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GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS IN ETHANOL LEAVES EXTRACT OF *SPHENOCENTRUM JOLLYANUM* AND THEIR BIOLOGICAL ACTIVITIES.

Emmanuel Uka[1], Queensley A. Eghianrunwa [2], Violet D.Akwo [3]

[1] *Emmanuel Uka Department of Biochemistry, Faculty of Science, University of Uyo*
[2] *Queensley A. Eghianrunwa Department of Biochemistry, Faculty of Science, University of Uyo*
[3] *Violet D.Akwo Department of Biochemistry, Faculty of Science, University of Uyo*

ABSTRACT

Sphenocentrum jollyanum is a plant genus of the family *Menispermaceae*. It has high medicinal importance as it is used traditionally to treat various diseases such as jaundice, breast engorgement related to the menstrual cycle, tumour, fibroids and improve the health of people. The present investigation was carried out to analyze the bioactive compounds present in ethanol crude extract of *Sphenocentrum jollyanum* leaves using GC-MS analysis. GC-MS analysis of ethanol extract *Sphenocentrum jollyanum* was done using a 7890A GC system (Agilent Technologies), coupled with 5977B MSD (Agilent Technologies) while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. A total of 45 bioactive compounds representing 99.98% of the total extract based on the retention time, peak area, molecular formula, molecular weight, and biological activities were identified by GC-MS which ranges from high molecular weight to low molecular weight compounds. The major compounds identified with their peak area percentages were 2,4-Di-tertbutylphenol, (21.05%), Z-8-Methyl-9-tetradecenoic acid (19.12), Hexadecanoic acid, ethyl ester (7.86%), Diisooctyl phthalate (7.13%), Phytol, Oleic Acid (7.03), 6,9,12-Octadecatrien-1-ol (6.65%), 3-Eicosene, (E)-(4.63%), 2-Methyl-Z, Z-3,13-octadecadienol (4.24%), n-Hexadecanoic acid (4.09%), trans-13-Octadecenoic acid (3.81%), Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl- (3.74%), Dibutyl phthalate (3.20%), and 9-Oxabicyclo (6.1.0) nonane, cis-(3.18%). The presence of these major phytoconstituents in the leaf extract provides various biological activities including antifungal, antibacterial, antioxidant, anti-inflammatory, and anti-tumour which supports the ethno-medicinal uses of the plant in curing diseases. We recommend further studies be carried out on the isolation, biosynthesis and characterization of the compounds.

Key words: GC-MS analysis, Bioactive compounds, *Sphenocentrum jollyanum*, Ethanol leaf extract, biological activity.

1. INTRODUCTION

Mankind since ancient times has depended upon the plant kingdom to meet all their medicinal needs. This prompted early men to explore their immediate natural surrounding and try many plants, animal products, mineral and develop a variety of therapeutic agents (Biren and Seth, 2017).

It is now advocated by the World Health Organization that universal health coverage and the integration of safe and effective traditional providers and complementary services into self-care practices and health service delivery, be focused on herbal medicine (Ipsos, 2008). Kalyany 2019 reported that over 4 billion people of the

world population presently use herbal medicine for primary health care. These medicinal plants are the most important source of organic compounds and one of such plants is *Sphenocentrum jollyanum*.

Sphenocentrum jollyanum, a perennial plant native to the tropical forest zone of West Africa, belongs to the family Menispermaceae. (Nia et al., 2004). It is widely distributed in Sierra Leone, Nigeria, Ghana, Ivory Coast, and Cameroun (Nia, 2004). *Sphenocentrum jollyanum* is a small erect sparsely branched shrub which grows up to 1.5m in height. It is locally known as "Aduro kokoo" (red medicine), "Okramankote" (dog's penis), Oban abe, and Ouse-abe among the people of Ghana, Republic of Benin, and Côte d'Ivoire respectively (Amidu, 2008). It is known locally in Nigeria as Oji-enyi, Ajo or Akerejupon and Ibong Isong among the Igbos, Yorubas and the Ibibios respectively (Nia et al., 2004).

Sphenocentrum jollyanum is an important medicinal plant that contains high level phytochemicals. These include flavonoids, saponins, terpenoids, isoquinoline alkaloids such as palmatine, columbamine and some other alkaloids. The most abundant of the phytochemicals are the alkaloids (Woode *et al*., 2009). Most of the biologic/ therapeutic effects of the plant are attributed to the chemical constituent. Many scientific research has reported the effects of the plant in respects to its sexual stimulant roles (Owiredu *et al.*, 2007), hepatoprotective effects (Olorunnisola *et al.*, 2011), wound healing property, antiviral and anti-inflammatory activities (Moody *et al.*, 2006), antidiabetic (Mbaka *et al*., 2011), antioxidant and analgesic properties (Nia et al., 2004), treating jaundice, breast engorgement related to the menstrual cycle, and tumour (Iwu, 1993). The charred fruit is used in treatment of fibroids in traditional Nigerian medicine (Egunyomi *et al.*, 2005) while the root hair is used with other anti-malaria plant as remedies against fevers, body pains and rheumatism (Burkill, 1985).

Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and identify compounds present in a plant sample (Uma and Balasubramaniam, 2012). It plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants containing biologically active components (Héthelyi *et al*., 1987). Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. However, fewer reports are available for the pharmacological properties of *Sphenocentrum jollyanum*. Keeping this in view, the present study has been undertaken to determine the bioactive compounds in crude ethanol leaf extract of *Sphenocentrum jollyanum.*

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

All the chemicals and reagents used for the research were of analytical grade

2.2 Collection, Identification and Authentication of Plant materials

Fresh leaves of *Sphenocentrum jollyanum* were harvested from Aka in Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria.

Fig: 1. Leaves of *Sphenocentrum jollyanum*

The Leaves were identified and authenticated by Mr. Etefia of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria. The plant was deposited at the herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Nigeria with the voucher No: NUUH: 040 (Uka *et al*., 2021a).

2.3 Preparation of Extract

Fresh leaves of *Sphenocentrum jollyanum* were thoroughly washed under running tap water to remove dust particles, air-dried for two weeks at ambient room temperature to obtain a fixed weight. The dried leaves were pulverized into a coarse powder using VTCL Solitaire mixer grinder (VTCL, India). Powdered leaf sample (400 g) was extracted in 60 % ethanol for 72 hours with intermittent stirring and filtered using Whatman No 1 filter paper. The filtrate obtained was concentrated in a water bath at 40 °C to completely remove the solvent (Uka *et al*., 2021b). The crude extract was subjected to GC-MS analysis.

2.4 GC-MS analysis of ethanol extract of *Sphenocentrum jollyanum* leaves

GC-MS technique was used in this study to identify the various compounds present in the ethanol leaf extract of *Sphenocentrum jollyanum*. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis was carried out at the Multi-User Science Research Laboratory, Ahmadu Bello University (ABU), Zaria, Nigeria. GC-MS analysis of this extract was performed using a 7890A GC system (Agilent Technologies), coupled with a Mass Selective Detector (MSD) 5977B (Agilent Technologies) driven by Agilent Chem-Station software package. The GC was equipped with an Agilent HP-5MS non-polar capillary column of 30 M length, 0.25 mm internal diameter and 0.25 µm film thickness as the stationary phase. The analysis was carried out using helium gas as the carrier gas as well as an eluent with a flow rate of 1.0 mL/minute in split less mode. About 1.0 µL of the ethanol extract of *Sphenocentrum jollyanum* (dissolved in methanol) was injected into the GC-MS using a micro syringe with the oven temperature programmed at 40 °C and then raised to 250 °C at the rate of 5 °C / min with a hold time of 3 min. The injector was held at 300 °C and the mass spectra were obtained by electron ionization voltage (EI) at 70 eV with a scan range of 40 to 650 mass-to-charge (m/z) ratio. The constituent compounds of the *Sphenocentrum jollyanum* were identified by direct comparison with that of the NIST database based on their retention time (RT), Molecular formula (MF), Molecular weight (MW), and mass spectral data with already known compounds in the National Institute of Standards and Technology 14 library (2018) database. This study was carried out to determine the classes of chemical compounds present in *Sphenocentrum jollyanum* expressed as a percentage based on peak area produced in the chromatogram.

2.5 Identification of compounds

GC-MS Chromatogram of *Sphenocentrum jollyanum* revealed fifteen peaks showing that forty-five different phytochemicals were present. Identification of phytochemical compounds and interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique 2014 (NIST-2014) having more than 243,000 patterns. The retention indices, peak area percentage and spectrum of the unknown components was compared with the spectrum of the known components of the NIST library and also with published literature. The name, molecular weight, molecular formula, structure and bioactivities of the phytochemicals of the components of the test materials were ascertained by the mass hunter software.

