Antioxidant Activity of Extracts of Defatted Seeds of Niger (*Guizotia abyssinica*)

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ABSTRACT: Niger (*Guizotia abyssinica*) seed was ground and then defatted with hexane. The meal remaining after oil extraction was tested as a source of antioxidants. Three solvent systems, A [80:20 (vol/vol) ethanol/water], B [80:20 (vol/vol) acetone/water], and C (water) were evaluated as extraction media. Crude extracts were examined for their antioxidant activity in a β-carotene–linoleate and a meat model system. Extract A exhibited superior antioxidant activity, compared to extracts B and C, and its composition was studied further by using column chromatography and HPLC. Four fractions (I–IV) were obtained, of which fractions III and IV showed activity in the β-carotene–linoleate model system. Fraction IV was also highly effective in scavenging the 2,2-diphenyl-1-picrylhydrazyl radical but was less active when used in a bulk oil model system. Preparative TLC showed fraction IV as consisting of two components. UV spectroscopy suggested that the major active component present was a chlorogenic acid-related compound. Furthermore, HPLC analysis established that chlorogenic acid was dominant in the free phenolics fraction (2.6 mg/g). Upon hydrolysis, however, a substantial amount of caffeic acid (42.8 mg/g) was released, presumably from esterified and glycosylated chlorogenic acid. Thus, niger extracts derive their antioxidant activity, at least in part, from the chlorogenic acid-related compounds.

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KEY WORDS: Antioxidant activity, chlorogenic acid, electron paramagnetic resonance, niger (*Guizotia abyssinica*), niger seed extracts.

One of the major problems encountered in the storage of foodstuffs is autoxidation of their lipid components. Oxidation limits the shelf life of many products including meats, edible oils, and cereal-based foods (1–3). In living organisms, oxidation is prevented by a number of endogenous systems such as enzymes, antioxidants, and chelators of metal ions (4). Once removed from the organism, lipids may be protected from oxidation in several ways (5). These include the use of low-temperature storage to slow down the oxidation process, the use of advanced packaging technologies to exclude oxygen and light, and the addition of antioxidants.

Many of the antioxidants in common use are of synthetic origin (6); these include BHA, BHT, TBHQ, and propyl gallate as well as both synthetic and natural tocopherols. Unfortunately, despite their effectiveness, synthetic antioxidants have raised health concerns because both BHA and BHT are suspected carcinogens (7). Thus, there is an interest in using natural antioxidants to protect food lipids from oxidation.

Natural antioxidants from a wide variety of plant sources, including soybean, rapeseed, sesame, peanut, cottonseed, rice, flax, borage, and evening primrose, have been studied (8). The use in food of these plant materials as well as many others dates back thousands of years; we also now know that they contain a number of antioxidants (9,10). The phenolic compounds present in plants, in addition to their antioxidant activity, may also serve as antimicrobial agents and/or colorants (9,10).

Phenolic compounds play a vital role in the biochemistry of plants (8,9). They serve as secondary metabolites and are responsible for the control of oxidative stress as well as for wound healing and as filters for protection against UV light. Once such plants are used as food, their phenolics retard oxidation both as primary antioxidants and as synergists and/or chelators of metal ions. Plant phenolics also have antipathogenic, antiherbivore, and allelopathic properties (10). In oilseeds, phenolic compounds are responsible for the dark color, bitter taste, and objectionable flavor of the resultant meal after oil extraction (9). Such compounds have been isolated from a variety of oilseeds, including canola, flax, sesame, borage, and evening primrose (8,11–14).

Niger (*Guizotia abyssinica*) is an oilseed that has been cultivated in East Africa and India for approximately 5000 years (15). Today it is particularly important to the economy of Ethiopia, where it accounts for 50–60% of its edible oil supply (15). Niger oil is also used in the manufacture of soap and paint and as a lubricant or lighting fuel. The remaining meal is used as feed, fertilizer, or fuel (16). In North America, niger seed is used as a birdseed. Previous research on niger seed has focused on studying the nutritional makeup of the seed and methods of efficient propagation of the plant (17–19). The whole seed contains up to 40% oil, 20.9% carbohydrate, and 27.8% protein.

The objectives of this study were to examine the antioxidant potential of defatted niger seed and its extracts. The extracts were then evaluated for their antioxidant and radical scavenging activities. Finally, isolation and tentative identification of the major antioxidants present in the extracts were attempted.

