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GC-MS Evaluation of the Phytoconstituents of the Ethanolic and N-Hexane Extracts of the whole Plant of Cleome Rutidosperma

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Abstract- Plants are the source of a great variety of phytochemicals, the valuable properties of which have been utilized by human being for centuries ever since the dawn of human civilization. Man has employed therapeutic plants as the greatest bio-resource of remedies for folk and contemporary treatments. Most plant species used as folklore medicine are claimed to retain the capacity to cure or mitigate some health disorders. Cleome rutidosperma has been reported for treatments as anti-inflammatory, antimicrobial, etc. agents. The present investigation was aimed to explore the phytoconstituents of the ethanolic and n-hexane extracts of the entire plant of Cleome rutidosperma employing the GC-MS technique. The extracts were analyzed with Thermo-Finnigan Trace GC Ultra (Waltham, MA, USA) equipped with a splitless injector, coupled to an ion trap mass spectrometer (MS) (Polaris Q) coupled to a Xcalibur data software processor. The GC-MS analysis of the crude ethanol and n-hexane extracts revealed mainly, esters, fatty acids, sterols, diketones and alcohols. Ethanolic and n-hexane extracts showed the presence of 22 and 25 phyto-compounds respectively, with a total of 40 pharmaco-bioactives put together. Some of the combined phytoconstituents are - (R)-(-)-14-Methyl-8hexadecyn-1-ol, hexadecanoic acid ethyl ester, 1-hexyl-2nitrocyclohexane and 3, 7, 11, 15-tetramethyl-2-hexadecen-1ol (Phytol). Major phytoconstituents in ethanol extract were phthalic acid, di(2-propylpentyl) ester (22.5 %), 8-lsopropyl-5methyl-5, 6.7,8-tetrahydro-2,4-quinazolinedione (14.82 %), nhexadecanoic acid (14.78 %), 1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (5.19 %), retinal (vitamin A aldehyde) (2.54%), while 2-methyl-5-5-diphenyl-4-(methylthio)imidazole (18.22 %), linoleic acid ethyl ester (8.11%), phthalic acid, isobutyl octadecylester (3.92 %), 4-fluoro-1-methyl-5carboxylic acid ethyl ester (3.69 %), octadecanoic acid 17methyl-methyl ester (3.08 %), Estra-1,3,5(10)-trien-17β-ol (2.25 %) and 2, 4-dimethyl-7-oxo-4,7-dihydro-triazolo (3, 2,-c)

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triazine (1.84%) were found in *n*-hexane extract. These compounds were confirmed using the NIST library. More bioactive constituents were identified in the *n*-hexane extract. The myriads of bioactive componenets present in the examined plant could have a synergistic effect which may boost its therapeutic capacity and could be valuable in the amelioration of various health disorders. Therefore, the therapeutic potentials of *Cleome rutidosperma* is very high.

Keywords: phytoconstituents, GC-MS, ethanolic and n-hexane extracts, cleome rutidosperma.