RESULTS

GCMS is one of the most precise techniques to separate and identify various secondary metabolites present in plant extract such as fatty acid esters, phenylpropane, aliphatic, fatty alcohol, aliphatic alcohol etc. The ethanol leaf extract of *Sphenocentrum jollyanum* was analyzed by GCMS to detect various compounds with the help of NIST database. The results of the GC-MS chromatogram showed 15 peaks with 45 chemical compounds corresponding to 99.98% of the entire extract (Table 1). The different bioactive compounds with the retention time and peak range between 5.255 to 22.685 and 21.05 to 1.47% respectively. The identified compounds were based on their peak, retention time, peak area (%), compound name, chemical structure, molecular formula, molecular weight and bioactivity confirmed by comparing the mass spectra obtained with literature mass spectra where available. The detailed phytocomponents and their biological activities obtained from GC-MS analysis of *Sphenocentrum jollyanum* are summarized in Tables 2, 3. Figures 2,3,4,5,6,7,8,9,10,11 and 12 showed the GCMS chromatogram and the spectra of different components of *Sphenocentrum jollyanum.*

The results revealed the presence of 2,4-Di-tert-butylphenol, Phenol, 3,5-bis(1,1-dimethylethyl) (21.05%), Cyclohexene, 6-butyl-1-nitro, Z-8-Methyl-9-tetradecenoic acid, Methyl 9,12-heptadecadienoate (19.12%), Hexadecanoic acid, ethyl ester, Undecanoic acid, ethyl ester (7.86%), Diisooctyl phthalate, Bis(2-ethylhexyl) phthalate, (7.13%), Phytol, Oleic Acid, cis-11-Hexadecenal (7.03%), 6,9,12-Octadecatrien-1-ol, Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)- (6.65%), 5-Eicosene, (E)-, 3-Eicosene, (E)-. 1-Octadecene (4.63%), 9,17-Octadecadienal, (Z)-, 2-Methyl-Z, Z-3,13-octadecadienol, cis-7, cis-11 Hexadecadien-1-yl acetate (4.24%), n-Hexadecanoic acid, n-Decanoic acid, L-Galactose, 6-deoxy-, (4.09%), trans-13-Octadecenoic acid, 1-Eicosene (3.81%), Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-,9-Octadecenoic acid (Z)-,2,3-dihydroxypropyl ester, 9-Oxabicyclo [6.1.0] nonane (3.74%), Dibutyl phthalate, 1,2-Benzenedicarboxylic acid, butyl 2 methyl propyl ester, 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (3.20%), 9-Oxabicyclo [6.1.0] nonane, cis-, 2-Methyl-Z, Z-3,13-octadecadienol, 8-Dodecen-1-ol, (Z)-(3.18%), Cetene,1-Hexadecanol, Trifluoroacetic acid, n-tridecyl ester, (2.78%), 9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12-Octadecadien-1-ol, (Z, Z)-,(1.47%). These phytochemicals are responsible for various biological activities like antifungal, antibacterial, antidepressant, antioxidant, anti-inflammatory, and anti-tumour activities of the leaf extract which supports traditional and modern use of *Sphenocentrum jollyanum* in curing various diseases.

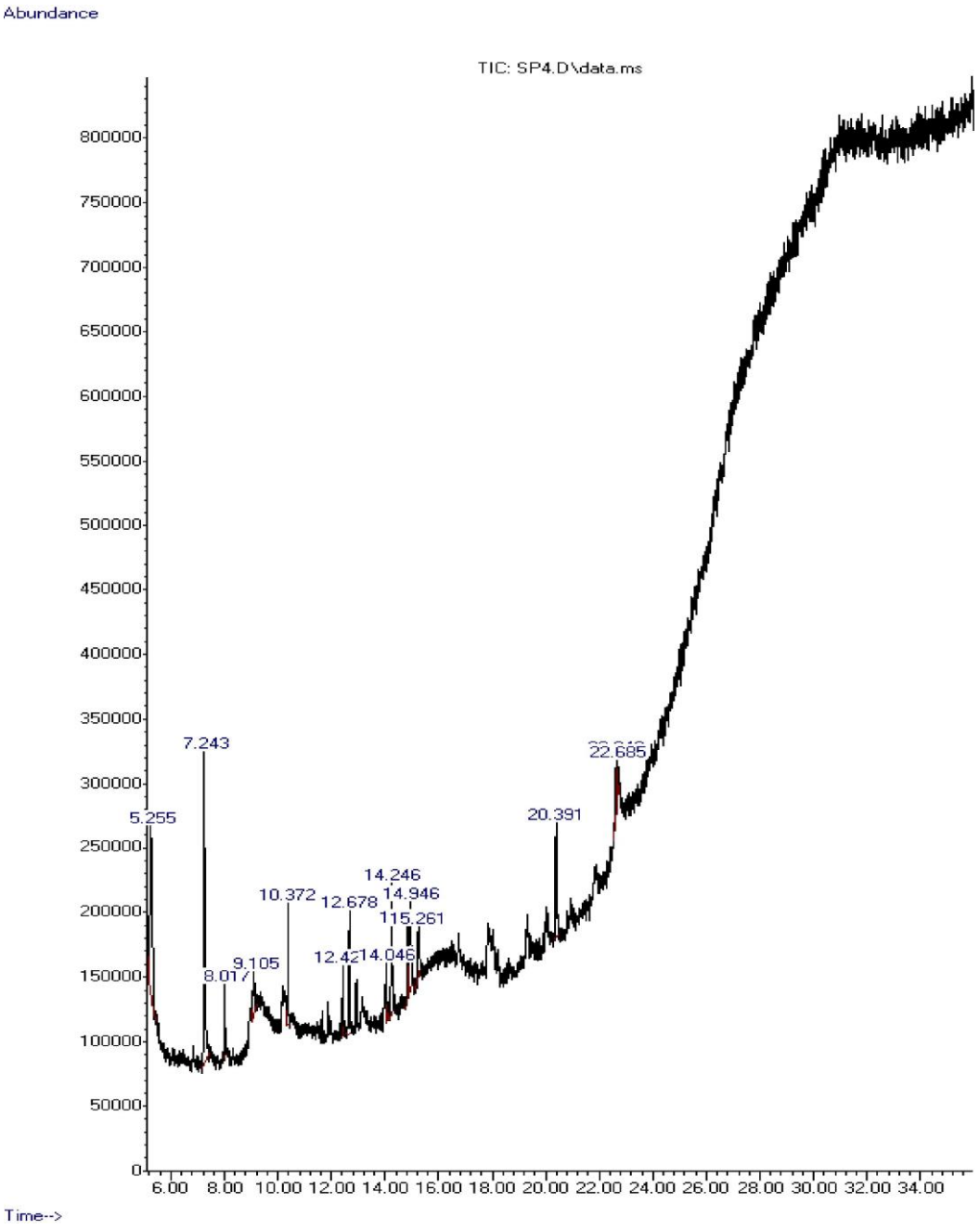
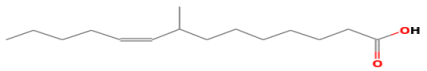
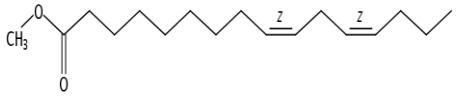
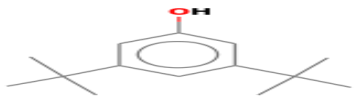
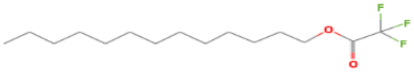
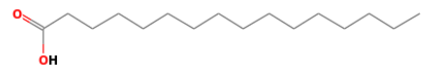
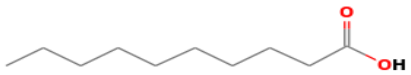
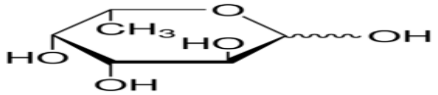
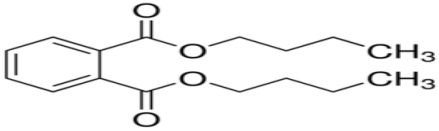
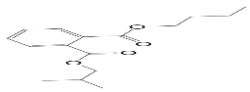
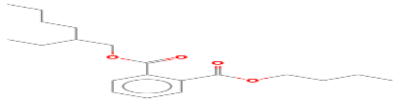
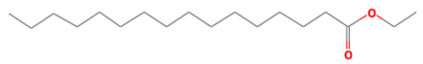
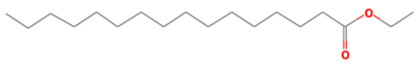
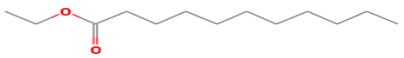
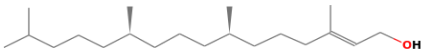
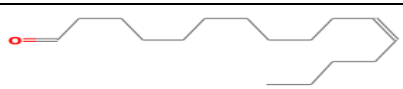
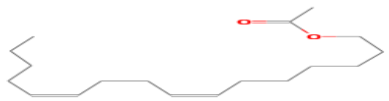


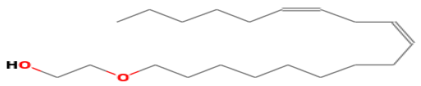
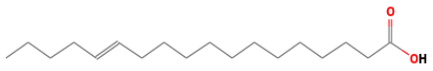
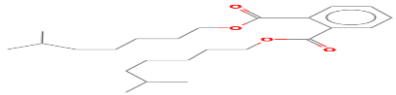
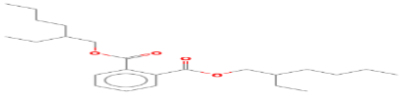
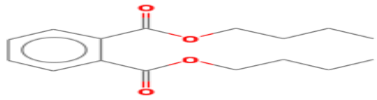
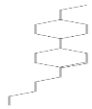
Figure 2. Typical GC-MS chromatogram of ethanol extract of *Sphenocentrum jollyanum*

Table 1: **Identified phytochemical compounds in crude ethanol leaf extract of *Sphenocentrum jollyanum***