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MATERIALS AND METHODS

Materials. Niger seeds (*G. abyssinica*) were obtained from a local supermarket. Seeds were stored at room temperature until used. Fresh pork shoulder meat (1 d after slaughter) was also acquired from a local supermarket and most of its surface fat was removed. The meat was ground twice in a meat grinder (Omega, Type 12; Larry Sommers Ltd., Toronto, Ontario, Canada) using a 0.79- and then a 0.48-cm plate. Ground pork was vacuum-packed in polyethylene pouches and stored in a freezer (Ultra Low; Revco Inc., West Columbia, SC) at –60°C until used. Bulk corn oil stripped of its natural antioxidants was purchased from Fisher Scientific (Neapon, Ontario, Canada).

Reagents, namely, 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropane, β-carotene, linoleic acid, Tween 40 emulsifier, BHA, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sinapic acid, ferulic acid, coumaric acid, and chlorogenic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Organic solvents, general chemicals, and inorganic acids were obtained from Fisher Scientific. Helium, hydrogen, and compressed air were obtained from Canadian Liquid Air Ltd. (St. John's, Newfoundland, Canada). Filter paper was obtained from Whatman International Ltd. (Maidstone, United Kingdom). Sephadex LH-20 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

Preparation of the meal. Niger seeds (50 g) were ground in a coffee grinder and defatted by blending the ground seeds with hexane (200 mL, 3 min, three times) in a Waring blender (Model 33BL73; Waring Products Division, Dynamics Corporation of America, New Hartford, CT) at ambient temperature. Defatted seeds were air-dried for 12 h and stored in vacuum-packed polyethylene pouches at –20°C until used.

Preparation of the crude extract. The meal (6 g) was extracted with different solvent systems [100 mL of 80:20 (vol/vol) ethanol/water (A), 80:20 (vol/vol) acetone/water (B), or water (C)] at 80°C for 30 min under reflux conditions. The slurry was filtered through a Whatman #4 filter paper and the filtrate was collected. This procedure was repeated two times and the filtrates were combined, after which the solvent was removed under vacuum at 40°C. The resulting concentrated solution was lyophilized for 72 h at –49°C and 0.062 mbar (Freezone 6, Model 77530; Labconco Co., Kansas City, MO).

β-Carotene–linoleate model system. This model system was modified from that originally reported by Miller (20). A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 mL of chloroform. A 2-mL quantity of this solution was pipetted into a 250-mL round-bottomed flask. After the removal of chloroform under vacuum at 40°C, 40 mg of purified linoleic acid, 400 mg of Tween 40 emulsifier, and 100 mL of aerated distilled water were added to the flask and shaken vigorously. Aliquots (4.8 mL) of this emulsion were transferred into a series of tubes containing 100 or 200 μL of an extract or BHA (in methanol) so that the final concentrations of extracts or BHA in the assy media were 0.5, 1.0, 1.5, and 2.0 mg/5 mL. BHA was used for comparative purposes. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a Hewlett-Packard diode array spectrophotometer (Model 8452A; Hewlett-Packard Co., Mississauga, Ontario, Canada). Subsequently, absorbance readings were recorded over a 2-h period at 15-min intervals by keeping the samples in a water bath at 50°C. Blank samples devoid of β-carotene were prepared for background subtraction.

Preparation of meat model systems. Meat model systems were prepared as described by Shahidi and Pegg (21). Ground pork was mixed with 20% (w/w) of deionized water in Mason jars. Crude extracts (A, B, and C) were added to the meat at 0.5, 1.0, and 2.0% (w/w) levels. The systems were thoroughly homogenized and cooked at 85 ± 2°C (internal temperature of 72 ± 2°C) in a thermostated water bath for 45 min while stirring occasionally with a glass rod. After cooling to room temperature, cooked meat samples were homogenized in a Waring blender for 30 s and then stored for 7 d at 4°C. Samples were removed for analysis on days 0, 1, 3, 5, and 7.

