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LIPID STUDIES OF *CUMINUM CYMINUM* FIXED OIL

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Abstract

The yield of solvent extracted oil of *Cuminum cyminum* was 18.7%. The oil was classified into hydrocarbon 1%, wax esters 1%, sterol ester 25%, triglycerides 55%, 1,3 diglycerides 1%, 1,2 diglycerides 1%, monoglycerides 2%, free fatty acid 10%, phosphatidyl-ethanolamines 2.0%, phospatidylcholine1.2%, lysophosphatidyl eth-anolamines 0.6% and phosphatidyinositol 0.2%. The fatty acid composition of all the lipid classes neutral as well as polar was determined by the application of thin layer and gas liquid chromatography.The oil is considered as a good source of petroselinic acid (51.7%) in the fatty acid composition. The range of fatty acid was found from C_{10} to $C_{20.}$

Introduction

Cuminum cyminum of the family Umbelliferae is a slender annual herb native of Egypt and Syria. It is extensively cultivated as a cold season crop on the plains and as summer crop on the hills in Northern India, Himalayas and the Punjab, Balochistan, Kashmir, Kumaon and Garhwal etc. The plants are annual or biennial herbs and cultivated in different parts of Pakistan for the recovery of their essential oil (Nasir & Ali, 1972). Cumin oil is employed advantageously in many types of flavouring preparations particularly in curries and culinary preparations of oriental type. It is also used to an extent in soap perfumery and in flavouring beverages. Cumin aldehyde has a powerful odour and is used only in traces in compounding synthetic floral perfumes such as cassie. *Cuminum cyminum* seeds are largely used as a condiment or spice in curries and pickles etc. It is also used in the indigenous medicines as a stimulant and carminative. Seeds have cooling affect and therefore form an ingredient of most prescriptions for gonorhoea, chronic diarrhoea and dyspepsia, externally they are applied in the form of poultice to allay pain and irritation of worms in the abdomen. Seeds reduced to powder, mixed with honey, salts and butter are applied to scropian bites (Al-Yahya & Colpharm, 1986; Johnson & Nam 1998, Kartikar & Basu, 1984). Studies have been carried out on the essential oils (Chopra, 1970) but no work has been done on fixed oil of this species. The present work reports on the fatty acid composition of neutral and polar lipids of *C. cyminum.*

Materials and Methods

a. Extraction of oil: The seeds from fresh crop of *Cuminum cyminum* (150g) were dried in an oven at 105^{o}C and then ground into fine powder. The oil was extracted with 300ml chloroform: methanol (2:1v/v) mixture (Akhtar *et al*., 1981) at room temperature by shaking on magnetic stirrer for 3–4 hours. After filtration the residual material was again treated three times with 100 ml portions of chloroform-methanol and 9% aqueous sodium chloride (3: 48: 47 v/v/v) solution (Folch *et al*., 1957).

After drying the extract over anhydrous sodium sulphate and removal of the solvent under reduced pressure, the lipids thus obtained were stored under an atmosphere of nitrogen. Triplicate samples were used in these studies.

b. Physico-chemical values: The physico chemical values like Iodine value, Saponification value, Ester value, Free fatty acids were determined according to the procedure of British Standard Specification 684 (Anon., 1958). Refractive index was determined with Abbe's refractometer.

c. Thin layer chromatography: The oil was fractionated qualitatively and quantitatively on 0.25mm and 0.5mm thick silica gel chromatoplates. Thin layer chromatograms of 0.25mm thickness were prepared by using 30 gm silica gel and 60 ml water while 0.5 mm silica gel chromatoplate were prepared by using 60 gm silica gel and 120ml water. These plates were activated at 105^{o}C for two hours. A known weight of oil (10% solution in cloroform) was loaded in a straight line about 3cm above the lower edge of chromatogram. The developing media for neutral and polar lipids were hexane: ether: acetic acid (80: 20: 2 v/v) and chloroform:methanol: 30 % ammonium hydroxide: water (60: 35: 5: 2.5 v/v) respectively (Akhtar *et al*., 1981). The saturated solution of antimony trichloride in chloroform was used for the identification of sterol and sterol ester. Appearance of red violet spot on TLC plate when kept at 100^{o}C for 10 min., confirmed the presence of these compounds. The reagent molybdenum blue dragendorff and ninhydrin were also used for the identification of phospholipids, phosphatidylcholine and lysophosphatidylethanolamine which showed blue, strand orange and red violet spot, respectively on thin layer chromatography (Lowsenstein, 1969).

