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ORIGINAL PAPER

Chemical Characterization of the Seed and Antioxidant Activity of Various Parts of *Salvadora persica*

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Abstract This study investigated the fatty acid, tocopherol, sterol and total phenolic compounds of *Salvadora percica* seeds as well as the potential antioxidant activity of the leaves, bark and seedcake extracts. Two samples of *S. persica* seed collected from Kordofan (sandy soil) and Gezira (heavy clay soil) states in Sudan were used. The predominant fatty acids were 14:0, 16:0 and 18:1 representing 45.50, 35.12 and 10.20% for Kordofan and 45.20, 34.49 and 10.66% for Gezira samples. Gamma-tocopherol was the predominant tocopherol in both samples representing 61.3 and 61.7% of the total tocopherols, respectively, followed by α-tocopherol at 21.1 and 20.2%, respectively. Total sterol content was 3399.6 and 3385.3 mg/kg for Kordofan and Gezira samples, respectively. Beta-sitosterol, campesterol, stigmasterol and Δ5-avenasterol were predominant. The content of total phenolic compounds was determined in *S. persica* bark (SPB), *S. persica* leaves (SPL), and *S. persica* seedcake (SPC) extracts of each sample according to the Folin–Ciocalteau method as 111.70, 132.60, and 66.10 mg GAE/g extract for the Kordofan sample. They were found to be 105.90, 129.10 and 62.90 mg GAE/g extract in the Gezira sample, respectively. The two samples were significantly ($P < 0.05$) different in total phenolic content with SPL as the highest in both samples. The methanolic extracts of SPL, SPB, and SPC in both samples were markedly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β-carotene in comparison with the control. But they were less effective than butylated hydroxyanisole.

Keywords Antioxidant activity · Fatty acid · Oxidative stability · *Salvadora persica* · Sterols · Tocopherols

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Introduction

Salvadora persica L. of the family Salvadoraceae is an evergreen shrub, known in Sudan as Arak, 4–6 m tall with a short trunk, white bark and smooth green leaves [1]. The plant, with a life span of 25 years, is popularly used as tooth brush material in Sudan and other Arab countries. It is widely scattered in the poor savanna areas in Western Sudan as a range crop having immense medicinal value with numerous uses. *S. persica* has been shown to contain trimethylamine and salvadorine which have antibacterial effects [2]. It also contains traces of tannins, saponins, flavonoids and sterols [3].

Arak tannic acid is a good blood anticoagulant. Arak oil can be used as a cure for joint pain, gall bladder diseases, polio, piles and gonorrhea, and intestinal worm problems. The oil has great potential for making soap and candles and can be used as a substitute for coconut oil [4]. *S. persica* seed contains about 40% oil with a fatty acid composition (lauric −20%, myristic −55%, palmitic 20% and oleic −5%) which can make an excellent soap. The oil is inedible

because of the presence of various substituted dibenzyl ureas. The oil also is used for making medicinal preparations and suppositories and curing rheumatic pains [5]. Mice injected with *S. persica* extracts showed significantly low exploratory locomotor activity and lower number of stereotype movements [6]. Many studies have demonstrated the antibacterial, anti-caries, anti-periopathic, and anti-fungal properties of aqueous extracts of various chewing sticks [7]. Radical reactions in vivo can damage life-essential molecules such as nucleic acids and proteins [8]. Phenolic compounds, particularly flavonoids, have been shown to possess an important antioxidant activity towards these radicals, which is principally based on their structural characteristics (number and position of phenolic hydroxyls, other groups, conjugation) [9]. This study investigated the fatty acid, tocopherol, sterols, and oxidative stability of *S. persica* oil and the antioxidant activity of leaves, bark and seedcake phenolic extracts of *S. persica.*

Materials and Methods

Materials

All solvents used were of analytical grade, including *n*-hexane, *n*-heptane, ethanol, methanol (HPLC grade), chloroform, Folin Ciocalteau phenol reagent and polyoxyethylene sorbitan monopalmitate (Tween 40) and were obtained from Merck (Darmstadt, Germany).

