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Analysis of glycolipids from black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) oilseeds

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Abstract

Edible plant glycolipids (GL) are anticipated to play a role in human nutrition. Total glycolipids (TGL) were separated from black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) seed oils by silica gel chromatography. Different GL subclasses were then identified and separated using high-performance liquid chromatography with ultraviolet adsorption (HPLC/UV). Separation was accomplished using Zorbax-Sil (5 μm) column with an isocratic elution by mixed solvents of isooctane/2-propanol (1:1, v/v) and detection at 206 nm. Methods are described for the analysis of GL constituents, sugar and sterols (ST), using gas–liquid chromatography equipped with flame ionization detector (GLC/FID). A relatively high level of TGL was found in all studied oilseeds. Six GL subclasses were detected in black cumin seed oil, wherein diglucosyldiacylglycerol (DGD) was the prevalent component, followed by glucocerebroside (CER). Among the TGL from coriander and niger oilseeds, acylated steryl glucoside (ASG), steryl glucoside (SG) and CER were detected. The fatty acid profiles of GL fractions from black cumin and niger seed oils was generally similar, wherein linoleic acid C18:2n-6 was the dominating fatty acid, followed by oleic acid C18:1n-9. Petroselinic acid C18:1n-12 was the fatty acid marker in GL subclasses obtained from coriander seed oil, followed by linoleic acid C18:2n-6. Four ST moieties were identified in black cumin and coriander SG and ASG fractions, while the fractions from niger oilseeds showed only three distinct ST peaks. As component sugar, glucose was the only sugar detected in all samples.

Keywords: *Nigella sativa* L.; *Coriandrum sativum* L.; *Guizotia abyssinica* Cass.; Glycolipids; Seed oil; Sugas; Sterols

1. Introduction

Oilseeds are important sources of oils of nutritional, industrial and pharmaceutical importance. Non-conventional oileeds are being considered because their constituents have unique chemical properties and may augment the supply of edible oils (Cherry & Kramer, 1989). So far, a large number of plants have been analyzed and some of these have been cultivated as new oil crops (Hirsinger, 1989). Discovery of niche markets will come from an understanding of what drives customer needs and wants (Joseph, 1992). The study of oilseeds for their minor constituents is useful in order to use both oil and minor constituents effectively. Natural fats and oils contain, apart from the triacylglycerols, a number of lipophilic materials of the most diverse chemical make up. Among the most interesting are the polar lipids, including glycolipids (GL) and phospholipids. Edible plant GL (Fig. 1) are thought to be nutrients in the human diet, but little is known about their intestinal digestion and absorption in mammals (Andersson, Bratt, Arnoldsson, Herslof, Olsson, Sternby et al., 1995). Information on GL of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) oilseeds is important in processing and utilizing the oil and by-products. Although the fatty acids composition of selected seed oils, has been reported (Babayan, Koothungal, & Halaby, 1978; Birgrit, Marion, & Eberhard, 1998; Dagne & Jonsson, 1997;