TBARS. Samples were analyzed for TBARS over a 7-d period according to the method of Siu and Draper (22). A 2-g quantity of each sample was placed in a centrifuge tube to which 5 mL of 10% TCA was added and vortexed (Fisher Vortex Genie 2; Fisher Scientific) at high speed for 2 min. A 5-mL quantity of a 0.02 M aqueous solution of TBA was then added to each centrifuge tube, which was then vortexed for an additional 30 s. The samples were then centrifuged at 3000 × *g* for 10 min, and the supernatants were filtered through Whatman # 3 filter paper. The tubes were placed in a boiling water bath for 45 min and cooled to room temperature, and the absorbance value of solutions was read at 532 nm. TBARS values were calculated by multiplying the absorbance readings at 532 nm by a factor of 3.4. This factor was determined from a standard curve prepared for 1,1,3,3-tetramethoxypropane, a precursor of malonaldehyde (23). Inhibition of TBARS formation was calculated using the following formula:

$$\% \text{ inhibition} = 100 \text{ (control value} - \text{sample value)/(control value)} \quad [1]$$

Column chromatography. Column chromatography was carried out according to the method of Shahidi *et al.* (8). A 1.0-g portion of crude extract was dissolved in 5 mL of ethanol and applied to a C16/100 column (Pharmacia) packed with Sephadex LH-20 and eluted with 80:20 (vol/vol) ethanol/water. Fractions (8 mL) were collected in test tubes placed in an LK13 Bromma 2112 Redira 6 fraction collector (Pharmacia), and their absorbance in ethanol was read at 280 nm using a Hewlett-Packard 8452A diode array spectrophotometer. Eluates were then pooled into four fractions based on their absorbance at 280 nm. Solvent was removed under vacuum at 40°C, and the resulting concentrated solutions were lyophilized for 72 h at –49°C and 0.062 mbar. Yield of the resultant solid was determined.

Phenolic content assay. A spectrophotometric method explained by Swain and Hillis (24) was adapted for the phenolic

content assay. Crude extracts, or fractions thereof, were dissolved in methanol to obtain a concentration of 0.5 mg/mL. Folin–Dennis reagent (0.5 mL) was added to a centrifuge tube containing the crude extract or its fractions (0.5 mL). Contents were mixed and a saturated sodium carbonate solution (1 mL) was added to the tube. Volume was adjusted to 10 mL by the addition of 8 mL of distilled water and the contents were mixed vigorously. Tubes were allowed to stand at ambient temperature for 25 min and then centrifuged for 5 min at $4000 \times g$. Absorbance of the supernatant was read at 725 nm. Blank samples of each extract or fraction were used for background subtraction. The total phenolic content in each sample was expressed as mg caffeic acid equivalents/g extract or fraction.

TLC. Each separated fraction was examined by TLC on silica gel plates (Sigma Chemical Co.) following a modified method explained by Shahidi *et al.* (8). Toluene/acetone/formic acid (30:30:10, by vol) was used as the developing system. After drying, bands were located by spraying with 1% (wt/vol) $FeCl_3$/1% (wt/vol) $K_3Fe(CN)_6$ in methanol. On a second plate, antioxidant activity of the bands was examined by spraying with a β-carotene–linoleate solution. Sinapic, caffeic, and chlorogenic acids were used as references along with the crude extract and fractions III and IV by using the same developing system. After drying, bands were located by viewing under a short- (254 nm) and a long- (365 nm) wavelength UV light.

Isolation and tentative identification of phenolic acids. Phenolic acids are present in the free, esterified, and glycosylated/bound forms. An aqueous suspension of the extract was prepared and its pH adjusted to 2 with 6 M HCl. The free phenolic acids were extracted into 5×10 mL diethyl ether using a separatory funnel. The aqueous portion was then hydrolyzed with 10 mL of 2 M NaOH over a 4-h period at room temperature under an atmosphere of nitrogen. After acidification to pH 2, the sample was extracted with 5×15 mL diethyl ether to isolate the phenolic acids released from the esters. To the aqueous layer a 7.5-mL quantity of 6 M HCl was added and allowed to hydrolyze over a 1-h period at 100°C. Phenolic acids released from the glycosides were then extracted into 5×22 mL diethyl ether. Each ether layer from the three operations was evaporated to dryness; the residue was then dissolved in 2–3 mL of methanol and filtered through a 0.45-μm nylon filter for HPLC analysis. A Shimadzu HPLC system equipped with a prepacked LiChrosphere 100 RP-18 (5 μm, 4×250 nm; Merck, Darmstadt, Germany) was used for separation of phenolic acids. The mobile phase was water/acetonitrile/acetic acid (88:10:2, by vol), flow rate was 1 mL/min, and the injection volume was 20 μL; peaks were monitored at 320 nm. Peaks representing different phenolics were tentatively identified based on comparison of their retention times with those of commercial standards. Details of the separation procedure were similar to those reported for rye caryopses (25).

