Lipid Composition of *Murraya koenigii* Seed

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Total seed lipids extracted from *Murraya koenigii* (Linn), Rutaceae amounted to 4.4% of the dry seed. The total lipids consisted of 85.4% neutral lipids, 5.1% glycolipids and 9.5% phospholipids. Neutral lipids consisted of 73.9% triacylglycerols, 10.2% free fatty acids and small amounts of diacylglycerols, monoacylglycerols and sterols. At least five glycolipids and seven phospholipids were identified. Sterylglucoside and acylated sterylglucoside were major glycolipids, while digalactosyldiacylglycerol, monogalactosyldiacylglycerol and monogalactosylmonoacylglycerol were present in small quantities. The phospholipids consisted of phosphatidylethanolamine, phosphatidylcholine, lysophosphatidylethanolamine and lysophosphatidylcholine as major phospholipids and minor quantities of phosphatidylinositol, phosphatidylglycerol and phosphatidic acid. The fatty acid composition of these different neutral lipids, glycolipids and phospholipids were determined.

KEY WORDS: Acylated sterylglucoside, digalactosyldiacylglycerol, monogalactosyldiacylglycerol, monogalactosylmonoacylglycerol, *Murraya koenigii* (Linn.), phosphatidylcholine, phosphatidylethanolamine, Rutaceae, sterylglucoside.

Murraya koenigii (Linn.), Spreng of the family Rutaceae, is distributed throughout India and the Andaman Islands (1). It is usually cultivated for its aromatic leaves, which are used as flavoring in curries and sauces (1,2). The fruit is edible (1), and consists of two seeds, containing 4.4% oil. Some of the physicochemical characteristics of *Murraya koenigii* leaves and fruits have been reported (1); however, information on lipid composition of its seeds is not available. Therefore, an investigation was carried out to determine the lipid composition of *Murraya koenigii* seeds.

MATERIALS AND METHODS

The seeds of *Murraya koenigii* were purchased locally. Standard triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, hydrocarbons, sterols, sterolesters, digalactosyldiacylglycerol, monogalactosylmonoacylglycerol, phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine, phosphatidylcholine, phosphatidylethanolamine and methyl palmitate, methyl stearate, methyl oleate, methyl linoleate were purchased from Sigma Chemical Co. (St. Louis, MO). Solvents used were of analytical grade and were distilled before use.

Lipid extraction. The total lipids from triplicate, 20-g samples of *Murraya koenigii* seeds were extracted and purified following the established procedure of Folch *et al.* (3). A measured portion of the purified lipid extract was used for gravimetric estimation of total lipids. Free fatty acids, refractive index, iodine value and saponifiable matter of the lipids were determined by AOCS methods Ca 5a-40, Cc 7-25, Cd 1-25 and Ca 6a-40, respectively (4).

Lipid classes and fatty acid analysis. The total lipids (TL) were fractionated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) on a silicic acid column (5) with chloroform, acetone and methanol, successively. NL were estimated gravimetrically, GL and PL were quantitated by total sugar estimation (6) and phosphorus estimation (7,8), respectively. NL were separated by thin-layer chromatography (TLC) with hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as solvent system. Individual components of NL were identified by comparison with standards and quantitated by photodensitometry (9). GL and PL were separated on TLC with chloroform/methanol/acetic acid/water (65:15:10:4, v/v/v/v) as the solvent system. Individual components of GL and PL were identified by cochromatography, comparison with authentic standards and by specific spray reagents (10,11). Quantitation of different components of GL and PL on preparative TLC was effected by estimation of sugar (6) and phosphorus (7,8), respectively.

Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transmethylation (12) of the lipids. The FAME were analyzed on a Shimadzu GC 9A chromatograph (Shimadzu Scientific Instruments, Columbia, MD) equipped with flame ionization detector (FID) and a stainless steel column (152.4 cm × 3.17 mm) packed with 20% diethyleneglycol succinate on 80-100 mesh Chromosorb W support, operated at a column temperature of 180°C, injection port and FID temperature of 210°C, under a nitrogen flow rate of 40 mL/min. The peak area and relative percentage of FAME were obtained with a Shimadzu integrator. The component of each peak was identified on the basis of a calibration curve of retention time *vs.* equivalent chainlength, and by comparison with those of authentic methyl ester standards. All determinations were performed in triplicate and mean values were reported.

RESULTS AND DISCUSSION

The seeds of *Murraya koenigii* contained 4.4% total lipids (dry basis). The purified lipids had the following physicochemical characteristics: dark yellow viscous liquid at ambient temperature (25-30°C) with strong spicy odor and pungent taste; refractive index at 25°C, 1.487; free fatty acids, 10.2 g oleic/100 g fat; unsaponifiable matter 4.2%. Fractionation of the lipids by silicic acid column chromatography showed that the total lipids consisted of 85.4% neutral lipids, 5.1% glycolipids and 9.5% phospholipids (Table 1).

