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Characterisation of fatty acids and bioactive compounds of kachnar (*Bauhinia purpurea* L.) seed oil

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Abstract

Information concerning the exact composition of kachnar (*Bauhinia purpurea*) seed oil is scare. In the present contribution, a combination of CC, GC, TLC and normal-phase HPLC were performed to analyse lipid classes, fatty acids and fat-soluble bioactives of kachnar seed oil. *n*-Hexane extract of kachnar oilseeds was found to be 17.5%. The amount of neutral lipids in the crude seed oil was the highest (ca. 99% of total lipids), followed by glycolipids and phospholipids, respectively. Linoleic, followed by palmitic, oleic and stearic, were the major fatty acids in the crude seed oil and its lipid classes. The ratio of unsaturated fatty acids to saturated fatty acid, was higher in neutral lipid classes than in the polar lipid fractions. The oil was characterised by a relatively high amount of phytosterols, wherein the sterol markers were β-sitosterol and stigmasterol. β-Tocopherol was the major tocopherol isomer with the rest being δ-tocopherol. In consideration of potential utilisation, detailed knowledge of the composition of kachnar (*B. purpurea*) seed oil is of major importance.

Keywords: Kachnar seed oil; *Bauhinia purpurea* L.; Chromatographic analysis; Fatty acids; Lipid-soluble bioactives

1. Introduction

A large quantity of oils and fats, whether for human consumption or for industrial purposes, is presently derived from plant sources. To meet the increasing demand for oils, improvements are being made, with conventional crops, as well as with selected plant species, that have the ability to produce unique desirable oils. Interest in newer sources of edible oils has recently grown. Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. On the other hand, no oil from a single source has been found to be suitable for all purposes because oils from different sources generally differ in their composition. This necessitates the search for new sources of novel oils. Several plants are now grown, not only for food and fodder, but also for a striking variety of products with applications in industry, including oils and pharmaceuticals.

The genus *Bauhinia*, consisting of 300 species, belongs to the family Leguminosae (Caesalpinioideae). *Bauhinia Purpurea* L. is found throughout peninsular India. It is a small evergreen medicinal and avenue tree. The root, stem, bark and leaves are being used against many diseases, such as jaundice, leprosy, cough, and also used in several Ayurvedic medicine formulations (Parrota,

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2001). The young pods and mature seeds of kachnar (*B. purpurea*) are known to be cooked and eaten by tribles such as the Kathkors and Gondas of India (Rajaram & Janardhanan, 1991).

The fatty acid composition and phenolics profile of the *B. purpurea* seeds have been reported (Badami & Daulatabad, 1969; Bharatiya & Gupta, 1981; Bharatiya, Dubey, Katiyar, & Gupta, 1979). The study of kachnar seed oil for its minor constituents, however, is useful in order to use both oil and the minor constituents effectively. Natural fats and oils contain, apart from glycerides, a number of lipophilic materials with a very diverse chemical make up. Among the most interesting are the glycolipids, phospholipids, sterols and fat-soluble vitamins. In this work, lipid classes, fatty acids and fat-soluble bioactives of kachnar seed oil have been analysed. The objective of this investigation was to understand the chemical nature of *B. purpurea* seed oil, which will serve as a basis for further detailed chemical investigation and nutritional evaluation of the kachnar (*B. purpurea*) seeds. The results, furthermore, will be important as an indication of the potentially nutraceutical and economical utility of kachnar (*B. purpurea*) seeds as a new source of edible oils.

2. Material and methods

2.1. Materials

Mature kachnar (*B. purpurea* L.) seeds were collected in February, 2004 from Gulbarga University Campus, Gulbarga (India) and the plant was identified with the help of the Flora of Gulbarga District (Seetharam, Kotresh, & Uplokar, 2000). A voucher specimen (No. HGUG 206) was deposited at the *Herbarium*, department of Botany, Gulbarga, University Gulbarga (India). Neutral lipid (NL) standards were from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for glycolipids (GL) identification, namely monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG) and esterified steryl glucoside (ESG), were of plant origin (plant species unknown) and purchased from Biotrend Chemikalien GmbH (Köln, Germany). Standards used for phospholipids (PL) identification, namely phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI) from Bovine liver and phosphatidylcholine (PC) from soybean, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for sterols (ST) characterisation were purchased from Supelco (Bellefonte, PA, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol) were purchased from Merck (Darmstadt, Germany). Reagents and chemicals used were of the highest purity available.

