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Lipid Studies of Carum Roxburghianum Seeds

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Abstract – Total lipids extracted from the powdered seeds of *Carum roxburghianum* were fractionated into hydrocarbons (0.30%), wax esters (0.30%), sterol esters (1.35%), triacylglycerols (72.41%), free fatty acids (6.06%), 1,3-diacylglycerols (4.60%), 1,2-diacylglycerols (0.64%), glycolipids (5.10%), sterols (1.20%), 2-monoacylgylcerols (3,18%), 1-monoacylglycerols (1.46%), phosphatidylethanolamines (1.08%) phosphatidylcholines (0.40%), lysophosphatidylethanolamines (1.48%) and phosphatidylinositols (0.44%) with the help of TLC. The fatty acid composition of all the lipid fractions was determined after converting them into their methyl esters with BF_3-methanol reagent and then analyzing them by GC. Oleic acid was found as a major component in all the lipid classes, whereas palmitic, linoleic and linolenic acids were present in lesser quantities. Arachidic acid was identified as a minor component in only seven out of twelve lipid classes.

Key words – *Carum roxburghianum*, Lipids, Fatty acid composition

Introduction

It is known that 174 species of the family *Umbelliferae* are present in Pakistan (Bhatty, 1977). The plants belonging to this family have been utilized locally in various ailments and their remarkable medicinal values are fully recognized by the Asian people of the subcontinent (Kirtikar and Basu, 1984). However no systematic research has been carried out on the fixed oils of these plants. Besides these medicinal applications, their seeds have been employed in baked foods and condiments. The present investigations are carried out in an effort to introduce non-conventional sources of vegetable oils in Pakistan. Different classes of polar and non-polar lipids have been separated and identified by the use of different locating reagents through thin-layer chromatography. The fatty acid compositions of all these classes are determined by gas chromatography after methylation and purification.

Materials and Methods

Extraction of Lipids – The lipids of ground seeds (40 g) were extracted with 300 ml chloroform: methanol (2:1 v/v) (Akhtar *et al*, 1980) mixture at room temperature by shaking on a magnetic stirrer for half an hour. After filtration, the residual material was further extracted with 3×100 mL portions of chloroform methanol mixture. All the extracts were combined and three consecutive washings with 100 mL chloroform; methanol and 0.9% aqueous sodium chloride (3:48:47 v/v) (Folch *et al*, 1957) solution were given to the lipids in a separating funnel to remove the non-lipid materials. The solvent was removed under reduced pressure at 40°C in a rotary evaporator and lipids were stored under nitrogen atmosphere.

Thin-layer chromatography – Sets of TLC glass plates of 0.25 mm and 0.5 mm thickness were prepared by using Silica gel G 60 (Merck Art No.7731) in water with the help of an applicator (Stahl, 1969). They were dried at room temperature and activated by heating at 105°C for 2 hours and used for qualitative and quantitative analysis of lipids. Lipids (200 mg) were separated into different classes of neutral and polar lipids by using hexane: diethylether: acetic acid (80:20:2 v/v) and chloroform methanol: 30% ammoniumhydroxide:water (60:35:5:2.5v/v) solvent systems (Akhtar *et al*, 1981), respectively. A locating reagent 2,7-dichlorofluorescein was used which gave purple yellow coloured bands under ultraviolet light at 360 nm. A saturated solution of antimony trichloride (Raie *et al.*, 1983) in chloroform was used for the identification of sterols and sterol esters. Appearance of red-violet spots on TLC plates when kept at 100°C for ten minutes confirmed the presence of these compounds. Similarly, hydroxylamine ferric chloride reagent (Raie *et al.*, 1983) was sprayed to locate and confirm the glycerides bands, which showed purple colour. Molybdenum blue solution (Colowick *et al.*, 1969a),

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Dragendorff reagent (Colowick *et al.*, 1969b) and ninhydrin solution (Colowick *et al.*, 1969c) were also used for the identification of phospholipids, phosphatidylcholines, and phosphatidylethanolamines giving blue, straw-orange and violet-red spots respectively on TLC layers.

Methylation – The different lipids separated by TLC except hydrocarbons and sterols were converted into their methyl esters by treating them with boron trifluoride-methanol reagent Morrison and Smith, 1984) (Merck Art no. 801663) in with Teflon lined screw capped test tubes. The methyl esters thus prepared were purified quantitatively by the application of thin layer chromatography using hexane: diethyl ether (9:1 v/v) as solvent system (Bhatty, 1977).

