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## LIPID STUDIES OF *CITRULLUS VULGARIS* OF THE FAMILY CUCURBITACEAE

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*Citrullus vulgaris* (watermelon seed oil (23.3%) was classified into hydrocarbons (1.4%), wax esters (2.2%), triglycerides (73.0%), free fatty acids (1.8%), 1:3 diglycerides (5.5%), 1:2 diglycerides (2.1%), alcohols (O.5%), sterols (2.0%), 2-monoglycerides (2.0%), 1-monoglycerides (4.0%), monogalactosyl diglycerides (0.5%) phosphatidyl ethanolamines (1.2%), phosphatidyl cholines (1.4%), lysophosphatidyl ethanolamines (1.0%), lysophosphatidyl cholines (0.6%), and phosphatidyl inositols (0.8%). Fatty acids ($C_{12}$-$C_{20}$) composition of all lipid classes, neutral as well as polar was determined by the application of thin layer and gas liquid chromatography.

***Key words.*** Lipid, *Citrullus vulgaris*, Cucurbitaceae.

### Introduction

Chahar maghaz, the seed kernels of water-melon, melon, cucumber and gourd are well known in the subcontinent for their nerve and brain tonic properties. These species belongs to an important family Cucurbitaceae consisting of 100 genera and 850 species [1]. These are grown in tropical and sub-tropical regions of the world and have been used since time immemorial. It creates an interest to study the chemistry of lipids obtained from the seeds which are abundantly available in Pakistan. The present work is about the investigation of the lipid compounds present in the seed oil of water-melon, variety sugar-baby. The work on water-melon lipids had been attempted previously [2-10] but no one had worked out thoroughly and systematically as it has been presented in this paper.

### Materials and Methods

*Extraction of lipids.* The total lipids were extracted from 20gm of powdered seeds with 450 ml mixture of chloroform: methanol (2:1, v/v) [11]. The solvent was removed at 45° under reduced pressure and the residue was given three consecutive washings with 100 ml chloroform: methanol: 0.9% aqueous sodium chloride (3:48:47, v/v) solution [12] in a separating funnel. After the removal of non lipid impurities, the pure lipids were dried over anhydrous sodium sulphate and weighed as 4.7 gm.

*Separation and identification of lipid classes.* The neutral and polar lipids were separated on 0.5 mm thick TLC plates using hexane: ether: acetic acid (80:20:2, v/v) and chloroform: methanol: 30% ammonium hydroxide: water (60: 35: 5: 2.5, v/v) solvent systems respectively [13]. The different components of the lipids were identified by comparing them with the standards and then varified by applying specific spray reagents [14] to the TLC plates. The saturated solution of antimony trichloride in chloroform was used for the identification of sterols by the appearance of red violet spot on TLC plate heated at 100° for 10 mins in an oven. The reagents molybdenum blue, Dragendorff and ninhydrin [14] were also used for the identification of phospholipids; phosphatidyl choline and lysophosphatidyl choline, phos-phatidyl ethanolamine and lysophosphatidyl ethanolamine which showed blue, straw orange and red violet spots respectively on TLC.

*Methylation and purification of methyl esters.* The classified lipids were respectively treated with boron tri fluoride-methanol reagent [15] for recommended time in a test tube with teflon lined screw cap for the formation of methyl esters. They were purified quantitatively on TLC plates using hexane: ether (9:1, v/v) solvent system [16]. The material ($R_f$ 0.6) was eluted with chloroform and the solvent was removed by distillation to get purified methyl esters prior to the application of gas liquid chromatography.

*Gas liquid chromatography.* A polar column (152.4x0.95cm) of polyethylene glycol succinate (10%) coated on "diatomite C" (80-100 mesh) at 200°, with the flow rate of 40 ml/minute of nitrogen as a carrier gas, was used for the identification of fatty acids as methyl esters by comparison of their retention times with those of authentic methyl esters under the same conditions. The percentage of each component was determined by calculating the peak areas by the application of gas liquid chromatography instrument (Pye Unicam 204 Series).

### Results and Discussion

Water melon belongs to the family Cucurbitaceae which is cultivated all over Pakistan. No exhaustive work has been carried on the lipids of the seeds. Therefore, the various classes of lipids were studied to investigate further and to know the reasons for the usefulness of the seeds.

