Chemistry of Natural Compounds, Vol. 46, No. 4, 2010

LIPIDS OF *Celastrus paniculatus* SEED OIL

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UDC 547.915

The genus *Celastrus* consists of about 100 species of shrubs, distributed over tropical Asia, China, Japan, Australia, and North America. Four species are confined to India, of which *Celastrus paniculatus* Willd. belongs to the family Celastraceae. *Celastrus paniculatus*, a plant known for centuries as the ''Elixir of life'', is a large woody, climbing shrub, height up to 10–18 m, and is common all over the hilly parts of India. The fruits are yellow when matured, and the seeds are reddish brown, covered with a scarlet aril [1, 2]. The plant exhibits therapeutic values and is used as a treatment for cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, and dyspepsia [1–3]. According to Ayurveda, *C. paniculatus* may be employed as a stimulant, nerve tonic, rejuvenant, sedative, and diuretic [4].

The petroleum ether extract of seeds yields a dark brown oil known as *Celastrus* oil or *Malkanguni* oil. Early reports on the seed oil using paper chromatography stated that the oil contains mainly palmitic, stearic, oleic, linoleic, and linolenic acids [5]. The oil acts as a powerful stimulant used in the treatment of scabies, body and rheumatic pains, wound eczema, and beriberi [6]. *Celastrus paniculatus* seed oil has been reported to exert pharmacological actions such as analgesic [7], antimalarial [8], and antispermatogenic [9]. Recently, *C. paniculatus* oil exhibited anxiolytic activity, and the oil was found to be safe [10]. Studies on *C. paniculatus* seed oil for its major and minor constituents are lacking. In this work, lipid classes, fatty acids, and fat-soluble bioactive components of *C. paniculatus* seed oil have been analyzed.

In the present investigation *C. paniculatus* seeds were found to contain about 46% crude oil. The proportion of lipid classes and subclasses presented in *C. paniculatus* 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 (0.55% of TL) and PL (0.34% of TL). Subclasses of NL in the crude oil contain triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DAG), esterified sterols (STE), and monoacylglycerol (MAG) in decreasing order. Significant amounts of TAG were found (ca. 97.1% of total NL), followed by FFA (ca. 1.08% of total NL), while DAG and STE were recovered in lower quantities. SG, ESG, and CER were the prevalent components of the total GL. Phosphorimetry of the TLC fractions (Table 1) revealed that the predominant PL subclasses were PC followed by PE, PI, and PS. About half of the total PL was in PC, and a quarter was in PE, while PI and PS were isolated in lower quantities.

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0009-3130/10/4604-0625

TABLE 1. Lipid Subclasses in *Celastrus paniculatus* Seed Oil, g/kg TL

Neutral lipid	R_f values $\times 100^a$	g/kg TL	Glycolipid	R_f values $\times 100^b$	g/kg TL	Phospholipid	R_f values $\times 100^b$	g/kg TL
MAG	14	3.87 ± 0.08	SQD	6	0.22 ± 0.02	PS	4.7	0.17 ± 0.01
DAG	39	7.55 ± 0.11	DGD	17	0.58 ± 0.04	PI	11	0.54 ± 0.01
FFA	56	10.2 ± 0.18	CER	29–35	1.01 ± 0.07	PC	20	1.90 ± 0.03
TAG	79	915 ± 4.09	SG	41	1.71 ± 0.05	PE	30	0.64 ± 0.07
STE	95	5.53 ± 0.07	MGD	64	0.16 ± 0.03			
			ESG	76	1.60 ± 0.06			

[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).
Results are given as the average 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.

TABLE 2. Fatty Acids of *Celastrus paniculatus* Oil and Its Lipid Classes

Fatty acid	Total lipids	Neutral lipids	Glycolipids	Phospholipids
	relative content (%)			
14:0	0.40 ± 0.88	0.39 ± 0.82	0.50 ± 0.03	0.46 ± 0.03
16:0	26.1 ± 0.02	26.0 ± 0.03	26.5 ± 0.33	26.7 ± 0.33
18:0	2.75 ± 0.36	2.55 ± 0.32	2.90 ± 0.55	2.84 ± 0.55
18:1	54.2 ± 0.39	54.3 ± 0.42	54.0 ± 2.30	54.0 ± 2.30
18:2	11.2 ± 1.25	11.3 ± 1.36	10.9 ± 1.22	10.9 ± 1.22
18:3n-3	5.35 ± 0.09	5.46 ± 0.02	5.20 ± 0.05	5.10 ± 0.05

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

TABLE 3. Sterols and Tocopherols in *Celastrus paniculatus* Seed Oil, g/kg

Compound	g/kg	Compound	g/kg
Brassicasterol	Nd	$\Delta^{5,24}$-Stigmastadinol	Nd
Campesterol	1.66 ± 0.08	Δ^{7}-Stigmastenol	0.43 ± 0.04
Stigmasterol	1.44 ± 0.10	Δ^{7}-Avenasterol	0.22 ± 0.02
Lanosterol	Nd	α-Tocopherol	0.52 ± 0.02
β-Sitosterol	4.90 ± 0.52	β-Tocopherol	Nd
Δ^{5}-Avenasterol	0.15 ± 0.01	γ-Tocopherol	1.04 ± 0.07
Sitostanol	Nd	δ-Tocopherol	Nd

Nd: not detected.
Results are given as the average of triplicate determinations ± standard deviation.

