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GC-MS Analysis of Fatty Acid Components in Ethanol Extracts of Two Varieties of Pumpkin Seeds

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Abstract

The two varieties of pumpkin seeds such as orange pumpkin seed (OPS) and white pumpkin seed (WPS) were analyzed with their ethanol extracts for the estimation of fatty acid components through GC-MS analysis. 37 compounds were analyzed in total in both OPS and WPS where about 19 compounds were pinpointed to be present in OPS, 25 compounds in WPS and about 8 compounds to be present common in both OPS and WPS. The peak area % concentration obtained from analysis were compared with the known peak area % concentrations of WILEY8.LIB and the results were interpreted. Some of the compounds determined to be present in higher concentration in OPS include were Propane 1,1,3-triethoxy- (6.58 %), Hexadecanoic acid, 1-(hydroxymethyl)-1,2 ethanediyl ester (9.38 %), 9,12-Octadecadienoyl chloride, (Z,Z)-propanetriyl ester, (E,E,E)- (17.59 %), 9-Octadecenoic acid, 1,2,3-propanetriyl ester – (19.19 %), Squalene (21.33 %) and those identified in WPS include Beta.-Sitosterol (5.10%), 9-Octadecenoic acid,1,2,3-propanetriyl ester (E,E,E) (5.65%), Chondrillasterol (7.30%), Propane, 1, 1, 3- triethoxy- (7.50%), Bicyclo[10.1.0]tridec-1-ene (40.55%). Among them biological activities of some of the important compounds are suggested. This study thus offers the base for using OPS and WPS as an herbal supplement.

Keywords: Pumpkin seeds, GCMS, fatty acids

1. INTRODUCTION

The pumpkin (*cucurbita*) belongs to the family *cucurbitaceae*. This family is one of the largest family in plant kingdom comprising of highest number of edible plant species. It is a high yield vegetable which is easy to grow and is consequently inexpensive [1]. The central cavity of pumpkin contains numerous off-white coloured seeds which are placed in net like structure. The seed content present varies from 3.52% to 4.27% [2]. The most valuable element of pumpkin are included in its part which is most commonly disregarded as waste, namely their seeds. These seeds are small, flat, edible seeds with varying colours and shape depending on the variety. These seeds are mostly covered by a white husk, although some of the pumpkin varieties produce seeds without the husk. Pumpkin seeds can be found hulled or semi–hulled at most grocery stores [3]. Pumpkin seed is one among the plant foods that contain high levels of bioactive components such as beta-carotene, unsaturated fatty acids, phenolic compounds, phytosterols and tocopherols. Some research suggest these functional components have antioxidant, anti-inflammatory activities [4]. The nutritional factors of pumpkin seed comprises of nutrients such as carbohydrates, fats and proteins, as well as minor components such as minerals, vitamins, fibres [5]. Research suggests that pumpkin seeds are an ample source of phytosterols, proteins, polyunsaturated fatty acids, antioxidants, vitamins, carotenoids, tocopherols and various other elements. It is also proved that these seeds contains fatty acid components such as palmitic, palmitoleic, steric, oleic, linoleic, gadoleic, total saturated fatty acids and total unsaturated fatty acids [6]. Seeds of pumpkin are submitted to be rich in oil and variability in the oil content which is due to their broad diversity. Due to the high content of unsaturated fatty acids, pumpkin seed oil is well-suited for enhancing nutritional benefits from foods [1]. They are rich in nutrients such as vitamin A, vitamin E, zinc. Seeds are proposed to contain Omega 3 and Omega 6 fatty acids. The Omega 3 fatty acids are known for their role in improving anti-inflammation property. Pumpkin seeds are high in zinc, which is mega-important for the healthy functioning of the brain, immune system, skin functions and more [7 and 8].

2. MATERIALS AND METHODS

2.1 Collection of samples

The orange pumpkins and white pumpkins were collected from the local markets of Coimbatore, Tamil Nadu during the month of December 2019, January 2020 and February 2020. The seeds from the respective pumpkins were separated manually, washed thoroughly, oven dried, powdered in a mixer grinder and stored in air tight plastic pouches. The OPS and WPS samples were authenticated by Dr. C. Murugan, Scientist 'E'-in-charge, Botanical Survey of India, Southern Regional Centre, Government of India, Coimbatore. The OPS was authenticated as *Cucurbita maxima* Dushesne- *CUCURBITACEAE* and WPS as *Cucurbita pepo* L. – *CUCURBITACEAE*.

