Phospholipids of Palash (*Butea monosperma*), Papaya (*Carica papaya*), Jangli Badam (*Sterculia foetida*), Coriander (*Coriandrum sativum*) and Carrot (*Daucus carota*) Seeds[1]

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Analyses of the phospholipids of palash *(Butea monosperma)*, papaya *(Carica papaya)*, jangli badam *(Sterculia foetida)*, coriander *(Coriandrum sativum)* and carrot *(Daucus carota)* seeds are reported in the present study. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol were identified as major components in all the seeds. Small amounts of lysophosphatidylcholine in palash and papaya, and cardiolipin in palash, papaya and carrot also were detected. The predominant fatty acids present in the total and individual phospholipids of the seeds examined were oleic, linoleic and palmitic. In the case of palash and papaya phospholipids, the profile of fatty acid distribution was similar to that of their respective oils. However, the unusual fatty acids present predominantly in jangli badam, coriander and carrot oils were found to be significantly reduced in the respective phospholipids.

Non-conventional oilseeds are now being considered as potential raw materials to augment the supply of edible oils. The study of these oilseeds for their minor constituents is useful in order to use both the oil and the minor constituents effectively. The information on phospholipids of these seeds is also important in processing and utilizing the oil and by-products (1-2). In continuation of our earlier studies on seed phospholipids, the present study pertains to analysis of phospholipids of five seeds, namely palash *(Butea monosperma)*, papaya *(Carica papaya)*, jangli badam *(Sterculia foetida)*, coriander *(Coriandrum sativum)* and carrot *(Daucus carota)* which belong to the Leguminosae, Caricaceae, Sterculiaceae and Umbelliferae families, respectively. The chemical characteristics and fatty acid composition of these seed oils were reported earlier by several authors (3-10).

EXPERIMENTAL

Materials. Papaya, coriander and carrot seeds were purchased from the local market. Jangli badam and palash kernels were collected from the local area. Reagents and chemicals used were of analytical grade (BDH and Indian Drugs and Pharmaceuticals Limited, India). Silica gel (finer than 200 mesh) for column chromatography and silica gel-G for thin layer chromatography were obtained from Acme Synthetic Chemicals, India. Lysophosphatidylcholine (egg yolk), phosphatidylinositol (soybean) and cardiolipin (bovine heart) were purchased from Sigma Chemical Company, St. Louis, Missouri, and TLC-pure phosphatidylcholine and phosphatidylethanolamine were isolated from egg yolk.

Methods: Extraction of phospholipids. Seeds (or kernels, 500 g) were powdered and extracted at room temperature with a mixture of chloroform and methanol (2:1, v/v) according to the procedure of Folch et al. (11). Acetone insolubles were obtained by repeated acetone precipitation of the total lipid extract (12). Pure phospholipids were separated from acetone-insoluble material by silica gel column chromatography (13) by using the following sequence of solvents: (i) chloroform, (ii) acetone, and (iii) methanol. The neutral lipids were eluted in chloroform, glycolipids in acetone and phospholipids in methanol.

Identification of phospholipids. The total phospholipids in each instance were spotted on the TLC plate (0.25 mm silica gel-G layer on 20 × 20 cm glass plates) along with authentic samples, and developed with (a) chloroform-methanol-water (65:25:4, v/v/v) and (b) chloroform-methanol-acetic acid-water (65:15:10:4, v/v/v/v) separately. The spots were visualized with different spray reagents such as (a) iodine vapors, which reveal all the lipid materials; (b) ammonium molybdate-perchloric acid reagent (14), specific for all phospholipids; (c) ninhydrin reagent (14), for ethanolamine- or serine-containing phospholipids, and (d) Dragendorff reagent (15), for choline-containing phospholipids.

Quantitative analysis of phospholipids (16). The total phospholipids in chloroform (16-19 μg) were spotted in triplicate on TLC plate (0.50 mm silica gel-G layer) and developed with chloroform-methanol-water (65:25:4, v/v/v). The individual phospholipids were located by exposure to iodine vapors, and the band corresponding to each phospholipid was scraped off separately. The phospholipids from scraped silica gel were extracted with a mixture of chloroform-methanol-water (10:10:9, v/v/v). The solvents were removed and the residues were transferred to 5-ml standard flasks. After adding chromogenic solution, the absorbance of the color was read at 710 nm (Shimadzu UV-visible spectrophotometer). The recovery studies of phospholipids from silica gel were first carried out with pure phosphatidylcholine and phosphatidylethanolamine and their mixtures and found to be quantitative.

