraction was carried out using magnetic stirring of the material in methanol/water (80:20 v/v) overnight followed by two ultra-sonic treatments for 45 min. (Overnight extract, ONEXT) and three ultra-sonic treatments for 45 min. only (Ultra-sonic extract, USEXT), respectively. Three fractions were obtained from each extract and the contents of total phenolic compounds were determined in each fraction according to the Folin-Ciocalteau method as 34.6, 54.8, and 58.6 mg/g of dry product in ONEXT and 29.6, 84.8, 143.9 mg/g in USEXT, respectively. The antioxidant activity of the extracts was evaluated according to the β-carotene-linoleic acid assay, where the extracts and their fractions showed significant effect ($p<0.05$). The antioxidative properties of the extracts obtained from the two extraction methods described were similar. The AAC (antioxidant activity coefficient) of these extracts and their fractions increased with an increasing concentration of the extract. The effect of ONEXT and USEXT at the 0.2 and 0.8 % levels on the oxidative stability of sunflower oil at 70°C was tested in the dark and compared with the commonly used synthetic antioxidant BHA. The oil peroxide values (PVs) were significantly ($p<0.05$) lower with the addition of extract in comparison to a control. In comparison to BHA (0.02%) the increase of PVs after the addition of ONEXT (0.2% and 0.8%) and USEXT (0.8%), respectively, was reduced. The oxidation of sunflower oil, treated with 0.2%, 0.5%, and 1.0% of ONEXT and USEXT, respectively, was tested using the Rancimat test at 120 °C. Both extracts increased the induction time compared to a control and BHA, and the stabilization factor F increased with the concentration.

KEY-WORDS: Antioxidant activity - ß-carotene-linoleic acid assay - Kernel oil meal - Methanolic extracts -Phenolic compounds - Sclerocarya birrea.

1. INTRODUCTION

Sclerocarya birrea subsp. caffera is a Savannah tree, belonging to the family Anacardiaceae. The common English name is Marula or Cider tree, and the tree is commonly known in Sudan as Homeid, where it is widely distributed in the western and southern regions. *S. birrea* requires sandy or alluvial soils and propagation is possible by seeds or cuttings (Voget, 1995). The seed encloses 2 - 3 soft white edible kernels (nuts), which are rich in oil and protein (Glew *et al*., 2004). The highly aromatic sweet-sour fruit can be eaten fresh, like a small mango, or used for the preparation of juices, jams, preserves, dry fruit rolls and alcoholic beverages. These nuts have a high nutritive value resulting from their high protein and oil content (Mizrahiy and Nerd, 1996). In our recent study the oil from *Sclerocarya birrea* kernels showed a high oxidative stability using the rancimat test (43hr) (Mariod *et al*., 2004).

The antioxidant activity of extracts of several plant materials has recently been reported (Velioglu et al., 1998; Tian and White, 1994; Duh and Yen, 1997; Leonardis *et al*., 2003; Marinova and Yanishlieva, 1997; Matthaüs, 2002; Wanasundara and Shahidi, 1994). The evaluation of the antioxidant activity of oil seed cake has been of interest in recent years (Amarowicz *et al*., 2001; Amarowicz *et al*., 2003), because it is useful as a natural antioxidant for the protection of fats and oils against autoxidation. The phenolic compounds quercetin and epicatechin derivatives were found in the methanolic extracts of wild and cultivated *Sclerocarya birrea* leaves (Braca *et al*., 2003).

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines) or carotenoids as well as ascorbic acid (Velioglu *et al*., 1998).

Yanishlieva and Marinova (1994) have studied the effectiveness of natural antioxidants containing two neighbouring phenolic groups, e.g. caffeic acid, esculetin and fraxetin, and the stabilization of sunflower oil was examined for different concentrations at 25°C and 100°C. They found that the stabilization factor F value (defined in materials and methods) of 0.02% BHT was 1.2 both at 100°C and 25°C. The effectiveness of fraxetin increased with rising temperature, while that of caffeic acid remained practically the same. Gamel *et al. (1999)* have established that a 0.02% rosemary methanolic extract increased the oxidative stability of sunflower oil at 63° and 120°C. The F value for 0.02% rosemary extract was 1.6 at 120°C, while Marinova *et al* [8] reported that the stabilization factor F for the ethanolic extract of summer savory (*Satureja hortensis* L.) at 0.1-0.5% in sunflower oil was 1.8-2.3 at 100°C, while F was also 1.2 for 0.02% BHT.