Methods

Sample Preparation

Salvadora persica dried leaves, bark and seeds were purchased from local stores in Medani, Gezira state and Ghibaish, Kordofan state, Sudan. Samples were cleaned under running tap water for 5 min, rinsed twice with distilled water and air-dried in an oven at 40 °C overnight. The leaves, bark and seeds were ground to a powder using an electric grinder (Petra electric, Burgau, Germany) for 10 min and then passed through a 35 mm (42 mesh) sieve which gave fine and homogenous SPL and SPB samples which were weighed and from which the phenolic compounds were extracted.

Oil Extraction

The oil was extracted from the ground seeds by extraction with petroleum ether in a Soxhlet apparatus for 6 h following the AOCS method [10]. The oil obtained was stored at 4 °C until further investigation. Drying the residue under a vacuum at 40 °C for 30 min led to a fine and homogenous fat-free powder (*S. persica* seedcake SPC) which was weighed and from which the phenolic compounds were extracted.

Fatty Acid Composition

The fatty acid composition of *S. persica* oil was determined following the ISO draft standard [11]. Briefly, one drop of the oil was dissolved in 1 mL of *n*-heptane, 50 μL 2 M sodium methanolate in methanol were 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 shortly mixed and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure, Merck, Darmstadt, Germany) were added, and after centrifugation at 4,500*g* for 10 min the top *n*-heptane phase was transferred into a vial and injected in 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 (TOC)

The tocopherol composition of *S. persica* oil was determined following Balz et al. [12]. A solution of 250 mg of *S. persica* oil 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 an L-6000 pump, a Merck-Hitachi F-1000 Fluorescence Spectrophotometer (The detector wavelength was set at 295 nm for excitation, and at 330 nm for emission) and a D-2500 integration system; 20 μL 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).

Sterols

The sterol composition of *S. persica* oil was determined following ISO draft standard [13]. In brief, 250 mg of oil was saponified with a solution of ethanolic potassium

hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column (Merck, Darmstadt, Germany) on which fatty acid anions were retained and sterols passed through. The sterol fraction from the unsaponifiable matter was separated by thin-layer chromatography on silica gel 20 × 20 cm, thickness of layer 0.25 mm using hexane/diethyl ether (1/1, v/v) as developing solvent (Merck, Darmstadt, Germany), reextracted from the TLC material and afterwards the composition of the sterol fraction was determined by GLC using betulin as the internal standard. The compounds were separated on an SE 54 CB column (Macherey-Nagel, Düren, Germany) (50 m long, 0.32 mm ID, 0.25 μm film thickness). Further parameters were: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature programme: 245 °C to 260 °C at 5 °C/min.

Oxidative Stability

The oxidative stability of *S. persica* oil was determined by the Rancimat method [14]. All experiments were carried out with a 743 Rancimat (Methrom AG, Herisau, Switzerland). In brief, 3.6 g oil was weighed into the reaction vessel, which was placed into the heating block kept at 120 °C. The air flow was set at 20 L/h for all determinations. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL distilled water. The conductivity of the distilled water was measured and recorded. The software of the Rancimat evaluated the resulting curves automatically. All determinations were carried out in duplicate.

Phenolic Compounds Extraction

Following the method of Silvia et al. [15] the phenolic compounds were extracted three times by sonication (Hwasin Technology, Seoul, Korea) using 150 mL of methanol/water (60:40, v/v) from 15 g air-dried, ground *S. persica* leaves, bark, and seedcake to obtain methanolic extracts (SPL, SPB and SPC, respectively) with solid to solvent ratio of 1:10 (w/v) at room temperature for 1 h. The extracts obtained were filtered through filter paper Whatman No 1. Then combined and concentrated by removing methanol by a rotary evaporator (Buchi, Flawil, Switzerland). The yield of each extract was measured before being kept at –80 °C for further analysis.