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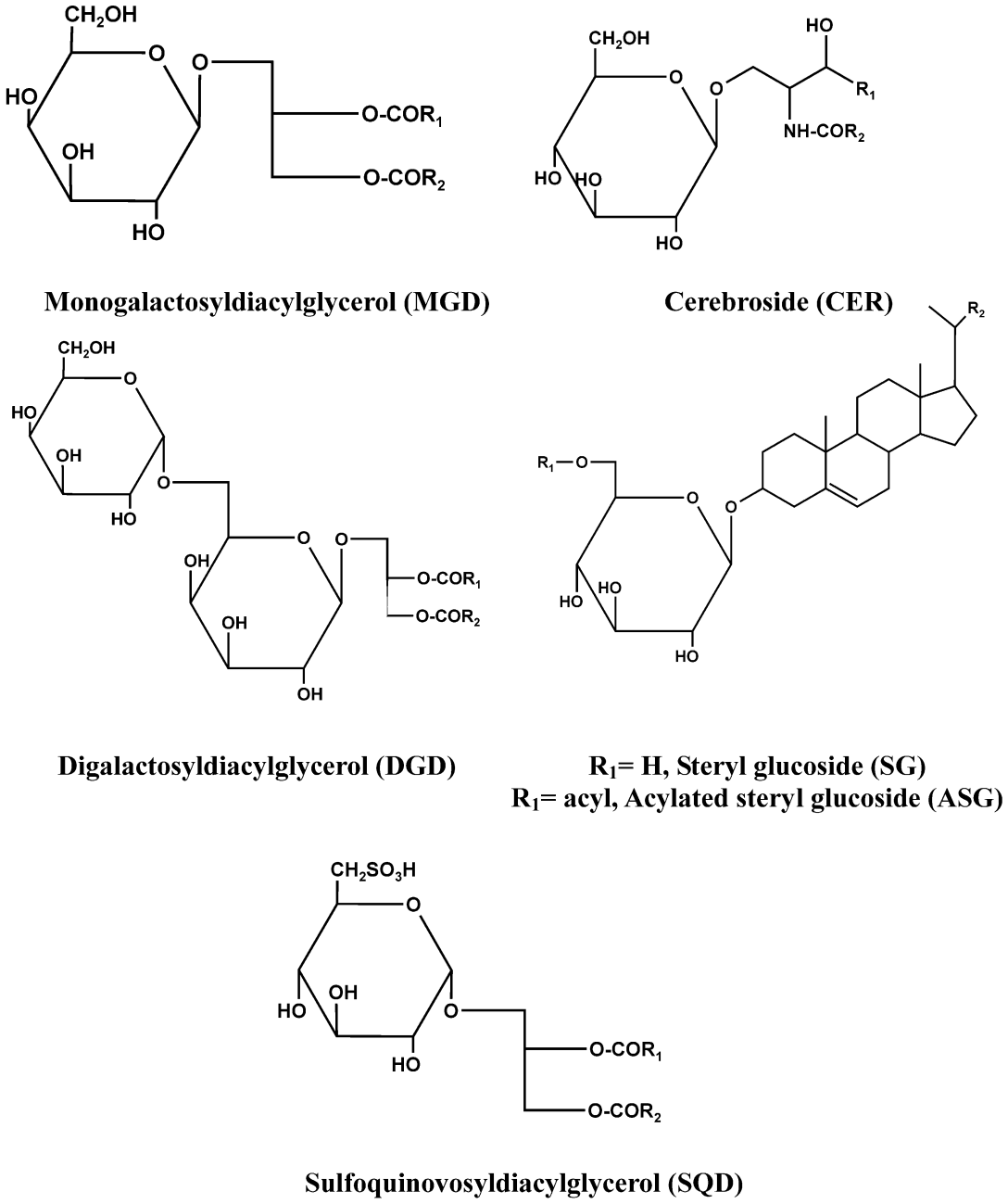

Fig. 1. Representative structures of glycolipid components investigated in the present study.

Dutta, Helmersson, Kebedu, Alemaw, & Appelqvist, 1994; Lakshminarayana, Rao, Devi, & Kaimal, 1981; Ramadan & Mörsel, 2002; Saleh Al-Jasser, 1992; Subbaram & Youngs, 1967), additional data on these oilseeds are needed, as part of the effort to assess the potential of these crops. No data about GL constitution of selected oilseeds are available.

Plant GL comprise compounds which can be divided into distinct groups with regard to their hydrophobic aglycones. In terms of quantitative abundance, glycosyl diacylglycerols usually rank first followed by glycosylated cereamides and sterols (Heinz, 1996). These GLs are widely distributed, if not universal, in edible plants (Allen, Good, Davis, Chisum & Fowler, 1966; Harwood, 1980; Mudd & Garcia, 1975). Analysis of GL requires methodology for isolation of the different compounds, elucidation of their chemical structures, release and separation of constituent building blocks and quantification. In previous studies (Choudhury & Juliano, 1980; Galliard, 1968; Kinsella, 1971; Morrison, Mann, Soon & Conentry, 1975; Walter, Hasen, & Purcell, 1971; Weber, 1979), phytoglycolipids have been quantified gravimetrically or colorimetrically after separating and isolating them by column chromatography or thin-layer chromatography (TLC). Apart form structural analysis, there is an

increasing demand for easy and reliable separation and quantification of various compounds, ideally by high-performance liquid chromatography (HPLC). HPLC is one of the most popular tools for the analysis of lipid classes and can be automated to a considerable degree to give much cleaner fractions in micropreparative applications. Recently (Lim, Park, & Suzuki, 1999; Picchinoi, Watada, & Whitaker, 1996; Sugawara & Miyazawa, 1999; Yamauchi, Aizawa, Inakuma, & Kato, 2001), plant GL have been identified and quantified by HPLC using different detectors, columns and solvent systems.

