GC-MS ANALYSIS OF BIO-ACTIVE COMPOUNDS IN ETHANOLIC EXTRACT OF DETARIUM SENEGALENSES

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ABSTRACT
Phytochemicals contain valuable therapeutic compounds which have been used in the treatment of several ailments. Some of which have been used as antibacterial, antifungal, anticonstipative, antispasmolytic, antiplasmodial and antioxidant agents as well as in the chemical and food industries. The aim of this study was to determine the chemical composition of the ethanol extract of the stem bark of D. senegalense using GC–MS. The stem bark was collected, air-dried, crushed and milled to obtain a powder. Standard extraction methods were used to obtain the extract. GC–MS analysis was carried out on the extract using GC 7890B, MSD 5977A, Agilent Tech. 16 compounds were identified among which were 3-ethyl-3-heptanol which showed the highest peak area (35.30 %) and the lowest peak area was dodecyl nonyl ether (0.31%). The other major compounds identified were 1,2,3,4-tetradecanetetrol, D-Fucose, 6-hepten-3-ol, 3-ethyl-3-heptanol, 4-heptenoic acid, methyl ester, 3-methyl-2-(2-oxopropyl) furan, docosanoic acid nonyl ester. These phytochemicals may be responsible for the medicinal efficacy of the stem bark of the plant as claimed by traditional practitioners and can they can be used as sources of therapeutic drugs as well as in the chemical industries.

Keywords: Detarium senegalenses; GC–MS; Phytochemicals; Therapeutic drug

INTRODUCTION

Phytochemicals are very important in plants because they play major roles in their growth and overall development. They are important in the protection mechanism of plants against harmful insects and microorganisms as well as the ultraviolet rays. Phytochemicals in plants also have physiological and biochemical functions. Some of the phytochemicals found in plants belong to some of these classes: flavonoids, alkaloids,

glycosides, tannins, saponins, and phenol (Abdulrahman, 2021; Savoia, 2012). Outside the plants, these chemicals can be used in a variety of ways in the chemical, pharmaceutical and in the food industries. Detarium senegalense is a deciduous tree. It has a short bole and a large very leafy crown. It can be up to 36 tall (Lebrun and Stork 2015). The fruit which may be toxic has a green flavoured mesocarp and it has been used as a sweetener or as a substitute for sugar. It also has medicinal properties. D. senegalense has rich alkaloid in its wood (Cisse et al., 2010; Akah et al., 2012). The stem decoction is taken as a remedy for gonorrhoea and the fresh stem is used for the treatment of snakebite (Afolabi and Afolabi, 2013). Studies on the nutritional and chemical composition of the fruits of D. senegalense have been reported (Cisse et al., 2010; Wang et al., 1996). The antitrypanosomal activity of the chemical compounds from the leaves of have been reported (Cisse et al., 2010; Afolabi, and Afolabi, 2013; Wang et al., et al., 1996). About 80% of the world population particularly in the rural areas depend on plant-based medicines as sources of primary health care (Gideon, 2015). Some phytochemicals possess valuable therapeutic activities and have been used as antibiotics, antifungal, anticonstipative, spasmolytic, antiplasmodial and antioxidant activities, (Manivachagam et al., 2008). Antiprotozoal activities of medicinal plants have also been reported (Avwioro, 2010). The aim of this study was to determine the chemical composition of the ethanol extract of stem bark of D. senegalense using GC–MS analysis to enable a clearer understanding of its application in the food and pharmaceutical industries. Ethanol solvent was purposely used for extraction in this study due to its high polarity in extracting polar phytochemicals.

MATERIALS AND METHODS

Collection of plant sample

Fresh stem bark of Detarium senegalense was collected from its growing habitat in February 2021 in Chaza village, Suleja Local Government Area, Niger State, Nigeria.

Identification of Plant

Detarium senegalense was identified and authenticated by Mallam Ibrahim Muazzam (an ethnobotanist) of the Department of Medicinal Plants and Traditional Medicine, National Institute of Pharmaceutical Research and Development, Abuja Nigeria. A voucher specimen number NIPRD/H/7082 was allocated to it and deposited at the Herbarium National Institute of Pharmaceutical Research and Development Abuja, Nigeria. The plants were transported to the Department of Pharmacology Laboratory, Rivers State University, Port-Harcourt for extraction process.

Preparation and extraction of plant sample

The stem bark of Detarium senegalense was rinsed in tap water, cut into tiny bits of about 2mm in diameter, air dried at room temperature (about 30 °C) for 28 days and milled to obtain a powdered sample. The pulverized plant sample (2000 g) was separately macerated successively in n-hexane, ethyl acetate and ethanol according to gradient polarity of the solvents. The maceration technique involved soaking the pulverize plant materials in an aspirator first with n-hexane (polarity = 0.009) and allowed to stand at room temperature for 3 days with frequent agitation. After exhaustive extraction with n-hexane, the procedure was repeated for ethyl acetate (polarity = 0.228) and ethanol (polarity = 0.762). The ethanol

extract was then used for the gas chromatography–mass spectrometry (GC–MS) analysis.