Determination of the Total Phenolic Compounds

The total phenolic compounds (TPC) in SPL, SPB and SPC methanolic extracts were determined following the method of Silvia et al. [15]. Dried samples (5 mg) and gallic acid as standard were diluted in 1.0 mL of 60:40 acidified methanol/water (0.3% HCl). Test solutions (samples or standards) of 100 μL were added to 2.0 mL of 2% Na_2CO_3. After 2 min, 100 μL of 50% Folin–Ciocalteau reagent were added and allowed to stand at room temperature for 30 min. A blank consisting of all reagents and solvents without extracts or standard was prepared. Absorbance was measured against the blank at 750 nm by a U-2000 Spectrophotometer (Hitachi, Ltd, Tokyo, Japan). The standard solution was prepared from gallic acid in concentrations of 0.05–0.4 mg/mL. The concentration of phenolic compounds in the extracts was calculated from a calibration curve of the standard, and expressed as gallic acid equivalents.

Determination of the Antioxidant Activity

β-Carotene–Linoleic Acid Assay

The antioxidant activity of the bark, leaves and the seed cake phenolic extracts of *S. persica* was evaluated using the *β*-carotene–linoleic acid assay [16]. 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 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 2 mg of each extract or 0.2 mg of butylated hydroxyanisole (BHA). The samples were put in a water bath at 50 °C over a period of 120 min. Subsequently; the absorbance was read at 470 nm, every 15 min. All samples were assayed in duplicate.

DPPH Method

Phenolic extracts of the leaves, bark and seed cake of *S. persica* were analyzed for antioxidant activities following the method of Gordon et al. [17]. Essentially, the hydrogen donating or radical scavenging ability was measured using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). A methanolic solution (100 μL) of the phenolic compounds extracted from the leaves, bark, and seedcakes was placed in a cuvette and 0.5 mL of a methanolic solution of DPPH (50 mg DPPH/100 mL MeOH) was added. Ascorbic acid, a known antioxidant, was used as a positive control. After incubation in darkness for 30 min and at ambient temperature (23 °C), the resultant absorbance was recorded at 515 nm. The decrease in absorbance at 515 nm was determined using a spectrophotometer (Hitachi, LTD, Tokyo, Japan). The absorbance of the DPPH radical without antioxidant, i.e. the control was measured. The

data is commonly reported as IC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period. All determinations were performed in triplicate.

Oxidation of Sunflower Oil in the Presence of *S. persica* Extract

The dried extracts of *S. persica* leaves, bark and seed cake were applied to sunflower oil at levels of 500, and 1,000 mg to examine their antioxidative activity. BHA was used for comparison at a level of 200 ppm. The dried extracts as well as the synthetic antioxidant were mixed with a minimum amount of absolute methanol in an ultrasonic water bath (Bandelin electronic, Berlin, Germany) and added to 100 g oil before mixing again for 10 min. A control sample was prepared using the same amount of methanol used to dissolve BHA and the extracts [18]. The peroxide value (PV) was used as indicator for the primary oxidation of the sunflower oil. The PVs were determined every 4, 8, 16, 24, 48, 72 and 96 h.

Statistical Analysis

The analyses were performed with three replicates. The mean values and standard deviation (means ± SD) were calculated and tested using Duncan's test ($P < 0.05$). Statistical analysis of variance (ANOVA) was performed on all values using the statistical program Statgrafics® Statistical Graphics System version 4.0 [19].

Results and Discussion

Oil Content, Fatty Acid, Tocopherol and Sterol Composition

The oil content of *Salvadora* seeds collected from Kordofan (sandy soil) and Gezira (heavy clay soil) states, Sudan, was found as 41.4 and 42.8% (Table 1), respectively, is comparable with that reported by FAO [4] which, reported less oil in the seeds (40%), and Reddy et al. [20] which showed high oil content 44.78–45.50% for seeds grown in alkali soils, and 43.28–44.36% for seed grown in saline soils. The oil content we showed in this study is less than the oil content reported by Tripathi and Rathore [21]. The oil content of the two samples was significantly different at $P < 0.05$. On the whole, the oil content of *S. persica* seeds is very high in comparison to common oil sources found in Sudan such as groundnut, sesame, cotton seed, and caster bean with oil contents between 20 and 45% [22, 23]. Therefore, from an economical point of view, the production of industrial oil from seeds of *S. persica* could be interesting.