I. Introduction

he exploitation of plants retaining therapeutic benefits has been a consistent practice by man all through history (Chen, et al; 2016). Plants have continued to be invaluable organic sources therapeutic constituents for folk and contemporary treatments, pharmaceutical intermediates, supplements and chemical entities for synthetic remedies (Alamgir, 2017; Dias, et al; 2012). There are more than 1300 healing plants utilized in Europe, of which 90 % are collected from wild resources; in the United States, around 118 of the top 150 medical preparations are naturally based (Balunas & Kinghorn, 2005). Moreover, up to 80 % of those in the developing nations are entirely reliant on herbal remedies for their major healthcare, and over 25 % of recommended medications in industrialized nations are derivative of wild plant species (Alves & Rosa, 2005; Hamilton, 2004). Through the growing request for herbal preparations, natural health products, and secondary metabolites of therapeutic plants, the application of curative plants is rising swiftly all over the sphere (Nalawade, et al; 2003; Cole, et al; 2007). Pharmaceutical plants inexpensive, freely accessible and have insignificant side effects. The strength, therapeutic and organoleptic properties of these natural products have been attributed to the occurrence of bioactive phytoconstituents. Hence there is a need to ascertain, isolate, characterize, quantify and validate these compounds which are frequently desirable by pharmaceutical establishments for the manufacture of novel therapeutic remedies for the management of several health disorders (Wadood, 2013; Altemimi, et al., 2017). Cleome rutidosperma DC (Family, Cleomaceae), an annual herbaceous, low growing plant reaching up to 100 cm tall. It is native to Tropical Africa, found in Guinea, Nigeria, Cameroons, Zaire and Angola. It thrives in waste grounds, humid or grassy places with trifoliate leaves and small blue, violet with whitish patches of flowers (Ghosh, et al., 2019). It has naturalized in diverse areas of Asia, Australia, America and the West Indies (Acevedo-Rodriguez & Strong, 2012). ethnomedicinal applications of Cleome rutidosperma have been documented and these have been accredited to the essential phytochemical constituents confined in them. Phytochemical evaluation of Cleome rutidosperma indicated the existence of tannins, lipids, amino acids, flavonoids, cardiac glycosides, alkaloids, steroids, saponins, terpenoids, polyphenols, phlobatannins, pentose and reducing sugars (Ibezim & Ugoeze, 2017; Ghosh, et al; 2019). Extracts of the entire plant or its parts have anti-convulsant, anti-inflammatory, anti-stimulant, anti-scorbutic, rubefacient, anti-diarrheal, vesicant and carminative properties. Anti-oxidant, antiplasmodial, analgesic, anti-microbial, diuretic, laxative, anthelmintic and anti-diabetic activities have well been documented (Bose, et al; 2006; Bose, et al; 2007; Chakraborty, et al., 2010; Bose, et al., 2017; Ibezim & Ugoeze; Akah & Nwambie, 1993). Solvent extraction is an essential approach in the isolation of these phytoconstituents in their original form. The extraction and yield of plant metabolites frequently hinge on the plant part as well as the nature, combination and concentration of extraction solvent (Ibezim & Ugoeze. 2017). Thus, diverse extraction solvent defines the presence of various phytoconstituents to be anticipated in an extract based on the variation in the solubility of the various phytochemical compounds existent in them. The subsequent extract with its bioactive constituent, in turn, defines the activity to be associated with the plant, thus, agreeing with the previous report that the phytochemical investigation of crude ethanolic extract of several parts of Cleome rutidosperma revealed different extents of steroids, saponins, triterpenoids and reducing sugars (Ibezim & Ugoeze, 2017). These were accountable for the detected microbiological activity related to the plant extract. It was reported that the methanol, chloroform and petroleum ether crude extracts of Cleome rutidosperma displayed substantial analgesic and depressed locomotor activity (Bose, et al; 2004). Also, petroleum ether, chloroform, methanol and aqueous extracts of Cleome rutidosperma has been verified to have wound healing property (Mondal & Suresh, 2012).

generally recognized as Fringed Spider Flower (FSF), is

In recent times, a detailed, modern analysis of bioactive, non-nutritive components of plants are undertaken in the isolation, identification and quantification of new therapeutic compounds of medicinal importance from plants for specific diseases (Altemimi, et al; 2017).

Gas chromatography-mass spectrometry (GC-MS) is a hyphenated analytical approach applied in the identification of various constituents contained by a test sample (Gabriella, et al; 2016). GC-MS can provide meaningful information for thermally stable, low molecular weight, volatile components (Kanimathi, et al; 2019). Qualitative confirmation of the divergent bioactive complexes from the chloroform and methanol crude extracts of Cleome species seeds employing GC-MS showed diverse categories of high and low molecular weight chemical entities with variable amounts (Kanimathi, et al; 2019). Their identification and characterization were based on their elution order in HP-5MS column. Although, there are reports on the GC-MS of Cleome spp. extracts and phytoconstituents present, but there is a paucity of information on their medicinal properties. Therefore, the present study employed GC-MS in the exploration of the phytoconstituents present in the ethanolic and *n*-hexane extracts of Cleome rutidosperma whole plant.