Isolation of major phospholipids. A combination of column (17) and preparative thin layer (solvent system, chloroform-methanol-water, 65:25:4, v/v/v) chromatographic procedures was used to obtain major phospholipids, namely phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, for analysis of their hydrolytic products and fatty acid compositions. The purity of the column fractions was checked by micro TLC using the above solvent system.

Fatty acid composition. Methyl esters of fatty acids from palash and papaya oil and phospholipids were prepared by treating them with 0.5 M sodium methoxide in methanol for about 30 min at 50 C (18). Sterculic and malvalic acids of jangli badam oil and phospholipids

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were estimated as their methyl esters via their keto and ether derivatives by treating the esters with methanolic silver nitrate as described by Schneider (19). Petroselinic and oleic acids in phospholipids and oil of coriander and carrot seeds were determined by potassium permanganate-sodium metaperiodate oxidation of methyl esters, followed by estimation of adipate and azelate by gas liquid chromatography (GLC) (20,21). GLC was carried out with a Hewlett-Packard 5840 A unit fitted with a hydrogen flame detector and data processor. A stainless steel column (1.8 m × 6 mm) packed with 10% Silar 10 C on Chromosorb W-HP (100-120 mesh) was used. The column temperature was maintained at 195 C for all fatty acid methyl esters, and for the adipate and azelate the column was operated with temperature programming from 150 to 180 C. The injection and detector temperatures were maintained at 250 C and 300 C, respectively. Flow rate of the carrier gas (nitrogen) was 35 ml/min. Standard fatty acid methyl esters were used to identify the peaks. Area percentage was recorded on the data processor.

Hydrolytic products: Bases. Strong acid hydrolysis (22) for the liberation of bases was carried out by heating each phospholipid sample (5-10 mg) with 6 N HCl (1-2 ml) for 12 hr at 100 C in a sealed tube. The fatty material was removed with chloroform. The aqueous layer was concentrated under reduced pressure and subjected to TLC on silica gel G according to the procedure of Kaufmann et al. (23), using the solvent system of 96% ethanol and 7% aqueous ammonia (1:2, v/v) followed by spraying with Dragendorff and ninhydrin reagents.

Glycerol and inositol. The sample (5-10 mg) was heated in a sealed tube with 6 N HCl (2 ml) for 24 hr at 100 C (24). Fatty material was removed with chloroform. The aqueous layer was concentrated and subjected to TLC with a solvent system of n-propanol-ethyl acetate-water-25% aq. ammonia (50:10:30:10, v/v/v/v) followed by spraying with sodium metaperiodate-benzidine reagent (Benzidine is a potent carcinogen. Avoid direct contact with skin).

RESULTS AND DISCUSSION

Crude phospholipids (acetone insolubles) were obtained from total lipid extract by repeated acetone precipitation, were further purified on silica gel column, and were found to contain traces of neutral lipid and glycolipids. Total contents of phospholipids in oils (chloroform:methanol extract) and seeds or kernels of palash, papaya, jangli badam, carrot and coriander were found to be 5.3, 0.84; 0.63, 0.15; 0.46, 0.2; 0.55, 0.12, and 0.56, 0.1%, respectively. The palash seeds contained a significant amount of phospholipids, hence it may be a good source of commercial lecithin.

Qualitative analysis based on response to specific spray reagents on TLC and comparison with authentic samples indicated the presence of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol as major phospholipids in all five seeds. However, small amounts of lysophosphatidylcholine in palash, and of cardiolipin in palash and carrot, also were detected. The presence of cardiolipin was confirmed by comparing the R_f value in the basic solvent system (25,26) in addition to acidic and neutral solvent systems.

The quantitation of phospholipids (Table 1) was carried out based on colorimetric estimation of phospholipid phosphorus without acid digestion (16) as used earlier for karanja seed phospholipids (27). Phosphatidylcholine, about 44% in palash and coriander; phosphatidylethanolamine, 35.4% in carrot, and phosphatidylinositol, 34% and 40.6% in papaya and jangli badam, were found to be major components. Cardiolipin was found only in palash (1.8%) and carrot (8.6%) phospholipids, and its presence was reported earlier in soybean (28) and neem (29) seeds. Lysophosphatidylcholine was detected in palash (4.9%) and papaya seeds. Cardiolipin and lysophosphatidylcholine could not be quantitated separately in papaya as they were present in very small quantities; their content was included in the unidentified fraction (19.2%). The composition profile of these seeds is comparable to the common oilseed phospholipids (27-30).

