Original Research Article

GC-MS analysis of the bioactive compounds in aqueous ethanol, dichloromethane, and n-hexane extracts of Pumpkin (*Cucubita pepo*) seed

Abstract

The study analysed the bioactive compounds (BACs) present in aqueous ethanol, dichloromethane, and n-hexane extracts of pumpkin *(Cucurbita pepo*)using Gas chromatography–mass spectrometry (GC-MS)technique. The dried grounded seed*C. pepo* were successivelyextracted using the three solvents and GC-MS analysis was performed to identify the differential quantitative BACs in the extracts of *C. pepo*seeds. The GC-MS results revealed the presence of 5, 22 and 17 for aqueous ethanol, dichloromethane, and n-hexane extracts respectively. Of the over 434 bioactive compounds present in *C. pepo*, palmitic, stearic, linoleic compounds and their derivatives were the bioactive compounds found in all three extracts. Scientific evidences suggest that palmitic, stearic, linoleic, barbituric acids, and their derivatives have significant biological effects. Compounds without documented scientific evidence such as Silane, dimethyl(2-methoxyethoxy)octadecylocy-, (22R)-6.alpha.,11.beta.,21 Trihydroxy-16.alpha.,17.alpha.-propylmethylenedioxypregna-1,4-diene-3,20-dione, and Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, .beta.-d- were found in n-hexane extract, while 2-hydroxy-1-(hydroxymethyl)ethyl ester E, Z-1,3,12-Nonadecatriene was found in dichloromethane extract. Results of this study may provide a foundation for the application of *C. pepo* in nonclinical setting and the designing of new drug for several clinical purposes.

Keywords: Extracts, pumpkin *(Cucurbita pepo)* seed, aqueous ethanol, dichloromethane, n-hexane, GC-MS analysis.

Introduction

Plants have countless bioactive compounds(Priya & Saravanan, 2017), which theirmetabolites have been used as therapeutic agents across the globe. According to WHO, traditional medicine remains the oldest and most widely consulted medical intervention in the world today because it dates back to the age of man(Petrovska, 2012; Tran et al., 2020). It is an inseparable part of health management and most often likened to African traditional medicine (Ahmed et al., 2018; Nsagha et al., 2020) because of its widespread use in most common cultures in the continent (Ahmed et al., 2018; Ezekwesili-Ofili & Okaka, 2019).

Cucurbita pepo(Image.1a & b) is an herbaceous plant that belongs to theCucurbitaceae family(Priya & Saravanan, 2017; Syed, 2019) commonly called 'marrow'(Jorjette, 2021). It is known as '*Ugbogulu*' in the Eastern Nigeria,'*Gamonfatake*' in Northern Nigeria, and 'Elegede' in Western Nigeria(Jorjette, 2021; Udomoh Eshemokha, 2020). The plant is known to be a good source of nutrients such as vitamin A and C(Priya & Saravanan, 2017). Several studies have provided insight into its use for health purposes such as urinary function and urodynamic effects (Damiano et al., 2016; European Medicines Agency, 2012; Nishimura et al., 2014; Perez Gutierrez, 2016), hyperplasia of the prostate gland(CARBIN et al., 1990; Damiano et al., 2016; Gažová et al., 2019; Nishimura et al., 2014; Perez Gutierrez, 2016), anti-inflammation and antioxidation (Almohaimeed et al., 2021; Bardaa, Ben Halima, et al., 2016; Bardaa, Moalla, et al., 2016; Nawirska-Olszańska et al., 2013), hypolipidemicand hepatoprotective activities(Abuelgassim & Al-showayman, 2012; Asgary et al., 2011; Makni et al., 2008), and antiparasitic effects (Beshay et al., 2019).

Image 1: The pumpkin fruit **(a)** unripe and **(b)** ripe

Specimens of the same plant species that are cultivated in different regions show significant differences in the presence of the primary and secondary metabolites(Gutbrodt et al., 2012; Pavarini et al., 2012; Sampaio et al., 2016). This is because of the chemical interaction with their environment that brings about the biosynthesis of secondary metabolites, resulting inadaptive response for survival (Gutbrodt et al., 2012; Miranda et al., 2015; Sampaio et al., 2016; Treutter, 2005). Studying the variation in the plant bioactive components is very usefulfor the scientific characterization and documentation of the plant species obtained from different regions (Sampaio et al., 2016). Therefore, it is imperative to evaluate the bioactive compounds present in this n-hexane, aqueous ethanol, and dichloromethane extracts of *C. pepo*seed obtained from South-South, Nigeria.

Materials and Methods

Collection of Plant material: The C. pepo (pumpkin) fruits were procured from Choba market (Latitude: 4°53'26"N and Longitude: 6°54'12"E in Obio/Akpor, Rivers State, and South-South, Nigeria. The fruit was identified and authenticated at the University of Port Harcourt herbarium with voucher specimen number UPH/PSB/2021/071.