In this connection, our investigation has been directed at the determination of GL in seeds of *N. sativa* L., *C. sativum* L. and *G. abyssinica* Cass. Total glycolipids (TGL) were separated from the seed oils under study by silica gel chromatography. Different GL subclasses were then identified and separated using HPLC with ultraviolet (UV) adsorption. Methods are described for the analysis of GL constituents, sugar and sterols (ST), using gas-liquid chromatography equipped with flame ionization detector (GLC/FID). The results are important as an indication of the potential economical utility of these seeds as a new source of edible oils.

2. Material and methods

2.1. Material

Mature black cumin (*N. sativa* L.), coriander (*C. sativum* L.) and niger (*G. abyssinica* Cass.) seeds were obtained from *Alfred Galke* GmbH (Gittelde, Germany) and stored at 4 °C until extraction. Standards used for GL identification, monogalactosyldiacylglycerol (MGD), digalactosyldiacylglycerol (DGD), cerebrosides (CER), steryl glucoside (SG) and acylated steryl glucoside (ASG) were of plant origin (plant species unknown) and purchased from *Biotrend* Chemikalien GmbH (Köln, Germany). Standards used for ST characterization, β-sitosterol, stigmasterol, lanosterol, ergosterol, campesterol, Δ5-avenasterol and Δ7-avenasterol were purchased from *Supelco* (Bellefonte, PA, USA). The boron trifluoride/methanol complex (BF_3 solution 10% in methanol), which was used for derivatization of the fatty acids, was purchased from *Merck* (Darmstadt, Germany). Reagents and chemicals used were of the highest purity available.

2.2. Methods

2.2.1. Extraction of total lipid (TL)

Seed material, finely ground in a mill, was Soxhlet-extracted using chloroform/methanol (2:1, v/v). The extracted lipids require an addition of 0.2 volume of 0.75% aqueous sodium chloride solution. The whole was thoroughly mixed without shaking, the layers were allowed to separate and the chloroform layer was recovered. The lipid extracts were collected in a flask and subsequently treated with sodium sulfate to remove traces of water; after filtration the extract was then taken to dryness on a rotary evaporator at 40 °C.

2.2.2. Separation of GL by column chromatography (CC)

TL in chloroform was separated into neutral lipids and GL by passing through 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) according to Rouser, Kritchevsky, Simon, and Nelson (1967). The eluting solvents for neutral lipids and GL were chloroform and acetone, respectively. Solvents were evaporated using a rotary evaporator and the percentage of each fraction was determined gravimetrically. The GL fractions in isooctane/2-propanol (1:1, v/v) were then subjected to HPLC/UV.

2.2.3. HPLC/UV analysis of GL subclasses

Normal-phase HPLC analysis of GL subclasses was performed with a Solvent Delivery Module LC-9A for *Shimadzu* (Kyoto, Japan). The chromatographic system included a Model 87.00 (*Knauer*; Berlin, Germany) Variable Wavelength Monitor detector as well as a 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, 5μm (*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, followed by regeneration of the column for the next anaylsis for 10 min. Prior to HPLC analyses, aliquots of the TGL (acetone fractions obtained from CC) were dried under N_2 and redissolved in the HPLC mobile phase. About 2 μg of each GL fraction were injected and 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 standards were injected individually, as well as in a mixture, to determine retention times and resolution of peaks. Standards were used to identify the components of seed oil GL subclasses via HPLC. 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. GL were quantified by isolation of the individual subclasses, followed by hexose measurement using the phenol/sulfuric acid method (Southgate, 1976) in acid-hydrolyzed lipids. The percentage distribution of each component was obtained from the hexose values.

