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ORIGINAL PAPER

Fat-Soluble Bioactives, Fatty Acid Profile and Radical Scavenging Activity of *Semecarpus anacardium* Seed Oil

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Abstract *Semecarpus anacardium* (family Anacardiaceae) has many applications in the *Ayurvedic* and *Siddha* systems of medicine in India. Detailed knowledge on the composition of *S. anacardium* oil, in consideration of potential utilization, is of major importance. In this investigation, column chromatography, gas chromatography, thin layer chromatography and liquid chromatography techniques were performed to analyze lipid classes, fatty acids and fat-soluble bioactives of *S. anacardium* crude seed oil. The amount of neutral lipids in the crude seed oil was the highest, followed by glycolipids and phospholipids, respectively. Linoleic followed by palmitic and oleic were the major fatty acids. The ratio of unsaturated fatty acids to saturated fatty acids was higher in neutral lipid classes than in the polar lipids. The main sterol compounds were β-sitosterol, campesterol and stigmasterol. δ-Tocopherol followed by β-tocopherol were the main tocopherols. When *S. anacardium* seed oil and extra virgin olive oil were compared for their radical scavenging activity toward 1,1-diphenyl-2-picrylhydrazyl radical and galvinoxyl radical (by electron spin resonance spectrometry), *S. anacardium* seed oil exhibited a stronger RSA.

Keywords *Semecarpus anacardium* · Seed oil · Lipid classes · Lipid-soluble bioactives · Sterols · Tocopherols · Radical scavenging activity

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Introduction

Interest in new sources of edible oils has recently grown. Plant seeds are important sources of lipids of industrial, nutritional and pharmaceutical importance [1]. Knowledge of medicinal plants has always guided the search for new cures [2]. *Ayurveda,* literally meaning ‘science of life’, is an ancient system of medicine practiced in India about 3,500 years ago. The *Ayurveda* system relies strongly on preventive medicine and the promotion of health [3]. There is a vast, promising and yet unexplored repository of plants, some of which are already in wide clinical use as crude drug preparations and formulations. Evaluation of the bioactive compounds of *Ayurvedic* medical plants might be helpful in modern drug development.

Semecarpus anacardium Linn. (Anacardiaceae), commonly known as ‘marking nut’, is a moderate-sized deciduous tree, reaching a height of up to 12–15 m, with rough dark brown bark. Commonly distributed in sub-Himalayan regions of India, *S. anacardium* is also found in Malaysia, Myanmar, Singapore, China, Northern Australia and Africa. The fruit is acrid, anthelmintic, and considered useful in the treatment of acute rheumatism, asthma,

neuralgia, epilepsy and psoriasis. It has been used therapeutically in neurological disorders, ulcers, corns, leprosy, leucoderma and arthritis [4, 5].

The nut of *S. anacardium* and its oil contain several phenolics including biflavonoids [6]. Trihydroxyflavone, semecarpol, anacardoside and bhilawanols have been identified as the main constituents of *S. anacardium* nut extract [7, 8]. Many medicinal properties such as acrid, digestive, antitumour, antimicrobial, antiinflammatory and antirheumatic have been attributed to the nut extracts [9, 10]. Studies have also established the anticancer potency of *S. anacardium* nut extract [11], by its immunomodulatory potency and by the prevention of enhanced lipid peroxidation [4]. Alkaloids isolated from *S. anacardium* nuts oil were capable of eliminating human tumour cells of diverse origin through induction of apoptosis. This is indicative of a potential therapeutic role of these ingredients in human cancer [12]. Against AFB_1 induced rat neoplasia in liver, the nut extract was non-toxic in quite large doses [11]. In an ethnopharmacological screening, the inhibitory effect on lipid peroxidation of *S. anacardium* methanolic extract was evaluated (IC_{50} value 120 μg/mL) using bovine brain phospholipid liposomes as model membranes [13]. Toxicological study on *Siddha* preparation of *S. anacardium* nuts showed that the drug is safe and does not induce any toxic manipulation on the biochemical parameters investigated in rats [14].

The seed kernel yields a reddish brown oil which is semi-dry with a pleasant taste. The seeds are eaten in India and are considered nutritious. A literature survey revealed that no detailed chemical investigations had been carried out on the seed oil of *S. anacardium*, while the nut shells had been investigated extensively. The contribution of dietary lipids to health is determined by their composition. The study of *S. anacardium* seed oil for its minor constituents, therefore, is useful in order to use both oil and the minor constituents effectively. Lipids contain, apart from triacylglycerols, a number of lipophilic bioactives with a very diverse chemical make up. Among the most interesting are the polar lipids, phytosterols and fat-soluble vitamins. On the other hand, the consumption of foodstuffs rich in antioxidants provides protection against cancer and cardio- and cerebrovascular diseases through the capacity of these antioxidants to scavenge free radicals. It is hard to find data on the radical scavenging activity (RSA) and antioxidant properties of *S. anacardium* oil.