Peak #	Retention Time (min)	Peak Area %	Library/ID	CAS #	Chemical Structure	Molecular Formula	Molecular Weight
			Cyclohexene, 6-butyl-1-nitro	084820-13-3		$C_{10}H_{17}NO_2$	183.25
			Z-8-Methyl-9-tetradecenoic acid	1000130-84-5		$C_{15}H_{28}O_2$	240.38
			Methyl 9,12-heptadecadienoate	1000336-36-2		$C_{18}H_{32}O_2$	280.45
			2,4-Di-tertbutylphenol	000096-76-4		$C_{14}H_{22}O$	206.32
			2,4-Di-tert-butylphenol	000096-76-4		$C_{14}H_{22}O$	206.32
			Phenol, 3,5-bis(1,1-dimethylethyl)	001138-52-9		$C_{14}H_{22}O$	206.32
			Cetene	000629-73-2		$C_{16}H_{32}$	224.43
			1-Hexadecanol	036653-82-4		$C_{16}H_{34}O$	242.44
			Trifluoroacetic acid,n-tridecyl ester	053800-02-5		$C_{15}H_{27}F_3O_2$	296.37

4	9.105	4.09	n-Hexadecanoic acid	000057-10-3		$C_{16}H_{32}O_2$	256.42
			n-Decanoic acid	000334-48-5		$C_{10}H_{20}O_2$	172.26
			L-Galactose, 6-deoxy-	002438-80-4		$C_6H_{12}O_5$	164.16
			5-Eicosene, (E)-	074685-30-6		$C_{20}H_{40}$	280.53
			3-Eicosene, (E)-	074685-33-9		$C_{20}H_{40}$	280.53
			1-Octadecene	000112-88-9		$C_{18}H_{36}$	252.48
			Dibutyl phthalate	000084-74-2		$C_{16}H_{22}O_4$	278.34
			1,2-Benzenedicarboxylic acid, butyl 2 methylpropyl ester	017851-53-5		$C_{16}H_{22}O_4$	278.34
			1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	000085-69-8		$C_{20}H_{30}O_4$	334.45
			Hexadecanoic acid, ethyl ester	000628-97-7		$C_{18}H_{36}O_2$	284.48

			Hexadecanoic acid, ethyl ester	000628-97-7		$C_{18}H_{36}O_2$	284.48
			Undecanoic acid, ethyl ester	000627-90-7		$C_{13}H_{26}O_2$	214.34
			9-Oxabicyclo [6.1.0] nonane, cis-	004925-71-7		$C_8H_{14}O$	126.19
			2-Methyl-Z, Z-3,13-octadecadienol	1000130-90-5		$C_{19}H_{36}O$	280.40
			8-Dodecen-1-ol, (Z)-	040642-40-8		$C_{12}H_{24}O$	184.32
			Phytol	000150-86-7		$C_{20}H_{40}O$	296.53
			Oleic Acid	000112-80-1		$C_{18}H_{34}O_2$	282.46
			cis-11-Hexadecenal	053939-28-9		$C_{16}H_{30}O$	238.41
			9,17-Octadecadienal, (Z)-	056554-35-9		$C_{18}H_{32}O$	264.45
			2-Methyl-Z, Z-3,13-octadecadienol	1000130-90-5		$C_{19}H_{36}O$	280.50
			cis-7, cis-11 Hexadecadien-1-yl acetate	052207-99-5		$C_{18}H_{32}O_2$	280.45
			2-Methyl-Z, Z-3,13-octadecadienol	1000130-90-5		$C_{19}H_{36}O$	280.50

			6,9,12-Octadecatrien-1-ol	056630-94-5		$C_{18}H_{32}O$	264.45
			Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-	017367-08-7		$C_{20}H_{38}O_2$	310.52
			Oleic Acid	000112-80-1		$C_{18}H_{34}O_2$	282.46
			trans-13-Octadecenoic acid	000693-71-0		$C_{18}H_{34}O_2$	282.46
			1-Eicosene	003452-07-1		$C_{20}H_{40}$	280.53
			Diisooctyl phthalate	000131-20-4		$C_{24}H_{38}O_4$	390.56
			Bis(2-ethylhexyl) phthalate	00117-81-7		$C_{24}H_{38}O_4$	390.56
			Dibutyl phthalate	000084-74-2		$C_{16}H_{22}O_4$	278.34
			Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-	301643-32-3		$C_{19}H_{34}$	262.50
			9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	000111-03-5		$C_{21}H_{40}O_4$	356.54
			9-Oxabicyclo [6.1.0] nonane	00286-62-4 5		$C_8H_{14}O$	126.19

15	22.685	1.47	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	003443-84-3		$C_{21}H_{38}O_4$	354.52
			9,12-Octadecadien-1-ol, (Z, Z)-	00506-43-4		$C_{18}H_{34}O$	266.46
			9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester				
		Total=99.98	**Total compounds = 45**				

∗Source: Dr. Duke's Phytochemical and Ethnobotanical databases (online database).

Table 2: Bioactivity of phytocomponents identified in ethanol leaf extract of *Sphenocentrum jollyanum*.

S/No.	RT	Identified Compound	Nature of Compound	Reported Biological Activity
		Cyclohexene, 6-butyl-1-nitro	c-nitro cycloalkenes	No bioactivity reported
		Z-8-Methyl-9-tetradecenoic acid	Fatty acid	Antifungal (Sathya *et al.*, 2016)
		Methyl 9,12-heptadecadienoate	Fatty acid esters	No bioactivity reported
		2,4-Di-tert-butylphenol	Phenylpropane	Antibacterial, Antioxidant (Ndiege *et al*.2021)
		Phenol,3,5-bis(1,1-dimethylethyl)	Phenylpropane	Antioxidant (Ndiege *et al*.2021)
		Cetene	Aliphatic	Antitumor and Antioxidant (Sunil *et al.*,2018)
		1-Hexadecanol	Fatty alcohol	Antioxidant, opacifier, emulsifier, and thickening agent, emollient (Amudha *et al.*, 2018)
		Trifluoroacetic acid, n-tridecyl ester	Fatty acid ester	Anti-inflammatory, antiarthritic, antimicrobial, anti-tumour, antiprotozoal and chemopreventive (Kalaivani *et al.*, 2018)
		n-Decanoic acid	Fatty acid	Antifungal (Sathya *et al.*, (2016).
		n-Hexadecanoic acid	Fatty acid	antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic and 5-alpha, reductase inhibitor Pauldasan *et al.*, 2020. Potent mosquito larvicide (Abubakar and Majinda, 2016), Anticancer, Antipesticide, Antimicrobial (Hameed et al., 2015).
		L-Galactose, 6-deoxy-	Sugar moiety	Flavouring agent (Azhagu,2021)
		5-Eicosene, (E)-	Fatty acid	antimicrobial activity (Yogeswari et al., 2012)
		3-Eicosene, (E)-	Fatty acid	Antimicrobial, Antihyperglycemic, Cytotoxic Activity, Antioxidant, Insecticidal activity (Banakar and Jayaraj, 2018)
		1-Octadecene	Alkene	Antibacterial, antioxidant (Indra et al., 2018)
		Dibutyl phthalate	Plasticizer compound	Antimicrobial, Antifouling (G. GnanaPriyanka Beulah et.al.2018)
		1,2-Benzenedicarboxylic acid, butyl 2 methyl propyl ester	Phthalate ester	Hypoglycemic Effect; α-glucosidase inhibitor (Bu et al., 2010)
		1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	Ester	antitumor activity; pro-inflammatory agent (El-Sayed et al., 2015). Antioxidant and/ or renal protective activity (Adeyemi et al. (2017)
		Hexadecanoic acid, ethyl ester	Fatty acid ester	Antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor (Adeniyi et al., 2019)
		Undecanoic acid, ethyl ester	Fatty acid esters	Antioxidant, Increase Aromatic Amino acid decarboxylase activity (Juliet et al., (2020).
		9-Oxabicyclo [6.1.0] nonane, cis-	Terpenoids	antibacterial, Anti-fungal and nematicidal activity (Sathya et al., (2016).
		2-Methyl-Z, Z-3,13-octadecadienol	Aliphatic Alcohol	Anticancer, Anti-microbial, Cytotoxic (Vikrama et al., 2018; Yirankinyuki et al. 2020)

		8-Dodecen-1-ol, (Z)-	Fatty alcohols.	No bioactivity reported
		Phytol	Diterpene alcohol	Anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (Islam et al., 2018)
		Oleic Acid	Monounsaturated fatty acid	5-α reductase inhibitor, allergenic, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-alopecic, anti-leukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic, insectifuge, perfumery, propecic and favour (Pauldasan *et al.*, 2020)
		cis-11-Hexadecenal	Fatty aldehydes	No bioactivity reported
		9,17-Octadecadienal, (Z)-	Unsaturated aldehyde	Antimicrobial (Karthika and Paulsamy 2014)
		cis-7, cis-11 Hexadecadien-1-yl acetate	Acetate compound	No bioactivity reported
		6,9,12-Octadecatrien-1-ol	Fatty acid	Antioxidant, Antibacterial (Elango V. et al., (2015).
		Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-	Alcoholic compound	Antimicrobial (Sana et al., 2019)
		trans-13-Octadecenoic acid	Fatty acid	Anti-inflammatory, antiamdrogenic, dermatitigenic, anaemiagenic, insecticides, flavour (Awonyemia et al., 2020)
		1-Eicosene	Alkene	Antimicrobial (Khurshid. et al. 2018)
		Diisooctyl phthalate	Benzoic acid ester.	Antimicrobial, Solvent, Plastilixer, Pesticide, Repellent (Mary and Giri (2018).
		Bis(2-ethylhexyl) phthalate	Benzoic acid esters.	Cytotoxic (Thenmozhi and Rajan (2015)
		Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl-	Cyclo hydrocarbon	No bioactivity reported
		9-Octadecenoic acid (Z)-, 2,3 dihydroxypropyl ester	Glycerol α-monooleate	Anticancer (Neeraj et al., (2019).
		9-Oxabicyclo [6.1.0] nonane	Oxabicyclic compound (Adeyemi et al., 2017)	No bioactivity reported
		9-Octadecenoic acid (Z)-, 2-hydroxy-1 (hydroxymethyl)ethyl ester	Fatty acid ethyl ester	Inhibition of proliferative effect in keloid fibroblasts (Anyasor, et al., 2014)
		9,12-Octadecadien-1-ol, (Z, Z)-	Fatty alcohol	Oligosaccharide Provider, Increase Zinc Bioavailability (Juliet et al., 2020)