II. Materials and Methods

a) Chemicals and reagents

The following reagents were used as procured and were of analytical grades: Ethanol (96 %), *n*-hexane (99 %) (Merck, Germany).

b) Collection of plant the sample

The whole plant of *Cleome rutidosperma* was collected from the premises of the University of Port Harcourt, Choba, Port Harcourt, Nigeria. The plant was identified and authenticated by a Taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt. The reference specimen was prepared and deposited in the University of Port Harcourt central herbarium with a voucher specimen no. UPH/3/107

c) Pre-extraction treatment of sample

The plant sample was washed with purified water, air-dried in the shade (2 - 3 weeks), pulverized into a coarse powder (Binatone, China).

d) Extraction of plant material

The method of Bose, et al., 2007 was involved with minor adjustment. The powdered plant material was extracted with 96 % ethanol and n-hexane successively by Soxhlet extractor at 78 and 69° C respectively. The respective extracts were concentrated to a semi-solid paste with the aid of a rotatory-evaporator. Each residue was transferred into a 50 ml beaker and further dried with the aid of nitrogen gas. The dried extracts were stored separately in air-tight universal bottles and labelled appropriately for GC-MS analysis.

e) GC-MS analysis

Extracts were analyzed with Thermo-Finnigan Trace GC Ultra (Waltham, MA, USA) equipped with a

splitless injector, coupled to an ion trap mass spectrometer (MS) (Polaris Q) and coupled to a Xcalibur data software processor. Chromatographic separation was done with a HP-5MS capillary column of 30 m length \times 0.25 mm i.d. \times 0.25 μm film thickness (Agilent J & W Scientific Co., Folsom, CA, USA). The oven temperature was automated, which was initially held at 80 °C for 5 min, and was increased to 200°C at a rate of 20 °C/min, held for 5 min and then raised to 280°C at a rate of 10 °C/min and held for 2 min. The flow rate of the carrier gas (Helium, 99.99 % purity) was kept constant at 1.18 mL/min. Splitless injection mode at an injection temperature of 250°C was carried out at a pressure of 79.5 kPa. The linear velocity and total flow were 10.0 cm/s and 32.7 mL/min, respectively. The interface line and ion source temperatures were 260°C and 250°C, respectively.

III. Results and Discussion

a) GC-MS Analysis

Figures 1 and 2 show the GC-MS spectra of ethanolic and *n*-hexane extracts of the whole plant of *Cleome rutidosperma* respectively. The phytochemicals identified and their retention times, molecular weight, molecular formula and percentage peak area (as percentage composition) are presented in Tables 1 and 2. Their identification was based on the National Institute of Standards and Technology (NIST) library similarity index. The GC-MS analysis of the ethanolic and hexane extracts displayed the existence of 22 and 25 compounds correspondingly. These compounds were mostly esters, fatty acids, sterols, diketones and alcohols.