2.2. Methods

2.2.1. Solvent extraction of total lipids

Seed material were finely ground (particle size = 2 mm) and Soxhlet-extracted with *n*-hexane for 8 h. Total lipids (TL) recovered were weighed and stored in chloroform at 4 °C for further analysis.

2.2.2. Column chromatography and thin-layer chromatography of lipid class

2.2.2.1. Fractionation of lipid classes and subclasses. TL were separated into the different classes by elution with different solvents over a glass column (20 mm dia × 30 cm) packed with a slurry of activated silicic acid (70–230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v). NL were eluted with 3-times the column volume of chloroform. The major portion of GL was eluted with 5 times the column volume of acetone and that of PL with 4 times the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of thin-layer chromatography (TLC) on silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany), a further characterisation of the GL and PL subclasses was carried out with the following solvent system: 25% chloroform/methanol/ammonia solution (65:25:4, v/v/v). For the characterisation of NL subclasses, silica gel F_{254} plates were developed in the solvent system *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For the detection of the lipids, the thin-layer plates were sprayed with the following agents: for the marking of all lipids with sulphuric acid (40%), for the marking of GL with α-naphthol/sulphuric acid and for the marking of PL with the molybdate-blue reagent (Kates, 1972). Each spot was identified with lipid standards as well as their reported retention factor (R_f) values. Individual bands were visualised under ultraviolet light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v). Fatty acid composition of NL, GL and PL were determined by GLC/FID as described below.

2.2.2.2. Quantitative determination of lipid subclasses. For the quantitative determination of NL subclasses, individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. Data presented are the averages of three gravimetric determinations. For the quantitative estimation of GL subclasses, the acetone fraction obtained by column chromatography (CC) was separated by TLC in the above given solvent system. The silica gel regions with the corresponding GL subclasses were scraped off followed by hexose measurement (photometrically at 485 nm using the phenol/sulphuric acid in acid-hydrolysed lipids) (Southgate, 1976). The percent distribution of each component was obtained from the hexose values. From the extinction values,

the quantitative amount was determined and related to their portion of the GL fraction. The determined portion was set into relation with the amount of TL, which had been separated by CC into the main lipid fractions. For the determination of the PL, the methanol fraction from CC was also separated by TLC in the above given solvent system and, after scraping off of the individual PL subclasses, brought to reaction with the hydrazine sulphate/sodium molybdate reagent at 100 °C for 10 min and photometrically analysed at 650 nm according to the AOCS method (1990). From the obtained extinction values, via a calibration chart for phosphorus, the amount of PL was calculated. The individual values were recorded in relation to the PL fraction (methanol fraction from CC) and to the amount of TL.

2.2.3. Gas chromatography analysis of fatty acid methyl ester

Fatty acids were transesterified into methyl esters (FAME), using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany), according to the procedure reported by Arens, Schulte, and Weber (1994). FAME were identified on a Shimadzu Gas chromatography (GC)-14A equipped with flame ionisation detector (FID) and 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 onto a 30 m × 0.25 mm × 0.2 μm film thickness, Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperatures were set at 250 °C. The initial column temperature was 100 °C, programmed by 5 °C/min to 175 °C and kept for 10 min at 175 °C, then by 8 °C/min to 220 °C and kept for 10 min at 220 °C. A comparison of the retention times of the samples with those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

2.2.4. Gas chromatography analysis of sterols

Separation of sterols (ST) was performed after saponification of the oil sample without derivatisation according to Ramadan and Mörsel (2003). TL (250 mg) were refluxed with 5 ml of ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were first extracted 3 times with 10 ml of petroleum ether; the extracts were combined and washed 3-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, then ether was completely evaporated under nitrogen. GLC analyses of unsaponifiable residues were carried out using a Mega Series instrument (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 (split-splitless injection was used). 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.

