J Am Oil Chem Soc (2010) 87:377–384
DOI 10.1007/s11746-009-1510-4

ORIGINAL PAPER

Fatty Acids, Tocopherols, Phenolics and the Antimicrobial Effect of *Sclerocarya birrea* Kernels with Different Harvesting Dates

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Received: 29 July 2009 / Revised: 23 October 2009 / Accepted: 5 November 2009 / Published online: 26 November 2009

Abstract Oil extracted from the kernel of *Sclerocarya birrea* with different harvesting dates was studied in terms of the oil content, fatty acids, tocopherols, phenolic compounds and antimicrobial activity. A quantitative increase in the oil content was observed to reach 63.0% at the end of the last harvesting date. The percentage of total fatty acids had altered and palmitic acid content was found to be 16.8% at the first date of harvesting and dropping for the rest of the dates to reach 14.6% by the end of the harvesting process. In the same manner, stearic acid was found to be 15.2% at the first date and this dropped dramatically to reach 8.8% by the end of the harvesting, while oleic and linoleic acids increased from 58.9 and 4.3% to 67.3 and 5.9%, respectively. Alpha and gamma tocopherols decreased rapidly, whereas the δ-tocopherol and δ-tocotrienol were 4.8 and 4.9 mg/100 g, respectively at the beginning and had disappeared completely by the last harvesting date. Total phenolic and flavonoid content increased continuously through the different harvesting dates. *Sclerocarya birrea* kernel oil was effective in inhibiting the growth of three out of four bacterial strains tested. This inhibitory effect was less than that of the control.

Keywords Antibacterial activity · Fatty acids · *Sclerocarya birrea* · Tocopherols · Total flavonoid · Total phenolic

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Introduction

Sclerocarya birrea subsp. *caffera* is a Savannah tree, belonging to the Anacardiaceae family. The common English name is Marula, the tree is commonly known in Sudan as Homeid, where it is widely distributed in western and southern areas [1]. Humans have used the marula tree as a source of nutrition for at least 10,000 years; the tree produces more than 600 kg of fruits per year with an average yield of 550–1000 kg with a current value of US$1 per kg of fruit [2]. The kernels contained 53.0, 28.0 and 8.0% of oil, protein and carbohydrate, respectively [3]. The oil contains 67.2, 5.9 and 14.1% oleic, linoleic and palmitic acid, respectively and 13.7 mg/100 g tocopherols [4]. Glew et al. [5] reported that fatty acids accounted for 47% of the pit, two-thirds of which was oleic acid. Recent studies on the oil from *Sclerocarya birrea* kernels showed a high oxidative stability even during deep frying due to its fatty acid and tocopherol composition [6], however, the level of total tocopherols decreased during processing by 38.7%, while no change in the fatty acid composition was observed [7].

In addition to its edible uses, marula oil is considered to be a nutritive skin care oil. It is rich in anti-oxidants and oleic acid, and it is an extremely stable cosmetic oil. Its high content of palmitic acid creates a protective coating on the surface of the skin. The oil absorbs

quickly, hydrates the skin, heals skin tissues, reduces redness, reduces trans-epidermal water loss, increases the smoothness of skin and conditions the hair [8]. In Sudan, people collect Homeid fruit for making jam and preparing beverages and selling kernels for snacks or oil pressing. Homeid has multiple uses in Sudan, including the fruits which are eaten fresh, the young leaves, which are used in salad dishes together with onion and groundnut butter, the kernels are eaten or the oil extractedfrom them. Because of the widespread occurrence and use of *Sclerocarya birrea* it has frequently been identified as a desirable species to support the development of rural enterprises based on the fruit or nuts and therefore as a species for potential domestication [9]. Marula oil is also reputed to have medicinal uses. It is used as a balm to treat ear, eye, and nose problems, especially in children. It can also be used to treat coughs, diarrhea, headaches and wounds when applied topically. The oil has also been reported to have food-preservative properties, and it has been used for dripping onto meat before it is air-dried for long-time storage [10].