2.2 Preparation of the extract

The OPS and WPS samples were oven dried at 110°C for about 4 hours. Then the dried seed samples were grinded to coarse powder in a mixer grinder so that this grinding process makes the parts exposed to the solvents for easy penetration to extract the phytoconstituents. The ethanol solution was selected for extraction because of the easy dissolving property. The powdered samples were stored in a sterile containers. The OPS and WPS samples of each 2.5 grams is weighed and immersed in 25 ml of ethanol solution each for 5 minutes and then filtered separately. The filtrates were kept in orbital shaker for 24 hours and then the extract obtained was subjected for analysis.

2.3 GC-MS analysis

The GCMS analysis of ethanol extracts of both OPS and WPS samples were performed in GC-MS analysis of the extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas with a column flow rate of 1.03mL/min. The column oven temperature was 70.0 °C Injection temperature was 280.00 °C and injection mode was split with a flow control mode of Linear Velocity with a split ratio of 10.1. The pressure being 63.7 kPa. The total flow was 14.4 mL/min, linear velocity: 37.2 cm/sec, Purge Flow rate: 3.0 mL/min.

2.4 Identification of obtained components

The components obtained in the chromatogram of OPS (figure 1) and WPS (figure 2) were identified by comparing the spectrum of the unknown components with the spectrum of known components stored in the WILEY8.LIB. The name, molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS AND DISCUSSION

The studies on the active components in the OPS and WPS ethanol extract by GC-MS analysis clearly showed the presence of 37 compounds in total. The OPS ethanol extract showed 19 compounds (table 1) and WPS ethanol extract showed 25 compounds (table 2). The results of the compounds in the table 1 and 2 were obtained by identifying, quantifying and comparing the peak results drawn from the figure 1 and 7 respectively with the known standards of WILEY8.LIB.

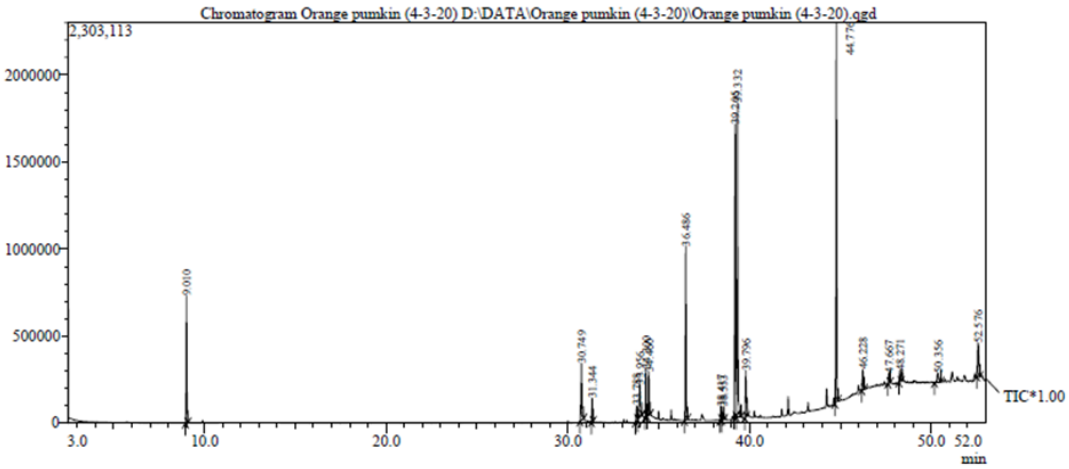

Figure 1 GCMS chromatogram of orange pumpkin seed

Table 1 GC-MS analysis of orange pumpkin seed

S. No	RT	Name of the compound	MF	Peak Area (%)
1	9.01	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	6.58
2	30.74	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	4.05
3	31.34	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	1.17
4	33.78	11, 14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	1.07
5	33.95	cis-Vaccenic acid	$C_{18}H_{34}O_2$	3.05
6	34.30	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	2.37