2.2.5. Normal-phase high performance liquid chromatography separation, identification and quantification of tocopherols

2.2.5.1. Procedure. Normal-phase high performance liquid chromatography (NP-HPLC) was selected to avoid extra sample treatment (e.g., saponification) according to Ramadan and Mörsel (2002). Analysis was performed with a solvent delivery LC-9A HPLC (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 backpressure 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 microliters of the diluted solution of TL in the mobile phase were directly injected into the HPLC column. Tocopherol isomers were identified by comparing their retention times with those of authentic standards.

2.2.5.2. Preparation of standard curves. Standard solutions were prepared by serial dilution to concentrations of approximately 5 mg/ml of each tocopherol isomer. Standard solutions were prepared from a stock solution which was stored in the dark at −20 °C. Twenty microliters were injected and peak areas were determined to generate standard curve data.

2.2.5.3. Quantification. All quantitation was by peak area using a Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions, repeated injections of different concentrations of the standard tocopherols were made, 3 times, onto the HPLC system. Injections in triplicate were made, at each concentration, for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation on any one was routinely less than 5%.

All experimental procedures were performed in triplicate and their mean values (±standard deviation) are given.

3. Results and discussion

3.1. General

In view of the good medicinal properties of kachnar (*B. purpurea*) and widespread distribution in the south Indian region, the seeds are being used by tribal people for edible purposes. However, a large quantity of the seed is wasted. In fact, for a plant to be suitable for oil production, it must meet the following two criteria: (i) the oil content must reach the minimum for commercially viable exploitation and (ii) the plant must be suitable for high acreage cultivation. The only exceptions are plants that contain lipids unique in their composition or with properties that can not be found elsewhere (Bockisch, 1998). In the present investigation kachnar (*B. purpurea*) seeds were found to contain about 17.5% crude seed oil.

3.2. Levels of lipid classes and subclasses

A suitable combination of chromatographic procedures on Silica gel was used to obtain major lipid classes and subclasses of kachnar seed oil. The proportions of lipid classes and subclasses presented in kachnar seed oil as well as R_f values of these subclasses are shown in Table 1. Among the TL present in the seeds, the level of NL was the highest (ca. 99% of TL), followed by GL (0.44% of TL) and PL (0.27% of TL), respectively. Subclasses of NL in the crude oil contained triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DAG), esterified sterols (STE), free sterols (ST) and monoacylglycerol (MAG) in decreasing order. A significant amount of TAG was found (ca. 93.0% of total NL), followed by a relatively low level of FFA (ca. 1.2% of total NL), while DAG and STE were recovered in approximately equal levels (ca. 0.7% of total NL). Subclasses of GL in the crude seed oil 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 sulpholipids 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). ESG, SG and CER were the prevalent components and made up about 93% of the total GL. The average daily intakes of GL in the human have 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 (Sugawara & Miyazawa, 1999). Therefore, it is noteworthy that kachnar crude seed oil could be a good source of GL in the diet. PL subclasses in oilseed extract were separated into four major fractions via TLC. Phosphorimetry of the TLC fractions (Table 1) revealed that the predominant PL subclasses were PC, followed by PE, PI and PS, respectively. About one half of total PL was PC and one quarter was PE, while PI and PS were isolated in smaller quantities.

3.3. Fatty acid profile of seed oil and its lipid classes

Fatty acid profiles of TL and lipid classes (NL, GL and PL) are presented in Table 2. According to the results shown in the table, twelve fatty acids were identified in kachnar seed extract, wherein the analysis of FAME showed that linoleic, followed by palmitic, oleic and stearic acids were the major fatty acids, which together comprised more than 97% of total identified FAME. A striking feature of the kachnar seed oil was

Table 1
Levels of lipid subclasses (g/kg TL) in kachnar (*Bauhinia purpurea* L.) crude seed oil

Neutral lipid subclass	R_f values × 100[a]	g/kg TL	Glycolipid subclass	R_f values × 100[b]	g/kg TL	Phospholipid subclass	R_f values × 100[b]	g/kg TL
MAG	14	2.97 ± 0.07	SQD	6	0.01 ± 0.03	PS	4.7	0.14 ± 0.02
DAG	39	6.93 ± 0.13	DGD	17	0.08 ± 0.05	PI	11	0.34 ± 0.03
FFA	56	11.8 ± 0.15	CER	29–35	1.19 ± 0.08	PC	20	1.50 ± 0.07
TAG	79	920 ± 2.09	SG	41	1.41 ± 0.08	PE	30	0.69 ± 0.06
STE	95	6.63 ± 0.09	MGD	64	0.15 ± 0.04			
			ESG	76	1.50 ± 0.07			

Results are given as the averages of triplicate determinations ± standard deviation.
Abbreviations: 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).
[b] Solvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v).