The objectives of this investigation were; (a) to obtain informative profile about the chemical composition of *S. anacardium* oil which will serve for further nutritional evaluation of *S. anacardium* seed oil, and (b) to study the antiradical action of *S. anacardium* oil. The results are important as an indication of the potentially nutraceutical and economical utility of *S. anacardium* seeds as a non-conventional source of edible oils.

Materials and Methods

Materials

Mature fruits of *Semecarpus anacardium* were collected in January 2006, 2007 and 2008 from Karkakpalli forest (Bidar district, India) and the plant was identified with the help of the Flora of Gulbarga District [15]. Because there were no significant differences in the results of the three seasons, the results of samples collected in 2008 were presented. A voucher specimen (No HGUG 206) was deposited at the Herbarium, Department of Botany, University Gulbarga (India). Neutral lipid (NL) reference compounds were from Sigma (St. Louis, MO, USA). Reference compounds used for glycolipids (GL) identification; monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG) and esterified steryl glucoside (ESG) were of plant origin and purchased from Biotrend Chemikalien GmbH (Köln, Germany). Reference compounds used for phospholipids (PL) identification; phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylcholine (PC) were purchased from Sigma (MO, USA). Fatty acids reference compounds were from Sigma (99% purity specific for GC). Reference compounds used for sterols (ST) characterization were purchased from Supelco (Bellefonte, PA, USA). Reference compounds used for α-, β-, γ- and δ-tocopherols were purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH, approximately 90%) was from Sigma (St. Louis, Mo, USA). Galvinoxyl radical were from Aldrich Chem. Co. (Milw., WI, USA). Extra virgin olive oil was from local market (Berlin, Germany). Reagents and chemicals used were of the highest purity available.

Methods

Extraction of Total Lipids

After carefully removal of the fruit shell, the seed kernels were finely ground (particle size = 2 mm) and Soxhlet extracted with *n*-hexane for 12 h. Total lipids (TL) recovered were dissolved in chloroform and stored 2 weeks in closed brown bottles at 4 °C for analysis.

Fractionation of Lipid Classes Using Column Chromatography

Total lipids (TL) were separated into the different classes by elution with different solvents over a glass column (20 mm dia × 30 cm) packed with slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in

chloroform (1:5, w/v). Neutral lipids (NL) were eluted with three-times the column volume of chloroform. The major portion of glycolipids (GL) was eluted with five-times the column volume of acetone and that of phospholipids (PL) with four-times the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. Fatty acid composition of NL, GL and PL was determined by GC/FID as described below.

Thin Layer Chromatography of NL Subclasses

By means of thin layer chromatography (TLC) on Silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany), a further characterization of the NL subclasses was carried out using a solvent system of *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For detection, the TLC plates were sprayed with sulphuric acid (40%). Each spot was identified with lipid reference compounds as well as their reported retention factor (R_f) values [16, 17]. For the quantitative determination of NL subclasses, individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether. Data presented are the average of three gravimetrical determinations.

Normal-Phase Liquid Chromatography of GL Subclasses

Normal-phase LC analysis of GL subclasses was performed according to the method described by Ramadan and Mörsel [18]. The chromatographic system included a Model 87.00 (Knauer; Berlin, Germany) Variable Wavelength Monitor detector, Rheodyna Model 7125 (Cotati California) injector and a 20-μL sample loop. The column was a stainless steel column, 25.0 cm × 4 mm i.d., packed with Zorbax-Sil, 5 μm (Knauer; Berlin, Germany). GL subclasses were separated with an isocratic elution by a mixed solvents of isooctane/2-propanol (1:1, v/v) and detected at 206 nm in 30 min then regeneration of the column for next analysis for 10 min. Prior to LC analysis, aliquots of the total GL [acetone fractions obtained from column chromatography (CC)] were dried under nitrogen and dissolved in the LC mobile phase. About 2 μg of the GL fraction was injected and the solvent flow was maintained at 0.5 mL/min at a column back-pressure of ca. 65 bar. About ten injections were necessary in order to obtain sufficient quantities of individual GL subclasses. GL reference compounds were injected individually as well as in a mixture to determine retention times and the resolution of peaks. Reference compounds were used to identify the components of seed oil GL subclasses via LC. The GL subclasses were manually collected and the purity of the individual GL subclasses was checked by TLC on silica gel 60 F_{254} plates (thickness = 0.25 mm; Merck; Darmstadt, Germany) using chloroform/methanol/ammonium hydroxide 25% (65:25:4, v/v/v) as the solvent system [19]. GL were quantitated by isolation of the individual subclasses followed by hexose measurement using the phenol/sulphuric acid method in acid-hydrolyzed lipids. The percentage distribution of each component was obtained from the hexose values.