Table 1 Oil content (%) and fatty acid composition (%) of *Salvadora persica* seed oil

Sample fatty acids	Kordofan mean ± SD	Gezira mean ± SD
12:0	3.31 ± 0.01	3.32 ± 0.02
14:0	45.50 ± 0.02	45.20 ± 0.02
16:0	35.12 ± 0.21	34.49 ± 0.32
18:1	10.20 ± 0.20	10.66 ± 0.12
20:0	0.70 ± 0.11	0.70 ± 0.22
20:1	3.82 ± 0.10	4.27 ± 0.07
22:0	0.89 ± 0.04	0.89 ± 0.06
24:0	0.46 ± 0.30	0.47 ± 0.20
∑SFA	85.98 ± 0.22	84.07 ± 0.11
∑MUFA	14.02 ± 0.10	15.93 ± 0.11
Ratio SFA/UFA	6.13 ± 0.21	5.27 ± 0.21
Oil content (g/100 g of seed)	41.4 ± 0.60	42.8 ± 0.50

All determinations were carried out in triplicate and mean values ± SD reported

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *UFA* unsaturated fatty acids

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) of crude *S. persica* seed oil are shown in Table 1. The major fatty acids were myristic acid ($C_{14:0}$) followed by palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$), representing 45.5, 35.1 and 10.2%, respectively, in oil extracted from the Kordofan sample, and 45.5, 34.4 and 10.6 in the Gezira sample. Besides these three main fatty acids, five more were identified and quantified in both samples. SFA was the main group of fatty acids, representing 85.9 and 84.0% in Kordofan and Gezira samples respectively, followed by MUFA 14.0 and 15.9%, respectively. The ratios of saturated/unsaturated acid are 6.1 and 5.2 respectively, which is very high because of the high level of saturated fatty acid such as C_{14}, and C_{16}. The fatty acid profile of *S. persica* oil is consistent with results published by other workers [4, 20, 21].

As further important criteria for the assessment of seed oils, the contents and composition of tocopherols, tocotrienols, and plastochromanol-8 (P-8) were determined; these data are presented in Table 2. The tocopherol content of *S. persica* seed oil showed a medium amount of tocopherols 46.3 and 45.7 mg/100 g in Kordofan and Gezira samples respectively, which was higher than the tocopherol amount of groundnuts oil (27.9 mg/100 g), and lower than the tocopherol amount of other common oils studied by Mariod et al. [23] such as sesame oil (64.7 mg/100 g),

Table 2 Tocopherol composition (mg/100 g) of *Salvadora persica* seeds from two Sudanese states

Sample tocopherol	Kordofan mean ± SD	Gezira mean ± SD
α-T	9.76 ± 0.12[e]	9.21 ± 0.13[e]
γ-T	28.23 ± 0.15[f]	28.20 ± 0.21[f]
β-T	0.72 ± 0.22[b]	0.80 ± 0.05[b]
P-8	1.99 ± 0.11[c]	1.95 ± 0.11[c]
γ-T_3	5.4 ± 0.11[d]	5.4 ± 0.20[d]
δ-T	0.17 ± 0.03[a]	0.20 ± 0.11[a]
Total amount of tocopherol	46.27 ± 0.11 [g]	45.76 ± 0.11 [h]

T tocopherol, T_3 tocotrienol, *P-8* plastochromanol-8

The superscript lettering indicates the statistical significance of differences at $P < 0.05$, the values in rows followed by the same letter are not significantly different. All determinations were carried out in triplicate and mean values ± SD reported

Table 3 Distribution of sterols (mg/kg oil) in *Salvadora persica* seed oil

Sample sterol	Kordofan mean ± SD	Gezira mean ± SD
Cholesterol	56.1 ± 0.11	57.3 ± 0.11
Campesterol	458.0 ± 0.20	460.0 ± 0.20
Stigmasterol	435.7 ± 0.32	448.7 ± 0.32
β-Sitosterol	2156.6 ± 0.51	2122.6 ± 0.52
Δ5-Avenasterol	134.0 ± 0.41	138.0 ± 0.42
Δ7-Avenasterol	50.0 ± 0.42	50.0 ± 0.43
Δ7-Campesterol	26.8 ± 0.41	26.2 ± 0.42
24-Methylenecholesterol	10.4 ± 0.12	10.0 ± 0.11
Δ7-Stigmasterol	72.0 ± 0.11	72.5 ± 0.13
Total	3399.6 ± 0.62	3385.3 ± 0.60

All determinations were carried out in triplicate and mean values ± SD reported. The superscript lettering indicates the statistical significance of differences at $P < 0.05$, the values followed by the same letter are not significantly different. All determinations were carried out in triplicate and mean values ± SD reported

cotton seed oil (77.8 mg/100 g) and sunflower oil (97.6 mg/100 g).