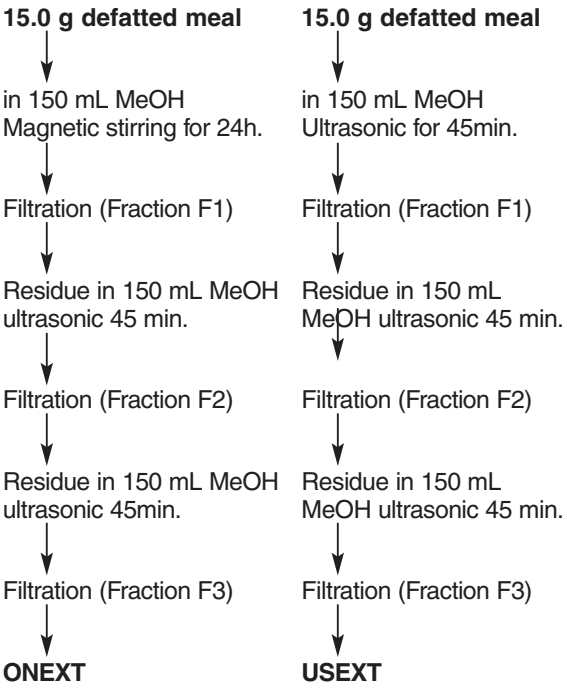
The aim of this study was to determine the content of total phenolic compounds and to examine the antioxidative activity of extracts obtained using two methods of extraction from *Sclerocarya birrea* kernel oil cake and their effect on the oxidation of sunflower oil.

2. MATERIALS AND METHODS

Materials

All solvents used were of analytical grade. Methanol, chloroform, butylated hydroxyanisole (BHA), β-carotene, linoleic acid and Folin-Ciocalteau reagent as well as polyoxyethylene sorbitan monopalmitate (Tween 40) were obtained from Merck (Merck, Darmstadt, Germany). Sunflower kernel oil was obtained from a local market (Teutoburger Ölmuhle, Ibbenbüren, Germany).

Dried seeds of *Sclerocarya birrea* were collected manually from Ghibaish and Abu Gibaiha provinces of western Sudan. Seeds were dehulled (decorticated) using a Vice model 2XFRONT equipment (Heuer, Plettenberg, Germany), crushed and ground with a grinding mill to pass through a 0.5 mm sieve (Petra electric, Burgau, Germany). The oil was extracted from the ground material by extraction with n-hexane (b.p 50-60°C) in a Soxhlet apparatus for 6 hr. following the AOCS method Aa 4-38 (1993), and the obtained cake was used for investigation.



Scheme 1
Extraction procedure

Preparation of extracts from the defatted residue of *Sclerocarya birrea*

Phenolic compounds were extracted from the defatted residues (15 g) of *Sclerocarya birrea* kernel meal using two different methods, with 80% methanol at a solid to solvent ratio of 1:10 (w/v) at 50° C following the method of Amarowicz *et al.* (2001) as shown in Scheme 1.

ONEXT extract

Fifteen grams of *S. birrea* seedcake were dissolved in 150 mL methanol 80% then extraction was carried out in a flask by magnetic stirring at 50° C overnight (24h) (ONEXT), then filtrated to obtain fraction F1. The residue was extracted twice by ultrasonic treatment for 45 min each using the same solvent with the same amount to obtain F2 and F3 respectively. Each fraction was collected individually and the solvent evaporated under vacuum at 40°C using a rotary evaporator (Büchi, Switzerland). All dried extracts were stored at 4°C until use.

USEXT extract

Fifteen grams of *S. birrea* seedcake were dissolved in 150 mL methanol 80% then extraction was carried out in a flask by ultrasonic treatment (USEXT) at 40° C for 45 min three times to obtain fractions F1, F2, and F3 respectively. Each fraction was collected individually and the solvent evaporated under vacuum at 40°C using a rotary evaporator (Büchi, Switzerland). All dried extracts were stored at 4°C until use.

Determination of total phenolics in extracts

The total amount of phenolic compounds in the individual fractions of the crude extract was determined following the method of Folin-Ciocalteau (Silvia *et al.*, 1984). From the dried fractions F_1, F_2 and F_3 5 mg were dispersed in 1 ml of 60:40 acidified methanol/water (0.3 % HCl). Test solutions of 100 µL were added to 2.0 ml of 2% Na_2CO_3 and mixed thoroughly. After 2 min., 100 µL of 50 % Folin-Ciocalteau reagent were added and the mixture was allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm by a U-2000 Spectrophotometer (Hitachi, LTD. Tokyo, Japan) against a blank consisting of all reagents and solvents without extracts. A standard solution was prepared from gallic acid and a curve was determined in a range from 0.05 mg/mL to 0.4 mg/mL. The concentration of phenolic compounds in the extracts was calculated from this curve. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