Normal-Phase Liquid Chromatography of PL Subclasses

Analysis of PL was performed according to the method described by Ramadan et al. [20]. A LiChrosorb Si-60, 5 μm (4 × 250 mm) column was used. Separation was monitored at 205 nm with a flow rate of 0.7 mL/min at room temperature. The elution system included solvent A (isooctane/isopropanol, 6:8, v/v), and solvent B (isooctane/isopropanol/water, 6:8:0.6, v/v/v), with a gradient transition from 100% solvent (A) to 100% solvent (B) over a period of 35 min followed by 15 min column regeneration. Twenty microlitres of solvent A containing approximately 1–2 μg of PL (fraction obtained from CC) was injected at a column back-pressure of approximately 105–120 bar. PL reference compounds were used to identify the components of seed oil PL via NP-LC. The PL fractions were collected, and the purity of the individual PL fractions was checked by TLC on silica gel 60 F_{254} plates (thickness = 0.25 mm; Merck; Darmstadt, Germany) using chloroform/methanol/ammonium hydroxide 25% (65:25:4, v/v/v) as the solvent system. The phosphorus content of the fractions collected from the LC was determined by using the AOCS method [21].

Gas Chromatography Analysis of Fatty Acid Methyl Esters

Fatty acids were transesterified into fatty acid methyl esters (FAME) using *N*-trimethylsulphonium hydroxide (TMSH, Macherey-Nagel, Düren, Germany) according to the procedure reported by Arens et al. [22]. In brief, 10 mg of oil sample was dissolved in 500 μL of *tert*-butyl methyl ether then 250 μL of TMSH was added and the mixture was vortexed for 30 s before injection. FAME were identified on a Shimadzu GC-14A instrument equipped with a flame ionization detector (FID) and a C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1 μL was injected in a 30 m × 0.25 mm × 0.2 μm film thickness Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250 °C. The initial column temperature was 100 °C programmed by 5 °C/min until

175 °C and kept 10 min at 175 °C, then 8 °C/min until 220 °C and kept 10 min at 220 °C. A comparison between the retention times of the samples with those of reference compounds mixture, run on the same column under the same conditions, was made to facilitate identification.

Gas Chromatography Analysis of Sterols

Separation of ST was performed after saponification of the oil sample without derivatisation [1]. TL (250 mg) were refluxed with 5 mL ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were firstly extracted three-times with 10 mL of petroleum ether, the extracts were combined and washed three-times with 10 mL of neutral ethanol/water (1:1, v/v) and then dried overnight with anhydrous sodium sulphate. The extract was evaporated in a rotary evaporator at 25 °C under reduced pressure, and then ether was completely evaporated under nitrogen. GC analyses of unsaponifiable residues were carried out using a Mega Series (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with FID. The following parameters were performed: DB 5 column (J&W scientific; Falsom, CA, USA) packed with 5% phenylmethylpolysiloxan, 30 m length, 0.25 mm i.d., 1.0 μm film thickness; carrier gas (helium) flow 38 mL/min. Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μL. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was <5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

Normal Phase Liquid Chromatography of Tocopherols

Procedure

NP-LC was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan and Mörsel [23]. Analysis was performed with a solvent delivery system LC-9A LC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 × 4 mm i.d. LiChrospher-Si 60, 5 μm, column (Knauer, Berlin, Germany). Separation of tocopherol isomers was based on isocratic elution when the solvent flow rate was maintained at 1 mL/min at a column back-pressure of about 65–70 bar. The solvent system selected for elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. Twenty microliter of the diluted solution of TL in the mobile phase were directly injected into the LC column. Tocopherol isomers were identified by comparing their retention times with those of reference compounds.

Preparation of Standard Curves

Standard solutions were prepared by series of dilutions ranging from 25 to 5 mg/mL with a stock solution of each tocopherol isomer. Standard solutions were prepared from a stock solution which was stored in the dark at −20 °C. Twenty microlitres was injected and peaks areas were determined to generate standard curve data.

Quantification

All quantitation was done by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentrations levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the standard tocopherols were made three times onto the LC system. Injections in triplicate were made at each concentration for both standards and samples.