Tocopherols are particularly important functional constituents of the unsaponifiable fraction of vegetable oils. These compounds display antioxidant properties and are active as vitamin E, which makes them particularly important for human health [24]. The main tocopherols of *S. persica* seed oil in the two samples were γ-tocopherol, α-tocopherol which, amounted to about 28.2, 9.76 and 28.2, 9.2% of the total tocopherols in Kordofan and Gezira samples, respectively.

Tocopherols were obviously the predominant group of vitamin E-active compounds in most of the seed oils. Nevertheless, some of the seed oils contained remarkable amounts of tocotrienols. In *S. persica* seed oil, instead of tocopherols appropriate unsaturated derivative γ-tocotrienol was found in an amount of 5.4 mg/100 g in the two samples (Table 2).

Phytosterols are of a great interest because some of them have antioxidant activity and an impact on health [25]. They are key components of the unsaponifiable matter of vegetable oils and fats. They represent 0.3–2% of the oil but it can reach more than 10% in certain plants [26]. The analysis of the sterols provides rich information about the quality and the identity of the oil investigated, and for the detection of oil and mixtures not recognized by their fatty acids profile [27]. This fraction has been considered as the major unsaponifiable fraction in many oils [28].

The values for sterols of both two *S. persica* seed oils are presented in Table 3. The most predominant sterol of the oil from two samples was β-sitosterol followed by campesterol and stigmasterol. Among the different phytosterols, sitosterol had been most intensively investigated with respect to its beneficial and physiological effects on health [29]. The next major components in both *S. persica* seed oils studied were campesterol constituting 13.5% in the two samples and stigmasterol constituting 12.8 and 13.3% of total sterols in Kordofan and Gezira, respectively.

In comparison to other oils (sesame, sunflower and cotton seed oils) usually used in human nutrition except groundnut, the oil of *S. persica* has lower amounts of total sterols. Mariod et al. [23] reported amount of 7,740, 2,940, 4,170 and 4,920 mg/kg for sesame, ground nut, sunflower and cotton seed oil, respectively. A remarkable high amount of Δ5-avenasterol was found in the oil of *S. persica* which came to 3.9 and 4.0% of the total sterols, in Kordofan and Gazira sample, respectively. This sterol class is known to act as an antioxidant and as an antipolymerization agent in frying oils [28]. Those authors White and Armstrong [28] pointed out that those sterols with an ethylidene group in the side chain are most effective as antioxidants and suggested that a synergistic effect of the sterols with other antioxidants may occur. The oxidative stability of *S. persica* oil is expressed as the induction period determined by the Rancimat method at 120 °C. The oil from Kordofan and Gezira samples showed a remarkably medium stability (3.5 and 3.1 h, respectively). These values are to some extent high in comparison to other edible oils (sesame and sunflower), but in comparison to other more stable oils such as rapeseed or olive oil *S. persica* oil has a low oxidative stability. The oxidative stability of such commonly used oils is 1.6 h for sesame oil, 5.4 h for sunflower oil, 4.0 h for peanut oil, 2.4 h for sunflower oil, 5.2 h for rapeseed oil and 3.7 h for olive oil [23, 29].

Total Amount of Phenolic Compounds

The results of using methanol as the solvent for the extraction of phenolic compounds from different parts (*S. persica* bark SPB, *S. persica* leaves SPL, *S. persica* seedcake SPC) of *S. persica* that were collected from the Kordofan and Gazira states of Sudan are given in Table 4. From this table it is evident that *S. persica* contains noticeable amounts of extractable compounds, and that the highest amount of total extractable compounds (TEC) was extracted from SPC followed by SPL and SPB in both samples. From Table 4 the TEC was found to be 405.66, 333.53 and 216.46 mg/g for SPC, SPL and SPB in the Kordofan sample, respectively, while it was found to be 402.90, 330.80 and 211.80 mg/g for SPC, SPL and SPB in the Gazira sample.