Two plates of 0.5mm thickness were used for neutral and one for polar lipid for preparative TLC in order to collect different fractions. These fractions in the form of bands were scratched from TLC plates in order to convert them into methyl ester.

d. Methylation and purification of methyl esters: The different purified lipids were treated with Borontriflouride – methanol (Morrison & Smith, 1964) for recommended time in test tube with Teflon lined screw cap for the formation of methyl esters which were purified quantitatively by the application of thin layer chromatography using hexane: ether (9:1 v/v) solvent system (Raie *et al*., 1983). The esters were extracted with hexane and stored at low temperature for GC analysis. This method of esterification is found to be the most useful and suitable for little amount of sample. The methyl ester thus obtained were extracted with hexane and then purified quantitatively by the application of thin layer chromatography (Raie *et al*., 1983). Fatty acid methyl esters were separated on 20% $AgNO_3$ impregnated thin layer chromatograms according to the degree of unsaturation. The esters of the mono-unsaturated acids were oxidized by the Von Rudloff's oxidation procedures (Hemilton & Raie, 1972) to liberate the mono and dicarboxylic acids which were methylated and separated on thin layer chromatograms. The presence of double bonds on 6 and 9 postions, respectively of the methylated monocarboxilic acids revealed the common occurrence of petroselinic acid and oleic acids when examined by gas liquid chromatography.

e. Identification of fatty acids by GC: The methyl ester of the whole oil and its lipid fractions were analysed for their fatty acid composition by gas chromatography. The

apparatus used for this purpose was Shimadzu GC-14A equipped with flame ionization detector (FID) and capillary column PEG (2.5m+0.2 mmi.d (Guenther, 1950). The column oven temperature was 190^{o}C and analysis was done without programming on 190^{o}C fixed temperature. Nitrogen gas was used as carrier gas with a flow rate 35ml /min. The temperature of injector and detector was set at 270^{o}C and 300^{o}C respectively. The peaks were recorded on Shimadzu CR- 4A chromatopac and were identified by comparing their retention times with those of standard methyl esters analysed under the same condition.

Results and Discussion

a. Physico-chemical properties: The lipids extracted from the seeds of *C. cyminum* were free from unwanted materials such as glucose, salts, urea, sucrose etc. The seeds contained moisture 4.87%, fat 20.42%, protein 17.0%, ash 3.98% and fiber 7.45%. The physicochemical properties of extracted oil were determined which showed Refractive Index 1.4681, Saponification value 142.09, Iodine value 101.63, FFA 1.4 and unsaponifiable matter 0.45 (Table 1) .The results indicate that free fatty acid value of the oil is low indicating that oil is rich in triglycerides. The high percentage of triglycerides is the basic property of the edible oil. Table 1 shows the iodine value (101.63) and the % age of unsaturated acids (85%) which is very high as compared to saturated acid (15%). It would appear that the composition of oil is very close to vegetable oil.

b. Fatty acid composition: The gas liquid chromatographic analysis of the total lipids indicates that the amount of oleic acid was maximum (65.87%), linolenic acid was the second major unsaturated acid (18.67%) present in the oil with the minor amount of saturated acid except palmitic acid (11.69 %) (Table 2). These results are in accordance to earlier reports which shows that palmitic oleic and linolenic acid are major fatty acids in most of the species of Umbelliferae. The monoenoic fractions were further established after oxidation and found that the amounts of petroselinic acid ($C_{18:1}$ $\triangle$6) were higher than those of oleic acid ($C_{18:1}$ $\triangle$9) in them, the occurrence of petroselinic acid ($C_{18:1}$ $\triangle$6) is known to be general characteristic of the seeds oils of Umbelliferae species.

c. Neutral and polar lipids: The neutral and polar lipids were identified by comparing their R_f values with those of standards and further confirmed by spraying specific reagents which gave spot tests in thin layer chromatography. After classification the neutral lipids found were hydrocarbon and wax (2%) sterol ester (25%) triglycerides (55%), free fatty acid (10%), 1,3 diglycerides (1%), and monoglycerides (2%) (Table 3). Polar lipids were 4% which were further fractioned by using polar solvent system (Akhtar *et al*., 1981). The composition of polar lipids showed posphatidylethanolamine (2%), phosphatidylcholine (0.2%), lysophosphatidyletanolamine (0.6%), phosphatidyl-choline (0.12%). The R_f values and percentage are given in Table 3.