The plant seeds contain tocopherols and tocotrienols, which are used as natural antioxidants and a source of vitamin E [11, 12]. In nature four different derivatives of tocopherols and tocotrienols (α-, β-, γ-, and δ) can be found, which differ in the methylation of the chroman ring. The antioxidant activity increases for tocopherols and tocotrienols in the order α to δ, whereas the biological activity is inversely proportional to the antioxidant activity [13]. Phenolic compounds are among the most important naturally occurring antioxidants present in plant-based foods. A major portion of naturally occurring phenolic compounds include free and bound forms of polyphenols, phenolic acids and flavonoids [14]. Various classes of flavonoids and carotenoids show quite different patterns of variation during ripening, achieving their maximum level at different harvesting dates [15]. The amount and composition of phenolic compounds in virgin olive oil depends on several factors such as olive cultivar, degree of maturation and agronomic and technological aspects of production [16]. Phenolic compound content is an important parameter for the evaluation of virgin olive oil quality as phenols largely contribute to oil flavor and taste as long as it is protected from autooxidation [17]. Phenolic compounds have possible beneficial pharmacological and antioxidative properties and have been reported to reduce the risk of cardiovascular disease, carcinogenesis, thrombotic tendency, and inflammation [18]. Flavonoids have long been recognized as possessing anti-inflammatory, antiallergenic, antiviral and antiproliferative activities [19, 20]. The antimicrobial effectiveness of fatty acids and their monoglycerides against *Salmonella typhimurium* and *Staphylococcus aureus*, *Chlamydia trachomatis* and food-borne molds *Aspergillus* spp. and *Penicillium* spp. have been reported [21–24].

There is still a need to expand the supply of processed goods with added value to wider markets locally, nationally, and internationally. There is an indication that marula products offer a promising economic alternative for the people in the rural areas of Sudan (Kordofan and Darfur states) and Africa as a whole. The cash injection earned from selling fresh marula products comes at a particularly crucial time of the year, when money is required for school fees, uniforms and books. Despite the accelerating interest in the commercialization prospects for *S. birrea* fruits, no data has been published about the fatty acid, tocopherol, and phenolic contents as well as the antimicrobial effects of *Sclerocarya birrea* kernels during harvesting. The aim of the present study was to provide basic information of changes occurring in the fatty acid, tocopherol and phenolic compounds composition, together with antimicrobial activity of developing *Sclerocarya birrea* kernels at different harvesting dates, and to contribute to a better definition of the optimal harvesting time in relation to the final kernel composition.

Materials and Methods

Materials

All solvents used were of analytical grade: *n*-hexane, *n*-heptane, diethyl ether, ethanol and methanol were acquired from Merck, Darmstadt, Germany.

Fruits (2.0 kg representing big, medium and small size fruits) of *Sclerocarya birrea* were collected manually from one tree from the orchard of the Faculty of Agriculture, University of Western Kordofan, Al-Fola, North Kordofan state, Sudan. The fruits were collected in the period between March and June 2006 at 15-day intervals, and each individual interval was considered as the harvesting date (SBK1–SBK8).

Following harvesting from the trees, the fruits were sun dried (day/night temperature regimes were maintained at 25/18 ± 2 °C (cool) and 34/25 ± 2 °C (warm). The average number of sunlight hours/day was 9.8–11.1 h, relative humidity varied from 18 to 21%, while the temperature varied from 25–34 °C (from January to June). The fruits were placed on plastic trays which, were placed on blocks to allow better air movement around the fruits. The trays were covered with cheesecloth to help protect the fruits from birds and insects. Fruits dried in the sun were brought under shelter at night. The outer dried tissue surrounding the pith of the fruit was removed and the nuts (seeds) were dehulled (decorticated) using a vice model

2XFRONT (Heuer, Germany), the kernels obtained were stored at 4 °C until further investigation.