7	34.46	Ethyl Oleate	$C_{20}H_{38}O_2$	2.58
8	36.48	Hexadecanoic acid, 1-(hydroxymethyl)-1,2 ethanediyl ester	$C_{35}H_{68}O_5$	9.38
9	38.41	9-hexadecyn-1-ol	$C_{16}H_{30}O$	0.73
10	38.55	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	$C_{17}H_{34}O$	0.71
11	39.20	9,12-Octadecadienoyl chloride, (Z,Z)-propanetriyl ester, (E,E,E)-	-	17.59
12	39.33	9-Octadecenoic acid, 1,2,3-propanetriyl ester	$C_{57}H_{104}O_6$	19.19
13	39.79	Octadecanoic acid, 2-hydroxy-1,3-propanetriyl propanediyl ester	-	2.76
14	44.77	Squalene	$C_{30}H_{50}$	21.33
15	46.22	1H-Purin-6-Amine,[(2Fluorophenyl)methyl	$C_{12}H_{10}N_5$	1.45
16	47.66	Gamma.-Tocopherol	$C_{28}H_{48}O_2$	0.72
17	48.27	2h-1-Benzopyran-6-Ol, 3,4-Dihyd-2,7,8-Trimethyl-2-(4,8,12,16,20,24,28,32-Octamethyl-3,7,11,15,19,23,27,31-Tritriacontaoctaeny	$C_{53}H_{82}O_2$	0.81
18	50.35	1-Heptatriacotanol	$C_{37}H_{76}O$	1.12
19	52.57	Stigmasterol	$C_{29}H_{48}O$	3.32

In the table 1, the RT is the Retention time, MF is the molecular formula, and Peak Area % is the concentration.

The table 1 denotes the compounds identified in OPS ethanol extract. The MF for compounds such as 9, 12-Octadecadienoyl chloride, (Z, Z) - propanetriyl ester, (E, E, E) - and Octadecanoic acid, 2-hydroxy-1, 3-propanetriyl propanediyl ester were unknown. From the table 1, the higher concentration compounds were Propane 1,1,3-triethoxy- (6.58 %), Hexadecanoic acid, 1-(hydroxymethyl)-1,2 ethanediyl ester (9.38 %), 9,12-Octadecadienoyl chloride, (Z,Z)- propanetriyl ester, (E,E,E)- (17.59 %), 9-Octadecenoic acid, 1,2,3-propanetriyl ester – (19.19 %), Squalene (21.33 %). The figures 2, 3, 4, 5, 6 shows the mass chromatogram of the above higher proportion compounds identified from OPS and table 1.

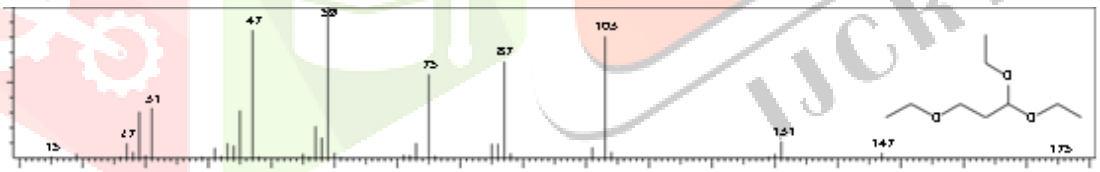

Figure 2 Mass chromatogram of Propane 1, 1, 3- triethoxy

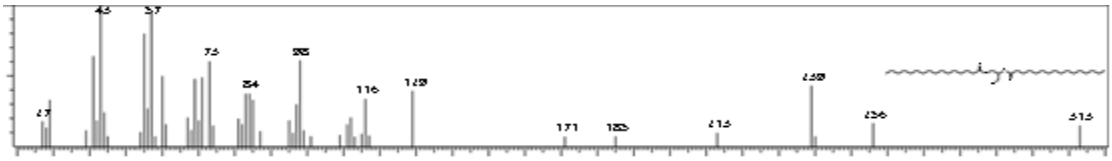

Figure 3 Mass chromatogram of hexadecanoic acid, 1-(hydroxymethyl)-1,2 ethanediyl ester

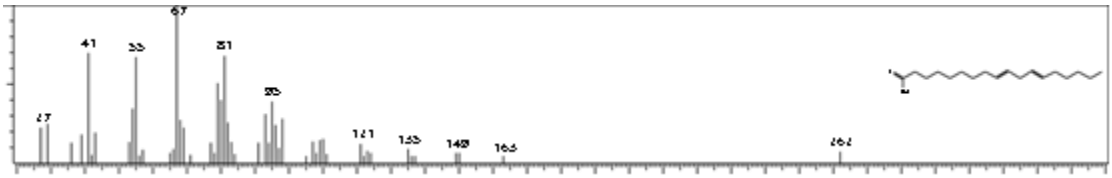

Figure 4 Mass chromatogram of 9, 12-Octadecadienoyl chloride, (Z, Z)- propanetriyl ester, (E,E,E)-