Extraction and Quantification of Phenolic Compounds

Aliquots of *Semecarpus anacardium* oil and extra virgin olive oil (2 g) were dissolved in *n*-hexane (5 mL) and mixed with 10 mL methanol–water (80:20, v/v) in a glass tube for 2 min in a vortex. After centrifugation at 3,000 rpm for 10 min, the hydroalcoholic extracts were separated from the lipid phase by using a Pasteur pipette then combined and concentrated in vacuo at 30 °C until a syrup consistency was reached. The lipidic residue was redissolved in 10 mL methanol–water (80:20, v/v) and the extraction was repeated twice. Hydroalcoholic extracts were redissolved in acetonitrile (15 mL) and the mixture was washed three times with *n*-hexane (15 mL each). Purified phenols in acetonitrile were concentrated in vacuo at 30 °C then dissolved in methanol for further analysis. Aliquots of phenolic extracts were evaporated to dryness under nitrogen. The residue was redissolved in 0.2 mL water and diluted (1:30) Folin-Ciocalteu's phenol reagent (1 mL) was added. After 3 min, 7.5% sodium carbonate (0.8 mL) was added. After a further 30 min, the absorbance was measured at 765 nm using UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). Caffeic acid was used for the calibration and the results of triplicate analyses are expressed as parts per million of caffeic acid.

Radical Scavenging Activity toward DPPH Radical (Spectrophotometric assay)

The RSA of *S. anacardium* seed oil and extra virgin olive oil was examined by the reduction of DPPH in toluene

according to Ramadan et al. [24]. A toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of oils (in 100 µL toluene) was mixed with 390 µL toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30 and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). The RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without a sample (control) and the inhibition percentage was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100.$$

Radical Scavenging Activity Towards Galvinoxyl Radical (Spectrometric Assay)

Miniscope MS 100 ESR spectrometer (Magnettech GmbH; Berlin, Germany) was used throughout the analysis. Experimental conditions were as follows: measurement at room temperature; microwave power, 6 db; centerfield, 3,397 G, sweep width 83 G, receiver gain 10 and modulation amplitude 2,000 mG. Ten milligrams of oils (in 100 µL toluene) was allowed to react with 100 µL of toluenic solution of galvinoxyl (0.125 mM). The mixture was stirred on a vortex stirrer for 20 s then transferred into 50 µL micro pipette (Hirschmann Laborgeräte GmbH, Ederstadt, Germany) and the amount of galvinoxyl radical inhibited was measured exactly 60 s after the addition of the galvinoxyl radical solution. The galvinoxyl signal intensities were evaluated by the peak height of signals against a control. Further ESR spectra have been recorded in intervals of 90 s for a total incubation time of 60 min. A quantitative estimation of the radical concentration was obtained by evaluating the decrease of the ESR signals in arbitrary units between 1 and 60 min incubation using the KinetikShow 1.06 Software program (Magnettech GmbH; Berlin, Germany). The reproducibility of the measurements was ±5 as usual for kinetic parameters [24].

All work was carried out under subdued light conditions. All the experiments were repeated at least three times when the variation on any one was routinely less than 5%. All experimental procedures were performed in triplicate and the mean values (± standard deviation) were given.

Results and Discussion

Lipid Classes and Subclasses

In the present study *S. anacardium* seeds were found to contain 36% crude seed oil, which confirms that *S. anacardium* seeds are a rich source of lipids. Combinations of chromatographic procedures were used to obtain major lipids classes and subclasses of *S. anacardium* seed oil. The proportion of lipid classes and subclasses presented in the seed oil as well as R_f values of NL subclasses are shown in Table 1. Among the TL present in the seeds, the level of NL was the highest (ca. 98.9% of TL), followed by GL (0.55% of TL) and PL (0.51% of TL), respectively. Subclasses of NL in the crude oil contained triacylglycerol (TAG), diacylglycerol (DAG), free fatty acids (FFA), monoacylglycerol (MAG) and esterified sterols (STE) in decreasing order (Table 1). A significant amount of TAG was found (ca. 97.1% of total NL) followed by a relatively low level of DAG (ca. 0.86% of total NL), while FFA, MAG and STE were recovered in lower levels. Subclasses of GL (Fig. 1) were sulphoquinovosyldiacylglycerol (SQD), digalactosyldiglycerides (DGD), cerebrosides (CER), sterylglycosides (SG), monogalactosyldiglycerides (MGD) and esterified sterylglycosides (ESG) as presented in Table 1. The proportion of each component was estimated by the lipid-carbohydrate determination. From the various reagents used in total carbohydrate estimation, phenol is most popular and apart from its high sensitivity, a further advantage is the equal response of hexose and sulfolipids when measuring the absorbance at 485 nm. In contrast, the colour developed with anthrone has different adsorption maxima for hexose (620 nm) and sulpholipids (590 nm). SG, ESG and CER were the prevalent components and made up about 75% of the total GL. The average daily intake of GL in human has been reported to be 140 mg of ESG, 65 mg of SG, 50 mg of CER, 90 mg of MGD and 220 mg of DGD [25]. Thus, *S. anacardium* seed oil could be used as a source of GL in diet. PL subclasses in oilseed extract were separated into four major fractions via NP-LC. Phosphorimetry of PL subclasses (Table 1) revealed that the predominant PL subclasses were PC followed by PE, PI and PS, respectively. About a half of total PL was in PC followed by PE (ca. 25%), while PI and PS were found in lower levels. In mango (*Mangifera indica* L., Family Anacardiaceae) kernel oil, NL represented 94.7% of the TL followed by PL (3.6%) and GL (1.7%), respectively [26].

