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LIPID STUDIES OF *ANNONA SQUAMOSA*

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The air dried seeds of *Annona squamosa* contain moisture 10.0%, oil 23.0% minerals 1.89% and proteins 24.05%. The fatty acid composition of light yellow coloured oil is C16:0 (13.51%), C18.0 (10.40%), C18.1 (46.96%), C18:2 (25.89%), C18:3 (1.39%) and an unknown acid 1.95% . The unsaponifiable is determined as sterols (25.1%), alcohols (54.1%) and hydrocarbons (20.8%). The hydrocarbons (C11- C31) and alcohols (C16-C18) have been separated, identified ad characterized by the application of thin layer and gas liquid chromatography.

Key words: Annona squamosa, Fatty acids, Hydrocarbons.

Introduction

The *Annona squamosa* locally known as "Sharifa" of the family Annonaceae is used as a fruit in Indo-Pak subcontinent, West Indies and also in other countries of the world. The medicinal importance of *Annona squamosa* fruit is well established for different diseases [1]. Keeping in view the nutritive value of *Annona squamosa,* it has been found out that it contains both reducing and non reducing sugars while seeds contain fixed oil which are all sources of energy [2,3].

Since no research work has been carried out on the lipids in Pakistan, hence, an attempt is here made not only to find out the fatty acid composition of the oil but hydrocarbons, sterols and alcohols have also been characterized. The characterization of hydrocarbons, sterols and alcohols is not available in literature. The physico-chemical values and fatty acid composition of the oil fully supports its status of being an edible oil.

Experimental

Extraction of oil. The air dried brown seeds of *Annona squamosa* were dried at 105°C for 4 hrs, crushed (100 g) and oil extracted in a Soxhlet with hexane [4]. The extract was dried over anhydrous sodium sulphate and filtered. The solvent was removed by distillation and the light yellow coloured oil (23.0g) was stored under nitrogen.

Physico-chemical values of the oil. The physico-chemical values were determined according to the procedure of British Standard Specifications 684 (1958).

Methylation of the oil. The oil (0.2 g) was treated with boron trifluoride methanol reagent for recommended time (1 hr), in a test tube with a teflon lined screw cap, for the formation of methyl esters which were extracted with hexane and then purified quantitatively by the application of thin layer chromatography using hexane: ether (9:1v/v) solvent system [5]. The material was eluted and the solvent was removed by distillation, to get purified methyl esters. Fatty acid composition was found out by gas liquid chromatography of these esters.

Hydrocarbons/alcohols/sterols separation by thin layer chromatography. The non saponifiable matter (300 mg) was separated by siliqa gel thin layer chromatograms (20 x 20 cm) of thickess (0.25 mm) into hydrocarbons (62.4 mg), alcohols (162.3 mg) and sterols (75.3 mg) using hexane/diethyl ether (1:1v/v) as a solvent system. The locating reagent 2,7-dichlorofluorescein was used which under UV at 254 nm showed purple yellow coloured bands of separated materials.

Identification of sterols. The saturated solution of antimony trichloride in chloroform was prepared for the qualitative check up of sterols. By spraying this solution on sterol spot on TLC and by heating it at 100°C for ten minutes, the red violet spot confirms the presence of sterols [6].

Acetylation of alcohols. The alcohols (100 mg) were strirred with acetic anhydride (8 ml), pyridine (24 ml) at room temperature for 18 hrs [7-9]. The acetates of alcohols were extracted with diethyl ether (3 x 75 ml) after the addition of water (60 ml). The acetates were throughtly washed to remove pyridine and acetic anhydride and were dried over anhydrous sodium sulphate prior to their recovery after distillation of diethyl ether. The alcohol acetates were purified by thin layer chromatography.

Gas liquid chromatography of methyl esters of oil. Methyl esters of the oil [10] were analyzed for the respective fatty acid composition by gas chromatography on a Shimadzu GC 14A apparatus equipped with a capillary column of PEG

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(25m x 0.22mm i.d.). The temperature was programmed at 180°C for 3 minutes with rise of 2°C/minute to 220°C. Injection and detector temperatures were 250°C and 300°C respectively with a flow rate of 2 ml/minute of nitrogen as a carrier gas. The methyl esters were identified by comparing their retention times with those of authentic methyl esters under the same conditions. The percentage of various fatty acids was determined with Shimadzu C-R4A Chromatopac Computing Integrator and has been reported in Table 2.