Methods

Oil Extraction

The stored kernels were crushed and ground using a grinding mill (Petra electric, Burga, Germany). The oil was extracted from the ground material by extraction with *n*-hexane at 50–60 °C in a Soxhlet apparatus for 6 h. following the AOCS method [25]. The oil content was determined as a percentage of the extracted oil to the sample weight (w/w). The samples were analyzed in triplicate, and then means and the standard deviations were calculated. The oil obtained was stored at 4 °C for further investigation.

Fatty Acid Composition

The overall fatty acid composition of the investigated *Sclerocarya birrea* kernels was determined following the ISO draft standard [26]. In brief, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 μL 2 M sodium methanolate in methanol was added, and the closed tube was agitated vigorously for 1 min. After addition of 100 μL of water, the tube was centrifuged at 4,500×*g* for 10 min and the lower aqueous phase was removed. After that 50 μL 1 M HCl were added to the heptane phase, the two phases were mixed for a short time and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulfate (monohydrate, extra pure, Merck, Darmstadt, Germany) was added, and after centrifugation at 4,500×*g* for 10 min the top *n*-heptane phase was transferred to a vial and injected into a Varian 5890 gas chromatograph with a capillary column, CP-Sil 88 (100 m long, 0.25 mm ID, film thickness 0.2 μm). The temperature program was: from 155 °C heated to 220 °C (1.5 °C/min.), 10 min isotherm; injector 250 °C, detector 250 °C; carrier gas 1.07 mL/min hydrogen; split ratio 1:50; detector gas 30 mL/min hydrogen; 300 mL/min air and 30 mL/min nitrogen; manual injection volume less than 1 μL. The integration software computed the peak areas and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization.

Tocopherols

For determination of tocopherols a solution of 250 mg of *Sclerocarya birrea* kernel oil (SBKO) in 25 mL *n*-heptane was directly used for the HPLC. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm) and a D-2500 integration system. Twenty microliters of the samples were injected by a Merck 655-A40 Autosampler onto a Diol phase HPLC column 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 mL/min. The mobile phase used was *n*-heptane/*tert*-butyl methyl ether (99 + 1, v/v) [27].

Extraction of Phenolic Compounds

The phenolic compounds were extracted following the method of Tsimidou et al. [28]. In brief: 50 g oil were dissolved in 50 mL petroleum ether, and then extracted three times with 30 mL of a mixture consisting of methanol:water (60:40 v/v). The three extracts were combined and treated once with 50 mL petroleum ether. The solvent was evaporated to dryness in a rotary evaporator (Büchi, Switzerland) at 40 °C.

Total Phenol Content

The total phenol content (TPC) extracts of SBKO obtained at different harvesting dates was determined by the Folin–Ciocalteau spectrophotometric method at 765 nm (Shimazu, Co., Ltd., Kyoto, Japan), the absorbance was measured after 2 h at 765 nm, the result was calculated as garlic acid equivalent (mg GAE/kg oil) [29]. The measurements were repeated three times. For the replicated samples, relative standard deviation (RSD) was reported.

Total Flavonoids

The total flavonoid content was determined following Chang et al. [30]. Briefly, 5 mL of 2% aluminum trichloride ($AlCl_3$) (Labosi, Paris, France) in methanol (Fluka Chemie, Switzerland) was mixed with the same volume of oil (0.01 or 0.02 mg/mL). Absorption readings at 415 nm with a spectrophotometer (Shimadzu Corp. Kyoto, Japan) were taken after 10 min against a blank sample consisting of a 5 mL oil solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with rutin (Sigma-Aldrich Chemie, Steinheim, Germany) (0–50 mg/L) as the standard. The mean of three readings was used and expressed as mg of rutin equivalents (RE)/100 g of oil.

Antimicrobial Activity of *Sclerocarya birrea* Kernel Oil SBKO

The antimicrobial activity of SBKO extract was evaluated using 2 Gram-positive bacteria, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29

and 2 Gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. All bacterial strains were obtained from the Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, Serdang, Malaysia.