Fatty Acid Profile of Seed Oil and Lipid Classes

Fatty acid profiles of TL and lipid classes (NL, GL and PL) are presented in Table 2. According to the results

Table 1 Lipid classes (g/kg TL) in *S. anacardium* crude seed oil

Lipid class	R_f values × 100[a]	g/kg TL	Glycolipid class	g/kg TL	Phospholipid class	g/kg TL
MAG	14	5.67 ± 0.23	SQD	0.35 ± 0.03	PS	0.25 ± 0.03
DAG	39	7.86 ± 0.33	DGD	0.64 ± 0.04	PI	0.74 ± 0.04
FFA	56	6.66 ± 0.21	CER	1.12 ± 0.04	PC	2.55 ± 0.09
TAG	79	885 ± 3.13	SG	1.55 ± 0.07	PE	1.22 ± 0.07
STE	95	5.43 ± 0.26	MGD	0.16 ± 0.02		
			ESG	1.32 ± 0.03		
Total glycolipids		5.14				
Total phospholipids		4.76				

Results are given as the average of triplicate determinations ± standard deviation

TL total lipids, *MAG* monoacylglycerols, *DAG* diacylglycerols, *TAG* triacylglycerols, *FFA* free fatty acids, *STE* sterol esters, *SQD* sulphoquinovosyldiacylglycerol, *DGD* digalactosyldiacylglycerol, *CER* cerebrosides, *SG* steryl glucoside, *MGD* monogalactosyldiacylglycerol, *ESG* esterified steryl glucoside, *PS* phosphatidylserine, *PI* phosphatidylinositol, *PC* phosphatidylcholine, *PE* phosphatidylethanolamine

[a] Solvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v)