Preparation and Extraction Process: The seeds were air dried on sacks in an aerated room for 2 weeks, then deshelled and ground into powder using a Vitamin E310 Explorian Blender. Fifty grams (50g) each of the powdered plant materials was soaked in 500ml of n-hexane, dichloromethane and aqueous ethanol in separate conical flasks and kept for 24hrs in a shaker, after which the mixture was filtered. The filtrate was evaporated at room temperature (Fatope et al., 1993).

GC-MS Analysis: The GC-MS analysis was carried out at Giolee Global Resources LTD, number 18 Uyo Street, Rumuomasi, Port Harcourt, Rivers State, Nigeria.The various solvent (aqueous ethanol, dichloromethane, and n-hexane) extracts of the biomass sample were analyzed using an Agilent 7890/5975 GC/MS. The equipment has the following features; a separation in Capillary column (HP-5), 30m x 0.25-0.32mm ID x 0.25µm film, and a 5% Phenyl Methyl Siloxane inner coasting. A 99.99% pure Helium gas was used as the carrier gas at flow rate of 2.54 ml per minute in split less mode. 1 microliter of the sample was injected to the column at 250^0C inlet Temperature. The oven temperature commenced at 50^0C holds for 2 minutes, and then ramped at 15^0C to 250^0C held for 10min, ramped again at 10^0C for 280^0C held for 25 minutes for 69minute run time. The auxiliary temperature was kept at 280^0C. The various mass spectrums detected were obtained by electron ionization at 70 eV and the detected operated in a scan mode 35 to 550Da atomic units. A 2 seconds scan interval and fragmentation were maintained at 35 to 550Da.

Identification of Compounds: Identification was based on the molecular structure, molecular mass, and calculated fragments elucidated by the MS Quadrupole mass analyser filtered on a mass to charge basis by the High energy diode (HE)(Miller & Denton, 1986). The compounds are qualitatively interpreted using the National Institute standard and Technology (NIST) spectrum database NIST 08 model. The name, molecular weight and structure of the components of the material were ascertained by use of the library(Epa et al., 2004).The spectrum of the unknown components was compared to the 2008 version, through which the various spectra extractions and interpretation were obtained. The relative percentage of amount of each component was estimated by comparing its average peak area to the total area(Epa et al., 2004; Wiley, 2016). To be assured of accuracy, the obtained compound data were compared to the NCBI pubchem data base (NIH U.S. National Library of Medicine, 2021), and in the event that the compound was yet to be properly identified, hyphen (-) was input, suggesting further investigation.

Results

The results from the GC-MS Analysis, showed various bioactive compound in the aqueous ethanol extract (Table 1), dichloromethane extract (Table 2), and n-hexane extract (Table 3) of *C. pepo* seed. This was evident from the peaks in the GC-MS chromatogram, which were identified according to their retention time (Figs. 1,2, & 3for aqueous ethanol, dichloromethane, and n-hexane extracts respectively).

The compounds identified in the aqueous ethanol seed extractswere n-Hexadecanoic acid (41.01%), Hexadecanoic acid, ethyl ester and Tetradecanoic acid, ethyl ester (9.7%), 9,12-Octadecadienoic acid (Z,Z) - (39.11), 9,12-Octadecadienoic acid, ethyl ester, Linoleic acid ethyl ester, and Ethyl 9.cis.,11.trans.-octadecadienoate (7.22%), Octadecenoic acid, ethyl ester and Heptadecanoic acid, 15-methyl-, ethyl ester (2.95).

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The compounds identified in the dichloromethane seed extracts were 2,4-Decadienal, (E,E)- and 4-Ethylcyclohexanol (0.07%); Tetradecanoic acid (0.40%);1-Hexadecanol, 2-methyl- , cis-11-Hexadecenal, and Cyclododecanol, 1-ethenyl- (0.11%);Hexadecanoic acid, methyl ester (Methyl-palmitate) and Pentadecanoic acid, 14-methyl-, methyl ester (2.3%);n-Hexadecanoic acid

(31.2%);9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl lineoleate), 11,14-Octadecadienoic acid, methyl ester, and 8,11-Octadecadienoic acid, methyl ester (3.56%); Octadecenoic acid, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester, and Octadecenoic acid, methyl ester (0.75%); 9,12-Octadecadienoic acid (Z,Z)- (46.58%); Octadecenoic acid (5.13%); Cyclopropaneoctanal, 2-octyl-, 11-Dodecen-1-ol trifluoroacetate (3.44%); Methyl 5,12-octadecadienoate (0.44%); (Z)6, (Z)9-Pentadecadien-1-ol (0.66%); 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (1.34%); Eicosanoic acid, Z,Z-10,12-Hexadecadien-1-ol acetate (0.90%); 9,17-Octadecadienal, (Z)- (0.27%); Quinoline, 1,2,3,4-tetrahydro-1-((2-phenylcyclopropyl) sulfonyl)-, trans-, Indan, 1-methyl-, and Silane, (1,2-dimethylpropoxy)trimethyl- (0.40%), Silane, dimethyl(2-methoxyethoxy)octadecyloxy-, Isophthalic acid, propyl undec-2-en-1-yl ester, and 1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-(0.28%); 4-(3,4,5,6-Tetrahydroxy-2-oxo-hexylamino)-benzonitrile, Cyclopropane, 2-bromo-1-methyl-1-phenyl-, and 5.beta.-Cholestane-3.alpha.,7.alpha.,12.alpha.,24.alpha.,25-pentol TMS (0.85%); 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Benzene, (2-methylcyclopropyl)-, and Benzene, 1-ethenyl-4-ethyl- (0.28%), 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester, and 2-hydroxy-1-(hydroxymethyl)ethyl ester E, Z-1,3,12-Nonadecatriene (0.30%); Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl)-, 5,6-Dihydroergosterol, and Benz[e]azulene-3,8-dione, 5-[(acetyloxy)methyl]-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-2,10-dimethyl-,3a.alpha.,6a.alpha.,10.beta.,10a.beta.,10b.beta.)-(+)- (0.10%), 3'-Chlorooxanilic acid N'-(3-ethoxy-4-hydroxybenzylidene)hydrazide, and 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, and Silane, 1,4-phenylenebis[trimethyl] (0.08%).