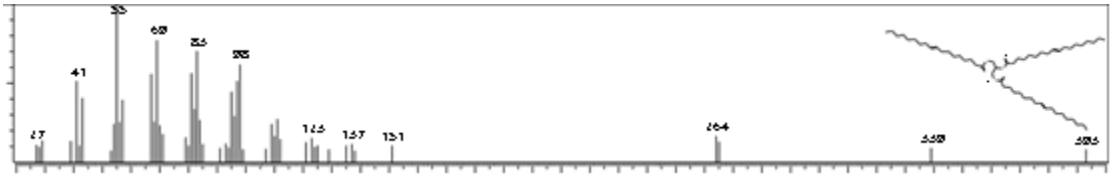

Figure 5 Mass chromatogram of 9-Octadecenoic acid, 1, 2, 3-propanetriyl ester

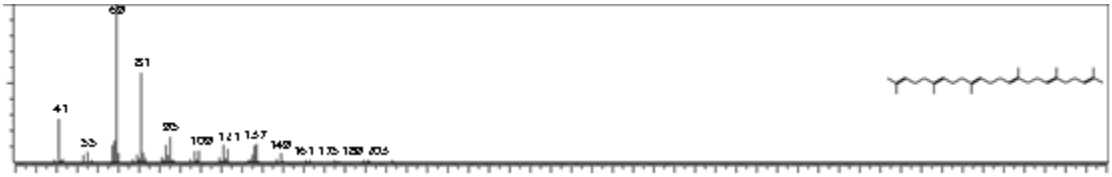

Figure 6 Mass chromatogram of Squalene

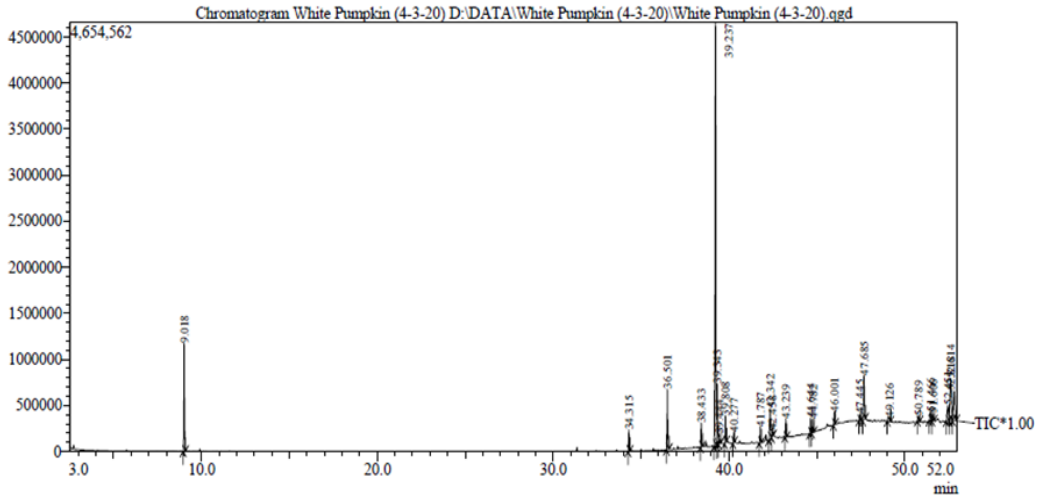

Figure 7 GC-MS chromatogram of white pumpkin seed

Table 2 GC-MS analysis of white pumpkin seed

S.No	RT	Name of the compound	MF	Peak Area (%)
1.	9.01	Propane, 1, 1, 3-triethoxy-	$C_9H_{20}O_3$	7.50
2.	34.31	Linoleic acid ethy1 ester	$C_{20}H_{36}O_2$	1.48
3.	36.50	Hexadecanoic acid, 1-(hydroxymethyl)-1,2)-ethanediyl ester	$C_{35}H_{68}O_5$	4.76
4.	38.43	9,12-Octadecadienoyl chloride,(Z,Z)-	$C_{18}H_{31}ClO$	1.83
5.	393	Bicyclo[10.1.0]tridec-1-ene	$C_{13}H_{22}$	4055
6.	39.34	9-Octadecenoic acid,1,2,3-propanetriyl ester(E,E,E)	$C_{57}H_{104}O_6$	5.65
7.	39.44	cis-9-Hexadecenal	$C_{16}H_{30}O$	1.11
8.	39.80	Triarachine	$C_{63}H_{122}O_6$	2.07
9.	40.27	Tetratriacontane	$C_{44}H_{90}$	0.75
10.	41.78	Eicosane	$C_{20}H_{42}$	1.33
11.	42.34	9,12-Octadecadienoic Acid (Z,Z)-, 2,3-Dihydroxypropyl Ester	$C_{21}H_{38}O_4$	4.00
12	42.45	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	0.81
13.	43.23	Octacosane	$C_{28}H_{58}$	1.41