i. GC-MS Analysis of ethanolic extract of Cleome rutidosperma

Amonast the 22 compounds identified in the ethanolic extract of Cleome rutidosperma, thirteen of them were predominant. These were phthalic acid, di(2propylpentyl) ester (22.5 %), 8-Isopropyl-5-methyl-5, 6,7,8-tetrahydro-2,4-quinazolinedione (14.82)%), *n*-hexadecanoic acid (14.78 %), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol) (9.96 %), 9,9-Dimethoxybicyclo[3.3.1] nona-2, 4-dione (5.5 %), 1, 2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (5.19 %), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (4.24 %), (1S, 15S)-Bicyclo[13.1.0] hexadecane-2-one (3.45 %), hexadecanoic acid ethyl ester (2.97 %), retinal (vitamin A aldehvde) (2.54 %), 3-Methyl-2-(2-oxopropyl)furan (2.25 %), 1-hexyl-2-nitrocyclohexane (2.13 %) and tetradecanoic acid, 12-methyl ester (1.88 %). The structures of these compounds are presented in Figure 3. Most of these phytoconstituents have been found to be bioactive against some pathogens and illnesses. For instance, phthalic acid, di (2-propylpentyl) ester has been reported to exhibit strong anti-bacterial and antifungi properties (Osuntokun et al., 2017; Osuntokun and Gamberini, 2019). Also, 8-Isopropyl-5-methyl-5,6,7,8tetrahydro-2,4-quinazolinedione as a derivative of quinazolinone is likely to possess a broad spectrum of biological actives such as antibacterial, antifungal, anticonvulsant, anti-inflammatory, anti-HIV, anticancer and analgesic activities (Jafari, et al., 2016). Jafari, et al., 2016, stated that quinazolines and quinazolinone derivatives have presented promises of antimicrobial and cytotoxic activities. The compound *n*-hexadecanoic acid (palmitic acid) has been used successfully for skin and anti-inflammatory purposes (Aparna, et al., 2012; Korbecki & Bajdak-Rusinek, 2019). Phytol or 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol is an acyclic diterpene alcohol, a key constituent of chlorophyll and a common precursor for the assembly of synthetic forms of vitamin E and vitamin K1. It is an antifungal and antimalarial agent which is very active against Salmonella typhi (Morah & Apebende, 2018). In addition, it is also recognized to have anti-ulcer, antioxidant, inflammatory and diuretic properties (Soyingbe, et al., 2013). Likewise, phytol has been made known to modulate transcription in cells via transcription factors PPAR-alpha and retinoid X receptor (RXR) and as well as a regulator of lipid metabolism (Adnan, et al., 2019). No biological activity have been reported for 9, 9-Dimethoxybicyclo [3.3.1] nona-2, 4-dione. Govindappa, et al., 2014 reported 1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester to have revealed robust α -Glucosidase inhibition and in-vivo hypoglycemic outcome, while 3-Methyl-2-(2-oxopropyl) furan - a furan derivative is antipyretic, anti-inflammatory and hepatoprotective (Borthakur, et al., 2020). The phytochemical (R)-(-)-14-Methyl-8-hexadecyn-1-ol, is an alkvnol. Although, there is no precise documentation on its bioactivity, but, it has been described as amongst predominant phytochemicals in plant extracts pharmacological responsible for actions like diabetics. antibacterial, anti-inflammatory, antihistaminic, hepatoprotective, hypocholesterolemic (Sellamuthu, et al., 2009; Vinay, et al., 2014). Retinal, also recognized as vitamin A -aldehyde - an oxidized metabolite of retinol, with a polyene chromophore, being the most vital chemical for animal vision (Tsin, et al., 2018) was also found in the whole plant of Cleome rutidosperma.

ii. GC-MS Analysis of n-hexane extract of Cleome rutidosperma

Eleven (11) phytochemicals were predominant amongst the 25 compounds identified in the *n*-hexane extract. The following phyto-compounds were also found in the ethanolic extract and have been discussed, they are - (R)-(-)-14-Methyl-8-hexadecyn-1-ol (22.91 %), hexadecanoic acid ethyl ester (14.52 %), 1-hexyl-2-nitrocyclohexane (4.97 %) and 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (Phytol) (4.30 %). The other compounds found in the *n*-hexane extract were 2-methyl-5-5-