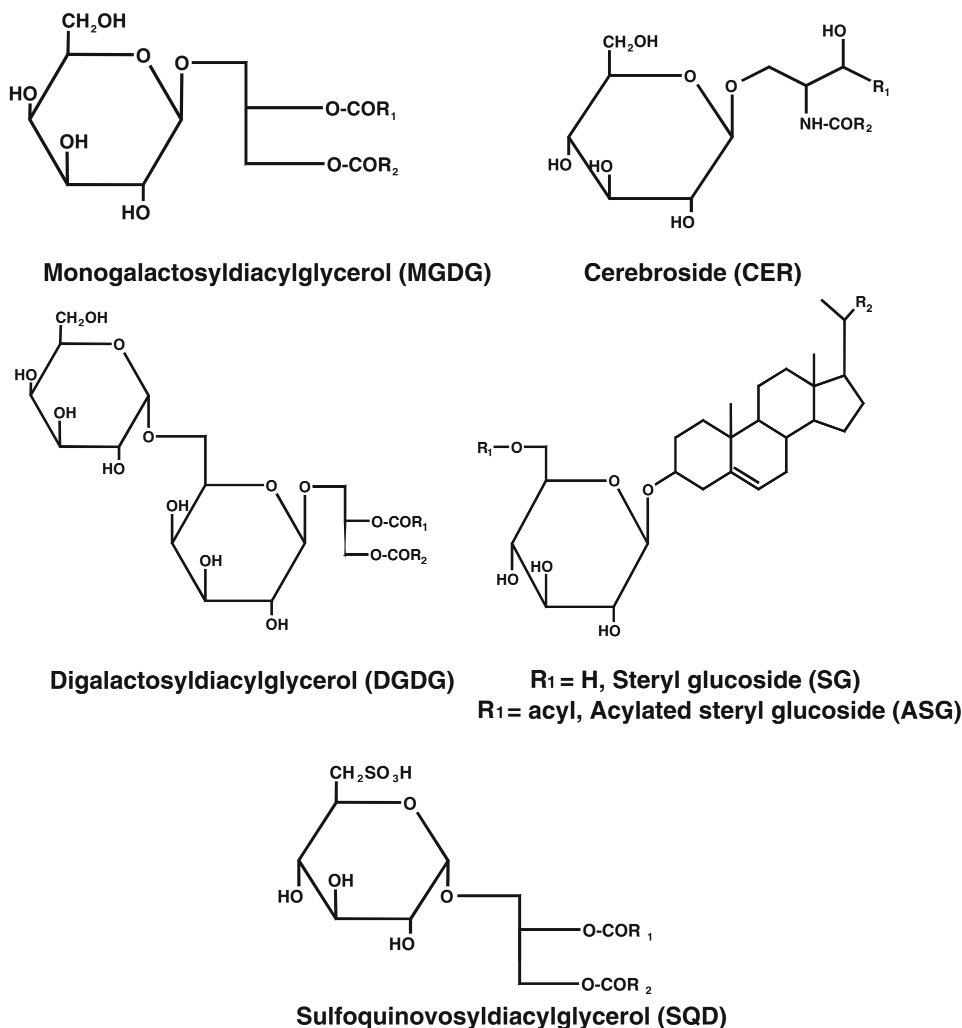

Fig. 1 Structures of glycolipids found in *S. anacardium*

shown in the table, eight fatty acids were identified in *S. anacardium* seed oil, wherein the analysis of FAME gave the proportion of linoleic followed by palmitic and oleic as the major fatty acids, which comprised together more than 90% of the total FAME. A striking feature of the *S. anacardium* seed oil was the relative high level of

Table 2 Fatty acid composition of *S. anacardium* seed oil and lipid classes

FAME compounds (fatty acid)	Total lipids Relative content (%)	Neutral lipids	Glycolipids	Phospholipids
Methyl hexadecanoate (C16:0)	12.6 ± 0.29	12.4 ± 0.32	12.7 ± 0.33	12.8 ± 0.31
Methyl *cis*-7-hexadecenoate (C16:1)	0.09 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.07 ± 0.01
Methyl octadecanoate (C18:0)	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.15 ± 0.02
Methyl *cis*-9-octadecenoate (C18:1n-9)	10.5 ± 0.43	10.7 ± 0.44	10.5 ± 0.49	10.5 ± 0.45
Methyl *cis*-9,12-octadecenoate (C18:2n-6)	68.8 ± 2.02	68.9 ± 2.06	68.6 ± 2.11	68.3 ± 2.15
Methyl *cis*-9,12,15-octadecatrienoate (C18:3n-3)	0.96 ± 0.02	0.98 ± 0.03	0.95 ± 0.03	0.93 ± 0.02
Methyl eicosanoate (C20:0)	3.42 ± 0.23	3.37 ± 0.26	3.46 ± 0.18	3.53 ± 0.15
Methyl docosanoate (C22:0)	2.83 ± 0.15	2.78 ± 0.14	2.87 ± 0.17	2.95 ± 0.14
Methyl tetracosanoate (C24:0)	0.69 ± 0.03	0.64 ± 0.04	0.71 ± 0.02	0.77 ± 0.02

Results are given as the averages of triplicate determinations ± standard deviation

polyunsaturated fatty acids (PUFA) which accounted for 70% of the total identified fatty acids. Fatty acids in neutral lipids and polar lipids were not significantly different from each other, wherein linoleic acid was the main fatty acid followed by palmitic acid. The ratio of unsaturated fatty acids to saturated fatty acid, however, was relatively higher in neutral fractions than in the corresponding polar fractions (GL and PL). Concerning saturated fatty acids (especially palmetic acid), GL resemble PL in the higher content of saturates, while saturated fatty acids were detected in lower levels in the corresponding NL. The fatty acid composition of TL and lipid classes of mango kernel oil revealed that the total saturated fatty acids of TL and NL of mango kernel oil were 44.6 and 46.5%, respectively, and the ratio of unsaturated to saturated fatty acids were 1.3 and 1.2, respectively. In general, stearic acid was the main saturated fatty acid, while oleic and linoleic were the major unsaturated fatty acids [26]. The fatty acid profile of *S. anacardium* seed oil points to the lipids as being a good source of the nutritionally essential fatty acids.

Sterols and Tocopherols Profile

β-Sitosterol was the sterol marker and comprised ca. 57% of the total ST content (Table 3). The next major components were campesterol and stigmasterol and these two major components constituted ca. 35% of the total ST. Other components, e.g., Δ7-avenasterol, Δ5-avenasterol and Δ7-stigmastenol, were presented at lower levels. Brassicasterol, lanosterol, sitostanol and Δ5, 24-stigmastadienol were not detected in the *S. anacardium* unsaponifiables. In mango kernel oil, *β*-Sitosterol was the most abundant sterol fraction followed by avenasterol, campesterol and stigmasterols [26]. The amounts of phytosterols in edible oils are used for the identification of oils, oil derivatives and for the determination of the oil quality. Furthermore, the concentration of ST has been reported to be little affected by environmental factors and/or by cultivation of new breeding lines [20]. Among the different plant sterols, sitosterol has been most intensively investigated with respect to its physiological effects in human. Many beneficial effects have been shown for the sitosterol. Phytosterols, in general, are of interest due to their antioxidant activity and impact on health. Recently, phytosterols have been added to vegetable oils for lowering the levels of cholesterol as an example of a successful functional food.