EPR spectroscopy. EPR spectroscopy was done according to a modified method reported by Sripriya *et al.* (26). A 600-μL quantity of 10 mM DPPH in toluene was mixed with 200 μL of crude extract or fraction so that the final concentration of the extract or fraction in the assay medium was 40, 80, or 160 ppm (as caffeic acid equivalents). The mixture was allowed to stand for 15 min, and a 50-μL quantity of it was transferred into a quartz capillary tube and sealed with sealing clay. The EPR spectrum was recorded in the EPR spectrometer (Bruker ESP 300; Bruker Instruments, Inc., Billeria, MA) set at 2×10^5 receiver gain, 1.0 G modulation amplitude, 200 s scan time, 3460 G center field, 100 G sweep width, and 0.5 s time constant.

Bulk stripped corn oil model system. A bulk stripped corn oil system was prepared using a modified version of that reported by Frankel *et al.* (27). Crude extract and fraction IV (80 ppm) were each dissolved in 500 μL of absolute ethanol and added to 25-g portions of stripped corn oil. Following thorough mixing, both solutions were divided into 5-mL portions and placed in 20-mL capped glass tubes. The tubes were stored in a 60°C forced-air oven (Thelco, Model 2; Precision Scientific Co., Chicago, IL) for 0, 3, or 7 d.

Conjugated diene test (bulk oil system). Each sample (20–40 mg) was weighed into a 25-mL volumetric flask. The mixture was then made up to the mark with isooctane (ACS grade) and mixed throughly. The absorbance was read at 234 nm using a Hewlett-Packard 8452A diode array spectrophotometer. Pure isooctane was used as a reference. The conjugated diene values were calculated according to the equation below and as explained by Wanasundara (28).

$$\text{CD value} = A/(C \cdot l) \quad [2]$$

where CD = conjugated diene; A = absorbance at 234 nm; C = concentration (g/100 mL); and 1 = path length (cm). Inhibition of the formation of conjugated dienes was determined using the following equation:

$$\%\ \text{inhibition} = 100\ (\text{control value} - \text{sample value})/(\text{control value}) \quad [3]$$

TBARS test (bulk oil system). Each sample (200 mg) was weighed into a 25-mL volumetric flask. It was then made up to the mark with 1-butanol (ACS grade) and mixed thoroughly. A 5-mL portion of this solution was transferred into a dry test tube, and a 5-mL quantity of fresh TBA reagent (200 mg TBA in 100 mL 1-butanol) was added to it. The tube was placed in a 95°C water bath for 120 min. The tube was cooled, and the absorbance of the solution was read at 532 nm. The TBARS value was calculated as follows:

$$\text{TBARS value} = (A^*0.415)/m \quad [4]$$

where A = absorbance at 532 nm and m = mass of the sample (g).

Preparative TLC. Preparative TLC was carried out using a modified version of the method described by Shahidi *et al.* (29). Separation of fraction IV into its components was accomplished using preparative silica gel TLC plates (Sigma Chemical Co.) with toluene/acetone/formic acid (30:30:10, by vol) as the eluting solvent. After drying, bands were located both visually and by viewing under a short- (254 nm) and a long- (365

nm) wavelength UV light. Bands were scraped off the TLC plate using a stainless steel spatula and placed in centrifuge tubes. Absolute ethanol (10–15 mL) was added to each tube followed by vortexing for 1 min. The tubes were then centrifuged for 10 min at 4000 × *g* and the supernatants were transferred into sample vials. The solvent was removed under a flow of nitrogen and the yield of solids recorded.

UV spectrophotometry. Samples of bands obtained by preparative TLC as well as chlorogenic acid were dissolved in methanol. UV spectra were recorded using a Hewlett-Packard 8452A diode array spectrophotometer as reported by Shahidi *et al.* (8). Pure methanol was used as a reference.

Statistical analysis. The significance of the differences in extract yield among solvent systems during extraction and crude extract, fraction IV, and control for antioxidant activity in the bulk stripped corn oil model system was evaluated using ANOVA and Tukey's Studentized test based on the data collected from triplicate determinations. The method used was that of McClave *et al.* (30).