GC–MS analysis

The GC–MS analysis of ethanol stem bark extract of Detarium senegalense was carried out on a HP-5ms Ultra Inert (Agilent 19091S-433UI). The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 m × 250 µm × 0.25 µm film. The carrier gas used is Helium with a low of 1.20 ml/min and an injection volume of 2µl was employed. The injector was operated at 325 °C at 70eV and the oven temperature was programmed as follows: 70 °C to 250 °C (5°C/minute), then gradually increased to 300 °C at 1min. (30°C/minute) and total run time was 65 min (Giera et al., 2012).

RESULTS

Identification of components

The result obtained from the GC–MS was interpreted by comparing it with known components in the database of National Institute Standard and Technology (NIST). The components identified are presented in table 1, while the GC chromatogram is presented in Figures. 1 through 13.

Table 1 Phytoconstituents of the ethanol stem bark extract of D. senegalense

S/N	Retention Time (RT)	Compounds	Molecular Formulae	Molecular Weight (g/mol)	Qual	Peak Area (%)
1	14.859	1,2,3,4-Tetradecanetetrol; Methyl 6-methyl heptanoate, Octanoic acid; 8-hydroxyoctanoate	$C_{14}H_{30}O_4$; $C_9H_{18}O_2$; $C_9H_{18}O_3$	262.39; 158.24; 174.24	14 12 12	13.46
2	23.941	D-Fucose; Thiazole; 4-Ethoxy-2-butanone	$C_6H_{12}O_5$; C_3H_3NS; $C_6H_{12}O_2$	164.16; 85.13; 116.16	35 30 25	10.68
3	24.486	D-Fucose; alpha -L-Galactopyranoside; methy l 6-deoxy-Heptanoic acid	$C_6H_{12}O_5$; $C_7H_{14}O_6$; $C_{15}H_{15}N_7O_2$	164.16; 194.18; 325.33	43 42 38	2.03
4	24.630	alpha -L-Galactopyranoside; methyl 6-deoxy-Heptanoic acid; o-Acetyl-L-serine	$C_7H_{14}O_6$; $C_{15}H_{15}N_7O_2$; $C_5H_9NO_4$	194.18; 325.33; 147.13	40 38 25	1.23
5	24.712	D-Fucose; Pentanoic acid; 3-methyl- Heptanoic acid	$C_6H_{12}O_5$; C5H10O; $C_8H_{16}O_2$	164.16; 102.1317; 144.21	43 38 32	0.83
6	24.740	alpha -L-Galactopyranoside; methyl 6-deoxy-Octanoic acid; 4,5-dihydro-2-methyl-	$C_7H_{14}O_6$; $C_9H_{16}O_2$; $C_{12}H_{20}N_2O_{12}$	194.18; 156.22; 384.29	33 27 25	0.42
7	24.863	2-Deoxy-D-glucose; alpha -L-Galactopyranoside; methyl 6-deoxy-Heptanoic acid	$C_6H_{12}O_5$; $C_7H_{14}O_6$; $C_{15}H_{15}N_7O_2$;	164.16; 194.18; 325.33	42 40 23	1.77
8	24.885	alpha -L-Galactopyranoside; methyl 6-deoxy-D-Fucose Inositol, 1-deoxy-	$C_7H_{14}O_6$; $C_6H_{12}O_5$; $C_6H_{11}O_8P$	194.18; 164.16; 242.12	50 43 33	0.46
9	24.930	D-Fucose alpha -L-Galactopyranoside, methyl 6-deoxy- Thiazole, 4,5-dihydro-2-methyl-	$C_6H_{12}O_5$; $C_6H_{10}O_2$	114.14; 164.16	43 40 38	0.47
10	27.283	6-Hepten-3-ol 11-(2-Cyclopenten-1-yl) undecanoic Acid Methyl 3-butynoate	$C_7H_{14}O$	114.19	17	15.53
11	27.406	3-Ethyl-3-heptanol 2-Furanethanol;	$C_9H_{20}O$; $C_{12}H_{20}O_3$;	144.25; 212.28;	16 16	35.30

		5-ethenyltetrahyd ro-. alpha.,. alpha.,5-trimethyl-, cis-cis-1-Methyl-2-(2'-propenyl) cyclopropane				
12	27.920	4-Heptenoic acid, methyl ester, (E)- 8-Nonynoic acid, methyl ester Butanamide, 3, N-dihydroxy-	$C_8H_{14}O_2$; $C_4H_9NO_3$	142.20; 119.12	32 23 12	10.76
13	35.281	3-Methyl-2-(2-oxopropyl) furan; 4-Methyl-1-heptyn-3-ol Dichloroacetic acid; 2,2-dimethylpropyl ester	$C_8H_{10}O_2$; $C_6H_{10}O_3$; $C_5H_{11}NO_2$	138.16; 130.14; 117.15	22 14 14	19.72
14	38.086	Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester Decane, 1-(ethenyloxy)- 2-Piperidinone, N-[4-bromo-n-butyl]-	$C_{12}H_{24}O_2$; $C_{23}H_{46}O_4$; $C_9H_{16}BrNO$	200.32; 386.6; 234.13	43 30 27	1.96
15	38.157	Dodecyl nonyl ether; Tridecane	$C_{21}H_{44}O$; $C_{13}H_{28}$	312.6; $C_{13}H_{28}$	18 14	0.31
16	38.519	Docosanoic acid; nonyl ester 3-Butenamide 2-Butyn-1-ol, 4-methoxy-	$C_{22}H_{44}O_2$; $C_{11}H_{22}O_2$; $C_5H_8O_2$	340.6; 186.29; 100.12	11 9 9	-15.40