Table 4 shows the results of total phenolic compounds of the methanolic extract from different parts of *S. persica*, these results show that SPL has the highest amount of total phenolic compounds 132.60 and 129.10 mg GAE/g extract in Kordofan and Gazira samples, respectively, followed by SPB and SPC and were significantly different at $P < 0.05$. It becomes clear that the total phenolic compounds were not related to the total extractable compounds. The relationship between the total extractable compounds and the content of phenolic compounds was represented in percentages of TPC/TEC. The total phenolics found in the total extractable compounds were high in SPB and low in SPC. The ratio of total phenolic compounds to the total extractable compounds ranged from 16.3 to 51.6% in the Kordofan sample and from 15.4 to 49.7% in the Gazira sample (Table 4). From these results it can be understood that in SPB, SPL and SPC of the Kordofan sample more than 49.4, 60.3 and 83.7% of the extractable compounds, respectively, were compounds other than phenolic compounds (Table 4). While in SPB, SPL and SPC of the Gazira sample more than 50.3, 61.0 and 84.6% of the extractable compounds, respectively, were compounds other than phenolic compounds.

Antioxidant Activity

DPPH Method

DPPH and β-carotene/linoleic acid methods were used to evaluate the antioxidant activity of *S. persica* extracts. The antioxidant activity of the extracts in sunflower oil was assessed using the peroxide value in comparison with a synthetic antioxidant. The DPPH radical has been widely used to test the free radical scavenging ability of different plant extracts [30–32]. The DPPH scavenging activities of different extracts of *S. persica* from Kordofan and Gazira are shown in Table 4. The DPPH values for investigated extract from different parts of *S. persica* were expressed as IC_{50}; the IC_{50} values for SPB, SPL and SPC from Kordofan were 1.73, 1.49, and 1.87, respectively. The DPPH values for SPB, SPL, and SPC from Gazira were 1.70, 1.43, and 1.82, respectively. Samples from Kordofan and Gazira were comparable concerning TEC, TPC, and IC_{50}. From Table 4, a significant correlation ($P < 0.05$) was found between the TPC and IC_{50} when the TPC level was high; the IC_{50} was low which indicates high antioxidant activity. Several studies showed a correlation between antioxidant activity and phenolic content [33, 34].

Table 4 Total extractable compounds (TEC) and total phenolic compounds (TPC) (expressed as gallic acid equivalents (%) in *Salvadora persica* extracts

Sample	TEC (mg/g)	TPC (mg GAE/g extract)	TPC/TEC (%)	IC_{50} (μg/ml)
Kordofan				
SPB	216.46 ± 0.12[a]	111.70 ± 0.21[c]	51.6	1.73 ± 0.13[c]
SPL	333.53 ± 0.21[b]	132.60 ± 0.50[d]	39.7	1.49 ± 0.12[b]
SPC	405.66 ± 0.31[c]	66.10 ± 0.32[a]	16.3	1.87 ± 0.11[d]
Ascorbic acid				0.02 ± 0.01[a]
Gezira				
SPB	211.80 ± 0.40[a]	105.90 ± 0.31[b]	49.7	1.70 ± 0.40[c]
SPL	330.81 ± 0.20[b]	129.10 ± 0.40[d]	39.0	1.43 ± 0.11[b]
SPC	402.90 ± 0.30[c]	62.90 ± 0.32[a]	15.4	1.82 ± 0.21[d]
Ascorbic acid				0.02 ± 0.01[a]

SPL Salvadora persica leaves, *SPB Salvadora persica* bark, *SPC Salvadora persica* seedcake, *IC_{50}* the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period. The superscript lettering in columns indicates the statistical significance of differences at $P < 0.05$, the values followed by the same letter are not significantly different. All determinations were carried out in triplicate and mean values ± standard deviation reported

It was clear that the antioxidant potential of *S. persica* extracts in DPPH assay was significantly correlated ($P < 0.05$) to its total phenolic compounds. The antioxidant activity increased proportionally to the total phenolic content, and a positive linear relationship between IC_{50} values and total phenolic compounds was found. This is due to the high amount of phenolic compounds present in *S. persica* extracts that act as free radical scavengers. However, the scavenging activity of ascorbic acid, a known antioxidant, used as positive control [35] was relatively more pronounced than that of *S. persica* extracts.