Table 3 Phytosterols and tocopherols (g/kg) in *S. anacardium* oil

Compound	g/kg
Campesterol	1.54 ± 0.05
Stigmasterol	1.16 ± 0.05
β-Sitosterol	4.65 ± 0.21
Δ5-Avenasterol	0.11 ± 0.03
Δ7-Stigmastenol	0.36 ± 0.02
Δ7-Avenasterol	0.21 ± 0.02
α-Tocopherol	0.12 ± 0.02
β-Tocopherol	0.36 ± 0.02
γ-Tocopherol	0.22 ± 0.03
δ-Tocopherol	1.32 ± 0.08

Results are given as the averages of triplicate determinations ± standard deviation

The levels and composition of tocopherols are summarized in Table 3. In our investigation, the NP-LC technique was used to eliminate column contamination problems and allow the use of a general lipid extract for tocopherols isolation (Fig. 2). Thus, saponification of oil samples was not required, which allowed shorter analysis time and greater vitamin stability during analysis [23]. Tocopherol isomers were present, wherein *δ*-tocopherol was the major compound and constituted ca. 65.3% of the total analytes. The rest of the compounds were

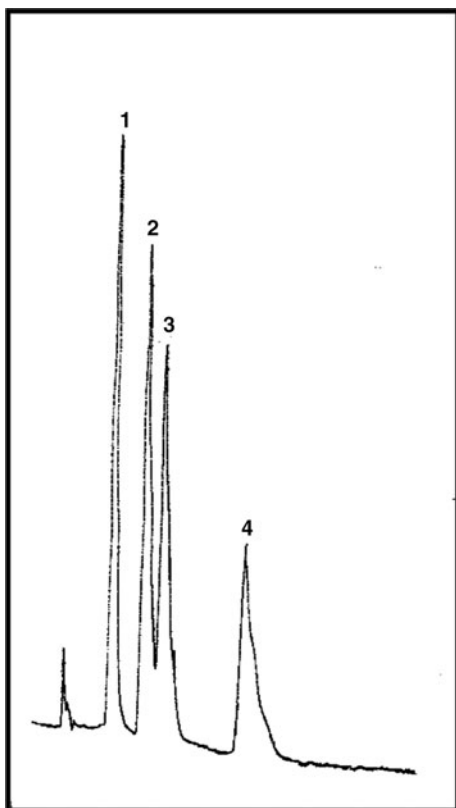

Fig. 2 Simultaneous isocratic normal-phase HPLC separation of tocopherols reference compounds mixture by direct injection into HPLC system. Detection was at 295 nm using isooctane/ethyl acetate (96:4, v/v) as the mobile phase. Key to peak identity: *1* α-tocopherol, *2* β-tocopherol, *3* γ-tocopherol, *4* δ-tocopherol (for chromatographic protocol see experimental section)

β-tocopherol (ca. 17.8 %), γ-tocopherol (ca. 10.8%) and α-tocopherol (ca. 5.9%). α-Tocopherol is the most efficient antioxidant of tocopherol isomers, while β-tocopherol has 25–50% of the antioxidative activity of α-tocopherol, and γ-isomer 10–35% [20]. Despite general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue in vivo, however, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in vitro, especially when compared to γ-tocopherol [27]. The nutritionally important components such as tocopherols (vitamin E) improve stability of the oil. Tocopherols are the major lipid-soluble, membrane-localized antioxidants in humans. A deficiency of these compounds affects many tissues in mammalian and bird models. Epidemiological studies suggest that people with lower vitamin E and other antioxidant intake may be at increased risk for certain types of cancer and atherosclerosis [1, 28]. Tocopherols in vegetable oils, moreover, are believed to protect PUFA from peroxidation. Levels of tocopherols detected in *S. anacardium* seed oil may contribute to the stability of the oil.

Radical Scavenging Activity of *S. anacardium* Oil in Comparison with Extra Virgin Olive Oil

Interest has increased in the free radical theory of disease causation, particularly in vascular diseases and cancer. These developments have led to the investigation of the role of antioxidants in disease protection. A free radical is defined as any chemical species that has one or more unpaired electrons. Oxidation is a natural and needed reaction in metabolism, wherein highly reactive hydroxyl radicals and peroxyl radical formed. These can attack DNA, protein and polyunsaturated fatty acids residues of membrane phospholipids. Antioxidants quench those radicals and if the supply of antioxidants is inadequate, a chain reaction takes place that may lead to damaged tissue [20, 29].