14.	44.64	Pentadecane, 8-Hexyl-	C21H44	1.24
15.	44.78	Squalene	$C_{30}H_{50}$	1.13
16.	46.00	Hexatriacontane	$C_{36}H_{74}$	1.03
17.	47.44	Celidoniol, deoxy-	$C_{29}H_{60}$	0.63
18.	47.68	Gamma.-tocopherol	$C_{28}H_{48}O2$	4.36
19.	49.12	Sulfurous acid, octadecyl1 2-propyl ester	$C_{21}H_{44}O_3S$	0.79
20.	50.78	Ergost-5-en-3-ol, (3.beta.)-	$C_{28}H_{48}O$	0.70
21.	51.46	Stigmasterol	$C_{29}H_{48}O$	1.17
22.	51.60	Pregnane-3,11,17,20,21-pentol,cyclic 17,21 -(methylboronate), (3.alpha.,5.beta.,20R)-	$C_{22}H_{37}BO_5$	0.90
23.	52.45	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	2.42
24.	52.61	Chondrillasterol	$C_{29}H_{48}O$	7.30
25.	52.81	Beta.-Sitosterol	$C_{29}H_{50}O$	5.10

In the table 2, the RT is the Retention time, MF is the molecular formula, and Peak Area % is the concentration.
The table 2 reveals the compounds identified in the WPS ethanol extract. According to table 2, some of the compounds which were present in greater concentration among all the 25 compounds were Beta.-Sitosterol (5.10%), 9-Octadecenoic acid,1,2,3-propanetriyl ester (E,E,E) (5.65%), Chondrillasterol (7.30%), Propane, 1, 1, 3- triethoxy- (7.50%), Bicyclo[10.1.0]tridec-1-ene (40.55%). Figures 8, 9, 10, 11, 12 represents the mass chromatogram of the above mentioned greater proportion compounds found in WPS ethanol extract expressed in table 2.

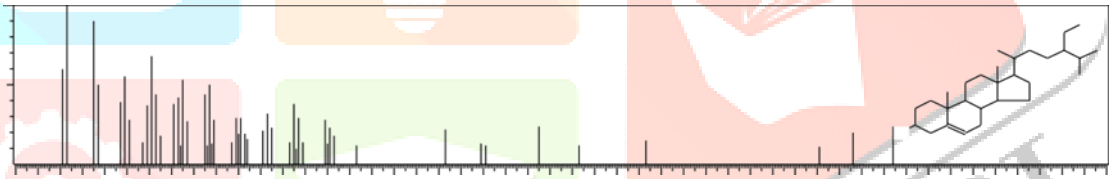

Figure 8 Mass chromatogram of Beta.-Sitosterol

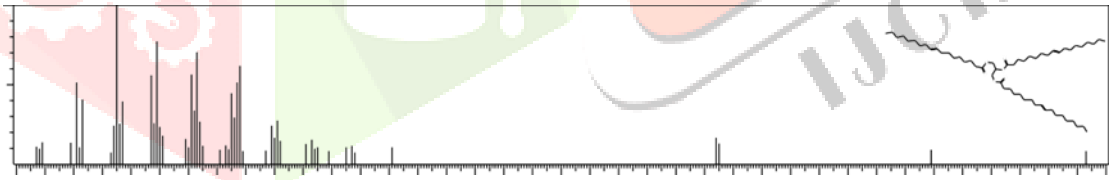

Figure 9 Mass chromatogram of 9-Octadecenoic acid,1,2,3-propanetriyl ester (E,E,E)