The mixture of chloroform and methanol was used for the complete extraction of neutral as well as polar lipids. The pure and dry lipids thus obtained counted to 23.3% were made free

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from unwanted material, such as glucose, salts, urea, sucrose, etc., by washing with a specific solvent mixture [12]. The classification of lipids into various neutral lipids 94.5% such as hydrocarbons, wax esters, triglycerides, 1:3 diglycerides 1:2 diglycerides, free fatty acids, alcohols, sterols, 2- monoglycerides and 1-monoglycerides, were accomplished by thinlayer chromatography using a solvent system, hexane-ether-acetic acid [13]. Similarly, polar lipid fractions 5.5%, monogalactosyl diglycerides, phosphatidyl ethanolamines, phosphatidyl cholines, lysophosphatidyl ethanolamines, lysophosphatidyl cholines and phosphatidyl inositols were fractionated by TLC in polar solvent system chloroform-methanol- ammonium hydroxidewater. The $R_f$ values and percentage composition of these lipids are given in Table 1.

TABLE 1. $R_f$ VALUES AND THE PERCENTAGE COMPOSITION OF (A) NEUTRAL AND (B) POLAR LIPIDS OF *CITRULLUS VULGARIS*.

| S.No. Lipids | ($R_f$) | ( %) |
|---|---|---|
| (A) Neutral Lipids | | |
| 1. Hydrocarbons | 0.96 | 1.4 |
| 2. Wax esters | 0.90 | 2.2 |
| 3. Triglycerides | 0.59 | 73.0 |
| 4. Free fatty acids | 0.40 | 1.8 |
| 5. 1:3-Diglycerides | 0.33 | 5.5 |
| 6. 1:2-Diglycerides | 0.27 | 2.1 |
| 7. Alcohols | 0.24 | 0.5 |
| 8. Sterols | 0.21 | 2.0 |
| 9. 2-Monoglycerides | 0.18 | 2.0 |
| 10. 1-Monoglycerides | 0.13 | 4.0 |
| (B) Polar Lipids | | |
| 1. Monogalactosyl diglycerides | 0.78 | 0.5 |
| 2. Phosphatidylethanolamines | 0.65 | 1.2 |
| 3. Phosphatidylcholines | 0.53 | 1.4 |
| 4. Lysophosphatidylethanolamines | 0.46 | 1.0 |
| 5. Lysophosphatidylcholines | 0.42 | 0.6 |
| 6. Phosphatidylinositols | 0.17 | 0.8 |

These results show that the percentage of polar lipids is very low as compared to neutral lipids, among which the percentage of triglycerides is the highest (73.0%). However, the preparative TLC was used for the accumulation of polar and neutral lipids of very low percentage for further experimental work.

The various classes of neutral as well as polar lipids separated by thin layer chromatography in minute quantities are efficiently converted into fatty acid and consequently methyl esters by reacting with boron trifluoride methanol reagent in a test tube. The methyl esters thus obtained were characterised by the use of gas liquid chromatography. The results are shown in Table 2.

The fatty acid range was $C_{12}$-$C_{20}$. The $C_{18:2}$ as the essential fatty acid has been found out as predominant one in neutral as well as in polar lipids due to the phylogenetic factor of this family[17]. Lauric and myristic acids ($C_{12:0}$ and $C_{14:0}$) were present in all the polar lipids except lysophosphatidyl ethanolamine in which myristic acid ($C_{14:}0$) was absent. In case of neutral lipids lauric acid found only in monoglycerides and $C_{14:0}$ was indicated in 1:3 diglycerides and wax esters. Among the unsaturated fatty acids, $C_{18:2}$ was higher in all the lipid classes than any other unsaturated acid. In case of saturated fatty acids, $C_{16:0}$ was predominant one in all the fractions except phosphatidyl inositol where $C_{12:0}$ and $C_{14:0}$ were higher than $C_{16:0}$. All fractions except 1:3 diglyceride were found to contain $C_{14:1}$ fatty acid. $C_{18:3}$ was absent in diglycerides, lysophosphatidyl choline and lysophosphatidyl

TABLE 2. FATTY ACID COMPOSITION (MOLE %) OF DIFFERENT LIPIDS IN *CITRULLUS VULGARIS* SEEDS OIL.