RESULTS AND DISCUSSION

To study the antioxidant efficacy of samples, the active components must be extracted effectively from the defatted seeds. A number of solvent systems have been used successfully for extraction of antioxidants and bioactives from selected oilseeds (11,12,29). Therefore, several solvent systems were evaluated for their effectiveness in the preparation of niger seed extracts. These were 80:20 (vol/vol) ethanol/water (A) and 80:20 (vol/vol) acetone/water (B). These systems were chosen because they were less polar than pure water and might extract more hydrophobic compounds. Water (C) was chosen because it might extract highly polar constituents and because it would certainly be the most practical solvent in any future industrial application. The yields obtained with the two extraction systems of A (10.6 ± 0.7%) and B (10.5 ± 0.9%) were not different; however, the yield obtained with C (4.0 ± 0.2%) was significantly lower than that obtained for other extraction systems.

To evaluate the antioxidant activity of the three crude extracts, a β-carotene–linoleate model system was used as a first step (31). The results are summarized in Figure 1. As oxidation progressed, the absorbance of β-carotene at 470 nm decreased and its yellow color faded. Clearly, all three extracts deterred oxidation to some extent, and their effectiveness increased at higher concentrations. However, extract C appeared inferior to extracts A and B at levels below 1.5 mg/5 mL assay medium; at higher concentrations differences were much less clear. Thus, it may be concluded that extract C had a lower content and/or different types of antioxidants. BHA was a more effective antioxidant than all the other extracts examined.

All three extracts were subsequently evaluated by the TBA test in a meat model system. Such systems have been used by a number of authors to evaluate natural antioxidants (1,2,21). The TBA test measures the formation of secondary oxidation products (TBARS) such as malondialdehyde, alkenals, and alkadienals and is in widespread use (4), despite the fact that it is a nonspecific test. As can be seen from Figure 2, the extent of oxidation in meat treated with extracts was lower than that of the controls, as reflected in TBARS values, and the observed differences were statistically significant ($P < 0.05$). As in the β-carotene–linoleate model system, the extracts exerted a concentration-dependent activity and displayed stronger effects at higher concentrations. At low concentrations (0.5% by wt), all three extracts performed erratically. However, it was clear that at 1.0 and 2.0% concentrations, extract A was superior to extracts B and C especially during extended storage. Thus, only

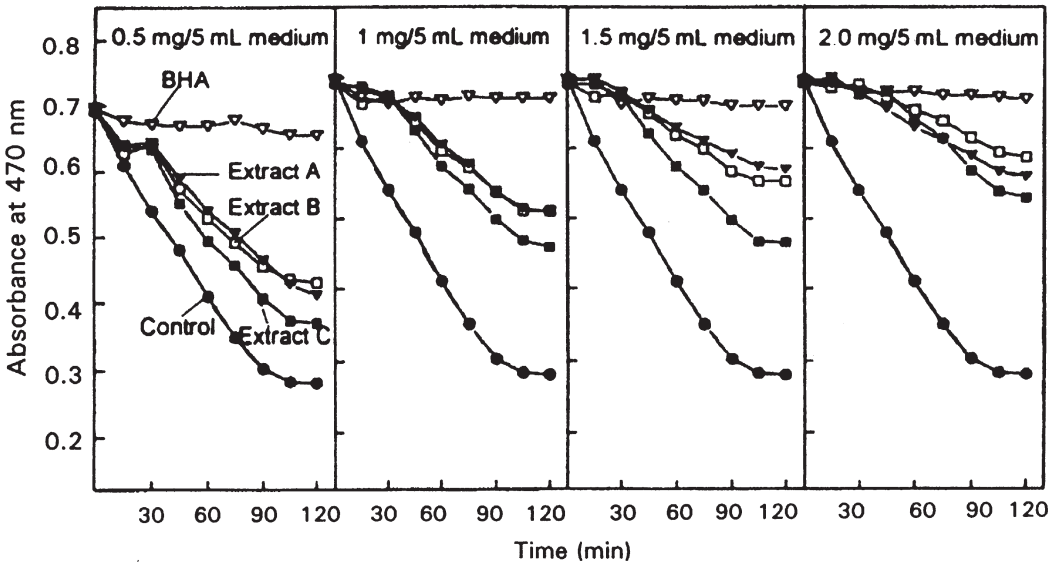

FIG. 1. Antioxidant activity of niger seed extracts A [80:20 (vol/vol) ethanol/water], B [80:20 (vol/vol) acetone/water], and C (water) in a β-carotene–linoleate model system.