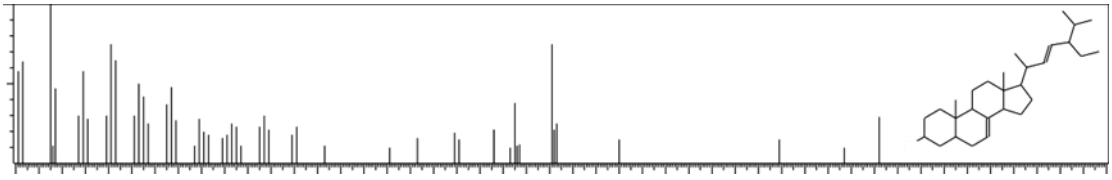

Figure 10 Mass chromatogram of Chondrillasterol

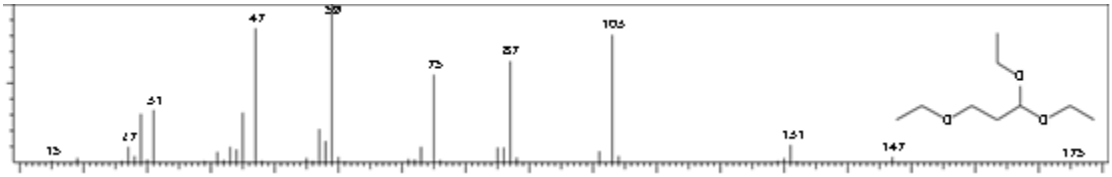

Figure 11 Mass chromatogram of Propane, 1, 1, 3- triethoxy

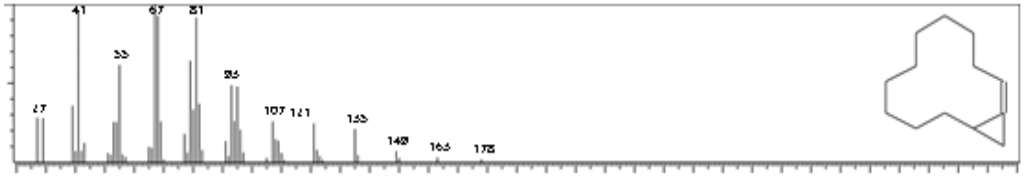

Figure 12 Mass chromatogram of Bicyclo[10.1.0]tridec-1-ene

As noted by Dar *et al*. (2017) important well known compounds such as Gamma-tocopherol with the common name vitamin E (0.72%), cis-vaccenic acid known as omega-7-fatty acid (3.055%), n- hexadecanoic acid known as palmitic acid (4.05%), Linoleic acid ethyl ester known as ethyl esters of omega-3-fatty acids and Ethyl oleate commonly called as ethyl oleic acid (2.58%) were found in the OPS ethanol extract analysis. Comparing the values of free fatty acid estimation of pumpkin seed oil by Devi *et al.* (2018) the values obtained in table 1 was less.

Some of the compounds such as beta-sitosterol, stigmasterol, gamma-tocopherol, linoleic acid ethyl ester, hexadecanoic acid and ethyl ester obtained in table 1 and 2 the values were less than the findings of Sudha *et al.* (2013) in GC-MS Analysis of Bioactive Components of Aerial parts of *Fluggea leucopyrus* Willd. (*Euphorbiaceae*) whereas beta-sitosterol value was similar.

From the tables 1 and 2, some of the compounds obtained commonly in both OPS and WPS ethanol extracts are included in table 3 with its activity.

Table 3 Activity of common compounds obtained in the GC-MS analysis of ethanol extracts of OPS and WPS

S. No	Name of the compounds	Nature of the compound	Activity
1	9-Octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E)	Esters of fatty acid	Anti-spasmodic and immune modulators [14]
2	9,12-Octadecadienoyl chloride, (Z,Z)-	Linolenic acid	Anti-inflammatory, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor [11]
3	Gamma-tocopherol	Vitamin E compound	Anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemia, cardio protective [14]
4	Hexadecanoic acid, 1-(hydroxymethyl)-1,2	Fatty acid	Cosmetic products, antioxidant [12]
5	Linoleic acid, ethyl ester	Linoleic acid ethyl ester	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge ,Antieczemic,Antiacne [9]
6	Propane, 1,1,3-triethoxy-	Ether compound	No Activity reported [13]
7	Squalene	Triterpene	Antiageing, Analgesic, Antidiabetic, Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary [9]
8	Stigmasterol	Steroid	Antimicrobial, Anticancer Antiarthritic, Antiasthma Diuretic, Anti-inflammatory [9]

In reference with the table 3, it may be suggested that the bioactivity of these pumpkin seeds may be due to the presence of good amounts of fatty acids and their activities as mentioned in table 3. Some of the commonly present activity among the compounds listed in table 3 were antioxidant, anticancer, antimicrobial, anti-inflammatory activity.