2.2.4. Compositional analysis of GL constituents by GLC/FID

The fatty acid composition was estimated after transmethylation with 10% BF_3-methanol. The sugar composition was determined as trimethylsilyl derivatives after acid-hydrolysis (methanolysis). The ST composition was determined after saponification of the SG and ASG samples without derivatization.

2.2.4.1. GLC/FID analysis of fatty acids. The fatty acids of TGL and GL subclasses were converted to methyl esters (FAME) by heating in 10% BF_3-methanol, according to the procedure reported by Metcalfe, Schmitz, and Pleca (1966). FAME were identified on a *Shimadzu* GC-14A equipped with FID and C-R4AX chromatopac integrator (Kyoto, Japan). The carrier gas (helium) had a flow rate of 20 ml/min, split 1:40. A sample of 1μl was injected on 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 until 175 °C and kept for 10 min at 175 °C, then 8 C/min until 220 °C and kept for 10 min at 220 °C. A comparision of the retention times of the samples with those of co-injected authentic standards, was made to facilitate identification.

2.2.4.2. GLC/FID analysis of sugar. TGL (100 mg) were hydrolysed in 5% anhydrous methanolic HCl at 100 °C in a sealed vials for 4 h. The residue was dried in vacuo over fresh KOH pellets and redissolved in 100 μl of dried pyridine (*Merck*; Darmstadt, Germany). Methylglycosides were derivatized to trimethylsilyl ethers by adding 100 μl trimethylchlorosilane (*Fluka*; Buchs, Switzerland) and 100 μl of N,O-bis-trimethylsilyltrifluoroacetamide (*Macherey-Nagel*; Düren, Germany); then vials were resealed and heated at 100 °C for 30 min. Trimethylsilyl derivatives were analyzed by GLC/FID in a *Mega Series* high resolution gas chromatograph (HRGC 5160; *Carlo Erba* Strumentazione, Milan, Italy) equipped with FID detector. The following parameters were tested and found useful: GLC column: ID phase DB 5, packed with 5% phenylmethylpolysiloxan (J&W Scientific, Falsom, CA, USA), 30 m length, 0.25 mm internal diameter, 1.0 μm film thickness; carrier-gas (helium) flow 38 ml/min (split-splitless injection was used). Detector and injector were maintained at 250 and 150 °C, respectively. The oven temperature was kept constant at 200 °C and injected volume was 2 μl. All aldoses as well as glucuronic acid (*Merck*; Darmstadt, Germany) derivatives eluted within 40 min. Qualitative analyses were performed with a C-R6A Chromatopac (*Shimadzu*, Kyoto, Japan) integrator.

2.2.4.3. GLC/FID analysis of sterol (ST) in SG and ASG subclasses. Characterization of the ST was performed after saponification of SG and ASG subclasses without derivatization according to Ramadan and Mörsel (2002). Data were acquired using the same *Carlo Erba Mega* GC apparatus and the same column used as for sugar analysis. Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μl. All ST homologues eluted within 45 min. The total analysis time was set at 60 min to assure the elution of all ST. Quantitative analyses were performed with a C-R6A Chromatopac (*Shimadzu*, Kyoto, Japan) integrator.

All extractions, CC, HPLC/UV and GLC/FID runs were performed in triplicate and mean values were calculated.

3. Results and discussion

3.1. General

With careful calibration, it is possible to use the UV response for direct quantification of lipid classes. There are many more published papers in which UV detection has however been used to enable collection of fractions for analysis by other methods, such as GLC of the fatty acid derivatives following transmethylation. Ultraviolet detection at 206 nm proved to be useful for qualitative evaluation in our laboratory, but not for quantitative analysis of GL subclasses. The method failed to separate all of the lipid classes present in the studied oilseeds. In addition, individual simple lipids were not resolved and emerged together at the start of the analysis when a complex lipid extract was analyzed. This made it necessary to extract the GL fractions prior to HPLC/UV analysis. The extracted residue was redissolved in isooctane/2-propanol (1/1, v/v) since the DGD was not soluble in chloroform alone.