RT: Retention time

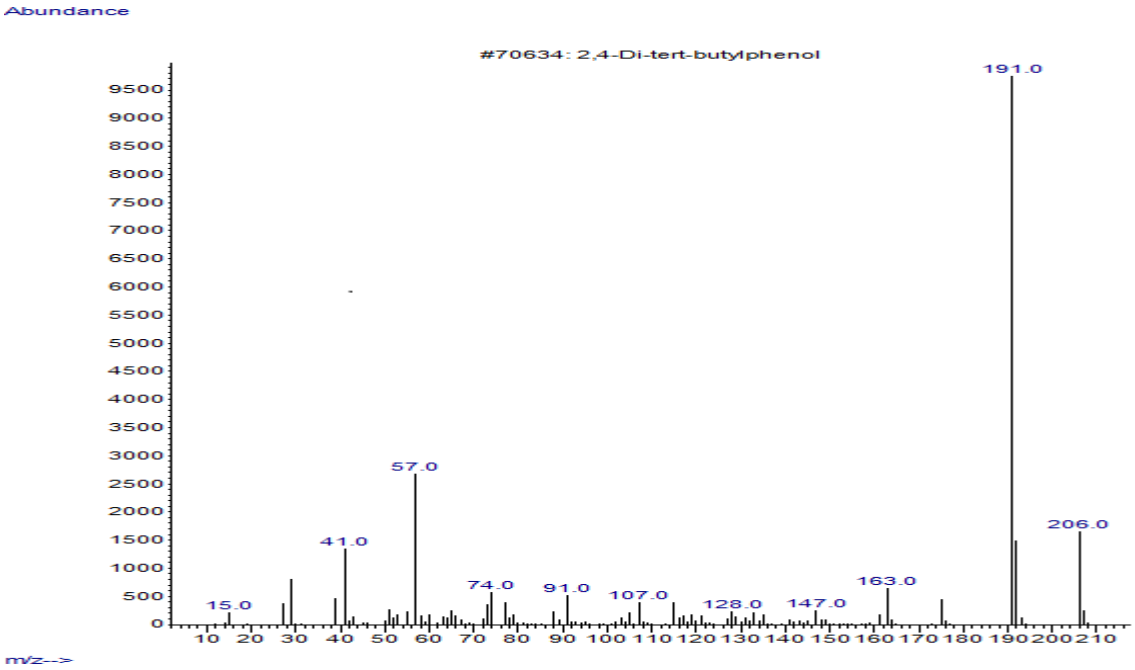


Figure 3: GC-MS spectra of 2,4-Di-tertbutylphenol (RT: 7.243, 21.05%) from *Sphenocentrum jollyanum* leaf extract.

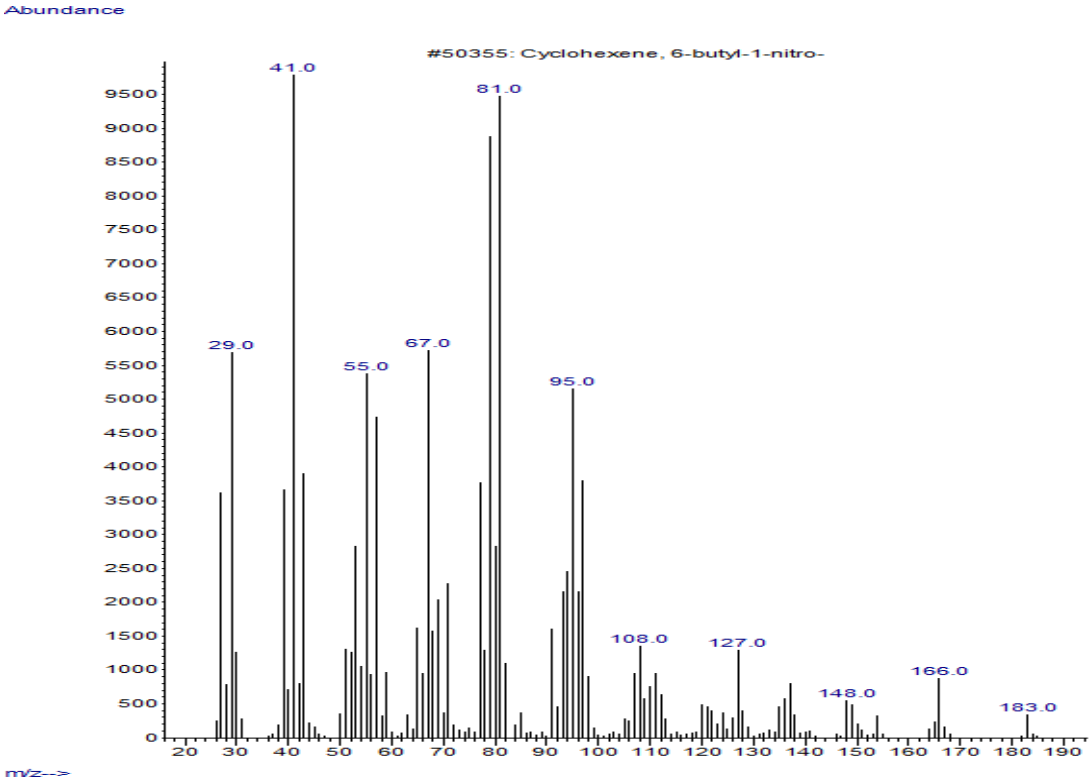


Figure 4: GC-MS spectra of Cyclohexene, 6-butyl-1-nitro (RT:5.255, 19.12%) from *Sphenocentrum jollyanum* leaf extract.

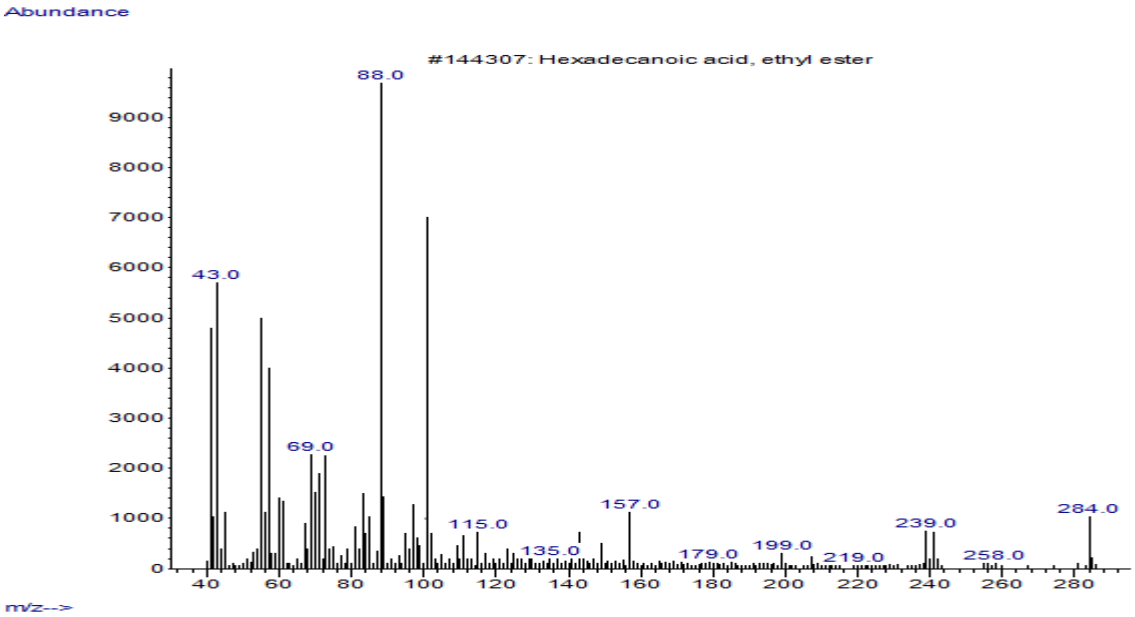


Figure 5: GC-MS spectra of Hexadecanoic acid, ethyl ester (RT: 12.678,7.86%) from *Sphenocentrum jollyanum* leaf extract.