As the results indicate the percentage of polar lipids 4% is lower as compared to neutral lipids (96%). However, the enriched technique was used for accumulation of polar and neutral lipids of very low percentage for further experimental work. The fatty acid moiety which plays a vital role for the formation of various classes of neutral and polar lipids is characterized by the use of gas liquid chromatography (Table 4).

Table 1. Physico-chemical characteristics of the oil from *Cuminum cyminum*.

Values	Units
Moisture	4.87 %
Fat	20.42 %
Ash	3.98 %
Protein	17.0 %
Refractive index	1.4681
Saponification value	142.09
Iodine value	101.63
FFA	0.14
Unsaponifiable matter	0.45 %

Table 2. Fatty acids composition of the oil of *Cuminum cyminum* seeds.

Fatty acids	%
Capric Acid $C_{10:0}$	0.32
Lauric Acid $C_{12:0}$	0.5
Myristic Acid $C_{14:0}$	2.0
Palmitic Acid $C_{16:0}$	11.69
Palmitolic Acid $C_{16:1}$	0.11
Stearic Acid $C_{18:0}$	0.4241
Oleic Acid $\triangle 9$ $C_{18:1}$	14.6
Petroselinic $\triangle 6$ Acid $C_{18:1}$	51.27
Linoleic Acid $C_{18:2}$	0.82
Linolenic Acid $C_{18:3}$	18.67
Arachidinic Acid $C_{20:0}$ & Higher	0.20

Table 3. % age lipids in the oil of *Cuminum cyminum* seeds.

Lipids	R_f	%
A. Neutral lipids		
Hydrocarbons & Wax ester	0.95	2
Sterol ester	0.75	25
Triglycerides	0.70	55
Free Fatty Acid	0.41	10
1,3 diglycerides	0.38	1
1,2 Diglycerides	0.30	1
Monoglycerides	0.15	2
B. Polar lipids		
Phosphatidylethanolamine	0.72	2.0
Phosphatidycholines	0.61	1.2
Lysophosphatidylethanolamine.	0.54	0.6
Phosphatidylanositols	0.17	0.2

$C_{18:0}$ was found predominant in neutral as well as polar lipids. Earlier studies have proposed octadecenoic acid as phylogenetic one which reflects the character of all species of this family. The difference between neutral and polar lipids may be made on the basis of fatty acid moiety (Table 4).

Table 4. Fatty acid composition of neutral and polar lipids in the oil of *Cuminum cyminum*.

Lipid fractions	Capric acid $C_{10:0}$	Lauric acid $C_{12:0}$	Myristic acid $C_{14:0}$	Palmitic acid $C_{16:0}$	Palmitoleic acid $C_{16:1}$	Stearic acid $C_{18:0}$	Oleic acid $C_{18:1}$	Linolic acid $C_{18:2}$	Linolenic acid $C_{18:3}$	Arachidinic acid $C_{20:0}$ & higher
	Fatty acid composition (%) of neutral lipids									
Wax	0.3794	2.8682	2.9972	14.60	—	—	63.98	13.07	5.079	2.3848
Sterol Ester	—	—	—	14.4237	—	—	65.58	13.4231	4.0852	2.4852
Triglycerides	0.8161	0.5661	0.2784	4.7893	0.0442	—	67.46	24.4987	0.3126	1.2022
FFA	2.4107	2.4484	4.1612	16.1133	1.6345	—	55.50	1.5427	6.9886	8.2971
I,3diglycerides	0.602	0.6023	0.786	7.668	1.4005	9.655	63.35	6.5039	4.962	5.092
1,2diglycerides	0.566	2.1858	3.1942	13.4586	0.5118	0.6029	59.64	5.1074	6.0882	7.2659
Monoglyc-eride	—	—	0.0668	5.2594	—	—	59.32	18.8476	8.84576	7.9929
	Fatty acid composition (%) of polar lipids									
Phosphatidylethanolamine	—	2.001	6.347	8.8307	—	0.7932	51.22	13.7932	13.0972	13.9284
Phosphatidylc-holine	0.9022	0.897	1.9147	6.2071	—	0.6722	56.15	17.8536	1.9840	5.3303
Lysophosphatidylethanolamine	—	0.020	0.9743	18.7911	—	—	46.67	10.8429	3.3621	19.2441
Phosphatidyinositol	2.001	4.495	4.4188	5.7491	0.5544	1.824	52.34	3.9172	8.525	15.6144