3.2. Distribution of individual GL subclasses

The present HPLC/UV procedure allows the identification and separation of six GL subclasses. The proportions of each component were 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 sulfoquinovose when measuring the absorbance at 485 nm. In contrast, the colour developed with anthrone has different adsorption maxima for hexose (620 nm) and sulfoquinovose (590 nm) (Heinz, 1996). These analyses are shown in Table 1 and a representative elution profile is shown in Fig. 2. GL subclasses (peaks 1–6) were completely separated from each other by the present HPLC/UV procedure. The 10-min re-equilibration between the end of one run and the injection of the next

Table 1
Glycolipids (GL) subclasses and their fatty acid composition (% of total FAME) in the studied seed oils

Fatty acid	Black cumin seed oil							Coriander seed oil				Niger seed oil			
	TGL 2.59[a]	ASG 9.95[b]	MGD 7.88[b]	SG 9.45[b]	CER 11.9[b]	DGD 55.6[b]	SQD 5.08[b]	TGL 4.14[a]	ASG 25.1[b]	SG 34.9[b]	CER 38.4[b]	TGL 4.90[a]	ASG 38.5 [b]	SG 29.5 [b]	CER 31.0 [b]
C16:0	18.4	19.9	20.1	nd	20.1	16.9	18.5	10.6	14.9	nd	7.52	23.5	21.4	nd	25.7
C16:1n-7	nd	nd	nd	nd	nd	nd	nd	0.85	0.81	nd	0.87	nd	nd	nd	nd
C18:0	2.07	1.99	1.60	nd	2.01	2.26	2.01	2.39	1.95	nd	2.55	7.80	8.42	nd	6.99
C18:1n-12	nd	nd	nd	nd	nd	nd	nd	55.2	53.0	nd	56.5	nd	nd	nd	nd
C18:1n-9	24.7	24.9	22.9	nd	27.2	26.1	23.9	4.67	3.97	nd	5.22	15.2	16.4	nd	14.3
C18:2n-6	51.8	50.9	52.7	nd	48.5	51.7	52.8	24.4	23.6	nd	25.5	50.9	51.2	nd	50.5
C18:3n-6	nd	nd	nd	nd	nd	nd	nd	0.57	0.49	nd	0.59	nd	nd	nd	nd
C20:0	nd	nd	nd	nd	nd	nd	nd	0.56	0.50	nd	0.58	0.41	0.55	nd	0.34
C18:3n-3	nd	nd	nd	nd	nd	nd	nd	0.11	0.10	nd	0.13	0.17	0.16	nd	0.16
C20:1n-9	0.64	nd	0.71	nd	nd	0.71	0.41	0.43	0.45	nd	0.39	nd	nd	nd	nd
C20:2n-6	2.39	2.31	1.99	nd	2.19	2.33	2.38	nd	nd	nd	nd	nd	nd	nd	nd
C22:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.42	0.41	nd	0.40
C22:1n-9	nd	nd	nd	nd	nd	nd	nd	0.05	0.05	nd	0.04	nd	nd	nd	nd
C23:0[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.28	0.22	nd	0.29
C20:5n-3[c]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.17	1.09	nd	1.19
C24:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.15	0.15	nd	0.13
C22:6n-3[c]	nd	nd	nd	nd	nd	nd	nd	0.17	0.18	nd	0.11	nd	nd	nd	nd

Results are given as the average of triplicate estimations (nd = not detected).
[a] g/100 g of TL.
[b] g/100 g TGL.
[c] Tentatively identified from the retention time.