Phytochemical analysis of ethanol bark extract of Deterium senegalense. Fri Apr 08 14:21:41 2022

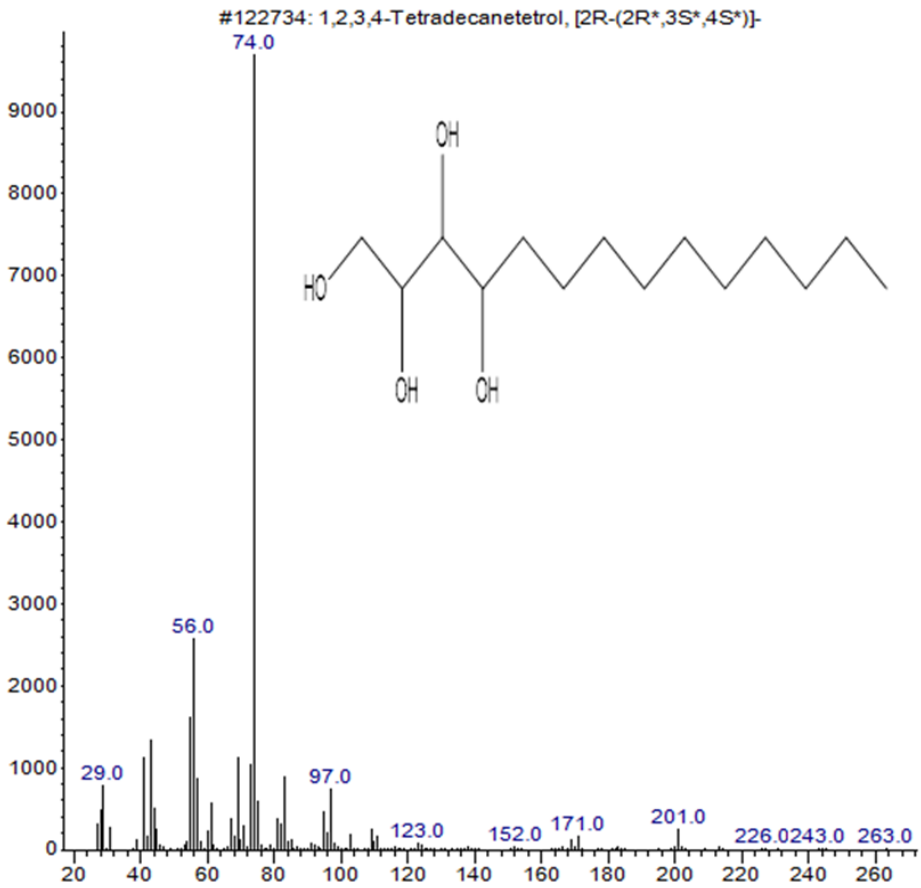

Figure 1. Spectral of 1,2,3,4-Decanetetrol, [2R-(2R*3S*,5S*)] in the Ethanol Bark Extract of Detarium senegalense.

