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LIPID STUDIES OF *CARUM CAPTICUM* SEED OIL

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The total lipids of *Carum capticum* were fractionated by thin-layer chromatography into hydrocarbons (1.2%), wax esters (2.0%), sterol esters (1.8%), triglycerides (54.1%), fatty acids (5.7%), 1:3 diglycerides (5.7%), 1:2 diglycerides (6.7%), glycolipids (7.2%), 2-monoglycerides (3.9%), 1-monoglycerides (7.4%), phosphatidyl ethanolamines (1.5%) phosphatidyl cholines (1.1%) lysophosphatidyl ethanolamines (0.6%) and phosphatidyl inositols (1.1%).

Key words: Lipids, Methylation, Thin-layer chromatography, Gas liquid chromatography.

Introduction

The plants of Umbelliferae family are found in abundance in Pakistan [1]. Since old times, these plants are being used in food and feed [2], in cosmetics and even in medicines [3]. In medicines active constituents are considered to be essential oils but little is known about the fixed oils. In the presented work, a systematic study is done on the separation, purification and identification of the lipids of *C. capticum*. Each separated lipid was then hydrolysed and methylated to determine their fatty acid composition with the help of gas liquid chromatography.

Materials and Methods

Extraction of oil. 40 Grams of dried and powdered seeds of *C. capticum* were shaken with 300 ml of chloroform and methanol (2:1 v/v) mixture for half an hour by a magnetic stirrer. After filtration, the residue was shaken with 100 ml of the above solvent mixture for 15 mins. The process was repeated 3 times and the filterates were combined. The solvent was removed with rotary evaporator and then the non-lipid impurities were removed by shaking with Folch washing consisting of chloroform: methanol: 0.9% sodium chloride (3:48:7v/v) [6]. The lower chloroform layer was separated and the solvent was removed with rotary evaporator.

Thin-layer chromatography. The neutral and polar lipids were identified by using solvent system hexane: diethyl ether: acetic acid (80:20:2) and chloroform: methanol: 30% ammonium hydroxide: water (60:35:5:2.5 v/v) respectively [7] by the application of thin-layer chromatography. The thin-layer chromatograms of 0.5 mm thickness were prepared by using 50 g of silica gel (Kiesel gel G type 60, Merck) and 100 ml water. The standards as well as different reagents were used to develop coloured spots [8-10] for the identification and confirmation of different classes of lipids. The standard solutions of antimony trichloride in chloroform was used for the identification of sterol and sterol esters. Appearance of red-violet spots on TLC plates when kept at 100° for 10 mins. confirmed the presence of these compounds. The reagents molybdenum blue, Dragendorff and ninhydrin were also used for the identification of phospholipids, phosphatidyl choline, phosphatidyl and lysophosphatidyl ethanolamine, which showed blue, straw-orange and red-violet spots respectively on TLC. Later on, 40mg lipids were separated quantitatively by using the above mentioned solvents into hydrocarbons (0.48 mg), wax esters (0.8 mg), sterol esters (0.72 mg), triglycerides (21.64 mg), free fatty acids (2.28 mg), 1:3-diglycerides (0.28 mg), 1:2-diglycerides (2.68 mg), glycolipids (2.88 mg), 2-monoglycerides (1.56%), 1-monoglycerides (2.96 mg), phosphatidyl ethanol amines (0.6 mg), phosphatidyl cholines (0.44 mg) lysophosphatidyl ethanolamines (0.24 mg) and phosphatidyl inositols (0.44 mg).

Gas liquid chromatography. The quantitatively separated each lipid fraction was methylated with boron trifluoride methanol reagent and the methylated fatty acids were purified by thin layer chromatography by using hexane: ether (9:1 v/v) solvent system [11]. The methylated margaric acid was used as an internal standard for the determination of fatty acid percentage by gas liquid chromatography. A polar column (152.4 x 0.95 cm) of polyethylene glycol succinate (10%) coated on diatomite "C" (80-100 mesh) at 200° with the flow rate of 40 ml/min. of nitrogen as a carrier gas was used in Pye Unicam 204 Series instrument for the identification of fatty acids as methyl esters by comparision of their retention times with that of standards under the same conditions [12].

Peak areas were obtained from the product of the retention times and the respective peak heights. The amounts of total fatty acids were determined by comparing the total peak area of all the fatty acids in each fraction with that of the internal

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standard. By calculating the average molecular weight of the fatty acids, in each of the fractions the amounts of the various fractions like triglycerides, monoglycerides, free fatty acids, phosphatidyl ethanol amine, phosphatidyl choline and P inositol were calculated.

Results and Discussion

The fresh, dried and powdered seeds of *C. capticum* were used for the extraction of lipids by chloroform: methanol (2:1 v/v). The solvent as well as essential oils, if present, are removed by rotary evaporator under reduced pressure. The lipids thus obtained (7.8 g) are made free from unwanted materials such as glucose, salts, urea, sucrose etc. by specific washing solvent mixture [6]. The polar as well as non polar lipids are separated by thin layer chromatography into a number of fractions as explained in the experimental work. The standards as well as different reagents are used to develop coloured spots for the identification of different classes of lipids. The sterol and sterol esters are detected as red spots by spraying with antimony trichloride solution in chloroform and by heating at 100° for 10 mins. [8]. The phosphatidyl choline shows orange spots with Dragendorff's reagent [9]. The phosphatidyl ethanolamine and its lyso derivative are identified as violet spots with ninhydrin [10]. The glycolipids are identified as blue violet spots with orcinol-sulphuric acid spray [13]. The R_f values and percentage composition of polar as well as a neutral lipids are given in Table 1. These results show that the percentage of polar lipids is very low as compared to neutral lipids. The phosphatidyl ethanolamine (1.5%) is of the highest percentage among polar lipids where as triglycerides (54.1%) is of the highest among neutral lipids. However, there are 10 and 4 fractions of neutral and polar lipids respectively.