The compounds identified in the n-hexane seed extracts were 2-Ethoxyethoxy-trimethylsilane 1-Butyl(dimethyl)silyloxypropane, and Silane, ethoxytrimethyl- (1.04%), Hexadecanoic acid, methyl ester (7.44%), n-Hexadecanoic acid (1.31%; 2.06%; and 1.50%); 9,12-Octadecadienoic acid (Z,Z) – and 9,17-Octadecadienal, (Z)- (14.5%; 11.3%), 9,12-Octadecadienoic acid (Z,Z) – (11.3%; 1.8%;), trans-13-Octadecenoic acid (1.80%), 1H-Indole-3-ethanamine, trans-N-tert-Butoxycarbonyl-4-hydroxy-l-proline, and 2,3-Difluorophenol (5.35%); Hexane, 2,5-bis[(trimethylsilyl)oxy]-, 3-Dimethylsilyloxy-6-ethyloctane, and Benzoic acid, 2-amino-, 3-phenyl-2 (3.62%); Silane, dimethyl(2-

methoxyethoxy)octadecylocy-, (22R)-6.alpha.,11.beta.,21-Trihydroxy-16.alpha.,17.alpha.-propylmethylenedioxypregna-1,4-diene-3,20-dione, and Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, .beta.-d- (2.70%); Cyclopropane, 2-bromo-1-methyl-1-phenyl-, 4-(3,4,5,6-Tetrahydroxy-2-oxo-hexylamino)-benzonitrile, and 7-Oxo-1,3,5-cycloheptatriene-1-carbonitrile (13.76%); 1H-Indole, 1-methyl-, .alpha.-Ethyltryptamine (8.30%); 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1- (hydroxymethyl)ethyl ester (2-Linoleoylglycerol), Methyl 9,12-heptadecadienoate, and Z,E-7,11-Hexadecadien-1-yl acetate (5.06%); Quinolin-5(6H)-one, 7,8-dihydro-2-hydroxy-4,7,7-trimethyl-, and Benzeneacetonitrile, 3,4-diethoxy- (5.42%); 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (14.06%); Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-, Quinazolin-4(1H)-one, 2,3-dihydro-3-(4-chlorophenyl)-2-ethyl-2 methyl-, and Chondrillasterol (1.14%).

Discussion

In this scientific report, some common bioactive compounds of significant therapeutic importance are highlighted.In this scientific report, some common bioactive compounds of significant therapeutic importance are highlighted. All three extracts had significant amount of Palmitic acid (n-Hexadecanoic acid, the -ethyl and -methyl esters with scientific evidences that show there potentiates inflammatory response through inhibition of the cyclooxygenase II and phospholipase A2 enzymes (Aparna et al., 2012a, 2012b; Hema. et al., 2011; J.-Y. Lee et al., 2010), as well as potentiates inflammatory response and apoptotic mechanisms through inflammasome-mediated secretion of IL-1β (Korbecki & Bajdak-Rusinek, 2019; Shirasuna et al., 2016) and TNF-α respectively (Belosludtsev et al., 2006). The antioxidant, hypocholesterolaemic, lubricant, and antiandrogeniceffects have also been reported (USDA, 2001). Studies also found that that palmitic acid (PA) blocks the entry of the virus through specific inhibition of HIV-1 fusion (D. Y. W. Lee et al., 2009; Lin et al., 2011).

Octadecenoic acid

Linoleic acid (9,12-Octadecadienoic acid) and associated compounds (the conjugated forms, cis-, and ester. Kiralan (2021) described the isomerization and biohydrogenation of linoleic acid to formation of cis,trans-9,11-octadecadienoic acid and (or) trans,cis-10,12-octadecadienoic acid, trans-11-

octadecaenoic acid and (or) trans-10-octadecaenoic acid, and lastly monoenoic acids to the saturated stearic acid (Octadecenoic acid)(Kiralan et al., 2021).α-Linoleic acid precursors that are members of ω-3 fats: eicosanoidseicosapentaenoic acid, and docosahexaenoic acid. The 3 series prostaglandins, 5 series leukotrienes, and thromboxane A3 from eicosapentaenoic acid exhibit anti-inflammatory, vasodilatory, and platelet anti-aggregatory abilities capable of controlling pulmonary and cardiac diseases(Adili et al., 2018; Goc et al., 2021).Docosahexaenoic acid producesresolvins, protectins, and maresins, which suppress inflammation and augment phagocytosis that lessens microbial loads; a very important activity in reducing the complications in COVID-19 and might be a preventive measure (Baral et al., 2021).