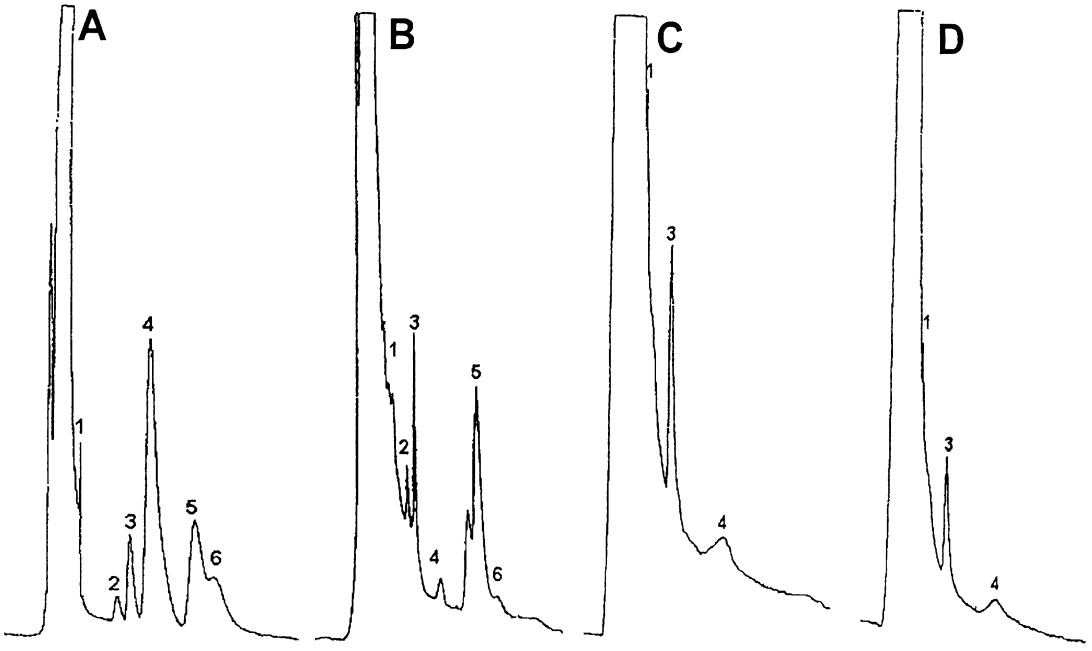

Fig. 2. HPLC/UV (206 nm) chromatograms obtained from the glycolipids (GL) standard mixture (A) as well as the GL subclasses of black cumin (B), coriander (C) and niger (D) oilseeds. A mixture of 2 μg each of standard GL was injected. Peaks: 1, ASG (R_t = 8 min); 2, MGD (R_t = 12 min); 3, SG 13 min); 4, CER (R_t = 15 min); 5, DGD (R_t = 19 min); 6, tentatively identified as SQD (R_t = 21 min).

sample is crucial to eliminate variations in the retention times and adequate separation of peaks. Regarding the polarity of eluting solvents to be used, it should be pointed out that ASG is more polar than MGD and CER travel between SG and DGD in most tested solvents. GL standard mixture model contained five major peaks, 1–5, which from their elution order were assumed to be ASG, MGD, SG, CER and DGD, respectively, while, a peak component, number 6, was tentatively identified as sulfoquinovosyldiacylglycerol (SQD) from its retention time (R_t = 21 min) as well as its behaviour on TLC plates (data not shown). Because of its small concentration,

sulfolipid cannot be clearly separated from DGD. A relatively high level of TGL was found in all studied seed oils. As can be seen, niger seed oil was the richest in GL fraction (4.90% of TL). All GL subclasses were detected in black cumin seed oil, wherein DGD was the prevalent component and made up more than one half of the TGL followed by CER as the second major subclass. ASG, MGD and SG were estimated in relatively equal amounts and comprised, together, about 30% of TGL. Moreover, sulfolipids (ca. 5% of TGL) were only detected in black cumin seed oil. The average daily intake of GL in human has been reported to be 140 mg of ASG, 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 black cumin seed oil could be an excellent as well as a complete source of GL in the diet. The GL subclasses, from both coriander and niger seed oils, had very similar chromatographic patterns and distributions. Among the TGL, ASG, SG and CER were the only detected components; each fraction comprised about one-third of TGL in both seed oils.