Determination of antioxidation activity by β-carotene bleaching method

The antioxidant activities of the different fractions were evaluated using the β-carotene–linoleic acid assay (Amarowicz *et al.,* 2003). Approximately 2 mg β-carotene were dissolved in 10 mL of chloroform. One milliliter of this solution was pipetted into a round-bottom flask. After the removal of chloroform using a rotary evaporator, 20 mg of linoleic acid, 200 mg of Tween 40, and 50 mL of distilled water were added to the flask with vigorous stirring. Aliquots (5 mL) of the prepared emulsion were transferred to a series of tubes containing 100 µl of each fraction (20 mg extract of each fraction in 1 ml of a mixture of methanol/water (60:40 v/v)). Subsequently, absorbance readings at 470 nm were recorded each 15 min by keeping the samples in a water bath at 50 °C over a period of 120 min. All samples were assayed in duplicate.

For the determination of the antioxidant activity coefficient (AAC) 0.1, 0.2 and 0.4 mg of the appropriate fractions of the two extraction methods were dissolved in 1 ml of a mixture of methanol/water (60:40 v/v). The antioxidant activity coefficient (AAC) of the extracts was evaluated in terms of bleaching of *β*-carotene using the following formula as described by Velioglu *et al.* (1998):

$$AAC = [A_s(120) - A_c(120) / A_c(0)-A_c(120)] \times 1000$$

With:

$A_s(120)$ = absorbance of the antioxidant mix at t = 120 min

$A_c(120)$ = absorbance of the control at t = 120 min

$A_c(0)$ = absorbance of the control at t = 0 min

Inhibitory effect of extract from *Sclerocarya birrea* oil cake on lipid oxidation by the Rancimat method

The combined fractions of each extraction method were applied to 100 g sunflower kernel oil at levels of 0.2 % and 0.8 % to examine their antioxidative activity. BHA at a level of 0.02 % was used as standard. The dried extracts as well as the synthetic antioxidant were mixed with 1 mL of absolute methanol in an ultrasonic water bath (Bandelin electronic, Berlin, Germany) and added to the oil before mixing again for 10 min. The oil was oxidized in the dark at 70 °C. A control sample was prepared by using the same amount of methanol used to dissolve the antioxidant and the extracts (Moure *et al.*, 2000).

The peroxide value (PV) and the inhibition of the oil oxidation (IO) were used as indicators for the primary oxidation of the sunflower oil. The PVs were determined every 4, 24 and 72 hours.

IO = 100% - (PV increase of samples/PV increase of control) X 100% (Duh and Yen, 1997).

For oxidation measurements by the Rancimat dried extracts of ONEXT and USEXT (200, 500 and 1000 mg) and BHA (20 mg) were dissolved in a minimum amount of absolute methanol in an ultrasonic water bath (Bandelin electronic, Berlin, Germany) and sonicated for 10 min. Afterwards the solutions were mixed with 100 g sunflower oil in a 250

ml glass flask, and again sonicated for another 10 min. 3.6 g of each mixture were placed in Rancimat tubes for the determination of oxidative stability.

All experiments were carried out with a 743 Rancimat (Metrohm AG, Herisau, Switzerland). The airflow rate was 20L/h, and temperature of the heating block was 120°C as recommended by Metrohm (1994).

The antioxidant efficiency was expressed as the stabilization (protection) factor:

$$F = IP_A / IP_0,$$

With:

IP_A = induction period of a stabilized sample

IP_0 = induction period of a control sample (Schmidt *et al.,* 2003).

All treatments were carried out in duplicate.

Statistical analysis

The analyses were performed with three replicates. The mean values and SD were calculated and tested using the Student-t-test (P > 0.05). A statistical analysis of variance (ANOVA) was performed using the statistical program Statgrafics® Statistical Graphics System version 4.0. (1985).

3. RESULTS AND DISCUSSION

The residues of the oil processing of seed kernels of *Sclerocarya birrea* were extracted according to two different methods. In Table 1 the content of the total phenolic compounds of the different fractions F1, F2, F3 of each extraction method, determined according to the Folin-Ciocalteau method is shown.

Table 1 shows that using successive ultrasonic extraction results in significantly increasing amounts of total phenolic compounds (P>0.05). Repeated ultrasonic treatment probably results in an easier penetration of the samples by the solvent.