Inhibition of β-Carotene Co-Oxidation in a Linoleate Model System

The antioxidant activity of phenolic compounds may inhibit free radicals from initiating the oxidation process and radical chain reaction. For this reason, two different methods were used for the determination of the antioxidant activity of the extracts: inhibition of β-carotene co-oxidation in a linoleate model system and DPPH free radical scavenging. Figures 1 and 2 reveal the decrease of absorbance of β-carotene in the presence of different methanolic extracts in the coupled system of β-carotene and linoleic acid at 50 °C. It shows that the control sample in both figures without addition of extract solution oxidized most rapidly. The methanolic extracts of SPL, SPB, and SPC were markedly effective in inhibiting the oxidation of linoleic acid and the subsequent bleaching of β-carotene in comparison with the control. In Figs. 1 and 2 (Kordofan and Gezira samples, respectively) SPB extract was the most effective followed by the SPL and SPC extract and the antioxidant activity of the extracts increased with concentration; there is no direct correlation between the level of phenolic compounds in the extracts and their antioxidant activity.

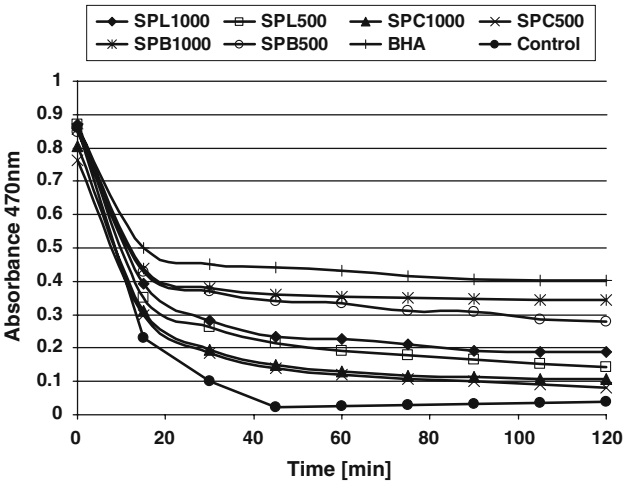

Fig. 1 Antioxidant activity of extracts obtained from *Salvadora persica* leaves (*SPL*), bark (*SPB*) and seedcake (*SPC*) from Kordofan and BHA: butylated hydroxyanisole measured by the bleaching time of β-carotene at 50 °C. All determinations were carried out in duplicate and mean values ± SD reported

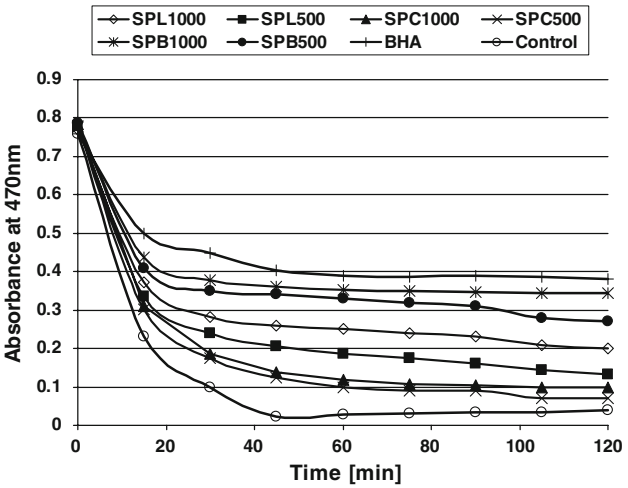

Fig. 2 Antioxidant activity of extracts obtained from *Salvadora persica* leaves (*SPL*), bark (*SPB*) and seedcake (*SPC*) from Gazira and BHA: butylated hydroxyanisole measured by the bleaching time of β-carotene at 50 °C. All determinations were carried out in duplicate and mean values ± SDs reported