3.3. Fatty acid profile of individual GL subclasses

The fatty acid composition of TGL, as well as individual GL subclasses, obtained from *N. sativa* L., *C. sativum* L. and *G. abyssinica* Cass. seed oils, is presented in Table 1. As expected among polar lipids, TGL and their individual subclasses in all studied seed oils contained more saturated and less unsaturated fatty acids than the corresponding triacylglycerols (data not shown). The ratio of saturated to unsaturated fatty acids (S/U%) was ca. 25.7, 15.6 and 48.3% in black cumin, coriander and niger seed oils, respectively. The fatty acid profiles of GL fractions from black cumin and niger seed oils were generally similar, wherein diunsaturated fatty acid C18:2n-6 (>50% of FAME) was the most abundant among GL acyl residues, followed by oleic acid, C18:1n-9, as the second unsaturated fatty acid. In both seed oils, palmitic acid, C16:0, was the major saturated fatty acid in all individual GL subclasses, while the second major saturated acid (stearic, C18:0) was detected at lower levels. Both saturated fatty acids, however, were estimated to be at higher levels in GL fractions obtained from niger seed oil and together comprised more than 30% of total FAME. On the other hand, GLC/FID analysis of FAME from GL subclasses obtained from coriander seed oil gave palmitic, stearic, oleic, linoleic and petroselinic esters as the main FAME. Twelve FAME were detected, wherein petroselinic acid, C18:1n-12, was the major fatty acid accounted for 55.2% of total FAME, followed by linoleic acid, C18:2n-6, The major saturated acids, namely palmitic and stearic, were found in small amounts in comparison with their amounts in black cumin and niger GL subclasses.

3.4. Sterols (ST) composition of steryl glucoside (SG) and acylated steryl glucoside (ASG)

In plants, subcellular membranes contain additional ST derivatives, sometimes in quantities exceeding free ST. These comprise steryl esters, SG and ASG. The knowledge of biological functions of SG is less comprehensive. It is well established that phytosterols, interfere with packing and ordering of acyl groups in lipid bilayers and membranes. The predominant SG is a β-D-glucopyranoside, but many additional SG with different glycosyl residues in α- or β-linkages have been isolated, including β-D-galactopyranosyl, β-D-glucuronopyranosyl, α-L-rhamnopyranosyl, α-D-riburonofuranosyl, β-D-xylopyranosyl and α-D-xyluronopyranosyl residues. Acylated derivatives of steryl glucosides usually carry a long-chain fatty acid at C6 of glucose. The normal C16 and C18 fatty acids known from membrane lipids are esterified in this position. Palmitic acid predominates, but oleic, linoleic and linolenic esters have been isolated also (Heinz, 1996). The ST components of SG and ASG subclasses obtained from black cumin, coriander and niger oilseeds were analyzed by GLC/FID after saponification. Table 2 summarizes the results of these analyses. Four ST moieties were detected in black cumin and

Table 2
Sterol (ST) composition of steryl glucoside (SG) and acylated steryl glucoside (ASG) in the studied seed oils

Compound	Black cumin seed oil		Coriander seed oil		Niger seed oil	
	SG	ASG	SG	ASG	SG	ASG
Ergosterol	nd	nd	nd	nd	nd	nd
Campesterol	nd	nd	42.1[a]	49.5	37.3	24.0
Stigmasterol	12.0	8.20	35.7	21.3	6.40	7.20
Lanosterol	nd	nd	nd	nd	nd	nd
β-Sitosterol	35.3	20.8	13.1	17.7	56.3	68.8
Δ5-Avenasterol	9.00	5.00	9.10	11.5	nd	nd
Δ7-Avenasterol	43.7	66.0	nd	nd	nd	nd

Results are given as the average of triplicate estimations (nd = not detected).
[a] g/100 g of total identified ST.