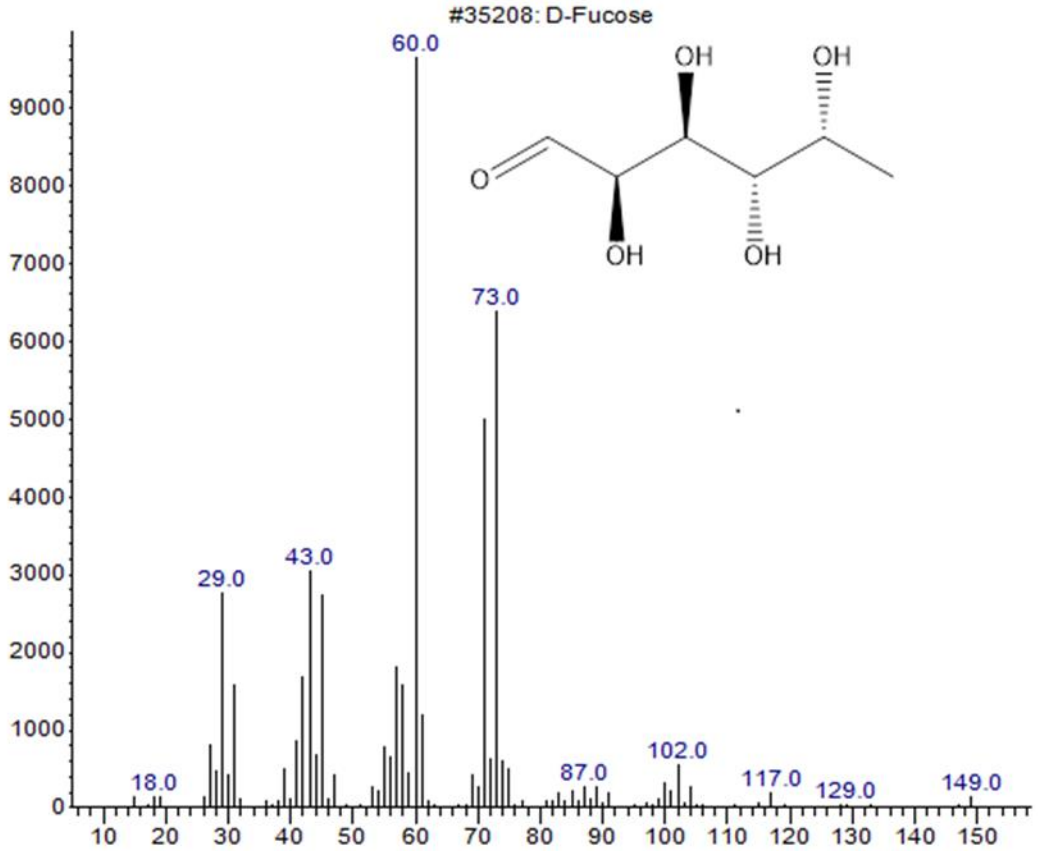

Figure 2. Spectral of D-Fucose in the Ethanol Bark Extract of Detarium senegalense

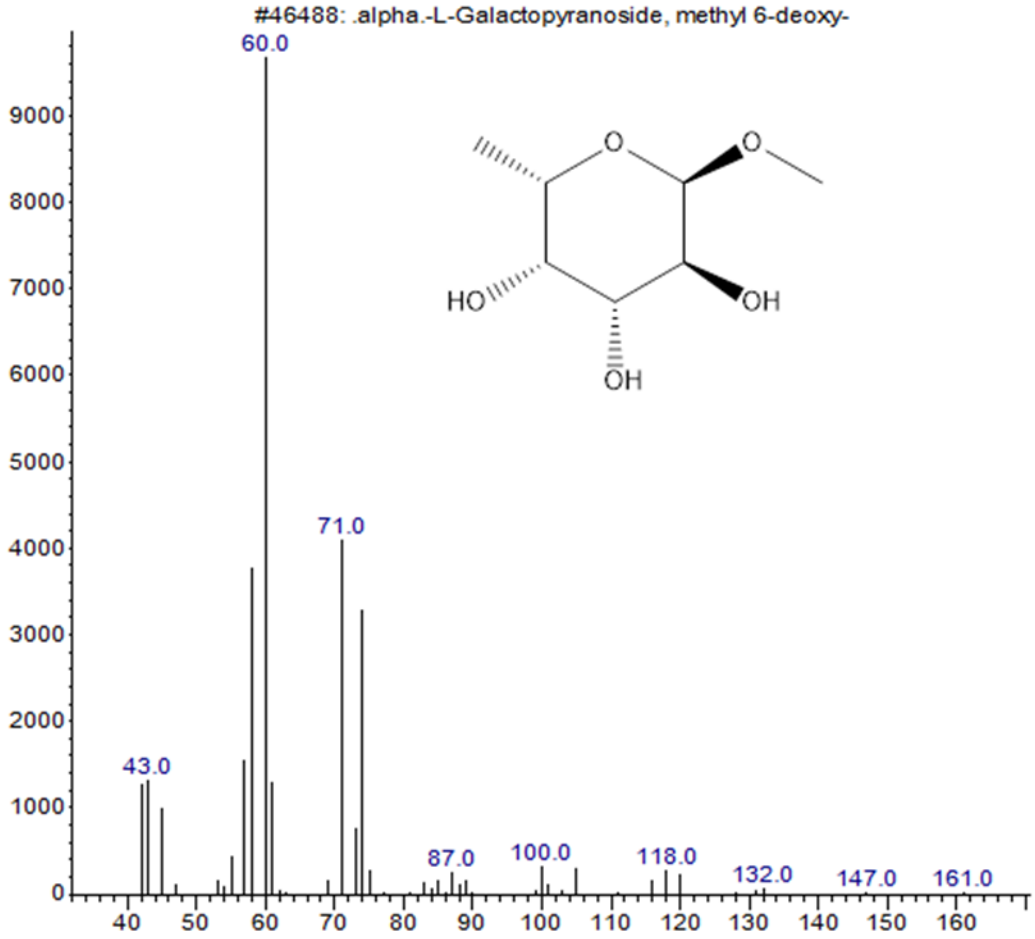

Figure 3. Spectral of the Alpha-L-Galactopyranoside, methyl 6-deoxy in the Ethanol Bark Extract of Detarium senegalense