Table 2
Fatty acid profile of kachnar (*Bauhinia purpurea* L.) crude seed oil and its' lipid classes

Fatty acid	Relative content (%)			
	Total lipids	Neutral lipids	Glycolipids	Phospholipids
C16:0	22.1 ± 0.88	21.1 ± 0.82	27.3 ± 0.79	27.8 ± 0.98
C16:1	0.15 ± 0.02	0.15 ± 0.03	0.17 ± 0.03	0.16 ± 0.02
C18:0	13.6 ± 0.36	13.7 ± 0.32	15.4 ± 0.25	15.0 ± 0.34
C18:1	16.3 ± 0.39	16.4 ± 0.42	16.6 ± 0.44	16.7 ± 0.49
C18:2	45.9 ± 1.25	46.8 ± 1.36	37.7 ± 1.21	38.0 ± 0.96
C20:0	0.26 ± 0.07	0.29 ± 0.05	0.24 ± 0.04	0.31 ± 0.06
C18:3 $n-3$	0.29 ± 0.09	0.12 ± 0.02	0.21 ± 0.01	0.19 ± 0.03
C18:3 $n-6$	0.16 ± 0.03	0.17 ± 0.04	0.13 ± 0.02	0.08 ± 0.03
C22:0	0.13 ± 0.01	0.11 ± 0.01	0.14 ± 0.03	0.12 ± 0.02
C20:2	0.33 ± 0.09	0.35 ± 0.08	0.83 ± 0.11	0.39 ± 0.09
C20:5 EPA	0.30 ± 0.07	0.21 ± 0.05	0.98 ± 0.15	0.59 ± 0.12
C24:1	0.32 ± 0.09	0.18 ± 0.05	0.28 ± 0.07	0.65 ± 0.06

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

the relatively high level of polyunsaturated fatty acids (PUFA). Trienes [(γ-linolenic acid, GLA, C18:3$n-6$) and (α-linolenic acid, ALA, C18:3$n-3$)] as well as EPA (C20:5), were also found in a relatively lower amounts. Fatty acids in neutral lipids and polar lipids did not differ significantly from each other; linoleic acid was the main fatty acid, followed by palmitic acid. The ratio of unsaturated fatty acids to saturated fatty acid, however, was higher in neutral fractions than in the corresponding polar fractions (GL and PL). Among the saturated fatty acids (especially palmitic and stearic), GL resemble PL in their higher contents of saturates, while saturated fatty acids were detected at lower levels in the corresponding NL. The fatty acid profile of kachnar seed oil shows that the lipids are a good source of the nutritionally essential fatty acids. A great deal of interest has been placed on the few oils that contain PUFA, especially GLA. The sources of natural GLA are few and, at present, only borage, evening primrose, hemp and hopseed oils are well known. The fruit seeds belonging to the Ribes family have recently been shown to contain significant levels of GLA; these include black currant oil (15–18%), red currant oil and gooseberry oil (Kamel & Kakuda, 2000). Moreover, interest in the PUFA as health-promoting nutrients has expanded dramatically in recent years. A rapidly growing literature illustrates the benefits of PUFA, in alleviating cardiovascular, inflammatory conditions, heart disease, atherosclerosis, autoimmune disorders, diabetes and other diseases (Finley & Shahidi, 2001; Riemersma, 2001). The fatty acid composition and high amounts of PUFA make the kachnar seed oil a special component for nutritional applications.