Disc Diffusion Method

The antibacterial effect of *Sclerocarya birrea* kernel oil on four strains of 2 Gram-positive bacteria, Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis* B29 and 2 Gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis* was performed by a standard disc diffusion technique following the method of Sahoo et al. [31]. A population of approximately 7.0 log CFU of each strain was inoculated onto duplicate plates containing antibiotic medium one agar (AM 1, Difco) by using sterile cotton swabs. The plates were allowed to dry at room temperature for 15 min. Paper discs of 6-mm diameter (Becton Dickinson Microbiology Systems, Maryland) were sterilized by autoclaving in a dry Petri dish. Ten microliter each of *Sclerocarya birrea* kernel oil and Streptomycin (10-μg disc) was added to separate paper discs and allowed to dry for 15 min. The density of the bacterial suspension was standardized by standard method and the concentrations of the cultures were adjusted turbidometrically at wavelength of 600 nm to 500,000–1000,000 Colony Forming Unit per mL (CFU mL). The *Sclerocarya birrea* kernel oil was dissolved in ethanol which was previously tested for antimicrobial activity against all test bacteria and found to have no antimicrobial activity. The extract was diluted to a concentration of 100 mg/mL and finally sterilized by filtration using 0.45 μm millipore filters. The discs were then placed aseptically over the bacterial cultures on AM1 plates, and incubated at 37 °C for 24 h. Sterile blank paper discs impregnated with sterile water served as a negative control. After incubation for 24 h, the zones of inhibition around the paper discs were measured accurately using a metric ruler to the nearest millimeter. Discs impregnated with each treatment and control was assayed on duplicate AM1 plates for each bacterial strain. The experiment was replicated three times and the data were analyzed using the mixed procedure of the Statistical Analysis Software (SAS Institute, Inc., Cary, NC) to determine significant differences ($P < 0.01$) in the antibacterial effects of SBKO and Streptomycin on four bacteria stains.

Statistical Analysis

Each value is a mean of three replications. Values of different parameters were expressed as the means ± standard deviations (mean ± SD). The discussion is based on the one-way analysis of variance (ANOVA; $P < 0.05$). All statistical analyses were performed using the SPSS of the windows statistical package (Release 8.0).

Results and Discussion

Oil Content

At the earliest dates of harvesting, the fruit of *Sclerocarya birrea* were small lemon-like and green in color, the fruits then grew rapidly over a 3-month period. During this period, the fruit increased in size and just before and during ripening noticeable changes occurred. The fruit changed in color from green to a mixture of green and pale yellow, denoting the onset of the ripening phase, with a noticeable mango-like flavor.

Previous studies [3, 5] showed that the oil content of *Sclerocarya birrea* was 53–63.0%. A quantitative increase in the content of oil content was observed as maturity progressed (Table 1). The oil content for different harvesting dates was significantly different ($P < 0.05$), at the last harvesting date, SBK8, the oil content of the kernel was 63.0% on a dry weight basis. The weight of the kernel increased significantly ($P < 0.05$) from 0.05 g at SBK1 to 0.32 g at SBK8, i.e., the last harvesting date. The kernel weight at the last harvesting date (0.32 g) is comparable to the 0.34 and 0.36 reported for South African and Namibian samples, respectively [32].