diphenyl-4-(methylthio)imidazole (18.22 %), linoleic acid ethyl ester (8.11 %), phthalic acid, isobutyl octadecylester (3.92 %), 4-fluoro-1-methyl-5-carboxylic acid ethyl ester (3.69 %), octadecanoic acid 17-methylmethyl ester (3.08 %), Estra-1,3,5(10)-trien-17β-ol (2.25 %) and 2, 4-dimethyl-7-oxo-4,7-dihydro-triazolo (3, 2,c)triazine (1.84 %). The phytochemical 2-methyl-5-5diphenyl-4-(methylthio) imidazole is an imidazole derivative. Imidazole and its derivatives are reported to be biologically active and have found use in the management of health disorders for their anti-fungal and anti-bacterial effects (Zampieri, et al., 2007; Shingalapur, et al., 2009; Sharma, et al., 2009), anti-inflammatory and analgesic activity (Puratchikodya & Doble, 2007; Achar, et al., 2010), anti-tubercular influence (Gupta, et al., 2004; Shingalapur, et al., 2009; Jyoti, et al., 2009), antidepressant actions (Hadizadeh, et al., 2008), anticancer activity (Congiu, et al., 2008; Ozkay, et al., 2010; Refaat, et al., 2010), antiviral effects (Sharma, et al., 2009; Tonelli, et al., 2010) and anti-leishmanial activity (Shalini, et al., 2010). The imidazole derivative constituted over 18 % of the *n*-hexane plant extract – being the second most predominant phytochemical found. Linoleic acid ethyl ester or Ethyl linoleate (ELA) - an essential fatty acid was also found. ELA is used as a vital constituent in many cosmetics, primarily due to its anti-inflammatory properties (National Center for Advancing Translational Sciences, NCATS). ELA is employed to aid neutrophils in the release of the reactive oxygen species (ROS) due to excess of bacteria and thus inhibits hyperkeratinization induced by lack of linoleic acid (Alarcon, et al., 2020). In addition it has been found to be used as parenteral injection for the treatment of ailments where there is a high plasma-cholesterol level of the blood (Feng, et al., 2020). 4-fluoro-1-methyl-5-carboxylic acid ethyl ester was reported by Hussein, et al., (2016) to have anti-protozoal and anti-mycobacterial.

GC-MS identification of phytochemicals in ethanolic and n-hexane extracts

The MS fragmentation patterns of key phytochemicals in the extracts were identified using the NIST library. Below are selected MS obtained for some compounds present in both ethanolic and n-hexane extracts, matched with that of the NIST library (Figures 4a-7b).

IV. CONCLUSION

This study showed the presence of biologically active components in Cleome rutidosperma through GC-MS analysis. Bioactives such as phthalic acid, di(2propylpentyl) ester, 8-Isopropyl-5-methyl-5,6,7,8tetrahydro-2,4-quinazolinedione, 3, 7. 11, tetramethyl-2-hexadecen-1-ol, 2-methyl-5-5-diphenyl-4-(methylthio)imidazole, 4-fluoro-1-methyl-5-carboxylic acid ethyl ester and others were identified by the GC-MS profiling of both ethanolic and n-hexane extracts of Cleome rutidosperma. Our findings has justified its use in traditional and herbal therapy. More bioactive compounds were identified through the *n*-hexane extractant. The myriads of bioactives existing in the investigated plant could have synergistic effect and this may boost its therapeutic ability and could also be utilized for the amelioration of different health disorders. Therefore, Cleome rutidosperma has high medicinal potentials.

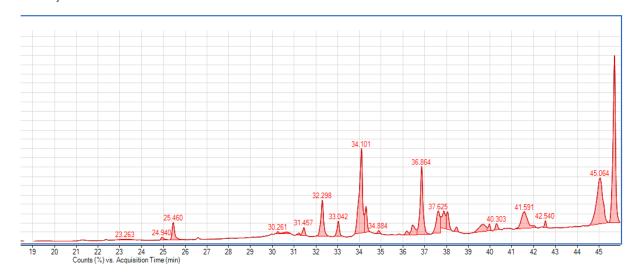


Figure 1: GC-MS chromatogram of ethanolic extract of Cleome rutidosperma whole plant