As against the understanding that saturated fatty acids in general, and palmitic acid (C16:0) in particular elevates LDL cholesterol and atherosclerosis risk (DiNicolantonio et al., 2016; Siri-Tarino et al., 2010a, 2010b). Dietary stearic acid (C18:0), on the other hand, is not associated with atherosclerosis risk, but with the reduction of LDL cholesterol by increasing endogenous cholesterol excretion, reducing reabsorption of cholesterol, without altering the bile concentration (Bonanome & Grundy, 1988; Imaizumi et al., 1993; Massel et al., 1997; Schneider et al., 2000).

Barbituric acid and its derivatives are well known for theiranticancer, antibacterial, and anti-sclerotic, anticonvulsant, antispasmodic, hypotensive hypnotic and sedative. There are recognised matrix metalloproteinase inhibitors, anticonvulsants, and anti-inflammatory and anxiolytic agents, as well as being used in local anaesthesia (Algar, 2010; Barakat et al., 2016; Singh et al., 2009; Wilbraham, 2008).

Conclusion

Of the over 434 activities present in C. *pepo*, palmitic, stearic, linoleic compounds and their derivatives were bioactive compounds found in all three extracts. Scientific evidences suggest that palmitic, stearic, linoleic, barbituric acids, and their derivatives have significant biological effects. Compounds without documented scientific evidence such as Silane, dimethyl(2-methoxyethoxy)octadecylocy-, (22R)-6.alpha.,11.beta.,21 Trihydroxy-16.alpha.,17.alpha.-

propylmethylenedioxypregna-1,4-diene-3,20-dione, and Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-, .beta.-d- were found in n-hexane, while 2-hydroxy-1-(hydroxymethyl)ethyl ester E, Z-1,3,12-Nonadecatriene was found in dichloromethane extracts. Results of this study may provide a foundation for the application *of C. pepo* in nonclinical setting and the designing new drug for several clinical purposes.

Table 1: Chemical composition of aqueous ethanolextracts of C. pepo seed.

Extracts	S/N	Compound(s)	Molecular weight	Formulae	Retention Time (min)	Peak Area (%)
Aqueous ethanol	1	n-Hexadecanoic acid (Palmitic acid)	256.4	$C_{16}H_{32}O_2$	13.18	41.01
	2	Hexadecanoic acid, ethyl ester (Ethyl-palmitate) Tetradecanoic acid, ethyl ester (Ethyl-Myristate)	284.5	$C_{18}H_{36}O_2$	13.351	9.7
	3	9,12-Octadecadienoic acid (Z,Z) - (Cis-Linoleic acid)	280.4	$C_{18}H_{32}O_2$	14.327	39.11
	4	9,12-Octadecadienoic acid, ethyl ester (Linolelaidic acid ethyl ester) Ethyl 9.cis.,11.trans.-octadecadienoate (Cla 9C,11TR free fatty acid)	308.5	$C_{20}H_{36}O_2$	14.457	7.22
	5	Octadecenoic acid, ethyl ester (Stearic acid) Heptadecanoic acid, 15-methyl-, ethyl ester (Margaric acid methyl ester)	312.5	$C_{20}H_{40}O_2$	14.645	2.95