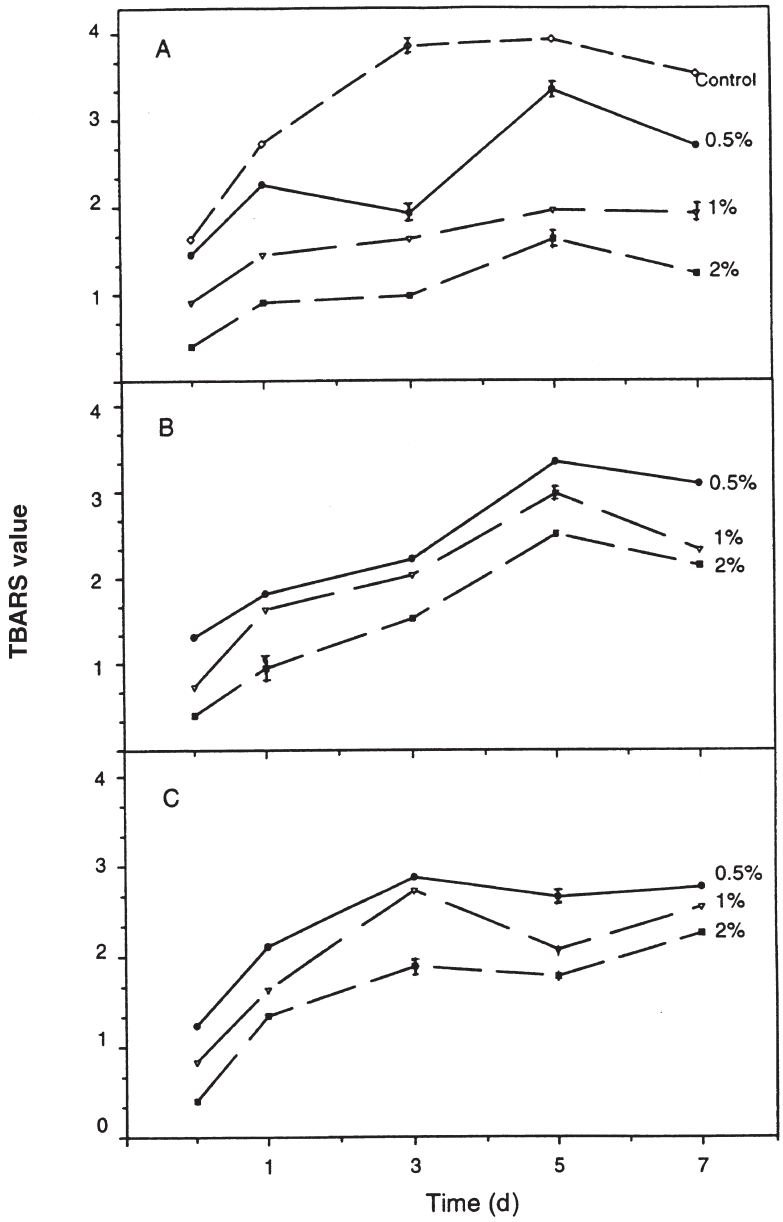

FIG. 2. Antioxidant activity of niger seed extracts A [80:20 (vol/vol) ethanol/water], B [80:20 (vol/vol) acetone/water], and C (water) in a meat model system. TBARS measured as mg malondialdehyde equiv/kg sample.

extract A was used in subsequent investigations for the isolation and identification of antioxidants present in it. Column chromatography was chosen for preliminary separation of components, as explained by other investigators (12,32,33). The fractions (I–IV) were pooled based on the absorbance of the eluates at 280 nm. Fractions I (tubes 1–25), II (tubes 25–55), III (tubes 55–75), and IV (tubes 76–120) contributed 10, 30, 35, and 25% to the total amount. The recovery yield from the column was 74%. The crude extract contained 15.2% phenolics as caffeic acid equivalents. Chemical tests were then used to study the chemical nature of the compound(s) involved. As a result, the vanillin assay did not show the presence of any measurable amount of condensed tannins in the crude extract. However, phenolic contents of fractions III and IV were quite high (approximately 47% each), whereas fractions I and II contained 2.0 and 3% phenolics, respectively.