In Figures 1 and 2 the antioxidative effect of the different fractions on a β-carotene linoleic acid system is shown. The examined fractions of the ONEXT and USEXT extracts exhibited good antioxidative activity based on the coupled oxidation of β-carotene and linoleic acid. The antioxidative effects of the fractions obtained by ONEXT were better than the effects of the fractions from USEXT; the antioxidative activity of the three fractions of both extracts were in the order F3 >F2> F1 (Figs. 1 and 2).

The calculation of the antioxidant activity coefficients (AAC) of the fractions obtained by the β-carotene-linoleate model system indicated that the AACs are correlated significantly *(P*> 0.05) with the concentration of the extract added to the solution and the content of phenolic compounds

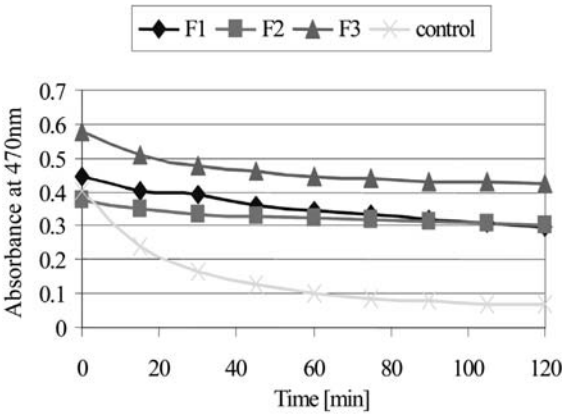


Figure 1
Effect of 0.4 mg/mL of the dried ONEXT fractions F_1, F_2 and F_3 on oxidation of β-carotene/linoleic acid at 50°C.

Table 1
Total phenolic compounds (PC) [mg of GAE/ g of extract]* and antioxidant activity coefficient (AAC) in *Sclerocarya birrea* seed cake

		AAC**		
Sample	**PC [mg of GAE/ g of extract]**	**0.1 mg/mL**	**0.2 mg/mL**	**0.4 mg/mL**
ONEXT				
F1	34.6±0.1	234.2	426.4	681.6
F2	54.8±0.2	540.0	681.3	711.5
F3	58.6±0.05	612.9	648.9	645.1
Total	148.0±0.2			
USEXT				
F1	29.6±0.1	205.0	434.7	529.6
F2	84.8±0.2	660.0	731.2	735.1
F3	143.9±0.06	760.2	841.6	850.6
Total	258.3±0.6			
BHA				

*PC, total phenolic compounds in the dried extracts as gallic acid equivalent.
**AAC, antioxidant activity coefficient. ONEXT, overnight extract. USEXT, ultrasonic extract. Data are means of triplicate results and mean value ± standard deviation (SD) reported.

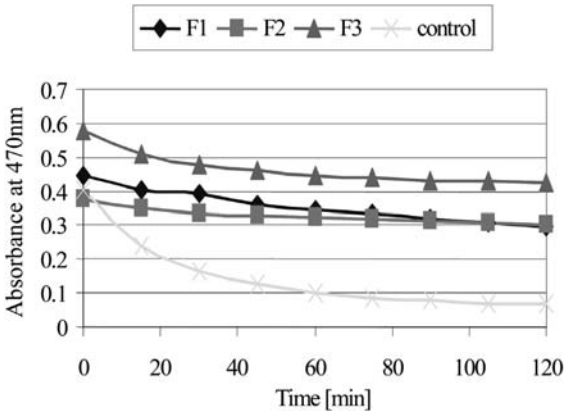


Figure 2
Effect of 0.4 mg/mL of the dried USEXT fractions F_1, F_2 and F_3 on oxidation of β-carotene/linoleic acid at 50°C.

Table 2
Effect of the extracts on the peroxide value (PV)* and inhibition of oxidation (IO) of sunflower oil after storage at 70 °C for 72 h

	Peroxide value (PV)	% Inhibition of oxidation (IO)
ONEXT (0.2 %)	22.4±0.1	81.2
ONEXT (0.8 %)	23.7±0.1	80.3
USEXT (0.2 %)	41.3±0.2	55.6
USEXT (0.8 %)	26.7±0.3	76.2
BHA (0.02 %)	35.4±0.3	68.1
Control	95.3±0.2	–

PV = meq O_2/Kg oil. Data are means of triplicate results and mean value ± standard deviation (SD) reported

(Table 1). With increasing concentration of the fractions in the β-carotene-linoleate model system the AAC increased. The highest increase of the AAC was found for fraction F1 depending on the concentration added to the solution, while the increase was remarkably smaller for the other fractions.