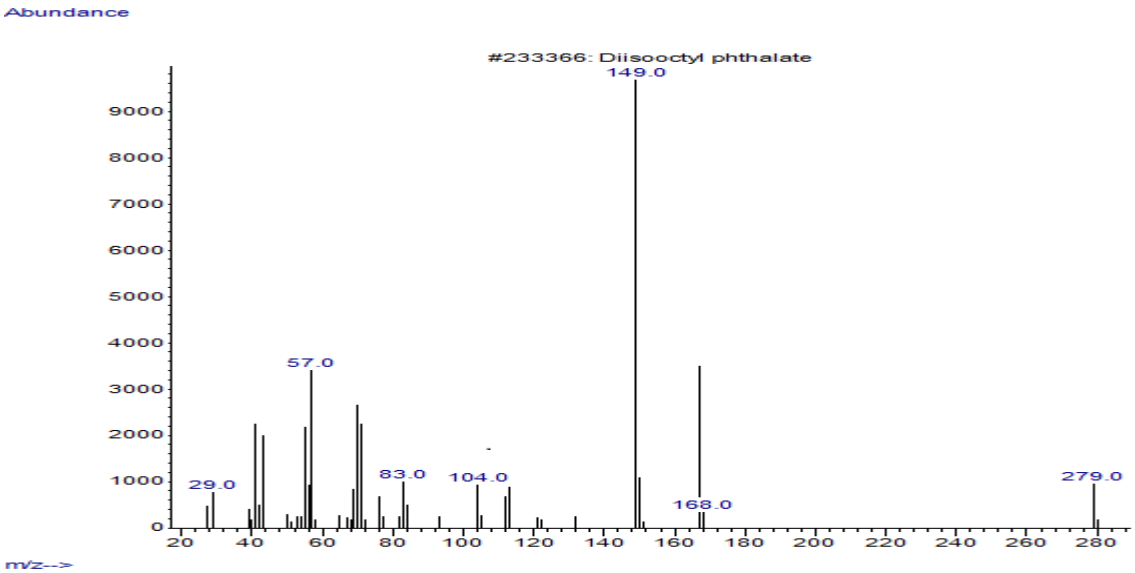


Figure 6: GC-MS spectra of Diisooctyl phthalate (RT: 20.391, 7.13%) from *Sphenocentrum jollyanum* leaf extract.

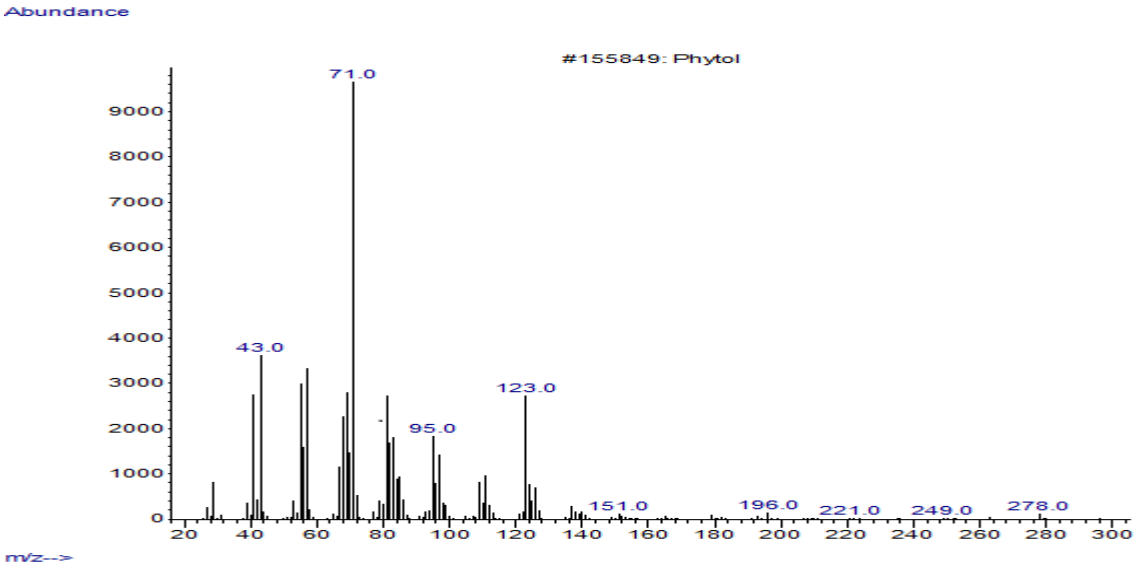


Figure 7: GC-MS spectra of Phytol (RT: 14.246, 7.03%) from *Sphenocentrum jollyanum* leaf extract.

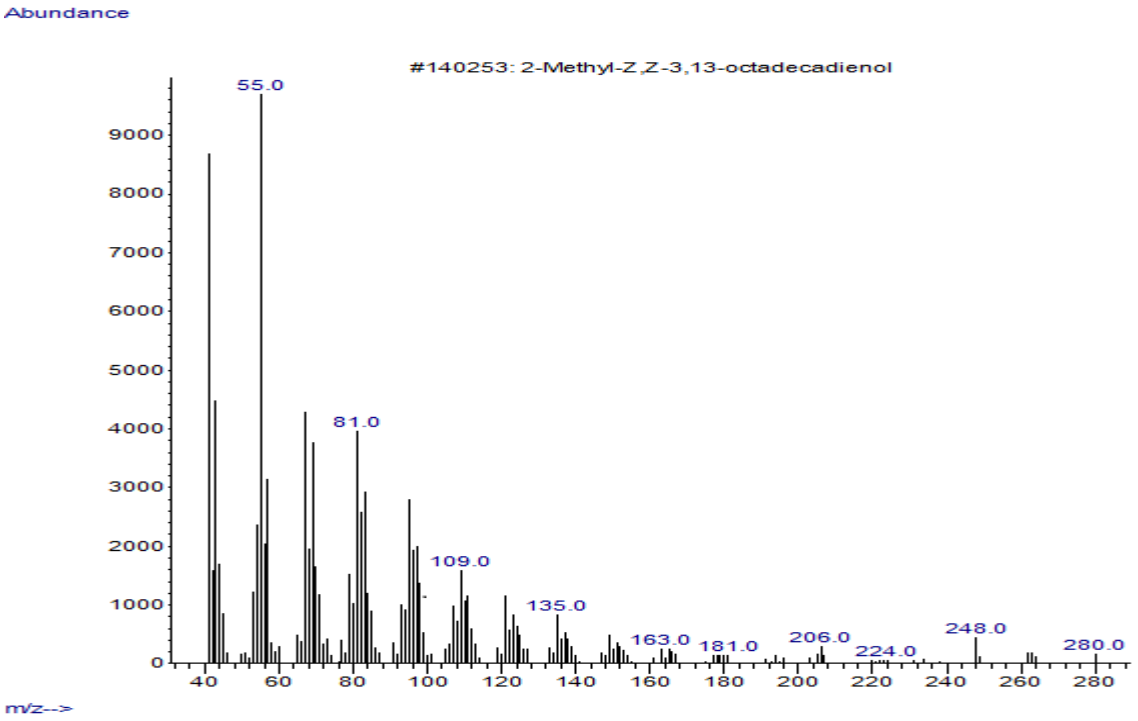


Figure 8: GC-MS spectra of 2-Methyl-Z, Z-3,13-octadecadienol (RT: 14.946, 6.65%) from *Sphenocentrum jollyanum* leaf extract.

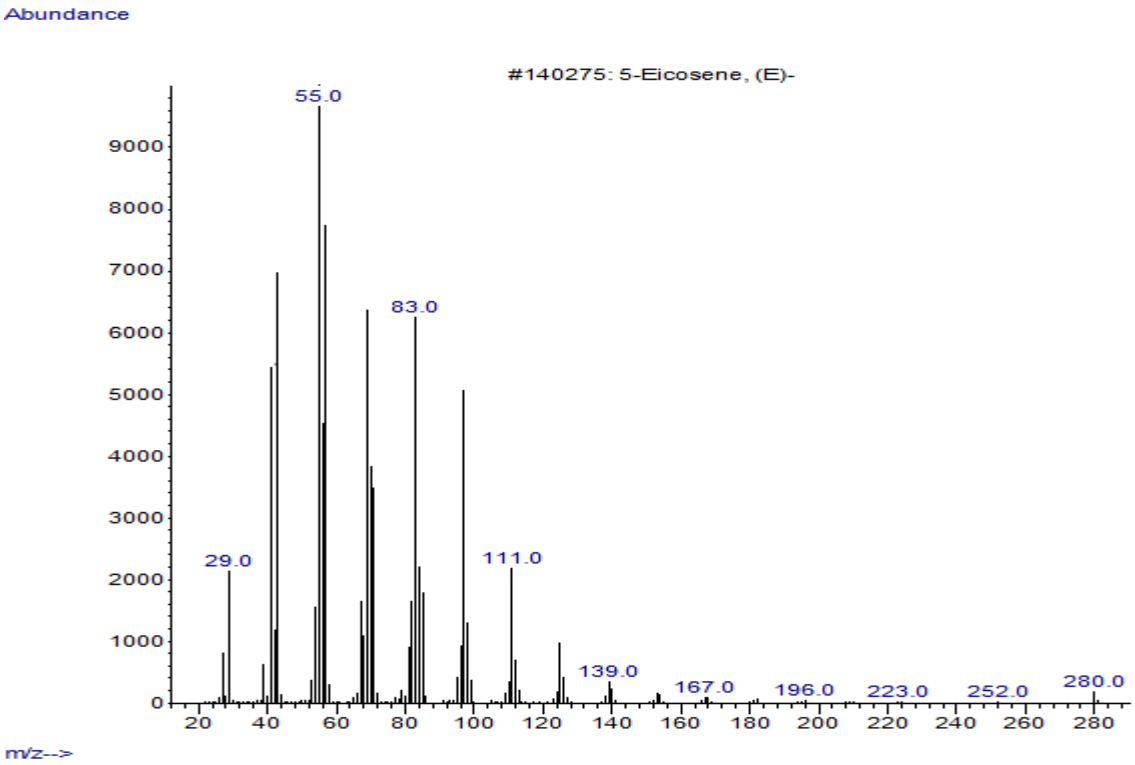


Figure 9: GC-MS spectra of 5-Eicosene, (E)- (RT: 10.372, 4.63%) from *Sphenocentrum jollyanum* leaf extract