Oxidation of Sunflower Oil in the Presence of *Salvadora persica* Extract

From the above results of TPC, DPPH, and the *Inhibition of β-carotene model system* it is clear that the phenolic compounds of *S. persica* contain effective antioxidants. Synthetic antioxidants, e.g. *tert*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and BHA are added to fats and oils to retard oxidation of the unsaturated fatty acids and to decrease the development of rancidity, natural phenolic antioxidants inhibit oxidation reactions when added to oils by acting as a hydrogen donor and afford relatively stable free radicals and/or non-radical products [36].

The effect of extracts from different parts of *S. persica* from Kordofan and Gazira samples and BHA on sunflower oil oxidation at 500 mg/100 g oil is shown in Fig. 3. The development of PV during the oxidation of sunflower oil was evaluated at 70 °C. This temperature was chosen, because at higher temperatures, the peroxides will decompose very fast [37]. In Fig. 3 the PV of sunflower oil (control) with and without *S. persica* extract (SPLK, SPLG, SPBK, SPBG, SPCK and SPCG) or BHA showed a gradual increase. As demonstrated in this figure, a maximum PV of 58.2 meq O_2/kg was reached after 96 h of storage in the control without addition of extract or BHA. Peroxide values of 42.5, 25.3 and 13.5 meq O_2/kg was reached after

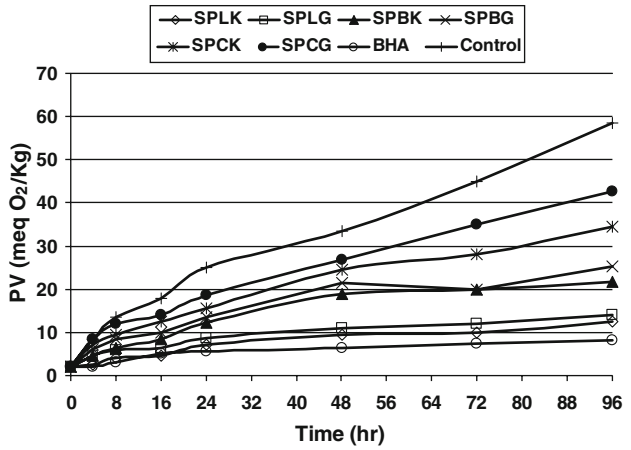

Fig. 3 Oxidation of sunflower oil treated with 500 mg *Salvadora persica* leaves (*SPL*), bark (*SPB*) and seedcake (*SPC*) and 20 mg of BHA: butylated hydroxyanisole in 100 g oil during storage at 70 °C. K: Kordofan sample, G: Gezira sample. All determinations were carried out in duplicate and mean values ± SD reported

96 h of storage in SPC, SPB and SPL of Gazira sample, respectively while it was 35.2, 21.7 and 12.4 meq O_2/kg in SPC, SPB and SPL from Kordofan sample, respectively. Significant differences ($P < 0.05$) were found between the control and different *S. persica* extracts or BHA, which decreased and slowed down the rate of peroxide formation, resulting in lower PVs after 96 h of storage at 70 °C. Extracts from Kordofan samples seem to be better in retarding oil oxidation than Gazira samples. The PVs of sunflower oil containing different *S. persica* extracts were found to be less effective than the synthetic antioxidant BHA. It can be concluded that methanolic extracts obtained from leaves, bark and seedcake of *S. persica* at concentrations of 0.5% were effective in stabilizing sunflower oil during storage at 70 °C.

In conclusion, results as shown in Tables 1, 2 and 3 demonstrate that *S. percica* seeds have a very high oil content (average 41% wt/wt) with highly saturated (~84%) fatty acids and a medium oxidative stability (~3.1 h). On the other hand this study provides data about phenolic compounds extracted from leaves, bark and seedcake of *S. persica* which can be used as natural antioxidant to retard edible oil oxidation which make them attractive candidates for commercial exploitation.

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