The development of the peroxide value (PV) during the oxidation of sunflower kernel oil was evaluated at 70°C. This temperature was used, because at higher temperatures the peroxides decompose very fast (Duh and Yen, 1997). The PV of sunflower kernel oil without ONEXT, USEXT or BHA showed a linear increase. A maximum PV value of 95.3 meq O_2/kg was reached after 72 hours of storage (Table 2). Significant differences (P>0.05) were found between the control and oil containing ONEXT, USEXT or BHA, which decreased and slowed the rate of peroxide formation. The PVs of sunflower oil containing 0.2 % USEXT, 0.2 % ONEXT or 0.02 % BHA were found to be 41.3, 22.4 and 35.4 meq O_2/kg, respectively. These samples showed an inhibition of oxidation (IO) after 72 hours of storage of 55.6, 81.2 and 68.1 % in comparison to a control. From this it can be assumed that all compounds added had a remarkable antioxidative activity to reduce oil degradation. ONEXT showed the highest activity, whereas the effect of USEXT was smaller than the effect of BHA.

In Table 2 the PV of sunflower oil with 0.8% ONEXT and 0.8% USEXT, were found to be 23.7 and 26.7, meq/kg, after 72 hours respectively. These samples had 80.3 and 76.2 % inhibition of oxidation IO after 72 hours of storage, compared with the control. These results indicate that ONEXT and USEXT of *Sclerocarya birrea* meal inhibited sunflower kernel oil oxidation. Furthermore, the antioxidative activity of 0.8 % USEXT and 0.8 % ONEXT were more effective than that of 0.02 % of BHA.

The oxidative stability of sunflower kernel oil supplemented by different concentrations of ONEXT and USEXT in comparison to BHA is shown in Table 3. The addition of antioxidants to the

Table 3
Oxidative stability of sunflower kernel oil after addition of phenolic extracts [mg/100 g] from *Sclerocarya birrea* meal

The oil treatment	IP [hour]	Increase of induction period [%]	Stabilization factor F	Amount of total phenolics (PC) present [mg/100 g oil]
Sunflower control	1.85±0.03[a]			–
Sunflower+BHA	2.32±0.03 [b]	25.4	1.25	–
Sunflower+0.2 % ONEXT	2.40±0.04[c]	29.8	1.29	29.6
Sunflower+0.5 % ONEXT	2.42±0.05[c]	30.8	1.31	74.0
Sunflower+1.0 % ONEXT	2.46±0.05 [c]	32.9	1.33	148.0
Sunflower+0.2 % USEXT	2.50±0.04[c]	35.1	1.35	51.7
Sunflower+0.5 % USEXT	2.53±0.03[c]	36.8	1.36	129.2
Sunflower+1.0 % USEXT	2.77±0.06[d]	49.7	1.50	258.3

IP = induction period data are means of triplicate results and mean value ± standard deviation
(SD) reported. Different characters (a,b,c,d) indicate significant statistically difference (P≤0.05) between the different samples

oil resulted in a significant improvement (P>0.05) of its oxidative stability. All the extracts with different concentrations showed a significantly better effect on the oxidative stability of the oil compared with BHA. While most of the extracts showed similar effects on the oxidative stability, the addition of 1.0 % USEXT resulted in a significantly better effect. The stabilization factor F was found to be 1.25 - 1.5, which increased with the concentration, which is in good agreement with the results of Gamel *et al.* (1999), who established that 0.02% rosemary methanolic extract increased the oxidative stability of sunflower oil at 120°C with an F value of 1.6.

4. CONCLUSION

Phenolic compounds were obtained from *Sclerocarya birrea* seedcake using two methods of extraction. Successive ultrasonic extraction results in increasing amounts of total phenolic compounds. Repeated ultrasonic treatment probably results in an easier penetration of the samples by the solvent. The antioxidative effects of fractions obtained by ONEXT were better than the effects of fractions from USEXT. The reducing power of each fraction correlated well (P>0.05) with the total content of phenolics present. The justification as to why ONEXT displayed stronger antioxidant activities than USEXT in spite of their higher phenolic amount of USEXT is a difficult task because (i) each extract is a complex mixture of phenolics with unknown structural characteristics; the difference in molecular structure may be in the arrangement of hydroxyl and methoxy groups, in the presence of ester and/or glycosidic bonds, and in the degree of association of the molecules involved; (ii) synergism of phenolics with each other as well as with non-phenolic compounds may also contribute to the total antioxidative activity of each fraction. Therefore, further work is required to isolate and identify the active component-(s) of *Sclerocarya birrea* seedcake phenolics present in ONEXT and USEXT fractions and to determine the kinetics and mechanism of their antioxidative activities

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