Gas Chromatography – The methyl esters were analyzed for fatty acid composition on Shimadzu GC-14A gas chromatograph equipped with flame ionization detector. A polar column (3.1 m×3 mm i.d.) of polyethylene glycol 20 M (10%) coated on diatomite "C" (80 100 mesh) was used for the analysis. The column oven temperature was programmed at 180°C-3min-2°C/min-210°C. Nitrogen was used as carrier gas with the flow rate of 40 ml/minute. The temperatures of detector and injector were maintained at 250°C and 220°C, respectively. The chromatograms were recorded on Shimadzu C-R4A Chromatopac and fatty acids were identified by comparing their retention times with those of authentic standard methyl esters run under the same conditions. The Chromatopac also computed the percentage of each component.

Results and Discussion

A polar solvent system (chloroform: methanol) was used to allow the maximum extraction of polar and neutral lipids from the *Carum roxburghianum* seed. The seed oil contained 3.40% polar lipids and 96.60% neutral lipids. The neutral lipids were fractionated into eleven classes whereas polar lipids were split into four classes by silica gel thin layer chromatography. The various lipid fractions identified by comparing their R_f values with those of the standards were hydrocarbons (0.30%), wax esters (0.30%), sterol esters (1.35%), triacylglycerols (72.41%), free fatty acids (6.06%), 1,3-diacylglycerols (4.60%), 1,2-diacylglycerols (0.64%), glycolipids (5.10%), sterols (1.20%), 2-monoacylglycerols (3.18%), 1-monoacylglycerols (1.46%) phosphatidylethanolamines, (1.08%), phosphatidylcholines (0.40%), lysophosphatidylethanolamines (1.48%) and phosphatidylinositols (0.44%) as shown in Table 1. the colour reactions were also performed for the identification of various lipid classes. The triacylglycerols were found as the predominant class in this oil.

Table 1. Weight Percentage of Different Lipid Classes Present in the Resting Seeds of Carum Proxburghianum

Lipid Classes	R_f Values	Percentage
NEUTRAL LIPIDS*		
Hydrocarbons	0.95	0.30
Wax esters	0.93	0.30
Sterol esters	0.72	1.35
Triacylglycerols	0.61	72.41
Free fatty acids	0.41	6.06
1,3-Diacylglycerols	0.34	4.60
1,2-Diacylglycerols	0.30	0.64
Glycolipids	0.25	5.10
Sterols	0.22	1.20
2-Monoacylglycerols	0.19	3.18
1-Monoacylglycerols	0.15	1.46
PHOSPHOLIPIDS**		
Phosphatidylethanolamines	0.70	1.08
Phosphatidylcholines	0.50	0.40
Lysophosphatidylethanolamines	0.54	1.48
Phosphatidylinositols	0.18	0.44

*The developing solvent mixture for neutral lipids was hexane: diethylether:acetic acid (80:20:12).
**The developing solvent mixture used for phospholipids was chloroform: methanol:ammonium hydroxide (30%): water (60:35:5:2.5).

Except hydrocarbons and sterols fractions, all the other lipid fractions were converted into their methyl esters by BF_3-methanol reagent and fatty acid composition determined by GC (Table 2). The fatty acid composition of all the lipid classes ranged from C_{12}-C_{20} fatty acid chain length. Lauric acid is found in highest percentage (10.66%) in phosphatidylcholines whereas it is present in lowest percentage (1.01%) in triacylglycerols fraction among the lipid classes analyzed. The percentage of myristic acid is maximum (15.03%) in phosphatidylinositols and minimum (2.58%) in glycolipids. Palmitic acid is found to be present at 17.48% in glycolipid fraction whereas it is present at 9.85% in sterol esters. Stearic acid is found at 10.0% in 1,2-diacylglycerols whereas it is present at 1.5% in triacylglycerols. Oleic acid is the predominant fatty acid found amongst all the lipid classes, however its highest percentage (59.20%) is found in triacylglycerols and lowest percentage (30.06%) in free fatty acids fraction. Lysophosphatidylethanolamines fraction, however, contains 20.42% linoleic acid and sterol esters fraction contains 7.04 % linoleic acid. Glycolipids fraction has 9.98% linolenic acid whereas 1,3-diacylglycerols contain the least proportion (1.03%) of this acid. The lipid fraction glycolipids contains the highest percentage (6.31%) of arachidic acid and traces of this acid is found in 1,3-diacylglycerols fraction, whereas 1-monoacylglycerols, phosphatidylethanolamines and phosphatidylcholines fraction do not contain arachidic acid.