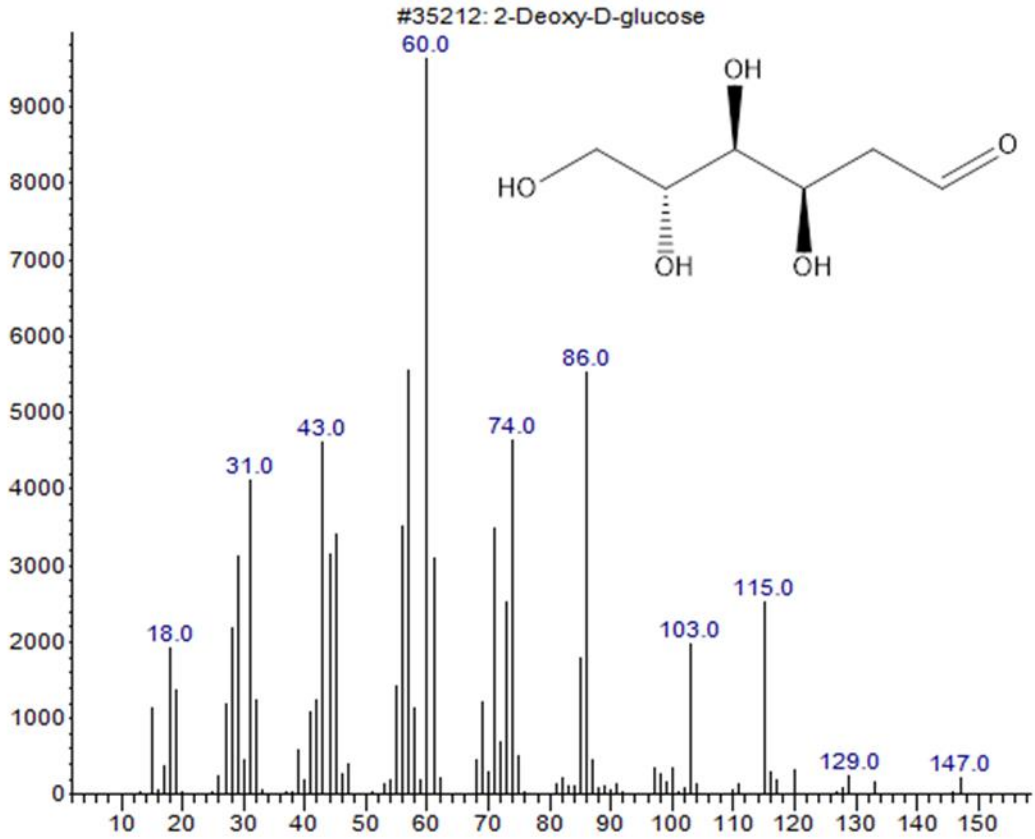

Figure 4. Spectral of the 2-deoxy-D-glucose in the Ethanol Bark Extract of Detarium senegalense

1,2,3,4-Decanetetrol
D-Fucose
Alpha-L-Galactopyranoside, methyl 6-deoxy
2-deoxy-D-glucose