3.4. Sterol analysis and composition

Levels of phytosterols (ST) in vegetable oils are used for the identification of oils, oil derivatives and for the determination of the oil quality (Artho, Grob, & Marianai, 1993; De-Blas & Del-Valle, 1996; Grob, Laufranchi, & Mariani, 1990; Homberg, 1991; Horstmann & Montag, 1987). Furthermore, the concentration of ST has been reported to be little affected by environmental factors and/or by cultivation of new breeding lines (Hirsinger, 1989; Homberg, 1991). Kachnar seed oil is characterised by a relatively high amount of unsaponifiables (11.9 g/kg TL), of which ca. 49% were phytosterols. Six compounds were postulated (Fig. 1), wherein the sterol marker was β-sitosterol which comprised ca. 64.5% of the total ST content (Table 3). The next major component was stigmasterol and these two major components were constituted ca. 85% of the total ST. Other components, e.g., campesterol and Δ7-stigmastenol, were in approximately equal amounts (ca. 6.0% of total ST). Δ7-Avenasterol and Δ5-avenasterol were at a lower levels, while brassicasterol, lanosterol, sitostanol and Δ5, 24-stigmastadinol were not detected in the kachnar unsaponifiables. Among the different plant sterols, sitosterol has been most intensively investigated with respect to its physiological effects in man. Many beneficial effects have been shown for the sitosterol (Yang, Karlsson, Oksman, & Kallio, 2001). Phytosterols, in general, are of interest, due to their antioxidant activity and impact on health. Recently, phytosterols have been added to vegetable oils as an example of a successful functional food (Ntanios, 2001).

3.5. Tocopherols profile

The nutritionally important components, such as tocopherols (vitamin E), improve stability of the oil. Tocopherols are the major lipid-soluble, membrane-localised antioxidants in humans. Deficiency of these compounds affects many tissues in mammalian and bird models (Nelson, 1980). Vitamin E deficiency in man causes defects in the developing nervous system of children and hemolysis in man (Sokol, 1996). Epidemiologic studies suggest that people with lower vitamin E and

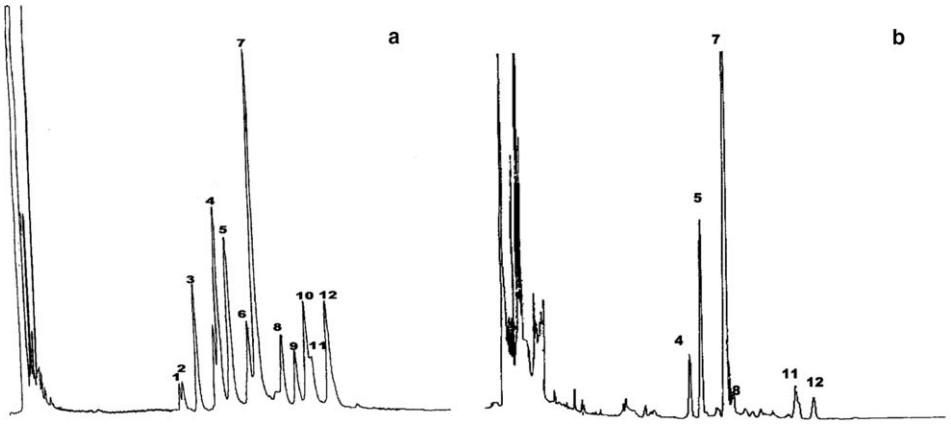

Fig. 1. GC/FID Separation of intact sterol standard mixture (a) and unsaponifiable matter of kachnar seed oil (b) by direct injection into the GC system without derivatisation. Key to peak identity: 1, cholesterol ($R_t = 21$ min); 2, ergosterol ($R_t = 22$ min); 3, brassicasterol ($R_t = 23.7$ min); 4, campesterol ($R_t = 26$ min); 5, stigmasterol ($R_t = 28$ min); 6, lanosterol ($R_t = 29$ min); 7, β-sitosterol ($R_t = 31.3$ min); 8, Δ5-avenasterol ($R_t = 33.6$ min); 9, sitostanol ($R_t = 34.5$ min); 10, Δ5,24-stigmastadienol ($R_t = 36.1$ min); 11, Δ7-stigmastenol ($R_t = 37.6$ min); 12, Δ7-avenasterol ($R_t = 40.3$ min), (for chromatographic protocol see Section 2).