The fatty acid composition of total lipids showed that oleic (18:1, 33.6%) and linoleic (18:2, 39.4%) acids were the predominant acids followed by palmitic acid (16:0, 17.9%), while stearic acid (18:0) was present to the extent of 8.6% (Table 1), similar to the seed lipids of other Rutaceae (13). The fatty acid profile of NL largely reflected that of TL, while GL and PL fractions had higher quantities of both oleic (18:1) and linoleic acids (18:2) (39.0%, 37.0% and 41.0%, 44.1%, respectively) compared to TL and NL. The fatty acid composition of NL, GL and PL and their different components of *Murraya koenigii* seed are reported here for the first time.

TABLE 1

Total Lipids, Neutral Lipids, Glycolipids and Phospholipids of *Murraya koenigii* Seed and their Fatty Acid Compositions[a]

Lipid class	Wt (%)		Fatty acid composition (%)			
			16:0	18:0	18:1	18:2
Total lipids	4.4		17.9	8.6	33.8	39.7
Neutral lipids[b]	85.4		19.0	9.3	32.9	38.8
sn-1,3-DG		2.4	35.4	1.9	36.8	25.9
sn-1,2(2,3)-DG		2.6	37.7	2.1	39.2	21.0
MG		5.2	44.2	2.0	31.6	22.2
FFA		10.2	22.4	5.0	29.6	43.0
TG		73.9	16.3	6.2	36.9	40.6
S, SE, HC		5.7	—	—	—	—
Glycolipids[c]	5.1		17.0	3.0	39.0	41.0
DGDG		6.5	25.9	1.0	39.2	33.9
MGDG		7.9	26.7	0.9	35.5	36.9
MGMG		9.8	27.0	0.8	31.4	40.8
SG		35.7	15.3	2.5	41.7	40.5
ASG		40.1	15.1	2.0	36.8	46.1
Phospholipids[d]	9.5		16.9	2.0	37.0	44.1
PA		3.0	28.0	1.0	36.9	34.1
PG		5.0	25.0	0.9	39.1	35.0
PI		7.1	29.0	1.0	12.2	57.8
LPC		11.1	24.0	1.8	17.2	57.0
LPE		14.0	25.0	1.7	24.3	49.0
PC		28.2	16.3	2.1	40.6	41.0
PE		32.1	14.8	2.1	41.1	42.0

[a] All values are means of three replicate analyses.

[b] DG, diacylglycerol; MG, monoacylglycerols; FFA, free fatty acids; TG, triacylglycerols; S, sterols; SE, sterolesters; and HC, hydrocarbons.

[c] DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; MGMG, monogalactosylmonoacylglycerol; SG, sterylglucoside; and ASG, acylated sterylglucoside.

[d] PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; and PE, phosphatidylethanolamine.

In regard to the neutral lipid fraction, triacylglycerols (TG) and free fatty acids (FFA) were found to be the major components present to the extent of 73.9% and 10.2%, respectively (Table 1). The quantity of *sn*-1,2(2,3)-diacylglycerol (2.6%) was slightly higher than *sn*-1,3-diacylglycerol (*sn*-1,3-DG) (2.4%), and was similar to results observed in peanut, chironji, cumin, fenugreek and rice bran oil (14–18). The fatty acid composition of different components of NL [except for sterols (S), sterolesters (SE), hydrocarbons (HC) and pigments] is presented in Table 1. Palmitic (16:0), oleic (18:1) and linoleic acids (18:2) together constituted 93.8% to 98.1% of the total fatty acids, while stearic (18:1) acid was present in small quantities (≤ 6.2%) in all the components of NL fraction.

The glycolipid fraction was resolved into sterylglucoside (SG), acylated sterylglucoside (ASG), monogalactosylmonoacylglycerol (MGMG), monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (Table 1). ASG and SG were the major glycolipids present at 40.1% and 35.7%, respectively, while MGMG, MGDG and DGDG were present in small quantities (≤ 9.8%). The fatty acid composition of these glycolipids (Table 1) showed that SG contained more oleic acid (41.7%), ASG contained more linoleic acid (46.1%), and DGDG, MGDG and MGMG showed more palmitic acid (25.9%, 26.7% and 27.0%, respectively) as compared to total glycolipids (Table 1).

The phospholipid fraction was resolved into seven components by TLC (Table 1). The major phospholipids were phosphatidylethanolamine (PE) (32.1%), phosphatidylcholine (PC) (28.2%), lysophosphatidylethanolamine (LPE) (14.0%) and lysophosphatidylcholine (LPC) (11.1%). Phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidic acid (PA) were present to the extent of ≤ 7.1%. The fatty acid composition of individual phospholipids (Table 1) shows that the PA, PG, PI, LPC and LPE fractions have higher levels of palmitic acid (28%, 25%, 29%, 24% and 25%, respectively) than that in the total phospholipids (Table 1). Also, PG, PC and PE have more oleic acid (39.1%, 40.6% and 41.1%, respectively) and PI, LPC and LPE contain more linoleic acid (57.8%, 57.0% and 49.0%, respectively) relative to the total phospholipid distributions (Table 1).

This preliminary investigation indicated that the lipid composition of *Murraya koenigii* seeds is similar to other seeds of Rutaceae. However, research on possible toxicity and economic feasibility for its utilization in various products should be conducted. This study provides increased knowledge concerning the lipid composition of *Murraya koenigii* seeds.

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