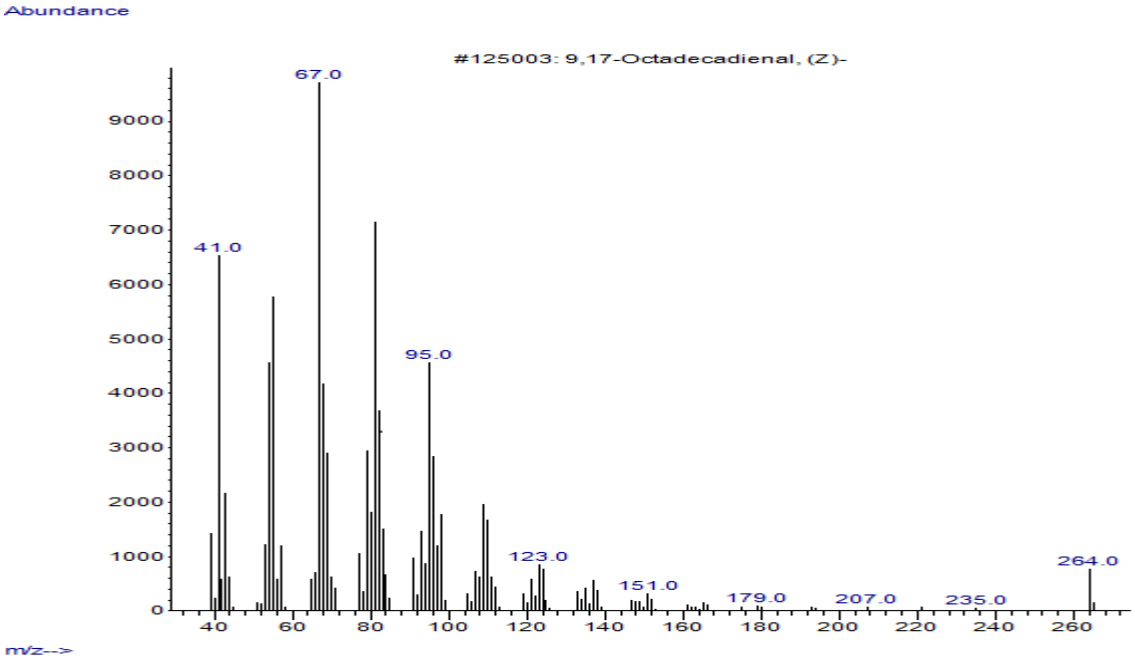


Figure 10: GC-MS spectra of 9,17-Octadecadienal, (Z)- (RT: 14.857, 4.24%) from *Sphenocentrum jollyanum* leaf extract.

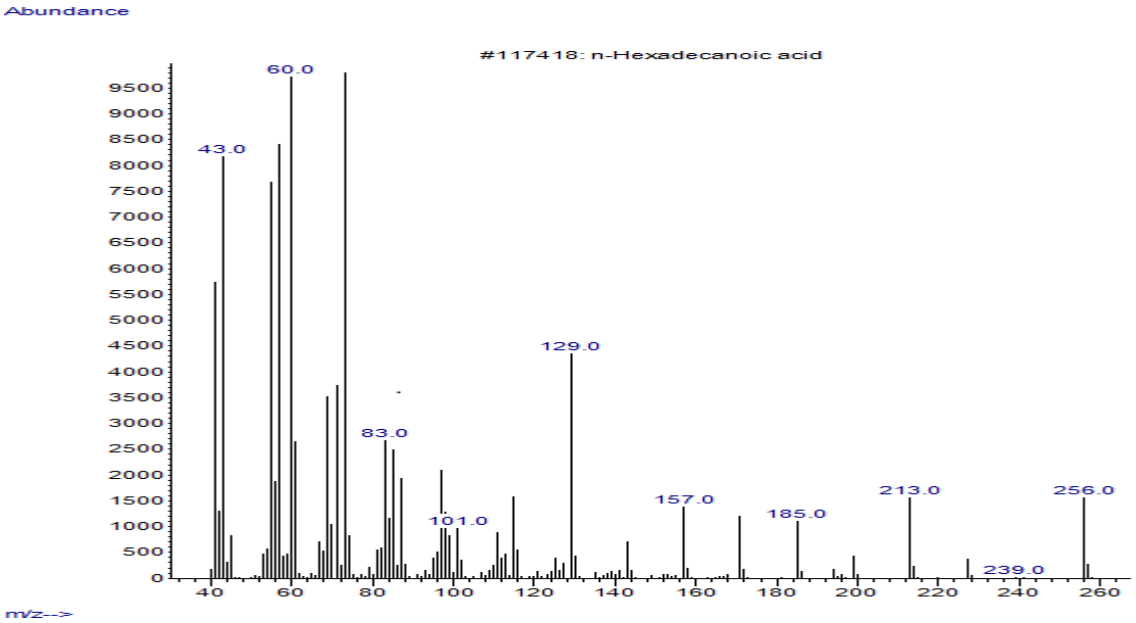


Figure 11: GC-MS spectra of n-Hexadecanoic acid (RT:9.105, 4.09%) from *Sphenocentrum jollyanum* leaf extract

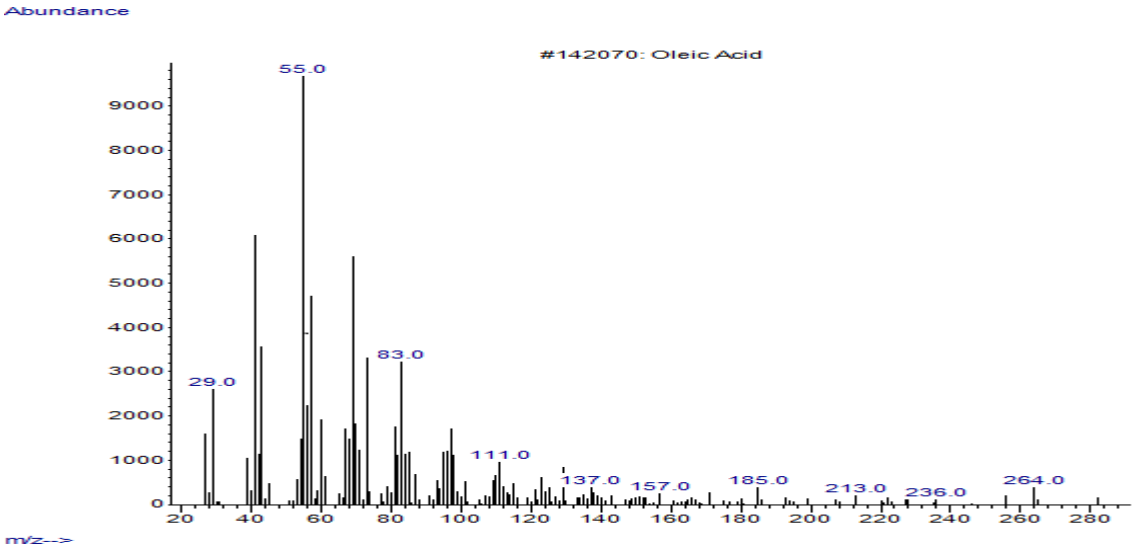


Figure 12: GC-MS spectra of Oleic Acid (RT: 15.261, 3.81%) from *Sphenocentrum jollyanum* leaf extract.

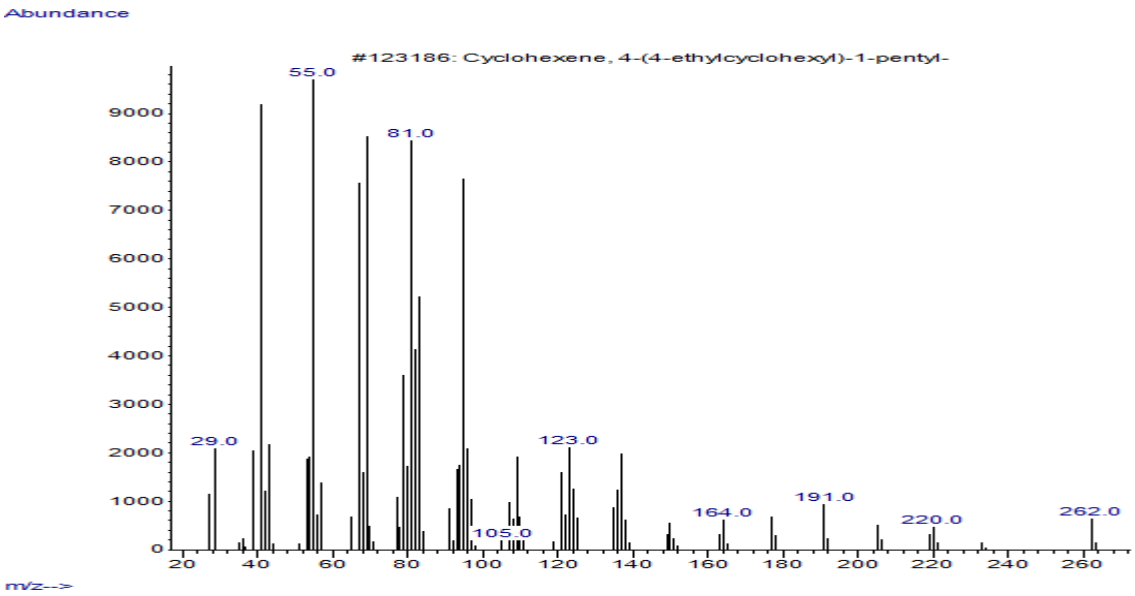


Figure 13: GC-MS spectra of Cyclohexene, 4-(4-ethylcyclohexyl) -1-pentyl- (RT: 22.648, 3.74%) from *Sphenocentrum jollyanum* leaf extract.