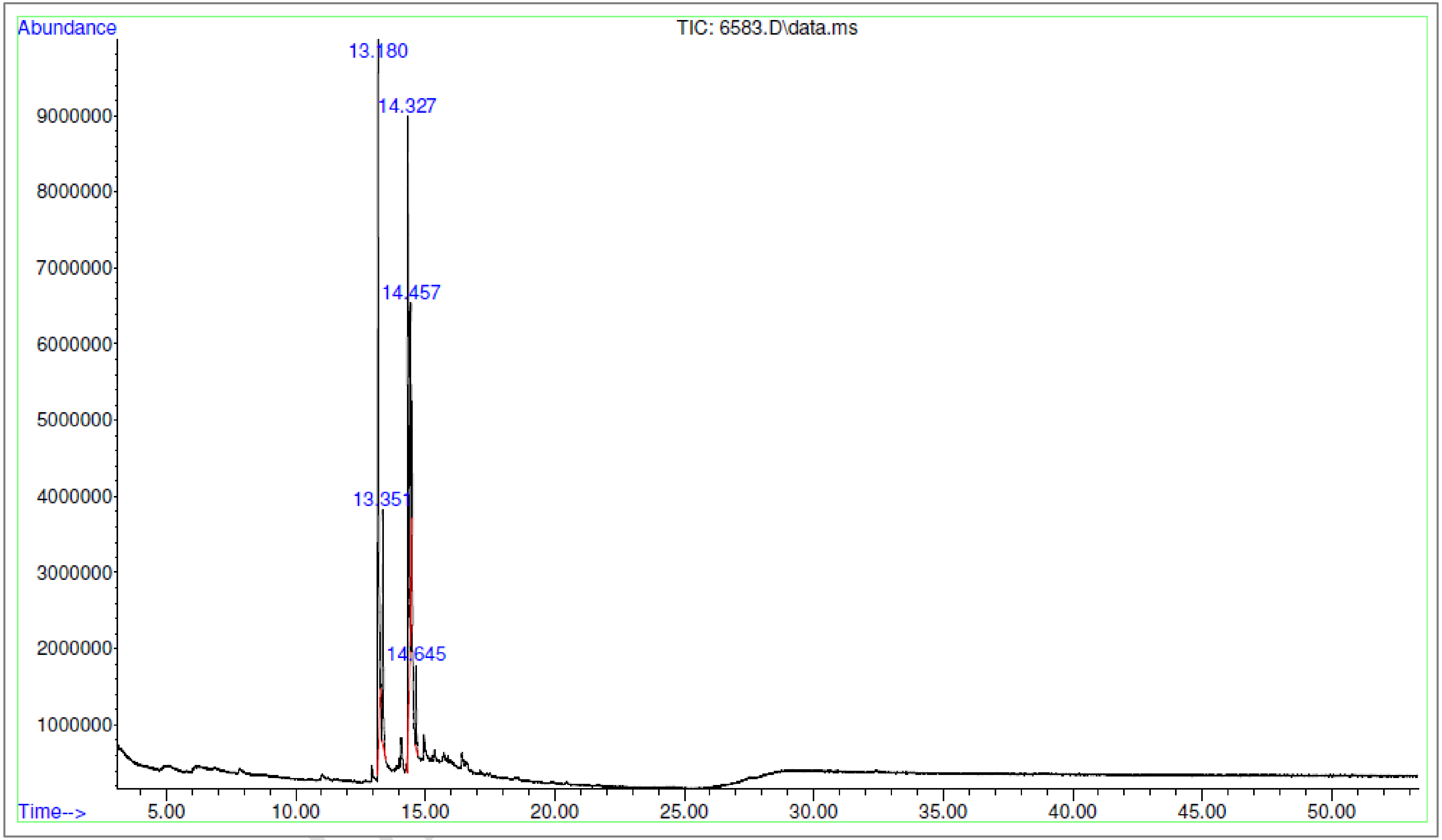

Figure 1:GC-MS chromatogram of aqueous ethanol extract of C. pepo seeds

Table 2: Chemical composition of aqueous dichloromethaneextracts of C. pepo seed.

Extracts	S/N	Compound(s)	Molecular weight (g/mol)	Formulae	Retention Time (min)	Peak Area (%)
Dichloromethane	1	2,4-Decadienal, (E,E)- 2,4-Decadienal 4-Ethylcyclohexanol	152.2 128.21	$C_{10}H_{16}O$ $C_8H_{16}O$	8.075	0.07
	2	Tetradecanoic acid (Mysteric acid)	228.37	$C_{14}H_{28}O_2$	11.769	0.40
	3	1-Hexadecanol, 2-methyl- cis-11-Hexadecenal Cyclododecanol, 1-ethenyl-	256.5 238.41 210.36	$C_{17}H_{36}O$ $C_{16}H_{30}O$ $C_{14}H_{26}O$	12.575	0.11
	4	Hexadecanoic acid, methyl ester (Methyl-palmitate) Pentadecanoic acid, 14-methyl-, methyl ester	270.5	$C_{17}H_{34}O_2$	12.875	2.03
	5	n-Hexadecanoic acid (Palmitic acid)	256.4	$C_{16}H_{32}O_2$	13.48	31.22
	6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Methyl lineoleate) 11,14-Octadecadienoic acid, methyl ester 8,11-Octadecadienoic acid, methyl ester	294.5	$C_{19}H_{34}O_2$	14.045	3.56
	7	Octadecenoic acid, methyl ester (Methyl stearate) Heptadecanoic acid, 16-methyl-, methyl ester Octadecenoic acid, methyl ester	298.5	$C_{19}H_{38}O_2$	14.227	0.75
	8	9,12-Octadecadienoic acid (Z,Z)- (Cis-Linoleic acid)	280.4	$C_{18}H_{32}O_2$	14.633	46.58
	9	Octadecenoic acid (Stearic acid)	284.5	$C_{18}H_{36}O_2$	14.874	5.13
	10	Cyclopropaneoctanal, 2-octyl- 11-Dodecen-1-ol trifluoroacetate	280.5 280.33	$C_{19}H_{36}O$ $C_{14}H_{23}F_3O_2$	14.992	3.44
	11	Methyl 5,12-octadecadienoate	294.5	$C_{19}H_{34}O_2$	15.404	0.44
	12	(Z)6, (Z)9-Pentadecadien-1-ol	224.38	$C_{15}H_{28}O$	15.492	0.66
	13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (alpha-Linolenic acid)	278.4	$C_{18}H_{30}O_2$	15.627	1.34
	14	Eicosanoic acid (Arachidic acid) Z,Z-10,12-Hexadecadien-1-ol acetate	312.5 280.4	$C_{20}H_{40}O_2$ $C_{18}H_{32}O_2$	15.821	0.90
	15	9,17-Octadecadienal, (Z)- (Linolenic acid)	264.4	$C_{18}H_{32}O$	16.186	0.27