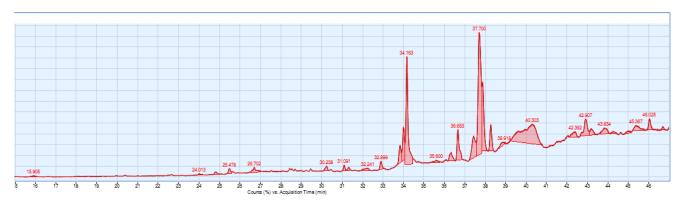
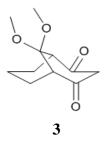
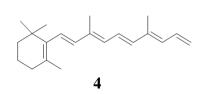


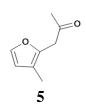
Figure 2: GC-MS chromatogram of hexane extract of Cleome rutidosperma whole plant



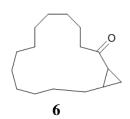
Phthalic acid, di (2-propylpentyl) ester 8-lsopropyl-5-methyl-5, 6, 7, 8-tetrahydro- 9, 9-Dimethoxybicyclo [3.3.1]

nona-2, 4-dione





2, 4-quinazolinedione

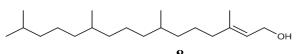


Retinal (Vitamin A aldehyde)

3-Methyl-2-(2-oxopropyl) furan hexadecane-2-one

(1S, 15S)-Bicyclo [13.1.0]

(R)- (-)-14-Methyl-8-hexadecyn-1-ol

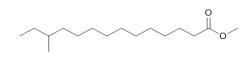


8 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol)

1-Hexyl-2-nitrocyclohexane

Hexadecanoic acid, ethyl ester

11 n-Hexadecanoic acid



12

Tetradecanoic acid, 12-methyl ester

1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester

15

17 Linoleic acid ethyl ester

19

2-Methyl-5, 5-diphenyl-4-(methythio)imidazole

0 21

7- Hexadecenal, (Z) -

16

2, 4-bis(1,1-dimethylethyl)-Phenol

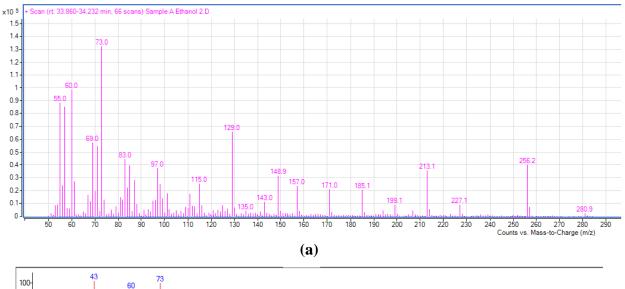
Octadecanoic acid, 17-methyl ester

18

4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester

2, 4-Dimethyl-7-oxo-4, 7-dihydro-triazolo (3,2-c)triazine

Figure 3: Chemical Structures of major constituents identified in ethanolic and hexane extracts of Cleome rutidosperma whole plant using GC-MS



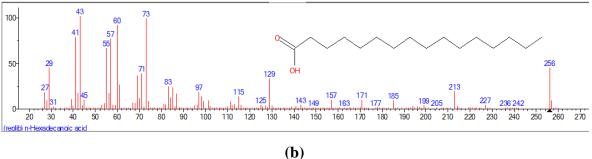


Figure 4: Mass spectra of n-hexedecanoic acid (a) in ethanolic extract (b) NIST library

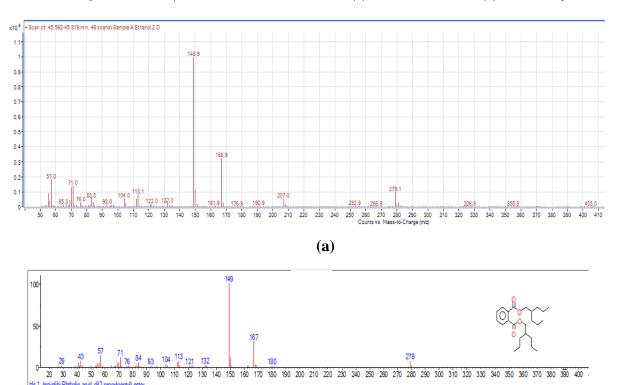
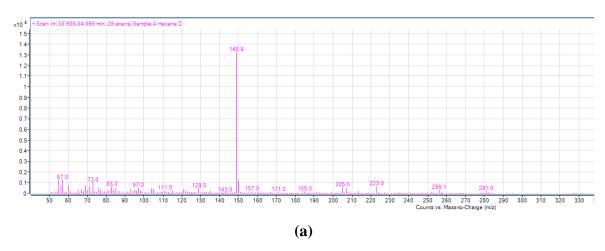