Changes in Individual Fatty Acids during Harvesting

Seed oils are composed primarily of triacylglycerols (TAGs), which are glycerol esters of fatty acids. The primary fatty acids in the TAGs of oilseed crops are 16–18 carbons in length and contain 0–3 double bonds. Palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) were predominant in *Sclerocarya birrea* kernel oil. The percentage of total fatty acids in *Sclerocarya birrea* kernels at different harvesting dates were analyzed (Table 2). The development of total fatty acids of SBKO over different harvesting dates are reported for the first time in this study, from Table 2, palmitic acid (16:0) was found to be 16.8% at the first harvesting date (SBK1) and dropped for the rest of the harvesting dates to reach 14.6% by the end of the last harvesting (SBK8). Stearic acid 18:0 was found to be 15.2% at the first date (SBK1) and dropped dramatically for the rest of the harvesting dates to reach 8.8% by the last harvesting, while oleic and linoleic acids increased from 58.9 and 4.3% to 67.3 and 5.9%, respectively. Rao et al. [33] reported an increase in linolenic and oleic acids of flax seeds at different developmental stages and then become steady until

Table 1 Kernel weight (g) and oil contents (%), total phenolics and total flavonoids of *Sclerocarya birrea* kernels (SBK) at various harvesting dates

Collecting time	Harvesting date code	Weigh g/kernel	Oil content (%)	Total phenolics (GAE)[a]	Total flavonoids (RE)[a]
15 March	SBK1	0.05 ± 0.2[a]	23.0 ± 0.21[a]	0.17 ± 0.21[a]	12.2 ± 0.32[a]
30 March	SBK2	0.08 ± 0.3[b]	25.0 ± 0.24[b]	0.19 ± 0.22[a]	14.5 ± 0.35[b]
15 April	SBK3	0.11 ± 0.5[c]	34.0 ± 0.32[c]	0.20 ± 0.20[b]	15.4 ± 0.61[b]
30 April	SBK4	0.15 ± 0.4[d]	37.0 ± 0.41[d]	0.22 ± 0.23[b]	16.0 ± 0.35[b]
15 May	SBK5	0.21 ± 0.6[e]	42.0 ± 0.65[e]	0.24 ± 0.22[c]	18.8 ± 0.40[c]
30 May	SBK6	0.27 ± 0.3[f]	50.0 ± 0.71[f]	0.25 ± 0.21[c]	21.4 ± 0.43[d]
15 June	SBK7	0.30 ± 0.6[g]	56.0 ± 1.2 [g]	0.26 ± 0.3[c]	22.2 ± 0.44[d]
30 June	SBK8	0.32 ± 0.5[h]	63.0 ± 1.6 [h]	3.57 ± 0.52[d]	22.6 ± 0.44[d]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) reported

Values in the same column with different superscript letters are significantly different ($P < 0.05$)

[a] The total phenolics are expressed in garlic acid equivalents mg/100 g oil, total flavonoids are expressed in rutin equivalents mg/100 g oil

Table 2 Change in fatty acid composition (%) of *S. birrea* kernel oil (SBK) at different harvesting dates

Fatty acid	SBK1	SBK2	SBK3	SBK4	SBK5	SBK6	SBK7	SBK8
Lauric acid 12:0	0.4 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.3 ± 0.2
Myristic acid 14:0	0.4 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2
Palmitic acid 16:0	16.8 ± 0.2	15.9 ± 0.2	16.1 ± 0.2	15.7 ± 0.2	14.6 ± 0.2	15.2 ± 0.2	14.7 ± 0.2	14.6 ± 0.2
Palmitoleic acid 16:1n-7	1.8 ± 0.2	0.2 ± 0.2	0.6 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Stearic acid 18:0	15.2 ± 0.2	13.6 ± 0.2	11.5 ± 0.2	12.8 ± 0.2	12.6 ± 0.2	13.0 ± 0.2	12.1 ± 0.2	8.8 ± 0.2
Oleic acid 18:1 n-9	58.9 ± 0.2	63.5 ± 0.2	63.4 ± 0.2	64.8 ± 0.2	65.7 ± 0.2	66.1 ± 0.2	67.2 ± 0.2	67.4 ± 0.2
Linoleic acid 18:2n-6)	4.3 ± 0.2	4.1 ± 0.2	5.0 ± 0.2	4.4 ± 0.2	4.1 ± 0.2	2.8 ± 0.2	3.0 ± 0.2	5.9 ± 0.2
Linolenic acid 18:3 n-3)	0.4 ± 0.1	0.3 ± 0.2	0.5 ± 0.2	0.0 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	0.9 ± 0.2
Eicosanoic acid 20:0	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.3	1.2 ± 0.1	1.1 ± 0.1
Behenic acid 22:0	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Lignoceric acid 24:0	0.3 ± 0.2	0.3 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1
SFA	34.6	32.1	30.5	30.7	29.4	30.6	28.9	25.8
USFA	65.4	67.9	69.5	69.3	70.6	69.4	71.1	74.2
Ratio USFA/SFA	1.8	2.1	2.2	2.3	2.4	2.3	2.5	2.9