Numerous methods are used to evaluate the antioxidant activities of natural compounds in foods or biological systems with varying results. The tests expressing antioxidant potency can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. Depending upon the reactions involved, furthermore, antioxidant capacity assays can be classified into two types: assays based on hydrogen atom transfer reactions and assays based on electron transfer. Galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH·) are commonly used to assess antioxidant activity in vitro. These methods are easy and accurate methods with regard to measuring the antioxidant capacity of both lipophilic and hydrophilic compounds found in fruit, vegetable juices or extracts and edible oils [24, 29, 30]. An organic solvent based oxygen radical absorbance capacity (ORAC) assay was developed whereby both the lipophilic and hydrophilic ORAC can be determined in the same sample to assess total antioxidant capacity [31]. However, fluorescein is not sufficiently lipid soluble, and its fluorescence intensity in nonpolar organic solvent is rather low [32].

Apart from the oxidative stability of vegetable oils and fats depends on the fatty acid composition, the presence of minor fat-soluble bioactives and the initial amount of hydroperoxides. Antiradical properties of the *S. anacardium* oil and extra virgin olive oil (as standard oil with respective high levels of nutritive antioxidants and bioactives) were compared using stable DPPH· and galvinoxyl free radicals. Figure 3 shows the RSA of *S. anacardium* oil and olive oil measured toward DPPH· and galvinoxyl radicals. It can be seen that *S. anacardium* oil has higher RSA than extra virgin olive oil. After 1 h of incubation, 35% of the DPPH radicals were quenched by *Celastrus paniculatus* oil, while olive oil was able to quench only 8.4%. ESR measurements also showed the same pattern, wherein *Celastrus paniculatus* oil quenched 31%

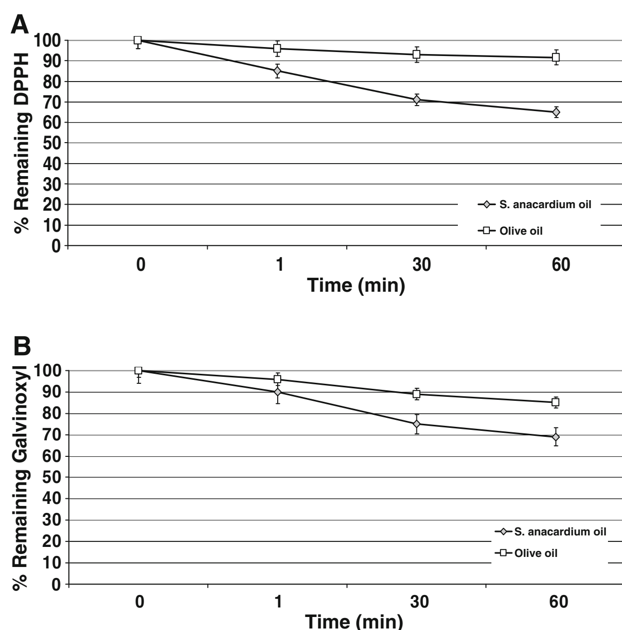

Fig. 3 Scavenging effect at different incubation times of *S. anacardium* crude seed oil and extra virgin olive oil on **a** DPPH radical as measured by changes in absorbance values at 515 nm and on **b** galvinoxyl radical as recorded by ESR. Values given are the mean of three replicates and *error bars* show the variations of three determinations in terms of standard deviation

galvinoxyl radical and extra virgin olive oil quenched 15% after 60 min of reaction.

Regarding the composition of *S. anacardium* oil and extra virgin olive oil, they have a different pattern of fatty acid and lipid-soluble bioactives. *S. anacardium* oil was characterized by a higher phenolic content (410 mg/kg) than extra virgin olive oil (350 mg/kg) as determined by the Folin-Ciocalteu method. The RSA of oils and fats can be interpreted as the combined action of different endogenous antioxidants. Moreover, the stronger antiradical action of *Celastrus paniculatus* oil compared to extra virgin olive oil may be due to (1) the differences in content and composition of polar lipids and unsaponifiables (2) the diversity in structural characteristics of potential phenolic antioxidants present, (3) a synergism of polar lipids with other components present, and (4) different kinetic behaviors of potential antioxidants. All these factors may contribute to the radical quenching efficiency of oils and fats.

Conclusions

Knowledge of the composition of *S. anacardium* seeds will assist in efforts for the industrial application of this plant. Data on *S. anacardium* seeds are very few; on the other hand, there are no reports in the literature about the detailed composition of *S. anacardium* seed oil. It may be concluded that the *S. anacardium* seeds give a considerable yield of oil and the oil seem to be a good source of essential fatty acids and lipid-soluble bioactives. The high linoleic acid content makes the oil nutritionally valuable. Tocopherols and sterols at the level estimated may be of nutritional importance in the application of the seed oil. *S. anacardium* seeds could be nutritionally considered as a new non-conventional supply for the pharmaceutical industries and edible purposes.

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