The conversion of fatty acids of neutral and polar lipids into their methyl esters is carried out by reacting very small quantities of lipid fraction with boron trifluoride methanol reagent in a test tube [14]. The methyl esters after extraction with hexane are purified by thin layer chromatography [11]. The fatty acids which are essential part of each lipid class are characterised by the use of gas liquid chromatography (Table 2). The methyl ester of each lipid fraction was further processed for the analysis and evaluation of fatty acids by the application of gas liquid chromatography. The fatty acid composition of polar as well as neutral lipids is given in Table 2. The oleic acid is of highest percentage among the fatty acids profile of $C_{12:O}C_{20:O}$. The highest percentage of oleic acid reflects the phylogenetic factor of Umbelliferae family [15]. However, each lipid class consists of saturated and unsaturated fatty acids. The range of saturated fatty acids in all fractions is $C_{12:O}C_{20:O}$. The percentage of palmitic acid is higher as compared to lauric acid and myristic acid. The percentage of palmitic acid, is higher in polar lipids in contrast to neutral lipids. The presence of stearic acid is not significant, but oleic acid among the unsaturated fatty acids is of highest percentage and secondly this monoenoic acid has been found out in highest percentage in neutral lipids as compared to polar lipids. So, it is evident that elongation process from palmitic to stearic acid is accompanied to desaturation in nature. The total unsaturation of each fraction of neutral lipid is higher with particular reference to triglycerides (79.0%) as compared to polar lipids. The importance and quality of essential fatty acids by the name linoleic acid which is the precursor of prostaglandins which play a defenite role in the health of a person by their existance in lung tissues, seminal plasma and accessory genital glands [16]. The study on the treatment of gastrointestinal diseases by *C. capticum* was carried out by Kirtikar, Basu and Nadkurni. The treatment was given to a patient either by the use of steam distilled compounds, being highly volatile in nature, were certainly essential oils in nature or the species under discussion was used as such for different ailments. It clearly indicates that by steam distillation organic compounds like lipids containing essential fatty acids and others are eliminated. So it is concluded on the basis of presented studies that the use of *C. capticum* as such may have better results as compared to steam distilled compounds for the treatment of diseases as mentioned above.

TABLE 1. R_f VALUES AND THE PERCENTAGE COMPOSITION OF (A) NEUTRAL AND (B) POLAR LIPIDS OF *CARUM CAPTICUM* OF THE FAMILY UMBELLIFERAE.

Lipids	R_f	% Yield
(A) NEUTRAL LIPIDS		
1. Hydrocarbon	0.95	1.2
2. Wax esters	0.93	2.0
3. Sterol esters	0.72	1.8
4. Triglycerides	0.61	54.1
5. Free fatty acids	0.41	5.7
6. 1:3-diglycerides	0.34	5.7
7. 1:2-diglycerides	0.30	6.7
8. Glucosides	0.25	7.2
9. 2-monoglycerides	0.19	3.9
10. 1-monoglycerides	0.15	7.4
(B) POLAR LIPIDS		
1. Phosphatidyl ethanolamine	0.70	1.5
2. Phosphatidyl choline	0.50	1.1
3. Lysophosphatidyl ethanol amine	0.54	0.6
4. Phosphatidyl inositol	0.18	1.1

TABLE 2. FATTY ACID COMPOSITION OF NEUTRAL AND POLAR LIPIDS OF *CARUM CAPTICUM* SEEDS.

Lipids	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Sterol esters	9.83	12.44	15.00	5.00	40.96	16.77	–	–
Triglycerides	3.30	2.00	8.80	4.10	63.50	9.30	6.20	2.80
Free fatty acids	4.98	7.86	13.62	6.09	43.05	14.14	5.59	4.67
1 : 3 -diglycerides	–	9.62	16.20	7.90	52.31	13.97	–	–
1 : 2-diglycerides	3.67	5.80	7.54	4.86	51.13	14.23	6.67	6.10
Glucosides	-	4.04	11.18	3.04	52.94	13.37	8.43	7.00
2-monoglycerides	2.00	3.35	8.78	6.41	52.02	10.87	7.43	9.14
1-monoglycerides	5.51	6.52	14.00	7.63	37.47	13.62	8.25	7.00
Phosphatidyl ethanolamines	10.18	14.25	19.90	6.65	29.53	14.85	4.64	-
Phosphatidyl cholines	10.47	15.52	18.42	8.33	23.71	13.46	6.52	3.57
Lysophosphatidyl ethanol amines	11.72	16.80	17.01	5.00	35.44	14.03	–	–
Phosphatidyl inositol	12.00	16.41	17.82	3.94	36.82	13.01	–	–

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