| Lipid classes | $C_{12:0}$ | $C_{14:0}$ | $C_{14:1}$ | $C_{16:0}$ | $C_{16:1}$ | $C_{18:0}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ | $C_{20:0}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Wax esters | – | 8.2 | 8.6 | 18.0 | 10.6 | 11.5 | 13.0 | 21.9 | 4.8 | 3.4 |
| Triglycerides | – | – | 3.3 | 21.9 | 3.3 | 9.2 | 16.0 | 45.2 | 1.1 | – |
| Free fatty acid | – | – | 3.6 | 19.3 | 3.5 | 12.9 | 12.2 | 44.2 | 4.3 | – |
| 1:3-Diglycerides | – | 12.1 | – | 20.0 | 11.1 | 16.9 | 17.3 | 22.6 | – | – |
| 1:2-Diglycerides | – | – | 2.5 | 19.6 | 2.5 | 11.2 | 8.8 | 55.4 | – | – |
| 2-Monoglycerides | 8.7 | – | 6.4 | 23.6 | 3.7 | 18.7 | 9.7 | 26.2 | 3.0 | – |
| 1-Monoglycerides | 10.8 | – | 14.5 | 15.1 | 5.6 | 11.3 | 9.3 | 16.8 | 9.3 | 7.3 |
| Monogalactosyl/diglycerides | 10.2 | 7.7 | 8.5 | 11.0 | 7.7 | 3.4 | 4.3 | 38.5 | 5.1 | 2.6 |
| Phosphatidyl ethanolamines | 11.7 | 9.2 | 12.5 | 13.5 | 7.8 | 5.7 | 13.2 | 18.9 | 7.5 | – |
| Phosphatidyl cholines | 8.0 | 12.2 | 12.5 | 14.1 | 11.9 | 8.9 | 12.2 | 20.2 | – | – |
| Lysophosphatidyl ethanolamines | 7.8 | – | 7.4 | 25.5 | 8.7 | 2.5 | 15.2 | 32.9 | – | – |
| Lysophosphatidyl cholines | 10.2 | 4.7 | 8.2 | 12.9 | 13.0 | 5.4 | 7.5 | 35.4 | 2.7 | – |
| Phosphatidyl inositols | 11.8 | 12.4 | 9.5 | 10.1 | 8.4 | 4.5 | 10.1 | 19.7 | 8.4 | 5.1 |

ethanolamine. $C_{20:0}$ was indicated in wax esters, 1-monoglyceride and monogalactosyl diglyceride. The dienoic fatty acid i.e. $C_{18:2}$ being and essential fatty acid lowers the serum cholesterol levels [18] and thus plays a vital role in human health.

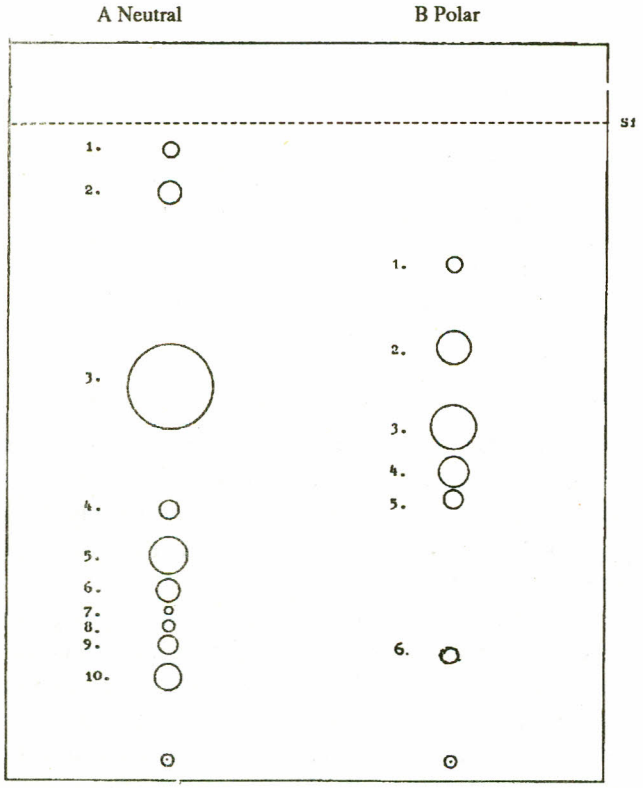


Fig. 1. Thin-layer chromatogram of neutral and polar lipids in the *citrullus vulgaris* seed oil.

(A) *Neutral lipids*. The solvent system used: hexane:diethyl ether: acetic acid (80:20:2), (1). Hydrocarbons, ( 2). Wax esters, (3). Triglycerides, (4). Free fatty acid, (5). 1:3 Diglycerides, (6). 1:2-Diglycerides, (7). Alcohols, (8). Sterols, (9). 2-monoglycerides, (10). 1-monoglycerides.

(B) *Polar Lipids*. The solvent system used:chloroform:methanol: ammonium hydroxide (30%): water (60:35:5:2.5); (1). monogalactosyl diglycerides, (2). Phosphatidyl ethanolamines, (3). Phosphatidyl cholines, (4). Lysophosphatidyl ethanolamines, (5). Lysophosphatidyl cholines, (6). Phosphatidyl inositols.

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