The fatty acid profiles of TL and lipid classes (NL, GL, and PL) are presented in Table 2. Six fatty acids were identified, in which oleic followed by palmitic and linoleic were the major fatty acids, which together comprise more than 90% of total FAME. α-Linolenic acid (C18:3*n*-3) was also found in relatively high amounts. The fatty acids in the neutral lipids and polar lipids did not differ significantly from each other. The ratio of unsaturated fatty acids to saturated fatty acid, however, was higher in the neutral fractions than in the corresponding polar fractions (GL and PL). Concerning the saturated fatty acids

(especially palmitic and stearic), GL resemble PL in the higher content of saturates, while saturated fatty acids were detected in relatively lower levels in the corresponding NL. A striking feature of *C. paniculatus* seed oil was the relative high level of monounsaturated fatty acids, which accounted for 54%.

Celastrus paniculatus seed oil is characterized by a relatively high amount of unsaponifiables (16 g/kg TL), of which ca. 55% are phytosterols (ST). Six compounds were detected, in which the sterol marker was β-sitosterol, which comprised ca. 64.5% of the total ST content (Table 3). The next major components were campesterol and stigmasterol, and these three major components accounted for more than 90% of total ST. Other components, e.g., Δ^7-avenasterol, Δ^5-avenasterol, and Δ^7-stigmastenol, were found in lower amounts.

The composition of tocopherols is summarized in Table 3. Two of the four tocopherol isomers were present, in which γ-tocopherol constituted 66.6% of the total analytes, the rest being α-tocopherol (ca. 33.3 %). α- and γ-Tocopherols proved to be the major tocopherols in vegetable oils and fats.

Celastrus paniculatus seeds were collected (October 2006) from the Khanapur Forest, Bidar district, Karnataka (India). The plant was identified with the help of the Flora of Gulbarga District [11]. A voucher specimen (No. HGUG-503) was deposited at the Herbarium, Department of Botany, Gulbarga University (Gulbarga, India). Neutral lipid (NL) standards were from Sigma Chemical Company. Standards used for glycolipid (GL) identification were purchased from Biotrend Chemikalien GmbH. Standards used for phospholipid (PL) identification were purchased from Sigma. Standards used for sterol (ST) characterization were purchased from Supelco. Standards used for tocopherols (α-, β-, γ-, and δ) were purchased from Merck.

Seeds were fine-milled into a fine particle sized (ca. 2 mm) meal, then subjected to Soxhlet extraction using *n*-hexane for 14 h.

Total lipids (TL) were separated into the different classes by elution with different solvents over a glass column (20 mm diameter × 30 cm) packed with a slurry of activated silicic acid (70 to 230 mesh) in chloroform (1:5, w/v). NL were eluted with three the column volumes of chloroform. The major portion of GL was eluted with five the column volumes of acetone, and that of PL, with four the column volumes of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of TLC on silica gel F_{254} plates (thickness 0.25 mm), a further characterization of the GL and PL subclasses was carried out with the solvent system chloroform–methanol–ammonia solution 25% (65:25:4, v/v/v). For the characterization of NL subclasses, TLC 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 TLC plates were sprayed with the following agents: for the marking of all lipids, with sulfuric acid (40%), for the marking of GL, with α-naphthol–sulfuric acid, and for the marking of PL, with molybdate-blue reagent [12].

Fatty acids were transesterified into methyl esters (FAME) using *N*-trimethylsulfonium hydroxide [13]. FAME were identified on a Shimadzu GC-14A equipped with a flame ionization detector (FID) and a C-R4AX chromatopac integrator.

Separation of sterols was performed after saponification of the oil sample without derivatization according to [14]. GLC analyses of unsaponifiable residues were carried out using a Mega Series chromatograph (5160, Carlo Erba) equipped with FID.

For tocopherols analysis, NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to [15]. Analysis was performed with a solvent-delivery HPLC chromatograph (Shimadzu). Separation of tocopherols 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 was isooctane–ethyl acetate (96:4, v/v) with detection at 295 nm [15]. All experimental procedures were performed in triplicate and their mean values (± standard deviation) are given.

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