$C_{14:0}$, $C_{16:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{20:0}$ were present in almost all lipid classes. $C_{16:1}$ was not present in polar lipids except Phosphatidylinositol. $C_{18:0}$ was also not present in neutral lipid except 1,3 diglycerides and 1,2 diglycerides. The percentage of $C_{18:1}$ was quite high in all lipid classes like neutral and polar lipids. The amount of $C_{18:1}$ was higher in the triglycerides of neutral lipids (67.46%) and (56.15%) in phosphatidylcholine of polar lipids (Table 4). The results indicate that the percentage of octadecenoic acid was greater in the neutral lipids and the percentage of hexadecenoic acid was higher in polar lipids.

$C_{14:0}$ and $C_{16:0}$ present in both polar and neutral lipids may be precursor for higher acids in both cases of lipids. The essential fatty acid $C_{18:2}$ may be precursors of postaglandins which are found in accessory genital glands, seminal plasma and lung tissues of human body and thus play a vital role in the health of a person (Gunstone, 1967).

The present study indicates that the seed oil is fit for edible purpose as it contains a high percentage of triglycerides and a low percentage of free fatty acids. The fatty acid composition of oil showing higher contents of unsaturated acids (85%) indicates its close resemblance to the vegetable oil. Such oils can be recommended for edible purpose on the basis of their fatty acid composition. The predominant saturated fatty acid was palmitic acid and among unsaturated acids the oil has been reported to contain a high percentage of petroselinic acid ($C_{18:1}$ 6) which is an isomer of oleic acid and is a general characteristic of seed oil of Umbelliferae family.

The base line data of fixed oil of different varieties *Cuminum cyminum* is not available to carry out further studies on the other aspects of these seed lipids. The present study therefore was an attempt to explore the parameters of the fixed oil of *C. cyminum* to know further possibilities of its application. The detailed chemical composition during these studies may help to correlate the medicinal properties with the chemical components of the *C. cyminum*.

References

Akhter, W.M., N. Kausar, M.N. Nawazish and Z. Hussain. 1981. Variation in composition of polar and non polar lipids and their fatty acids in germinating seeds of *Cucumis melo*. *Pak. J. Biol*., 16(2): 71-81.

Al–Yahya, M. and A. Collpharm. 1986. Phyto Chemical studies of plant used in traditional medicine in Saudi Arabia. *Fitoterapia*, 57(3): 179-82.

Anonymous. 1958. British Standard Specification. Methods of analysis of oils and fats. British Standard house, 2 Park Street, London W.I.

Chopra, G.L. 1970. *Indigenous Drugs of India. Augiosperms*. Unique Publishers, Lahore 9th Edn. pp. 45.

Folch. J., M. Lees and G.H. Solane Stanley. 1957. Isolation and purification of total lipids from tissues. *J. Biol Chem*., 226: 497

Guenther, E. 1950. *The Essential Oils.* IV D – Van Nostrand Company Inc. New York.

Gunstone, F.D. 1967. *An introduction to the chemistry and biochemistry of fatty acids and their glycerides.* 2nd edition, Chapman and Hall, 11. New Fette Lane BC.

Hamilton, R.J. and M.Y. Raie. 1972. Analysis of Free Fatty Acids. *J. Chem. Educ*., 49: 507.

Johnson, L. and Y. Nam. 1998. Hepatitis C Protocols, 621.

Kartikar, K.R. and B.D. Basu. 1984. *Indian Medicinal Plants*, II, 1190-1231, 2nd. Edition, Lalit Mohan Basu, Allahabad, India.

Lowsenstein, J.M. 1969. In: *Method in Enzymology. Lipids*, XIV. Academic Press.

Morrision, W.R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethyl acetate from lipid with boron triflouride – methanol. *J. Lipid Res*., 5: 600-608.
Nandkarni, A.K. 1954. In: *Indian Materia Medica*, 3rd. Edition, 936.
Nasir, E and S.I. Ali. 1972. Flora of West Pakistan, Stewart Herbarium, Gordon College Rawalpindi, No 20.
Raie, M.Y., M. Ahmad, S.A Akhter and S.A. Khan. 1983. Fette Serfen Anstrichmittel. 7, 279-280.
William, K.A. 1986. *Oils, Fats and fatty foods*, 4th Edition.

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