Table 3
Levels of sterols and tocopherols (g/kg) in kachnar (*Bauhinia purpurea*) crude seed oil

Compound	g/kg
Brassicasterol	nd
Campesterol	0.36 ± 0.04
Stigmasterol	1.22 ± 0.10
Lanosterol	nd
β-Sitosterol	3.83 ± 0.12
Δ5-Avenasterol	0.02 ± 0.01
Sitostanol	nd
Δ5, 24-Stigmastadinol	nd
Δ7-Stigmastenol	0.32 ± 0.03
Δ7-Avenasterol	0.17 ± 0.01
α-Tocopherol	nd
β-Tocopherol	2.57 ± 0.08
γ-Tocopherol	nd
δ-Tocopherol	0.99 ± 0.07

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

other antioxidant intakes and plasma levels may be at increased risk for certain types of cancer and for atherosclerosis (Gey, Puska, Jordan, & Moser, 1991; Rimm et al., 1993). It is also suggested that supplementation with antioxidants may decrease the risk of these and other degenerative processes (Kallio, Yang, Peippo, Tahvonen, & Pan, 2002). Tocopherols in vegetable oils, moreover, are believed to protect PUFA from peroxidation (Kamal-Eldin & Andersson, 1997). Data about the qualitative and quantitative composition of vitamins E are summarised in Table 3. In our investigation, the NP-HPLC technique was used to eliminate column contamination problems and to allow the use of a general lipid extract for tocopherol isolation. Thus, saponification of oil sample was not required, which allowed shorter analysis time and greater vitamin stability during analysis. Two of the four tocopherol isomers were present (Fig. 2), and β-tocopherol constituted ca. 72.2% of the total analytes, the rest being δ-tocopherol (ca. 27.8%). α-Tocopherol is the most efficient antioxidant of the tocopherol isomers, while β-tocopherol has 25–50% of the antioxidative activity of α-tocopherol, and the γ-isomer 10–35% (Kallio et al., 2002). Despite general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue in vivo, other studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in vitro, especially when compared to γ-tocopherol (Kamal-Eldin & Appelqvist, 1996). Levels of tocopherols detected in kachnar seed oil may contribute to protect oil against oxidation.

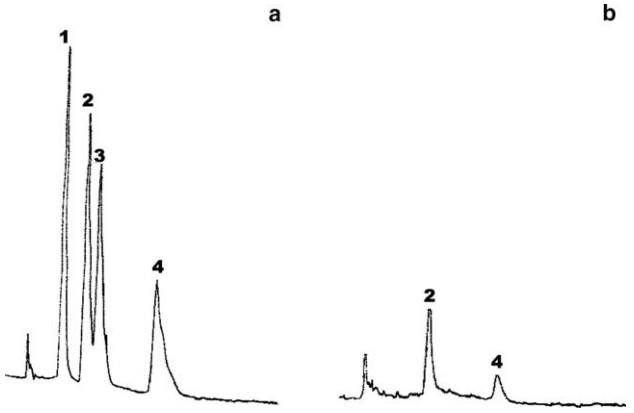

Fig. 2. Simultaneous isocratic normal-phase HPLC separation of tocopherols reference mixture (a) and tocopherol isomers present in kachnar crude seed oil (b) by direct injection of seed oil into HPLC system. Detection was at 295 nm using isooctane/ethyl acetate (96:4, v/v) as a mobile phase. Key to peak identity: 1, α-tocopherol; 2, β-tocopherol; 3, γ-tocopherol; 4, δ-tocopherol (for chromatographic protocol see Section 2).

4. Conclusions

Improved knowledge on the composition, analysis and properties of kachnar seeds would assist in efforts to achieve industrial application of this plant. Data about kachnar seeds are very few, and there are no reports of the detailed composition of kachnar seed oil. In concluding this investigation, it is clear that the performed chromatographic techniques constituted a flexible analytical system, which gave valuable information about the structure of the seed oil. The kachnar seeds give a considerable yield of oil and the oil seems 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. Kachnar seeds could be nutritionally considered as a new non-conventional supply for the pharmaceutical industries and for edible purposes.

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