4. CONCLUSION

This study concludes the results of GC-MS analysis in which 19 compounds were obtained from the OPS ethanol extract and 25 compounds from the WPS ethanol extract. Thus, the presence of various bioactive compounds in the OPS and WPS justifies the future scope for the use of this seed samples for ailment purposes. However, more study must be conducted to understand the mechanism for the development of drugs. Therefore from the results chalked from this study, OPS and WPS can be recommended as a food supplement with phytopharmaceutical importance.

5. REFERENCE

1. Aamir Hussain Dar, SA Sofi, Shafiya Rafiq. Pumpkin the functional and therapeutic ingredient: A review. International Journal of Food Science and Nutrition. 2017; 2(6): 165-170.
2. N Manda Devi, R V Prasad, Gaibimei Palmei. Physico-chemical characterization of pumpkin seeds. International Journal of Chemical Studies. 2018; 6(5): 828-831.
3. Gitika Sharma and Sarla Lakhawat. Development, quality evaluation and acceptiblity of pumpkin seed flour incorporated in gravy: Food Science Research Journal. 2017; 8(2): 319-325.
4. S.Y. Al-Okbi, D.A. Mohamed, E. Kandil, M.A.Abo-Zeid, S.E. Mohammed and E.K. Ahmed. Anti-inflammatory activity of two varieties of pumpkin seed oil in an adjuvant arthritis model in rats. GRASAS Y ACEITES. 2017; 68(1): 1-12.
5. Danai Sakka and Haralabos C. Karantonis. *Invitro* health beneficial activities of Pumpkin seeds from *Cucurbita moschata* cultivated in Lemnos. International Journal of Food Studies. 2015; 4: 221-237.
6. Neel Ratnam, Vandana, Md Najibullah, Md Ibrahim. A Review on *Cucurbita pepo.* International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(9): 1190-1194.
7. Qamar Abbas Syed, Mafia Akram and Rizwan Shukat. Nutritional and Therapeutic Importance of the Pumpkin Seeds. Biomedical journal of scientific and technical research. 2019; 21(2): 15798-15803.
8. N Manda Devi, RV Prasad and Nukasani Sagarika. A review on health benefits and nutritional composition of pumpkin seeds. International Journal of Chemical Studies. 2018; 6(3): 1154-1157
9. Sudha T, Chidambarampillai S and Mohan V.R. GC-MS Analysis of Bioactive Components of Aerial parts of Fluggea *leucopyrus* Willd. *(Euphorbiaceae)*. Journal of Applied Pharmaceutical Science. 2013; 3(5): 126-130
10. B. Ezhilan and R. Neelamegam. GC-MS Determination of Bioactive Compounds of *Polygonum glabrumi.* Journal of Phytology. 2011; 3(9): 23-25
11. Babu A, Sheik Noor Mohamed M, Jaikumar K, Anand D, Saravanan P. In vitro Anti-Helicobacter pylori Activity and GC-MS Analysis of *Enicostemma axillare* (Lam). Raynal. International Journal of Pharmaceutical Sciences and Drug Research. 2017; 9(3): 100-104
12. Devi. R and P. Serfoji. Gcms Analysis Of Bioactive Compounds In Ethyl Acetate Extract Of Earthworm Gut *Streptomyces Fulvissimus* (Jenson, 1930). International Journal of Pharmacy and Biological Sciences. 2018; 8(4): 200-205
13. M. Alagammal, P. Tresina Soris and V.R. Mohan. GC-MS analysis of *Polygala rosmarinifolia* Wights & Arn. Journal of Applied Pharmaceutical Science, 2012; 2(4):188-190.
14. K Gokul and V Priya. Phytochemical and GC-MS analysis of *Ziziphus glabrata* Heyne ex Roth (*Rhamnaceae*). International Journal of Fauna and Biological Studies. 2019; 6(2): 42-51
15. M. Abdalla Elhassan, H. S. Ahmed Hoyam, H. Abdalrahman Samia, M. E. Khogali Salwa. GCMS Analysis of Pumpkin Seeds (*Cucurbita maxima, Cucurbitaceae*). Archives of Business Research. 2018; 6(8): 1-9.