Resolution of hydrocarbons and alcohols as acetates. The hydrocarbons and alcohol acetates were analyzed for the composition of the respective hydrocarbons and alcohols by gas chromatography on a Shimadzu GC-14A apparatus equipped with FID and a capillary column of SE-30 (25 m x 0.22 mm i.d). The temperature programming of the column oven was 150°C-5 minutes -5°C- 300°C and nitrogen was used as a carrier gas with a flow rate of 2 ml per minute. The injector and detector temperatures were 250° and 300°C respectively. The peaks were recorded on Shimadzu C-R4A Chromatopac and were identified by comparison of their retention times with those of the standard samples.

Results and Discussion

The dried seeds of *Annona squamosa* contain protein (24.05%), ash (1. 89%) and oil (23.0%) whereas previous workers [3] claim oil (25.0%_) which does not differ much from the presented results. Physico-chemical values (Table 1) are determined by British Standard Specifications No. 684 (1958) and fatty acid composition (Table 2) is determined by the application of thin layer and gas liquid chromatographies. The oil contains saturated fatty acids (23.91%) and unsaturated fatty acids (76.09 %). It contains the highest percentage of oleic acid (46.96%) which is very close to ground nut oil (47%) [11]. Previous workers [12] also claimed the presence of oleic acid as the highest which is supported by the presented work. The characterization of non saponifiable is not available in the previous literature, so main emphasis is given on the separation and identification of the components of the non-saponifiable matter. The oil contains 0.97% non-saponifiable material which is composed of hydrocarbons (20.8%), alcohols (54.1%) and sterols (25.1%) having Rf values 0.65, 0.45 and 0.25 respectively. The hydrocarbons (C11 - C31) are analysed by SE-30 capillary column (Table 3). The highest percentage of C23 (36.5%) and the next higher C25 (13.9%) among the hydrocarbons is found out. It is the usual pattern of hydrocarbon distribution in the vegetable kingdom. The alcohols as acetates after purification are characterized (Table 4) by gas liquid chromatography. The highest percentage is of oleyl alcohol (53.6%) and oleic acid (46.90%). The next highest percentage of linolyl alcohol (22.90%) and linoleic acid (25.89%) having the same chain length C18 is supported by the theory of biosynthesis of fatty alcohols and fatty acids in nature [13].

TABLE 1. PHYSICO CHEMICAL CHARACTERIZATION OF THE ANNONASQUAMOSA SEED OIL.

1.	Moisture	10.0 %
2.	Ash	1.89 %
3.	Protein	24.05%
4.	Oil	23.00%
5.	Non-saponifiable matter	0.97%
6.	Iodine value	76.75
7.	Saponification value	150.57
8.	Ester value	149.37
9.	Free fatty acids	0.60%
10.	Specific gravity	0.8956
11.	Refractive index at 33°C	1.4677

TABLE 2. THE FATTY ACID COMPOSITION OF THE ANNONA SQUAMOSA SEED OIL

	Fatty acids	Percentage (%)
1.	$C_{16:0}$	13.51
2.	$C_{18:0}$	10.40
3.	$C_{18:1}$	46.96
4.	$C_{18:2}$	25.89
5.	$C_{18:3}$	1.39
6.	$C_{20:0}$	1.95

TABLE 3. HYDROCARBONS OF ANNONA SQUAMOSA SEED OIL.

	Chain length	Percentage
1.	$C_{11:0}$	5.4
2.	$C_{13:0}$	10.5
3.	$C_{15:0}$	3.4
4.	$C_{17:0}$	5.0
5.	$C_{23:0}$	36.5
6.	$C_{24:0}$	3.4
7.	$C_{25:0}$	13.9
8.	$C_{27:0}$	9.7
9.	$C_{29:0}$	4.2
10.	$C_{31:0}$	7.8

TABEL 4. FATTY ALCOHOLS OF ANNONA SQUAMOSA SEED OIL.

	Alcohol acetates	Percentage
1.	$C_{16:0}$	10.0
2	$C_{18:0}$	7.80
3.	$C_{18:1}$	53.60
4.	$C_{18:2}$	22.90
5.	$C_{18:3}$	5.70

The sterols are separated by thin layer chromatography and the presence of sterols is confirmed by the colour test [6]. Further work for their characterization may be carried out.

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