16	Quinoline, 1,2,3,4-tetrahydro-1-((2-phenylcyclopropyl) sulfonyl)-, trans-	313.4	$C_{18}H_{19}NO_2S$	16.692	0.40
	Indan, 1-methyl-	132.2	$C_{10}H_{12}$		
17	Silane, (1,2-dimethylpropoxy)trimethyl-	160.33	$C_8H_{20}OSi$	17.715	0.28
	Silane, dimethyl(2-methoxyethoxy)octadecyloxy-	402.7	$C_{23}H_{50}O_3Si$		
	Isophthalic acid, propyl undec-2-en-1-yl ester	360.5	$C_{22}H_{32}O_4$		
	1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	206.32	$C_{14}H_{22}O$		
18	4-(3,4,5,6-Tetrahydroxy-2-oxo-hexylamino)-benzonitrile	280.28	$C_{13}H_{16}N_2O_5$	18.151	0.85
	Cyclopropane, 2-bromo-1-methyl-1-phenyl-	211.1	$C_{10}H_{11}Br$		
	5.beta.-Cholestane-3.alpha.,7.alpha.,12.alpha.,24.alpha.,25-pentol TMS	813.6	$C_{42}H_{88}O_5Si_5$		
19	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (2-Linoleoylglycerol)	354.5	$C_{21}H_{38}O_4$	18.374	0.28
	Benzene, (2-methylcyclopropyl)- Benzene, 1-ethenyl-4-ethyl-	132.2	$C_{10}H_{12}$		
20	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	354.5	$C_{21}H_{38}O_4$	19.845	0.3
	2-hydroxy-1-(hydroxymethyl)ethyl ester E, Z-1,3,12-Nonadecatriene	-	-		
21	Barbituric acid, 5-allyl-5-(cyclohex-2-en-1-yl)-	248.28	$C_{13}H_{16}N_2O_3$	29.985	0.1
	5,6-Dihydroergosterol	398.7	$C_{28}H_{46}O$		
	Benz[e]azulene-3,8-dione, 5-[(acetyloxy)methyl]-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-2,10-dimethyl-,3a.alpha.,6a.alpha.,10.beta.,10a.beta.,10b.beta.)-(+)-	348.4	$C_{19}H_{24}O_6$		
22	3'-Chlorooxanilic acid N'-(3-ethoxy-4-hydroxybenzylidene)hydrazide	361.8	$C_{17}H_{16}ClN_3O_4$	30.768	0.08
	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	282.29	$C_{17}H_{14}O_4$		
	Silane, 1,4-phenylenebis[trimethyl	222.47	$C_{12}H_{22}Si_2$		