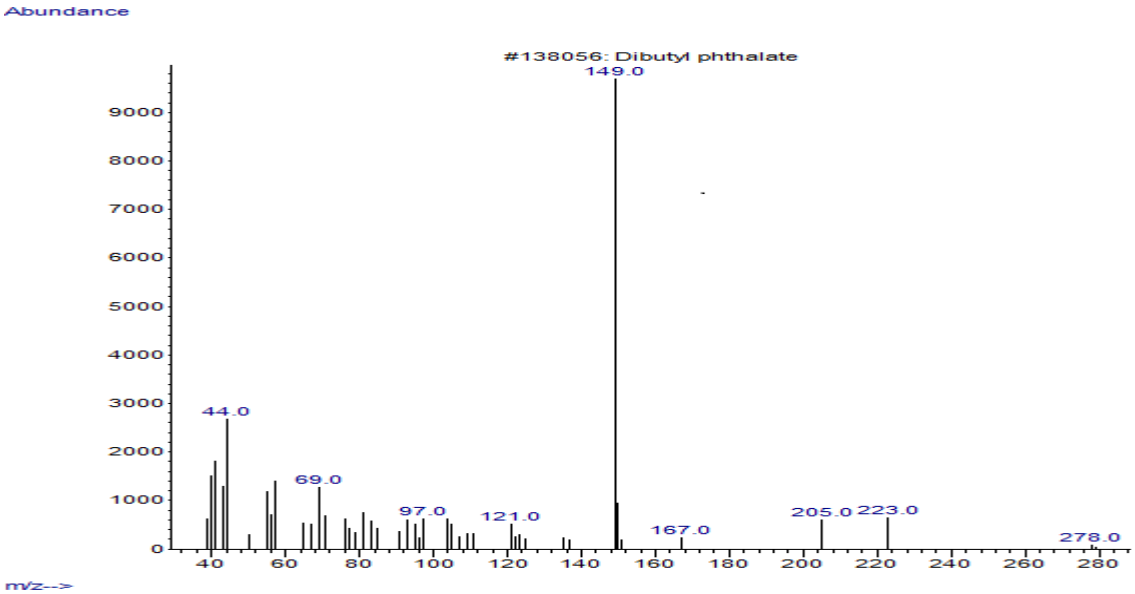


Figure 14: GC-MS spectra of Dibutyl phthalate (RT: 12.426, 3.20%) from *Sphenocentrum jollyanum* leaf extract

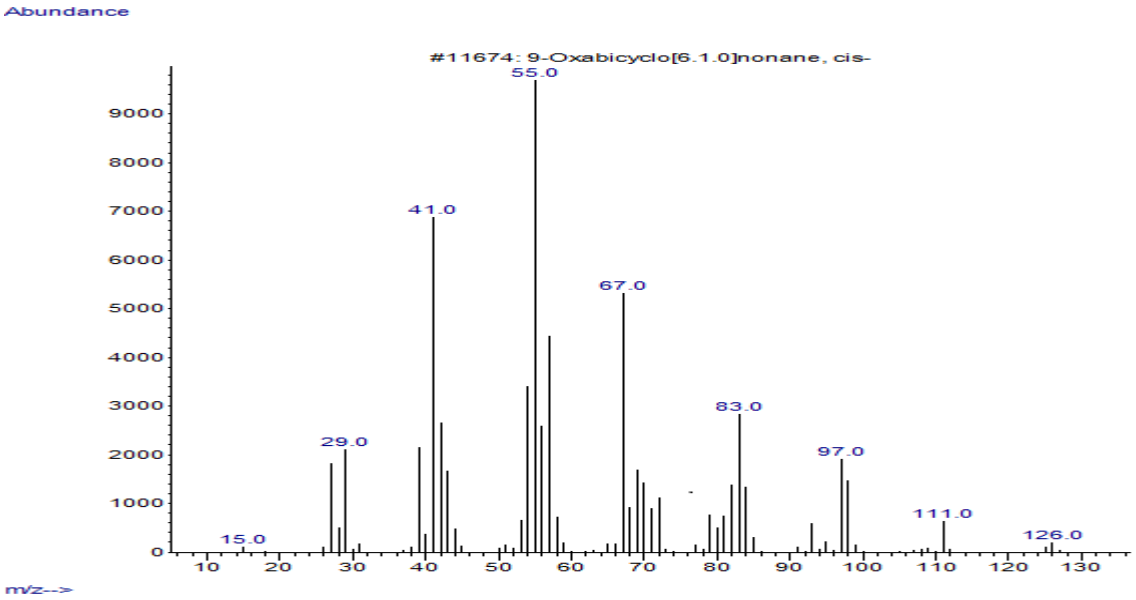


Figure 15: GC-MS spectra of 9-Oxabicyclo [6.1.0] nonane, cis- (RT: 14.046,3.18%) from *Sphenocentrum jollyanum* leaf extract.

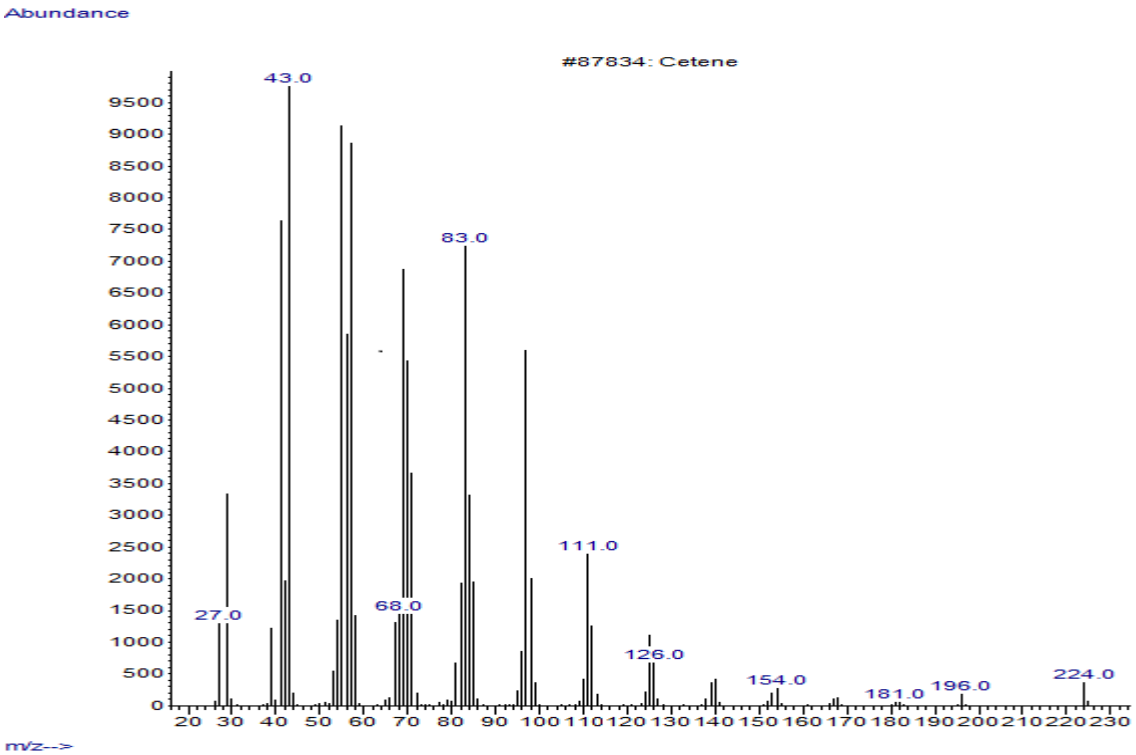


Figure 16: GC-MS spectra of cetene (RT:8.017, 2.78 %) from *Sphenocentrum jollyanum* leaf extract.