It is interesting to note that lauric acid is present in almost the same percentage (7.0%) in sterols esters fraction and phosphatidylinositols fraction. The same is true for myristic acid which is found to be almost in equal percentage (15.0%) in 2-monoacylglycerols and phosphatidylinositols. Palmitic acid is found to be present in the same range of percentage (~15.0%) in 2-monoacylglycerols, phosphatidylethanolamines and lysophosphatidylethanolamines fractions. Again palmitic acid is in almost equal proportions (9.85-9.97%) in sterol esters fraction and triacyglycerols fraction. Phosphatidylinosetols fraction also contain almost equal proportions (16.43-16.66%) of palmitic acid. It is also found that stearic acid is present in exactly the same percentage (8.0%) in free fatty acids fraction and phosphatidylethanolamines fraction. The percentages of linoleic acid are fairly close (33.66%, 33.88%) in phosphatidylethanolamines fraction and phosphatidylcholines fractions, respectively. Similarly the percentage of linoleic acid is equal in 2-monoacylglycerols fraction and phosphatidylchlolines fraction. Again it is almost equal (13.11-13.12%) in 1,3-diacylglycerols and triacylglycerols fractions.

Linolenic acid is found to be almost equal in percentage proportions in sterol esters (7.0%), free fatty acids (7.03%) and lysophosphatidylethanolamines fractions (7.0%). Linolenic acid is in equal proportions (6.0%) in 1-monoacylglycerols fraction and phosphatidylcholines fraction. Arachidic acid is not present in 1-monoacylglycerols, phosphatidylethanolamines and phosphatidylcholines fractions, however it is present in 4.0% proportions in lysophosphatidylethanolamines fraction and phosphatidylinositols fractions (Table 2).

The percentages of saturated and unsaturated fatty acids of each lipids fraction are given in Table 3. It is seen that the highest percentage (49.46%) of saturated fatty acids and lowest percentage (50.54%) of unsaturated fatty acids are found in phosphatidylinositols. Again it is shown that triacylglycerols has the highest percentage (77.52%) of unsaturated fatty acids and lowest percentage (22.48%) of saturated fatty acids. The results also reflected that oleic acid was the major fatty acid among the unsaturated fatty acids whereas palmitic acid was the major fatty acid among the saturated fatty acids moiety.

Previously (Chaudri *et al.*, 1999) reported the distribution of fatty acids at 1,2 and 3 positions in the triacylglycerols of *Carum roxburghianum* seeds. The positions of fatty acids at 1,2 and 3 carbons of triacylglycerols molecules in each group were determined by lipolytic hydrolysis by pancreatic lipase and GC of methyl esters. The 2-position of these glycerols was found to be occupied by the unsaturated acids preferentially. The results of the study allow possibility of predicting the distribution pattern of fatty acids in different triacylglycerol fractions.

Table 3. Percentage of Saturated and Unsaturated Fatty Acids in Different Lipid Classes of C. Roxburghianum

Lipid Fractions	Saturated fatty Acids (%age)	Unsaturated Fatty acids (%age)
Sterol esters	28.45	71.55
Triacylglycerols	22.48	77.52
Free fatty acids	42.84	57.16
1,3-Diacylglycerols	36.84	63.03
1,2-Diacylglycerols	43.28	56.72
Glycolipids	33.87	66.13
2-Monoacylglycerols	35.38	64.62
1-Monoacylglycerols	32.33	67.67
Phosphatidylethanolamines	42.11	57.89
Phosphatidylcholines	46.12	53.88
Lysophosphatidylethanolamines	42.03	57.97
Phosphatidylinositols	49.46	50.54

Table 2. Fatty acid Composition of Different Lipids in the Resting Seeds of C. roxburghianum

LIPIDS	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Sterol esters	7.04	2.81	9.85	8.45	57.81	7.04	7.00	0.30
Triacylglycerols	1.01	5.70	9.97	1.50	59.20	13.12	5.20	4.30
Free fatty acids	6.01	11.54	12.78	8.00	30.06	20.07	7.03	4.51
1,3-Diacylglycerols	9.95	9.33	10.66	7.80	49.02	13.11	1.03	*T
1,2-Diacylglycerols	6.81	9.66	13.11	10.0	42.61	11.11	3.00	3.70
Glycolipids	2.81	2.58	17.48	4.69	36.34	19.81	9.98	6.31
2-Monoacylglycerols	1.44	15.03	15.22	3.62	46.31	14.01	4.31	0.14
1-Monoacylglycerols	5.23	10.00	14.10	3.00	45.00	16.67	6.00	0.00
Phosphatidylethanolamines	9.65	9.39	15.07	8.00	33.66	16.20	8.03	0.00
Phosphatidylcholines	10.66	11.03	16.66	7.77	33.88	14.00	6.00	0.00
Lysophosphatidylethanolamines	4.02	13.24	15.22	5.55	30.55	20.42	7.00	4.00
Phosphatidylinositols	7.00	15.03	16.43	7.00	32.54	13.00	5.00	4.00

*T = Traces.

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