Figure 5: Mass spectra of Phthalic acid, di (2-propylpentyl) ester (a) in ethanolic extract (b) NIST library

(b)



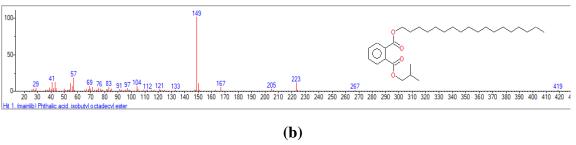
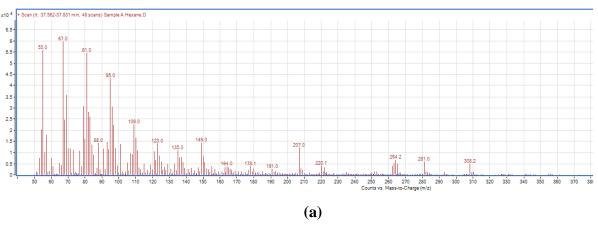


Figure 6: Mass spectra of Phthalic acid, isobutyl octadecyl ester (a) in hexane extract (b) NIST library



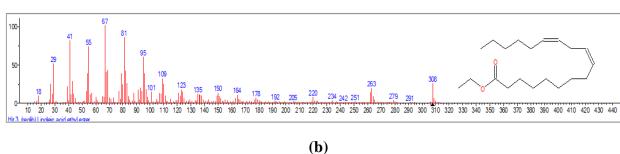


Figure 7: Mass spectra of Linoleic acid ethyl ester (a) in hexane extract (b) NIST library

Table 1: Compounds identified in ethanolic plant extract by GC-MS

S/N	RT	Name of compound	M. formula	M.wt	Peak Area (%)
1	23.263	S(+)-1-Cyano-2-methyl-azetidine	$C_5H_8N_2$	96.068	0.35
2	24.94	2, 5-bis(1, 1-dimethylethyl)-Phenol	C ₁₄ H ₂₂ O	206.167	0.45
3	25.46	2, 4- bis(1, 1-dimethylethyl)-Phenol	C ₁₄ H ₂₂ O	206.167	2.32
4	30.261	6, 10, 14-Trimethyl-pentadecan-2-ol	C ₁₈ H ₃₈ O	207.292	0.27
5	30.667	Carbamic acid, hydroxyl-, ethyl ester	$C_3H_7NO_3$	105.043	0.42
6	31.228	14-Heptadecenal	$C_{17}H_{32}O$	252.245	0.23
7	32.298	1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	C ₁₆ H ₂₂ O ₄	278.152	5.19
8	33.042	Tetradecanoic acid, 12-methyl ester	$C_{16}H_{32}O_2$	256.240	1.88
9	34.100	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.240	14.78
10	34.306	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.271	2.97
11	36.183	2-Methyl –E-7-octadecene	C ₁₉ H ₃₈	266.297	0.57
12	36.447	1-Hexyl-2-nitrocyclohexane	C ₁₂ H ₂₃ NO ₂	213.173	2.13
13	36.864	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296.308	9.96
14	37.625	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252.245	4.24
15	37.883	(1S, 15S)-Bicyclo[13.1.0] hexadecane-2-one	C ₁₆ H ₂₈ O	236.214	3.45
16	38.054	3-Methyl-2-(2-oxopropyl)furan	$C_8H_{10}O_2$	138.068	2.25
17	38.461	3-Acetoxypentadecane	$C_{17}H_{34}O_2$	270.256	0.51
18	39.685	Retinal (Vitamin A aldehyde)	C ₂₀ H ₂₈ O	284.214	2.54
19	40.303	7-Hexadecanal, (Z)	C ₁₆ H ₃₀ O	238.230	0.73
20	41.591	9,9-Dimethoxybicyclo[3.3.1] nona-2, 4-dione	C ₁₁ H ₁₆ O ₄	212.105	5.50
21	45.064	8-Isopropyl-5-methyl-5, 6,7,8-tetrahydro-2,4-quinazolinedione	$C_{12}H_{18}N_2O_2$	222.137	14.82
22	45.716	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.277	22.05