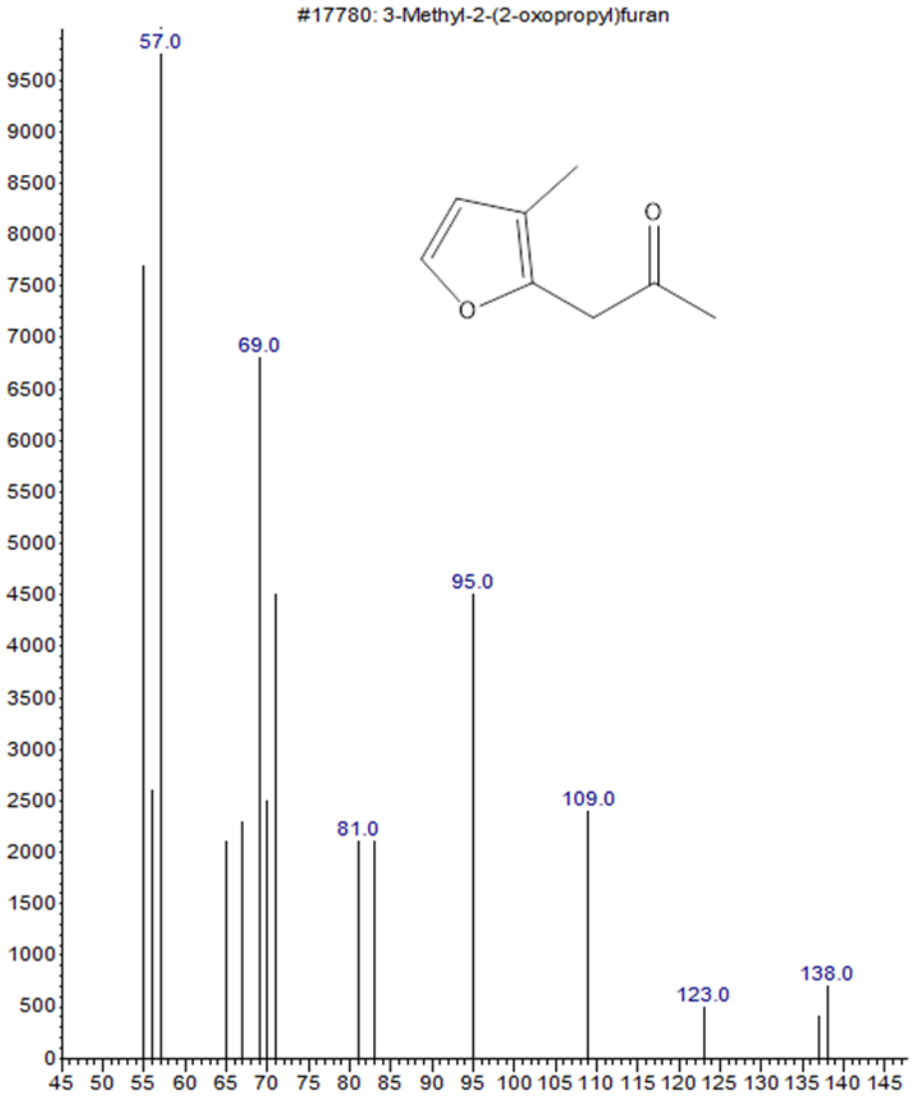

Figure 5. Spectral of the 3-methyl-2-(2-oxopropyl) furan in the Ethanol Bark Extract of Detarium senegalense

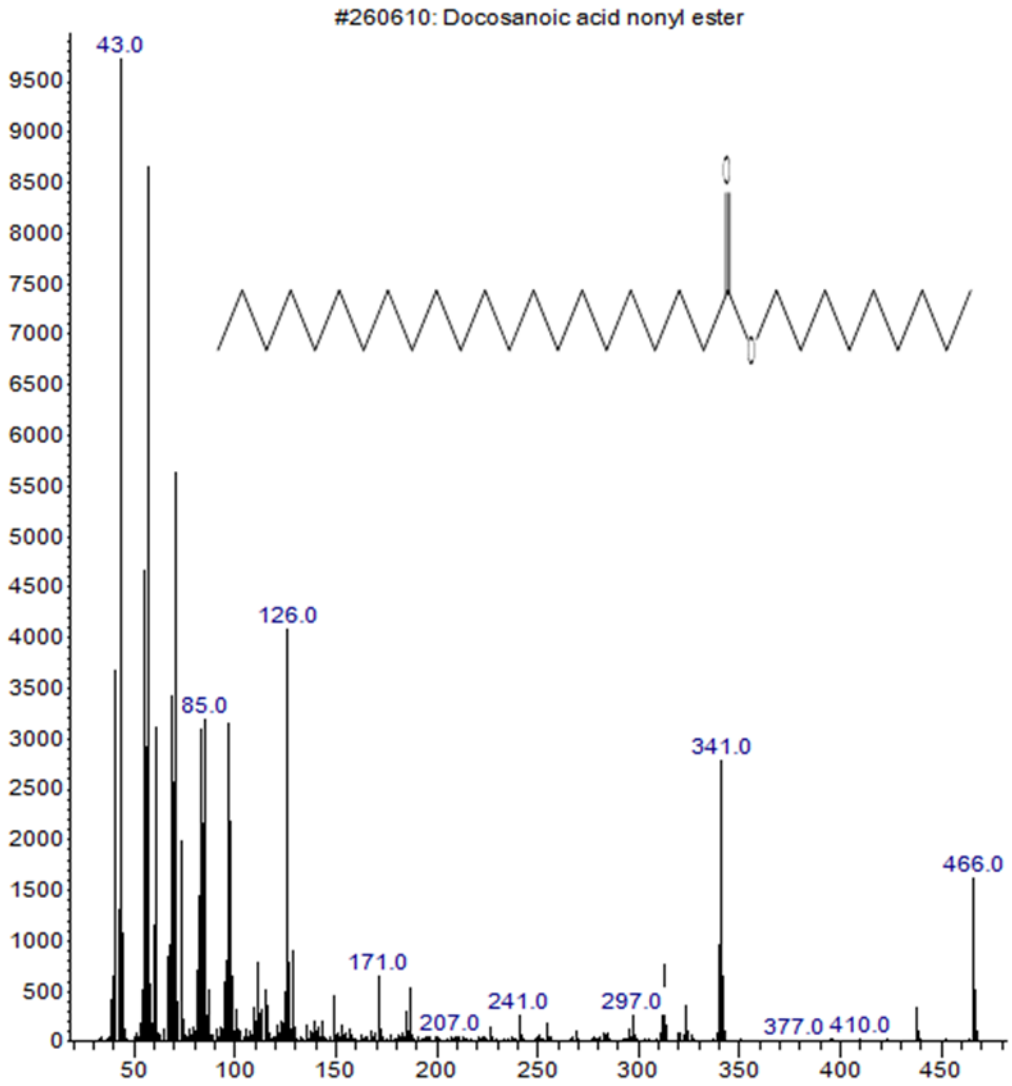

Figure 6. Spectral of Docosanoic acid, nonyl ester in the Ethanol Bark Extract of Detarium senegalense