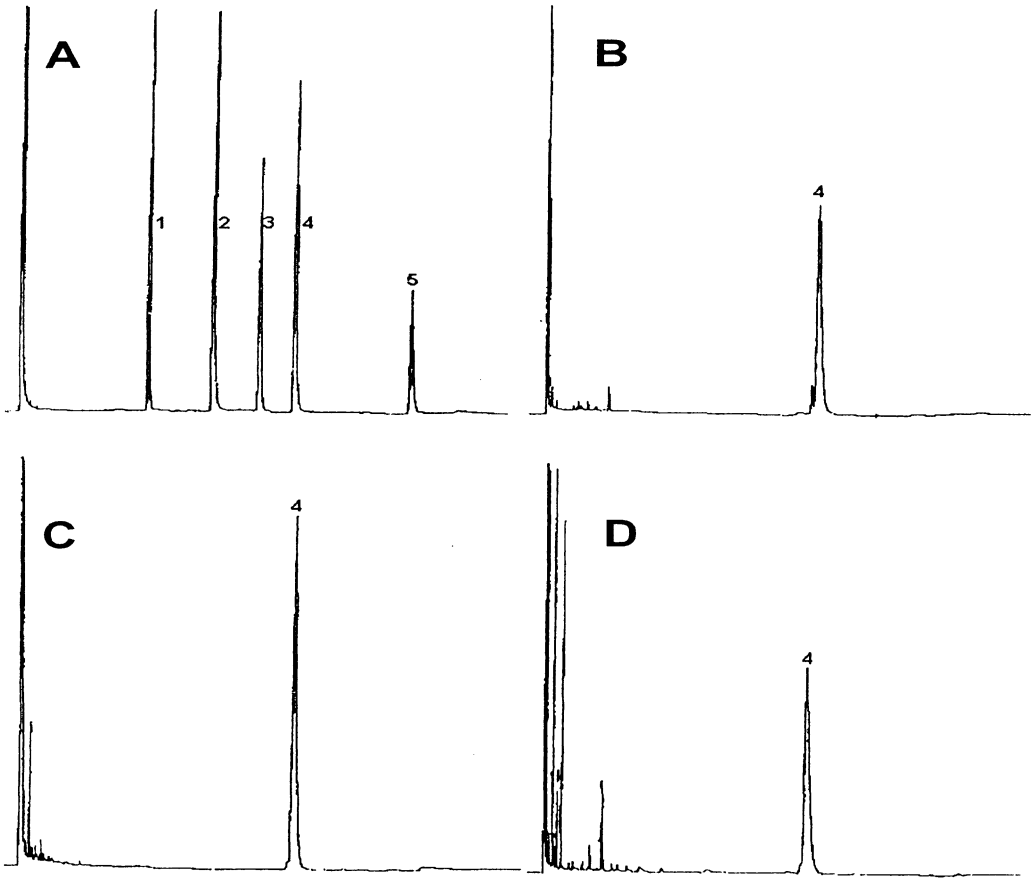

Fig. 3. GLC/FID chromatograms obtained from *O*-trimethylsilyl derivatives of monoaldoses and glucuronic acid model mixture (A), as well as sugar produced from methanolysis of total glycolipids (TGL) in black cumin (B), coriander (C) and niger (D) oilseeds. A DB5 column was packed with 5% phenylmethylpolysiloxan and the column temperature was set at 200 °C. The injector and detector were kept at 150 °C and 250 °C, respectively. Peaks: 1, xylose ($R_t = 14$ min); 2; mannose ($R_t = 20$ min); 3, galactose ($R_t = 25$ min); 4, glucose ($R_t = 28$ min); 5, glucuronic acid ($R_t = 40$ min).

coriander SG and ASG fractions, while the fractions from niger oilseeds showed only three distinct ST peaks. Δ7-Avenasterol represented the dominant ST of the total ST pool in black cumin subclasses, followed by β-sitosterol. In coriander SG and ASG fractions, campesterol was the major ST, component, followed by stigmasterol, while β-sitosterol and Δ5-Avenasterol were detected at lower levels. In order of decreasing prevalence, β-sitosterol > campesterol > stigmasterol were the major ST found in niger SG and ASG fractions. Ergosterol in all analyzed samples was typically below detection limits.

3.5. Sugar profile of TGL

After methanolysis and silylation of TGL from different seed oils under study, *O*-trimethylsilyl derivatives were injected into capillary column DB5 (5% phenylmethylpolysiloxan, 30 m×0.25 mm i.d., 1.0 μm film thickness; *J&W* Scientific, Falsom, CA, USA), which to the best of our knowledge has not been used before in the analysis of sugar derivatives. Glucose was the only sugar detected in all analyzed TGL samples (Fig. 3). Therefore, it has been suggested that, in addition to the structures shown in Fig. 1 further compounds do exist in some mature oilseeds in which more or even all galactosyl residues are replaced by glucosyl groups.

In conclusion, phytoglycolipids from the examined oilseeds, could be successfully separated and analyzed using conditions mentioned. High levels of GL suggest that these oils could be a suitable and valuable source to obtain corresponding GL concentrates as well as essential fatty acids. The presence of all GL fractions in black cumin oilseeds, in appreciable concentrations, makes it an excellent complete source of GL in the human diet. It is anticipated that comercial exploitation of GL from *N. sativa* L. seed oil will soon be realized.

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