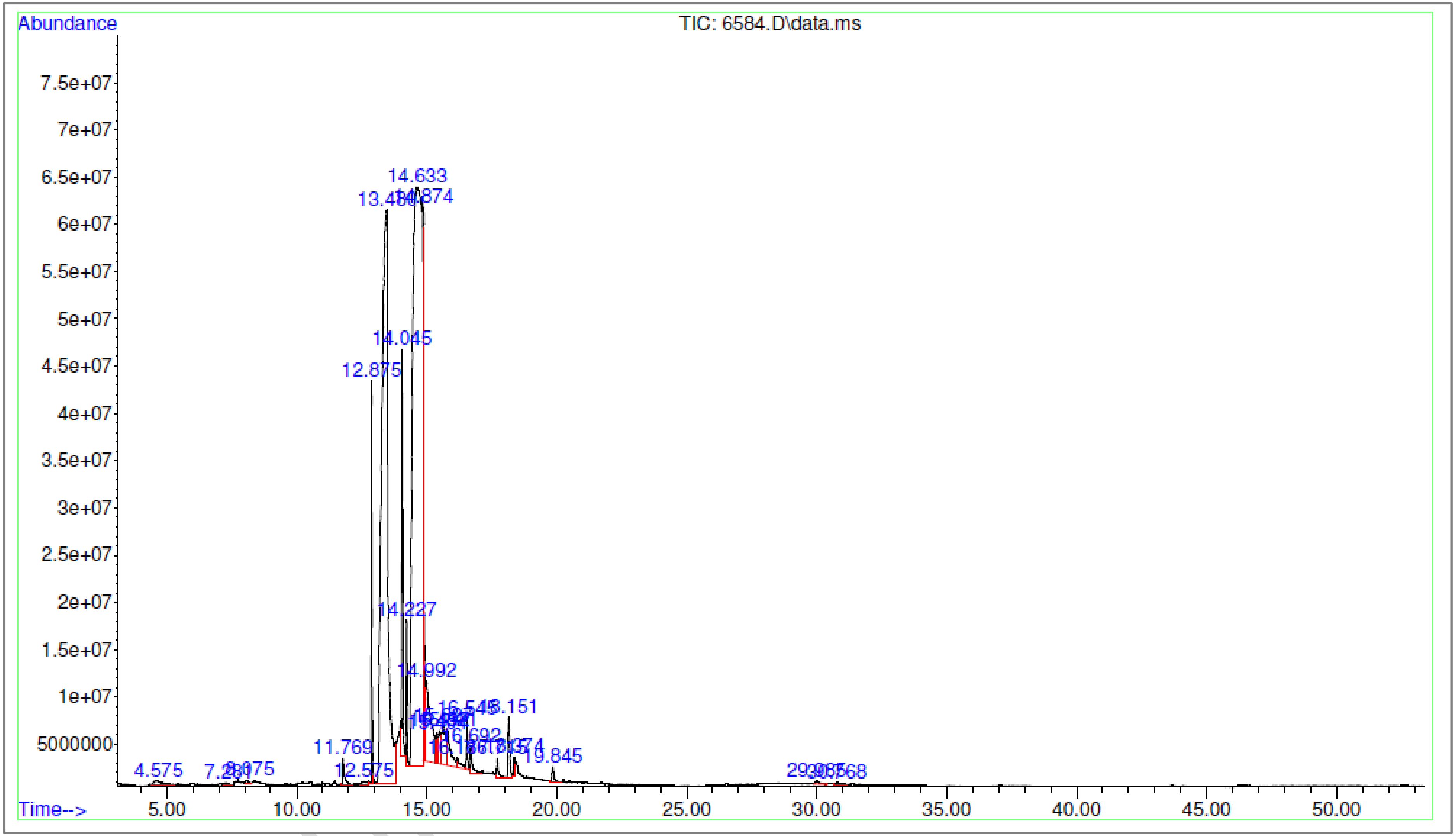

Figure 2:GC-MS chromatogram of dichloromethane extract of C. pepo seeds

Table 3: Chemical composition of aqueous n-hexane extracts of C. pepo seed.

Extracts	S/N	Compound(s)	Molecular weight (g/mol)	Formulae	Retention Time (min)	Peak Area (%)
n-hexane	1	2-Ethoxyethoxy-trimethylsilane	162.30	$C_7H_{18}O_2Si$	4.240	1.04
		1-Butyl(dimethyl)silyloxypropane	174.36	$C_9H_{22}OSi$		
		Silane, ethoxytrimethyl-	118.25	$C_5H_{14}OSi$		
	2	Hexadecanoic acid, methyl ester (Methyl-palmitate)	270.50	$C_{17}H_{34}O_2$	12.886	7.44
		Pentadecanoic acid, 14-methyl-, methyl ester				
		Hexadecanoic acid, methyl ester				
	3				13.374	1.31
	4	n-Hexadecanoic acid (Palmitic acid)	256.40	$C_{16}H_{32}O_2$	13.527	2.06
	5				13.757	1.50
	6	9,12-Octadecadienoic acid (Z,Z) - (Cis-Linoleic acid)	280.40	$C_{18}H_{32}O_2$	14.598	14.15
		9,17-Octadecadienal, (Z)-	264.40	$C_{18}H_{32}O$		
	7	9,12-Octadecadienoic acid (Z,Z) - (Cis-Linoleic acid)	280.40	$C_{18}H32O_2$	14.704	11.30
	8		280.40	$C_{18}H_{32}O_2$	15.239	1.8
		trans-13-Octadecenoic acid	282.50	$C_{18}H_{34}O_2$		
	9	1H-Indole-3-ethanamine	160.22	$C_{10}H_{12}N_2$	16.662	5.35
		trans-N-tert-Butoxycarbonyl-4-hydroxy-l-proline	231.25	$C_{10}H_{17}NO_5$		
		2,3-Difluorophenol	130.09	$C_6H_4F_2O$		
	10	Hexane, 2,5-bis[(trimethylsilyl)oxy]-	262.54	$C_{12}H_{30}O_2Si_2$	16.815	3.62
		3-Dimethylsilyloxy-6-ethyloctane	215.43	$C_{12}H_{27}OSi$		
		Benzoic acid, 2-amino-, 3-phenyl-2	253.29	$C_{16}H_{15}NO_2$		
	11	Silane, dimethyl(2-methoxyethoxy)octadecylocy-	-	-	17.809	2.7
		(22R)-6.alpha.,11.beta.,21-Trihydroxy-16.alpha.,17.alpha.-propylmethylenedioxypregna-1,4-diene-3,20-dione	-	-		
		Galactopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-,.beta.-d-	-	-		
	12	Cyclopropane, 2-bromo-1-methyl-1-phenyl-	211.10	$C_{10}H_{11}Br$	18.256	13.76
		4-(3,4,5,6-Tetrahydroxy-2-oxo-hexylamino)-benzonitrile	280.28	$C_{13}H_{16}N_2O_5$		
		7-Oxo-1,3,5-cycloheptatriene-1-carbonitrile	131.13	C_8H_5NO		
	13	1H-Indole, 1-methyl-	131.17	C_9H_9N	18.527	8.3