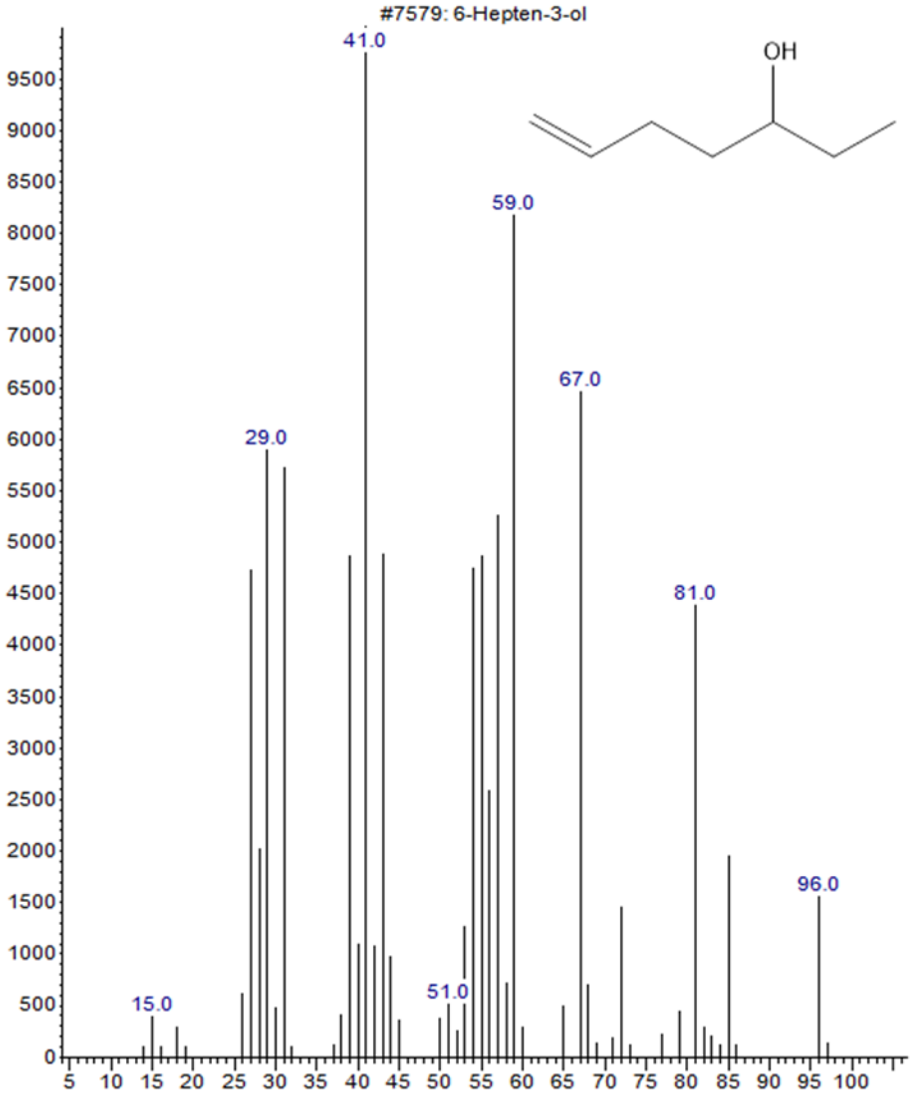

Figure 7. Spectral of the 6-Hepten-3-ol in the Ethanol Bark Extract of Detarium senegalense