TLC was subsequently used to investigate each fraction for its constituents. The results of the first plate, using $FeCl_3/K_3Fe(CN)_6$ as the spray, and the second plate, sprayed with a β-carotene–linoleate, are shown in Table 1. Antioxidative components (three in each of the fractions I–III and two in fraction IV) delayed the bleaching of β-carotene, as shown by the shaded spots. In this model system, fraction I had no antioxidative activity and the compound at R_f 0.15 in fraction II displayed a very weak activity. All spots in fractions III and IV, located in the previous plate, proved to be strongly antioxidative;

TABLE 1
R_f Values of Different Spots in Crude Extract and Fractions of Niger Seeds and Standard Phenolics on TLC Plates Sprayed with a $FeCl_3/K_3Fe(CN)_6$ (system 1) or β-Carotene–Linoleate (system 2) Solution or Under UV Light[a]

Fraction	R_f (spray system 1)	R_f (spray system 2)	R_f (under UV light)
I	0.62, 0.75, 0.87	ND	—
II	0.15, 0.24, 0.46	0.15	—
III	0.62, 0.65, 0.73	0.62, 0.65, 0.73	0.21, 0.52, 0.73
IV	0.65, 0.73	0.62, 0.73	0.52, 0.73
Crude extract	—	—	0.21, 0.52, 0.73
Sinapic acid	—	—	0.88
Caffeic acid	—	—	0.85
Chlorogenic acid	—	—	0.54

[a]ND, not detected.

both fractions were also shown to have a very high content of phenolics with strong antioxidant activity (12,24,31). On the basis of these results, fractions I and II were not studied further.

The third and last TLC plate compared the crude extract and fractions III and IV with three known phenolic compounds, namely, sinapic, caffeic, and chlorogenic acids (Table 1). Under UV light, the crude extract showed three spots (R_f 0.21, 0.52, and 0.73). Fractions III and IV showed three and two spots, respectively, as seen in the previous plates. However, their R_f values were somewhat different: R_f 0.21, 0.52, and 0.73 for fraction III, and R_f 0.52 and 0.73 for fraction IV. These are probably the same components seen earlier, but they appear in different positions. This is most likely due to slight changes in the solvent system or some existing differences in the thickness and other variables in the plates used. Sinapic and caffeic acids were located at R_f 0.88 and 0.85, respectively. Therefore, these compounds do not appear to contribute to the antioxidant activity of the extracts to any significant degree but may exist in the glycosylated or esterified forms. Chlorogenic acid appeared at R_f 0.54, close to the middle spot of fraction III and the lower spot of fraction IV; thus, these compounds could potentially be related.

The crude extract, following appropriate workup, was subjected to HPLC analysis. Results in Table 2 indicate the presence of a number of phenolic acids in the free form. Very small amounts of sinapic and caffeic acids were also present, but these were not detected to any significant degree by the TLC procedure under the experimental conditions used. However, most phenolics were present in the esterified form, presumably in the form of esterified chlorogenic acid, which produced caffeic acid upon hydrolysis. Meanwhile, glycosylated caffeic acid was also present in the extracts.

The radical scavenging activity of the crude extract and fractions II and IV was studied using EPR spectroscopy. The radical chosen was DPPH because of its relative stability and its common use in similar studies (34,35). The results of this experiment are illustrated in Figure 3. The control sample had a strong and clear DPPH signal. At 40, 80, and 160 ppm, the DPPH signal for the crude extract was much weaker than that for the control, demonstrating partial quenching of this radical. At 40 ppm, fraction III was less effective in quenching the DPPH radical than the crude extract at the same level. However, its effectiveness increased at higher concentrations. On the other hand, for fraction IV at all levels, the DPPH signal could barely be seen and was almost completely quenched. The crude extract did not perform as well as fraction IV because it contained more nonphenolic constituents. Fraction IV may have outperformed Fraction III because it contained more effective radical-scavenging compounds or fewer nonphenolic constituents. The latter reason seems more likely since all the components of fraction IV seen on the TLC plates also appeared in fraction III along with a few other constituents. Based on this experiment, fraction IV was chosen for further studies. Phenolics are known to scavenge DPPH radicals effectively, and caffeic acid has been shown to scavenge DPPH radicals better than other phenolic acids or phenyl propenoids (35).

Since the crude extract was effective as an antioxidant mixture in an emulsion (β-carotene–linoleate) and in a meat model system, the next step was to test the activity of both the crude extract and fraction IV in bulk oil under accelerated conditions. Similar accelerated storage tests have been used by other authors to evaluate the efficacy of antioxidants (24,31). The percentage inhibition of formation of both the conjugated dienes and TBARS can be seen in Table 3; the crude extract was

TABLE 2
Phenolic Acids in Niger Seed Crude Extract (mg/g extract)[a]

Phenolic acids	Chlorogenic	Caffeic	*p*-Coumaric	Ferulic	Sinapic
Free	2.6	0.6	Trace	—	0.1
Esterified	—	40.7	0.2	0.2	Trace
Glycosylated	—	2.1	—	—	—
Total	2.6	43.4	0.2	0.2	0.1

[a]The total of all phenolic acids in the free, esterified, and glycosylated forms in defatted seeds of niger was 46.3 mg/g extract.