All determinations were carried out in triplicate and mean values ± standard deviations (SD) reported. The SKB numbers 1–8 are codes for the different maturity stages

SFA saturated fatty acids, *UFA* unsaturated fatty acids

the end of the maturation. While linoleic and palmitic acids content decreased and dropped dramatically during the developmental stages to reach only 10% by the end of the maturation process.

The fatty acid composition of the oil from *Sclerocarya birrea* kernels exhibited greater variation during harvesting dates (SBK1 to SBK8). Although alterations were clear between total saturated and total unsaturated acids, differences were quite pronounced in palmitic, stearic, oleic and linoleic acids (Table 2).

Changes in Tocopherols during Harvesting

Tocopherol isomers and total tocopherol content varied significantly ($P < 0.05$) between various harvesting dates (Table 3). In the first harvesting date all four isomers of tocopherols (α, β, γ, and δ) were observed; however, α-tocopherol was the predominant form, comprising about 68.7% of the total tocopherol concentration. Alpha and gamma tocopherols decreased rapidly, whereas the δ-tocopherol and δ-tocotrienol were 4.8 and 4.9 mg/100 g, respectively, at the beginning and disappeared completely until there was none at the last harvesting date. At the first three harvesting dates (SBK1, SBK2 and SBK3), α-tocopherol was the predominant form of tocopherol, whereas in the subsequent harvesting dates up to the frinal harvesting date (SBK8), the γ-isomer constituted the maximum proportion of the total tocopherol content (Table 3). From this table, significant differences were found within the tocopherol profile among the SBK1 to

Table 3 Tocopherol [mg/100 g] analysis of *Sclerocarya birrea* kernel oil during different harvesting dates

Sample	α –Tocopherol Mean ± SD	γ -Tocopherol Mean ± SD	δ-Tocopherol Mean ± SD	δ-Tocotrienol Mean ± SD	Total amount Mean ± SD
SBK1	115.6 ± 0.6[a]	36.9 ± 0.2[a]	4.9 ± 0.1[a]	4.8 ± 0.1[a]	168.2 ± 0.7[a]
SBK2	71.5 ± 0.4[b]	18.4 ± 0.4[b]	0.0 ± 0.0[b]	2.0 ± 0.2[b]	91.9 ± 0.5[b]
SBK3	14.8 ± 0.2[c]	12.8 ± 0.1[c]	0.0 ± 0.0[b]	0.0 ± 0.0[c]	27.6 ± 0.3[c]
SBK4	1.2 ± 0.2[d]	12.6 ± 0.3[d]	0.0 ± 0.1[b]	0.0 ± 0.0[c]	13.8 ± 0.2[d]
SBK5	0.4 ± 0.1[e]	12.7 ± 0.2[d]	0.6 ± 0.2[c]	0.0 ± 0.0[c]	13.7 ± 0.3[d]
SBK6	0.4 ± 0.0[e]	12.6 ± 0.2[d]	0.0 ± 0.0[b]	0.0 ± 0.0[c]	13.0 ± 0.2[d]
SBK7	0.4 ± 0.1[e]	11.2 ± 0.2[e]	0.0 ± 0.0[b]	0.0 ± 0.0[c]	11.6 ± 0.1[e]
SBK8	0.3 ± 0.1[e]	3.1 ± 0.1[f]	0.0 ± 0.0[b]	0.0 ± 0.0[c]	3.4 ± 0.1[f]