	.alpha.-Ethyltryptamine	188.27	$C_{12}H_{16}N_2$		
14	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1- (hydroxymethyl)ethyl ester (2-Linoleoylglycerol)	354.50	$C_{21}H_{38}O_4$	18.874	5.06
	Methyl 9,12-heptadecadienoate	280.40	$C_{18}H_{32}O_2$		
	Z,E-7,11-Hexadecadien-1-yl acetate	280.40	$C_{18}H_{32}O_2$		
15	Quinolin-5(6H)-one, 7,8-dihydro-2-hydroxy-4,7,7-trimethyl-	205.25	$C_{12}H_{15}NO_2$	19.939	5.42
	Benzeneacetonitrile, 3,4-diethoxy-	205.25	$C_{12}H_{15}NO_2$		
16	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (Squalene)	410.70	$C_{30}H_{50}$	20.597	14.06
	Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-	412.7	$C_{29}H_{48}O$		
17	Quinazolin-4(1H)-one, 2,3-dihydro-3-(4-chlorophenyl)-2-ethyl-2 methyl-	300.8	$C_{17}H_{17}ClN_2O$	29.991	1.14
	Chondrillasterol	412.7	$C_{29}H_{48}O$		

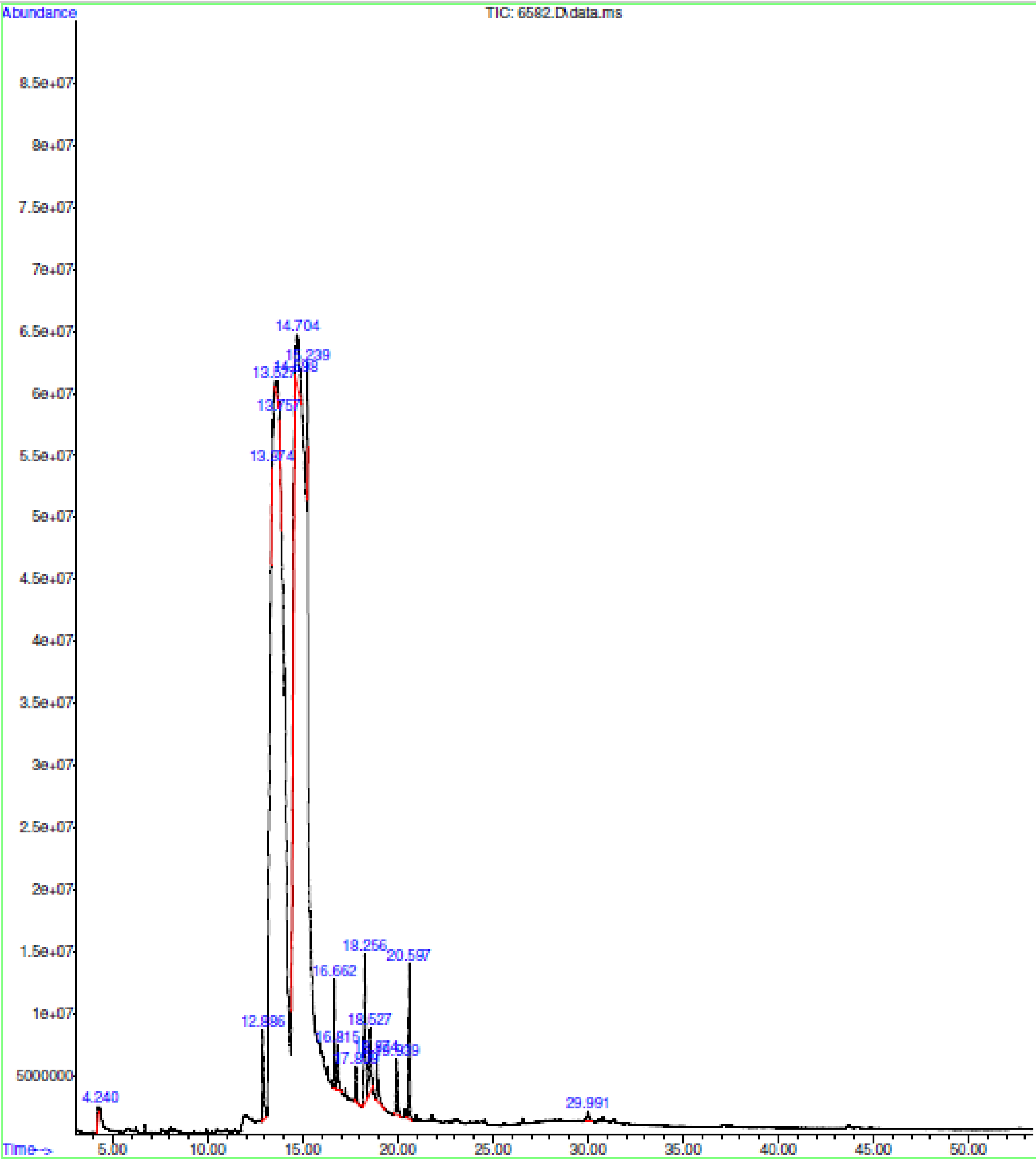

Figure 3:GC-MS chromatogram of n-hexane extract of C. pepo seeds

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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