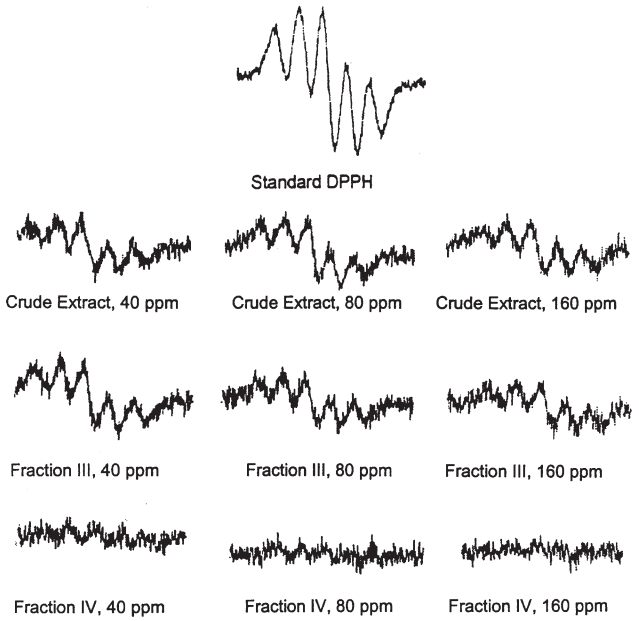

FIG. 3. Quenching activity of extract A of niger seed and fractions III and IV for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as shown by EPR spectroscopy.

clearly superior to fraction IV in inhibiting oxidation on both day 3 and day 5. However, inhibition of TBARS for this fraction was similar to that of α-tocopherol (15% on day 3 and 10% on day 5) at a 200-ppm level of addition. Thus, the crude extract may contain components that work well in bulk oil but that are not present in fraction IV. Finally, the other components present in the crude extract may act synergistically with the phenolics to reduce oxidation of the oil. Lipid oxidation proceeds through a number of steps (4), and fraction IV may be excellent in quenching radicals but may not inhibit other steps, contrary to that observed for the crude extract.

The final phase of this study involved the isolation of active compounds from fraction IV using preparative TLC, as explained elsewhere (29). The preparative plate matched the previous analytical plates and led to the separation of two components (R_f 0.65 and 0.73). The total yield was 97%, and the relative proportions of the two constituents were 74.5 and 25.5%, respectively.

The compounds from spots of fraction IV with R_f 0.65 and 0.73 were subjected to UV spectroscopy, as phenolic compounds have previously been shown to absorb in this region (10). The compound with R_f 0.65 showed absorbances at 245 and 330 nm with a shoulder at 308 nm; these values correspond exactly with those of chlorogenic acid. The compound with an R_f value of 0.73 showed absorbance at 282 nm with a shoulder at 308 nm and was different from the standards used. Chlorogenic acid was chosen as a standard because its R_f was similar to that of one of the spots of fraction IV. The spectrum of the R_f 0.65 component and its similarity to that of chlorogenic acid strongly suggest that the two compounds are very similar in structure. Upon hydrolysis, however, compound R_f 0.65 produced a considerable amount of caffeic acid. Thus, one may conclude that chlorogenic acid (R_f 0.54) might have existed in esterified and glycoslyated/bound forms, as evidenced from HPLC studies performed directly on both the crude extract and on compound R_f 0.65 upon hydrolysis.

TABLE 3
Inhibition of Oxidation (%) of Meat Lipids with Crude Extracts of Niger Seed and Fraction IV Upon Storage

	Storage time (d)	
Extract	3	5
Conjugated dienes		
Crude extract	74	42
Fraction IV	19	14
TBARS		
Crude extract	25	20
Fraction IV	15	12

Based on the results presented above, defatted niger seed and its extracts provide a natural source of antioxidants. Although the antioxidant activity of the compounds involved is equal or superior to that of tocopherols, their efficacy is lower than that of commonly used synthetic antioxidants. Hence, use of niger seed is recommended in applications where functionality of seed components, such as their fat- and moisture-binding ability and textural effects, are important in addition to their antioxidant activity.

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