All determinations were carried out in triplicate and mean values ± standard deviations (SD) reported

Values in the same column with different superscript letters are significantly different ($P < 0.05$)

SBK4 harvesting dates of *Sclerocarya birrea*, while no significant differences among the SBK5 to SBK7 harvesting dates. Alpha tocopherol decreased drastically with later harvesting from 115.6 mg/100 g at the first date of harvesting (SBK1) to only 0.3 mg/100 g at the last date of harvesting (SBK8), in the same manner the γ-tocopherol decreased with later harvesting date from 36.9 to 3.1 mg/100 g. Hashim et al. [34] found significant differences in tocopherol content between two peanut cultivars, and these differences were also affected by the date of harvesting. Goffman et al. [35] reported a maximum increase followed by a drastic decrease to zero for tocopherol accumulation in developing pods of rapeseed, while in the seeds, tocopherols accumulated linearly to a maximum content at the mid point of maturity, then remained constant until the end of the maturation process. The decrease in tocopherol with later harvesting can be the result of consumption of tocopherol to protect lipids (an increase in oil content means greater tocopherol consumption) as the major function of the lipophilic tocochromanols in seeds may be the protection of stored lipids from oxidation. However, attempts to verify a positive correlation between the accumulation of tocopherol and tocotrienol and that of lipids or polyunsaturated fatty acids in seeds gave inconsistent results [12].

Changes in Phenolic and Flavonoid during Harvesting Dates

The amount of total phenolic compounds in the oil extracts from *Sclerocarya birrea* kernels was determined by the Folin–Ciocalteau assay for different harvesting dates. The results of this colorimetric method, expressed as gallic acid equivalents are shown in Table 1. The concentrations of total phenolic content and total flavonoid changed significantly ($P < 0.05$) with later harvesting dates. Total phenolics content increased continuously and was at a maximum (3.5 GAE mg/100 g oil on a dry weight basis) at the last harvesting date SBK8. In the same manner the total flavonoids content also increased from 12.2 ± 0.32 at the first harvesting date to 22.6 ± 0.44 mg/100 g oil at the last harvesting date. In contrast, Kumar et al. [36] reported that bioactive constituents other than isoflavones and tocopherols may decline with the advancement of maturity in soybean seeds. While Gimeno et al. [37] reported that olive oil phenols decrease during ripening.

Antimicrobial Activity of *Sclerocarya birrea* Kernel Oil SBKO

Studies on the antibacterial activities of biologically active plants have clearly become an ongoing research topic using different screening methods. The disc diffusion method (DDM) was the first method of choice, possibly due to its simplicity and its ability to analyze a large number of test samples. These could be supported by that many publications utilized DDM as a means of determining antibacterial activity [31, 38]. The growth inhibition zones measured by disc diffusion method are presented in Table 4. The antibacterial activities of oils of *Sclerocarya birrea* kernel during different harvesting dates were evaluated using Gram-positive and Gram-negative bacteria. The solvents used to dissolve the oils of SBK and used alone as the control did not show any antibacterial activity (data not shown). In Table 4, the screening of the oils antibacterial effects was summarized. The average zone of inhibition of *Sclerocarya birrea* kernel oil at different harvesting dates (SBK1-SBK8) against methicillin resistant *S. aureus* (MRSA) ranged from 4.5 ± 0.3 to 10.0 ± 0.5 mm, and against *P. aeruginosa* ranged from 7.0 ± 0.5 to 14.0 ± 1.5 mm, while against *S. choleraesuis* it ranged from 1.2 ± 0.1 to 6.0 ± 0.5 mm. From these results, it was clear that SBKO had an explicit low antibacterial activity towards these three bacterial strains in comparison to