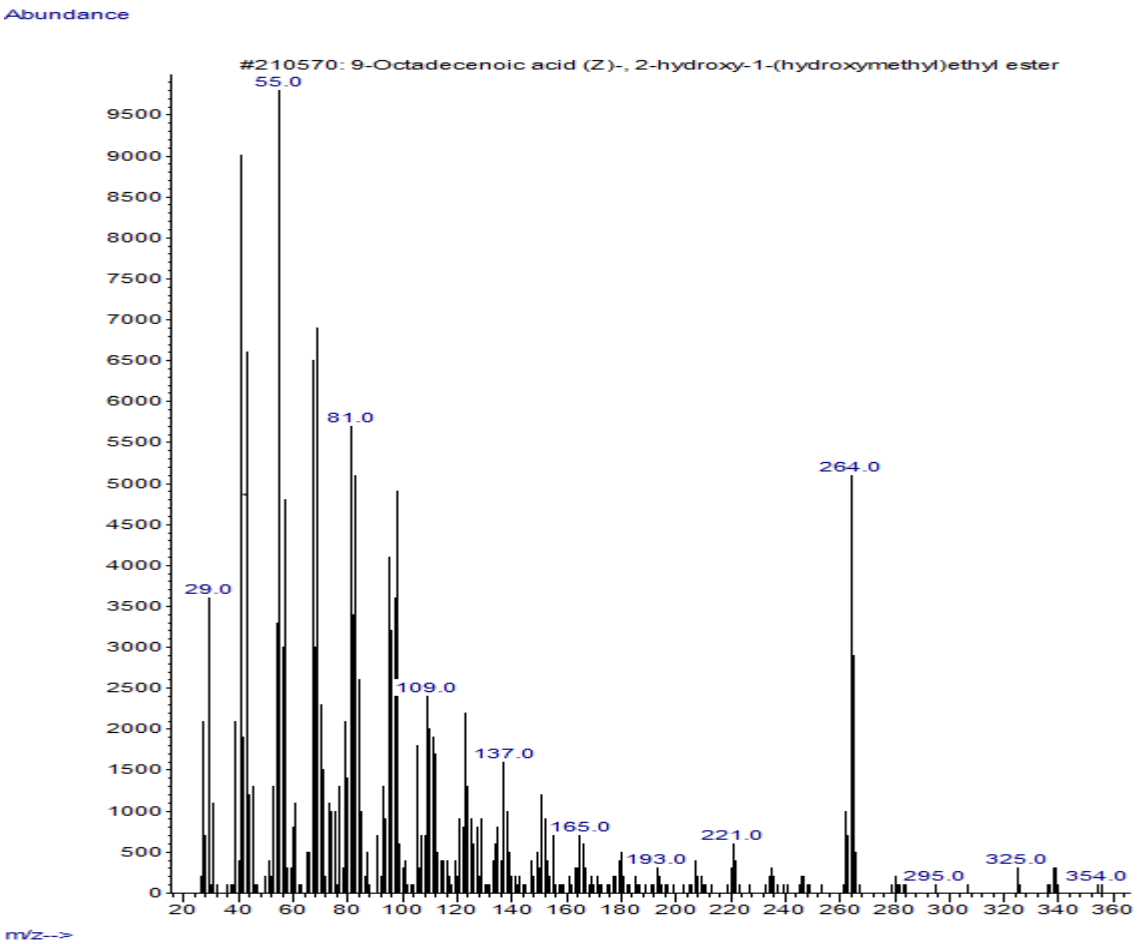


Figure 17: GC-MS spectra of 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester (RT: 22.685, 1.47%) from *Sphenocentrum jollyanum* leaf extract.

DISCUSSION

Gas Chromatography–Mass Spectrometry (GC-MS) is a hybrid analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas MS helps fragment the analyte to be identified on the basis of its mass(Sahil *et al*., 2011; Jenke, 1996). GC-MS provides enhanced sample identification, higher sensitivity, an increased range of analyzable samples, and faster results, which enable a whole new range of applications for GC-MS in several areas (Susha, 2019).

In the present study, the GC-MS chromatogram detected 15 peaks representing 45 compounds from the ethanol leaf extract of *Sphenocentrum jollyanum* (Table 1., Figure 1). Among the identified bioactive components, 2,4-

Di-tert-butylphenol, also known as 2,4-DTBP and Phenol, 3,5-bis(1,1-dimethylethyl) with retention time (RT) of 7.243 has the highest peak area of 21.05%. They belong to the class of organic compounds known as phenylpropanes. They have antibacterial and antioxidant properties. They are used industrially as UV stabilizers and as antioxidants for hydrocarbon-based products ranging from petrochemicals to plastics. They have also been reported to prevents gumming in aviation fuels (Ndiege *et al*.2021).

Z-8-Methyl-9-tetradecenoic acid with RT of 5.255 and a peak area of 19.12% has antifungal properties (Sathya *et al.*, 2016).

Hexadecanoic acid, ethyl ester and Undecanoic acid, ethyl ester are fatty acid esters. They have RT of 12.678 with a peak of 7.86%. Hexadecanoic acid, ethyl ester has antioxidant, flavour, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor (Adeniyi *et al.*, 2019) while Undecanoic acid, ethyl ester exhibits antioxidant, and increases aromatic amino acid decarboxylase activity (Juliet *et al.*, (2020).

Di-iso-octyl phthalate, and Bis(2-ethylhexyl) phthalate are a class of organic compounds known as benzoic acid esters. It has a RT of 20.391 and peak of 7.13%. Di-iso-octyl phthalate has been reported to possess antimicrobial, solvent, plasticizer, pesticide, repellent (Mary and Giri (2018). Bis(2-ethylhexyl) phthalate is a primary metabolite that are directly involved in an organism's growth, development or reproduction and are potentially toxic compound. Thenmozhi and Rajan (2015) reported the cytotoxic properties of Bis(2-ethylhexyl) phthalate.

Phytol, Oleic acid and cis-11-Hexadecenal are diterpene alcohol, monounsaturated fatty acid and fatty aldehydes respectively. They have RT of 14.246 and peak of 7.03 %. Phytol is the product of chlorophyll metabolism in plants. It is used in the manufacturing of Vitamin E and K1 which are important in the many functions of the human body. It is used along with simple or corn syrup as a hardener in candies (Inoue *et al.*, 2005, Sathiyabalan *et al.*, 2014). Phytol has been reported to show vast biological activities like anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects (Islam *et al.*, 2018). It has also been reported to have anti-cancer properties with effects on both Gastric Adenocarcinoma Cells (AGS) (Song and Cho, 2015), Glioblastomas (Gustavo *et al.*, 2017) and antischistosomal properties (Josue´ *et al.*, 2014). Oleic Acid has 5-α reductase inhibitory, allergenic, anti-inflammatory, anti-androgenic, cancer preventive, anemiagenic, anti-

alopecic, anti-leukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic, insectifuge, perfumery, propecic and flavour activities (Pauldasan *et al*., 2020).

6,9,12-Octadecatrien-1-ol, and Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)- are fatty acid and alcoholic compound with RT of 14.946 and peak of 6.65 % respectively. It has antioxidant, antibacterial (Elango *et al.*,2015) and antimicrobial properties (Sana *et al*., 2019)

5-Eicosene, (E)-, 3-Eicosene, (E)-, and 1-Octadecene are fatty acids and alkene compounds respectively. They have RT of 10.372 and peak of 4.63 %. The fatty acids have antimicrobial, antihyperglycemic, cytotoxic activity, antioxidant, and insecticidal activities (Yogeswari *et al.*, 2012; Banakar and Jayaraj, 2018). Indra et al., 2018 reported the antibacterial, and antioxidant properties of 1-Octadecene.

9,17-Octadecadienal, (Z)- and cis-7, cis-11 Hexadecadien-1-yl acetate are unsaturated aldehyde and acetate compounds. It has RT of 14.857 and peak of 4.24 %. 9,17-Octadecadienal, (Z)- has been reported to have antimicrobial (Karthika and Paulsamy 2014). n-Hexadecanoic acid, n-Decanoic acid, and L-Galactose, 6-deoxy- are fatty acids and sugar compounds respectively. They have RT of 9.105 and peak area of 4.09 %. The n-Hexadecanoic acid has been reported to possess antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic and 5- alpha, reductase inhibitor (Pauldasan *et al.*, 2020). It has also been reported to be a potent mosquito larvicide (Abubakar and Majinda, 2016), anticancer, anti-pesticide, antimicrobial activities (Hameed *et al.*, 2015). n-Decanoic, also known as capric acid been reported to possess antifungal (Sathya *et al.*, (2016). It is used in the manufacture of esters for artificial fruit flavors and perfumes. It is also used as an intermediate in chemical syntheses. Capric acid is used in organic synthesis and industrially in the manufacture of perfumes, lubricants, greases, rubber, dyes, plastics, food additives and pharmaceuticals. Capric acid may be responsible for the mitochondrial proliferation associated with the ketogenic diet, which may occur via PPAR gamma receptor agonism and the targeting of genes involved in mitochondrial biogenesis (PMIDL 24383952). The L-Galactose, 6-deoxy- has been reportedly used as Flavouring agent (Azhagu,2021)

Trans-13-Octadecenoic acid and 1-Eicosene are fatty acid and alkene compounds with RT of 15.261 and peak of 3.81 %. Trans-13-Octadecenoic acid has been reported to possess anti-inflammatory, antiandrogenic, dermatitigenic, anaemiagenic, insecticides, flavour properties (Awonyemia *et al.*, 2020). Khurshid. *et al.*, 2018 has also reported the antimicrobial property of and 1-Eicosene.

3. CONCLUSIONS

The GC-MS analysis of ethanol leaf extract of *Sphenocentrum jollyanum* has revealed the presence of various secondary metabolites with various degrees of biological activities. The presence of these various bioactive compounds confirms the application of *Sphenocentrum jollyanum* leaves for various ailments by traditional practitioners which includes antibacterial, antioxidant, antitumor and antifungal activities. However, further investigation is required for the possible isolation and development of novel drugs using some of the phytochemicals and bioactive compounds found in *Sphenocentrum jollyanum*.

CONFLICT OF INTERESTS

There is no conflict of interest between authors.

ACKNOWLEDGEMENTS

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CONFLICT OF INTERESTS

We have no conflict of interests.

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