Table 2: Compounds identified in hexane plant extract by GC-MS

S/N	RT	Name of compound	M. formula	Mw.t	Peak Area (%)
1	15.905	2-Methyl-1-undecanol	C ₁₂ H ₂₆ O	186.198	0.02
2	24.808	2-Hexyl-1-octanol	$C_{14}^{}H_{30}^{}O$	214.230	0.38
3	25.478	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.167	0.69
4	26.702	2-Methyl-E-7-octadecene	C ₁₉ H ₃₈	266.297	1.21
5	30.238	2-Hexyl-1-octanol	$C_{14}^{}H_{30}^{}O$	214.230	0.65

6	31.091	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296.308	0.53
7	32.241	(Phytol) 3-Methyl-2-(2-oxopropyl)furan	C ₈ H ₁₀ O ₂	138.068	0.66
8	32.899	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.261	0.98
9	33.832	Estra-1,3,5(10)-trien-17β-ol	C ₁₈ H ₂₄ O	212.105	2.25
10	33.998	Phthalic acid, isobutyl octadecyl ester	$C_{30}^{}H_{50}^{}O_{4}^{}$	474.371	3.92
11	34.163	Hexadecanoic acid, ethyl ester	$C_{18}^{}H_{36}^{}O_{2}^{}$	284.272	14.51
12	36.326	1-Hexyl-2-nitrocyclohexane	C ₁₂ H ₂₃ NO ₂	213.173	1.48
13 14	36.653 37.419	3,7,11,15-Tetramethyl-2-hexadecen-1-ol 1-Hexyl-2-nitrocyclohexane	$C_{20}H_{40}O$ $C_{12}H_{23}NO_{2}$	296.308 213.173	4.30
15	37.700	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252.245	4.97 22.91
16	37.871	Linoleic acid ethyl ester	$C_{20}^{}H_{36}^{}O_{2}^{}$	308.272	8.11
17	38.272	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}^{}H_{40}^{}O_{2}^{}$	312.303	3.08
18	38.918	Acetic acid, trifluoro-, 3,7-dimethyloctyl ester	$C_{12}H_{21}F_3O_2$	254.149	0.93
19	40.303	2-Methyl-5,5-diphenyl-4-(methylthio)imidazole	C ₁₇ H ₁₆ N ₂ S	280.103	18.22
20	42.392	7-Hexadecenal, (Z)-	C ₁₆ H ₃₀ O	238.230	1.38
21	42.907	4-Fluoro-1-methyl-5-carboxylic acid, ethyl ester	C ₇ H ₉ FN ₂ O ₂	172.065	3.68
22	43.193	exo-1,2-O-Ethylidene- α -d-erythrofuranose	$C_{6}H_{10}O_{4}$	146.058	1.29
23	43.834	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c) triazine	$C_6H_7N_5O$	165.065	1.84
24	44.228	Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-benzodioxol-5-yl)-6-methyl-5-(2-propenyl)-, [1R-(6-endo,7-exo,8-syn)]-	C ₂₁ H ₂₄ O ₇	388.152	0.28
25	45.001	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	C ₁₄ H ₂₂ O	206.167	0.53

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Conflict of interest

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