Table 4 Antibacterial activity of *Sclerocarya birrea* kernel oil (SBKO) against different bacterial strains using the disc diffusion method

	Bacterial Strains			
	Diameter of inhibition zone (mm) (Mean ± SD)			
Maturity stages	MRSA	PA	SC	BS
SBK1	5.5 ± 0.5	7.2 ± 0.6	2.2 ± 0.2	–
SBK2	4.5 ± 0.3	8.1 ± 0.4	1.2 ± 0.1	–
SBK3	6.3 ± 1.1	9.3 ± 0.3	3.2 ± 0.2	–
SBK4	10.0 ± 0.5	14.0 ± 1.5	6.0 ± 0.5	–
SBK5	9.4 ± 1.0	7.0 ± 0.5	6.0 ± 0.5	–
SBK6	9.0 ± 1.1	14.0 ± 1.2	–	–
SBK7	7.0 ± 1.1	11.0 ± 1.1	–	–
SBK8	6.3 ± 0.4	9.0 ± 0.4	–	–
Streptomycin	20 ± 1.7	20 ± 1.7	23 ± 2.1	23 ± 2.1

The screening of the extracts antibacterial effect was carried out by determining the zone of inhibition using the paper disc (6 mm in diameter, Whatman No. 1) diffusion method ($n = 3$)

MRSA Methicillin resistant *Staphylococcus aureus*, *PA Pseudomonas aeruginosa*, *SC Salmonella choleraesuis*, *BS Bacillus subtilis*

streptomycin which was used as the control. SBKO showed a broad spectrum activity against all tested bacteria except for *B. subtilis* which showed resistance to all the oils of SBK. The highest antibacterial activity observed in this investigation was observed for oil from SBK4 against MRSA and *P. aeruginosa*, and from SBK6 against *P. aeruginosa*. Both MRSA and *P. aeruginosa*, which are well noted for their insusceptibility to most antibiotics [39], were inhibited by SBK4 and SBK6 with a remarkable activity. The above-mentioned findings give a substantial explanation for the chemical diversity of the oils of SBK collected at different harvesting dates, the antibacterial effect can be related to oil composition and can mainly be related to phenolic compounds as phenolics possess many interesting biological activities. Their antimicrobial activity has gained importance as phenolic berry extracts, for example, inhibit the growth of selected Gram-negative intestinal bacteria and work as strong inhibitors of a non virulent Salmonella strain [40]. The extract from different parts of *Sclerocarya birrea* has been reported to possess antibacterial activity [41]. Although the various bacterial strains varied in their antimicrobial susceptibility, SBKO was seemed to be effective in inhibiting the growth of three of the tested bacterial strains. The inhibitory effect of SBKO on the three bacterial strains was less than that of streptomycin which was used as a positive control in the study because these three bacteria were reported to be highly sensitive to the antibiotic [40]. Results of the study indicate that *Sclerocarya birrea* kernel oil possesses significant antimicrobial activity against three bacterial strains, and experiments evaluating the antifungal properties of *Sclerocarya birrea* kernel oil are under way in our laboratory.

Conclusions

The oil content of *Sclerocarya birrea* kernels increased with later harvesting dates, from 23.0% at the first harvesting date to a value of 63.0% at the last date of harvesting. With early harvesting dates of the *Sclerocarya birrea* kernels, the content of total phenolics and total flavonoids amounted to 0.17 ± 0.21 and 12.2 ± 0.32, and then increased sharply to reach 3.57 ± 0.52 and 22.6 ± 0.44 mg/100 g oil, respectively. The palmitic and stearic acids decreased when harvesting of *Sclerocarya birrea* kernel was later, while oleic and linoleic acids increased. Significant differences were found within the tocopherol profile among harvesting dates of *Sclerocarya birrea*, where both α-tocopherol and γ-tocopherol decreased drastically with later harvesting dates. *Sclerocarya birrea* kernel oil possesses significant antimicrobial activity against three bacterial strains.

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