The major phospholipids isolated were subjected to acid hydrolysis. TLC of the hydrolytic products indicated the presence of choline, ethanolamine and inositol along with glycerol in phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, respectively, which confirms their identity and purity.

Fatty acid compositions of the individual and total phospholipids along with those of the oils are given in Tables 2 and 3. The predominant fatty acids present in the total and individual phospholipids of the seeds examined were oleic, linoleic and palmitic. The contents of palmitic and linoleic acids were found to be significantly higher in the phospholipids than in the corresponding oils. In the case of papaya and palash phospholipids, the pattern of fatty acid distribution was similar to that of the oils. Sterculic acid was found to be less in jangli badam total phospholipids (14.6%) than in the oil (51.0%), while

TABLE 1

Phospholipid Composition of Palash, Jangli Badam, Papaya, Carrot and Coriander Seeds

Phospholipid	Palash	Jangli badam	Papaya	Coriander	Carrot
Phosphatidylcholine	44.6	30.0	28.1	44.0	29.1
Phosphatidylethanolamine	14.8	23.0	18.7	29.3	35.4
Phosphatidylinositol	27.0	40.6	34.0	23.1	23.1
Lysophosphatidylcholine	4.9	—	—	—	—
Cardiolipin	1.8	—	—	—	8.6
Unidentified	6.9	6.4	19.2	3.6	3.8

TABLE 2

Fatty Acid (wt %) Composition of Jangli Badam, Palash and Papaya Seed Phospholipids

	Jangli badam					Palash					Papaya				
Fatty acid	Oil	PL	PC	PE	PI	Oil	PL	PC	PE	PI	Oil	PL	PC	PE	PI
14:0	—	—	—	—	—	—	—	—	—	—	0.2	0.3	1.7	1.8	0.8
16:0	16.6	38.3	39.3	36.8	30.4	18.9	26.5	22.4	29.0	32.9	16.3	24.4	21.1	30.8	21.2
18:0	3.6	4.8	4.5	5.0	12.6	5.8	3.6	3.6	1.1	6.1	5.7	6.9	5.7	5.6	7.2
18:1	10.1	16.9	17.8	15.2	17.4	23.0	33.2	41.8	33.1	25.9	73.7	61.4	68.2	56.6	68.2
18:2	9.1	23.8	22.6	25.6	23.9	34.8	35.4	32.2	34.3	34.2	4.1	7.0	3.3	5.2	2.6
18:3	2.4	1.6	1.6	1.6	2.0	—	—	—	—	—	—	—	—	—	—
Sterculic acid	51.0	14.6	14.2	15.8	13.7	—	—	—	—	—	—	—	—	—	—
Malvalic acid	7.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20:0	—	—	—	—	—	1.9	0.6	—	2.5	0.9	—	—	—	—	—
22:0	—		—	—	—	11.2	0.4	—	—	—	—	—	—	—	—
24:0	—	—	—	—	—	4.4	0.3	—	—	—	—	—	—	—	—

PL, Total phospholipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine, and PI, phosphatidylinositol.

TABLE 3

Fatty Acid (wt %) Composition of Carrot and Coriander Seed Phospholipids

	Carrot						Coriander				
Fatty acid	Oil	PL	PC	PE	PI	CL	Oil	PL	PC	PE	PI
16:0	6.7	19.1	18.0	16.8	28.7	15.8	3.4	24.3	13.7	23.4	32.9
18:0	0.5	0.9	1.4	1.2	1.3	0.5	0.4	1.8	2.2	1.3	0.5
18:1 (Δ6)	68.9	24.7	—	—	—	—	72.4	8.6	—	—	—
18:1 (Δ9)	11.3	20.0	—	—	—	—	9.1	17.6	—	—	—
18:1 (Total)	80.2	44.7	50.2	41.3	42.6	39.4	81.5	26.2	42.1	24.3	22.9
18:2	12.2	34.4	30.4	39.4	27.0	43.5	13.9	42.2	42.0	49.9	42.5
18:3	0.4	0.9	—	1.3	0.4	0.8	0.8	5.5	Tr.	1.1	1.2

malvalic acid was completely absent in the phospholipids as against 7.2% in the oil. However, it was reported earlier that cyclopropene fatty acids were completely absent from the jangli badam phospholipids (31).

Petroselinic acid was determined only in the oil and total phospholipids of coriander and carrot seeds; in the individual phospholipids the content of petroselinic and oleic acids was reported together as 18:1 (total). The content of petroselinic acid was found to be lower in total phospholipids than in the respective oils for both species. These observations are in close agreement with earlier reports on the presence of unusual fatty acids in seed phospholipids (21,32-34).

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