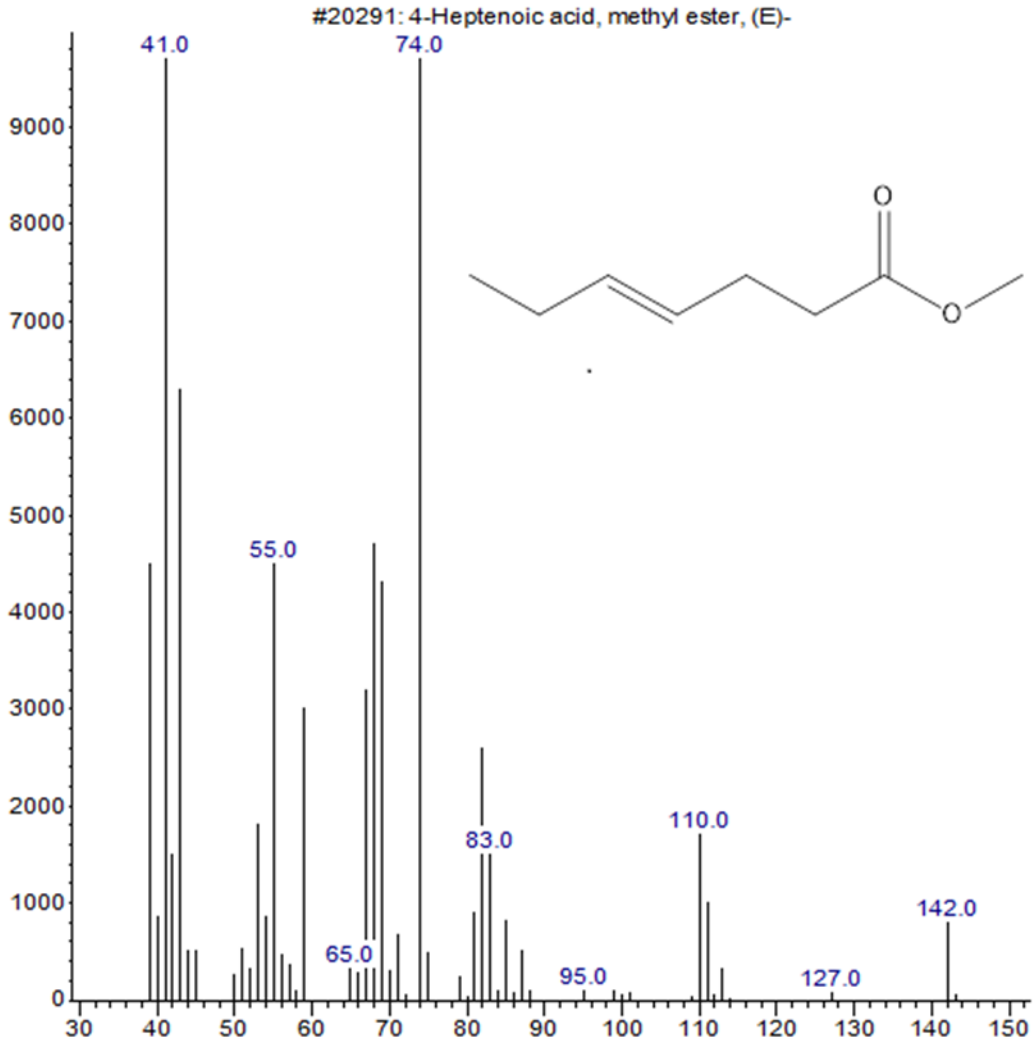

Figure 8. Spectral of the 4-Heptenoic acid, methyl ester in the Ethanol Bark Extract of Detarium senegalense

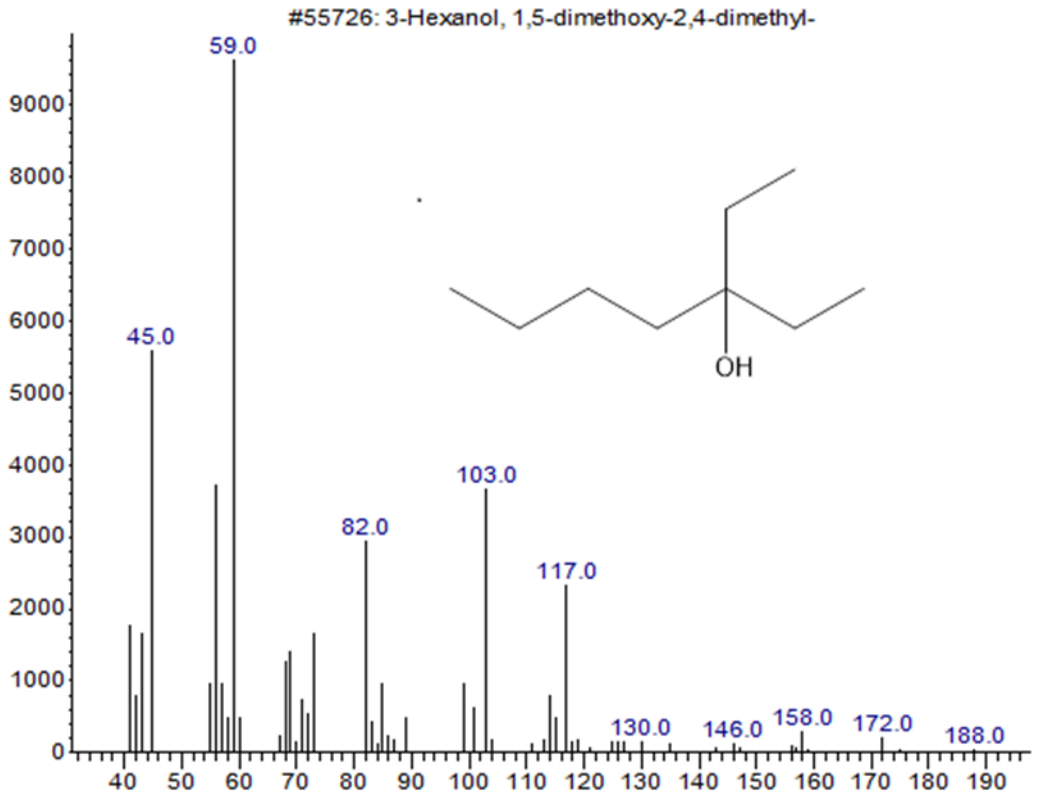

Figure 9. Spectral of the 3-Hexanol,1,5-dimethoxy-2,4-dimethyl in the Ethanol Bark Extract of Detarium senegalense

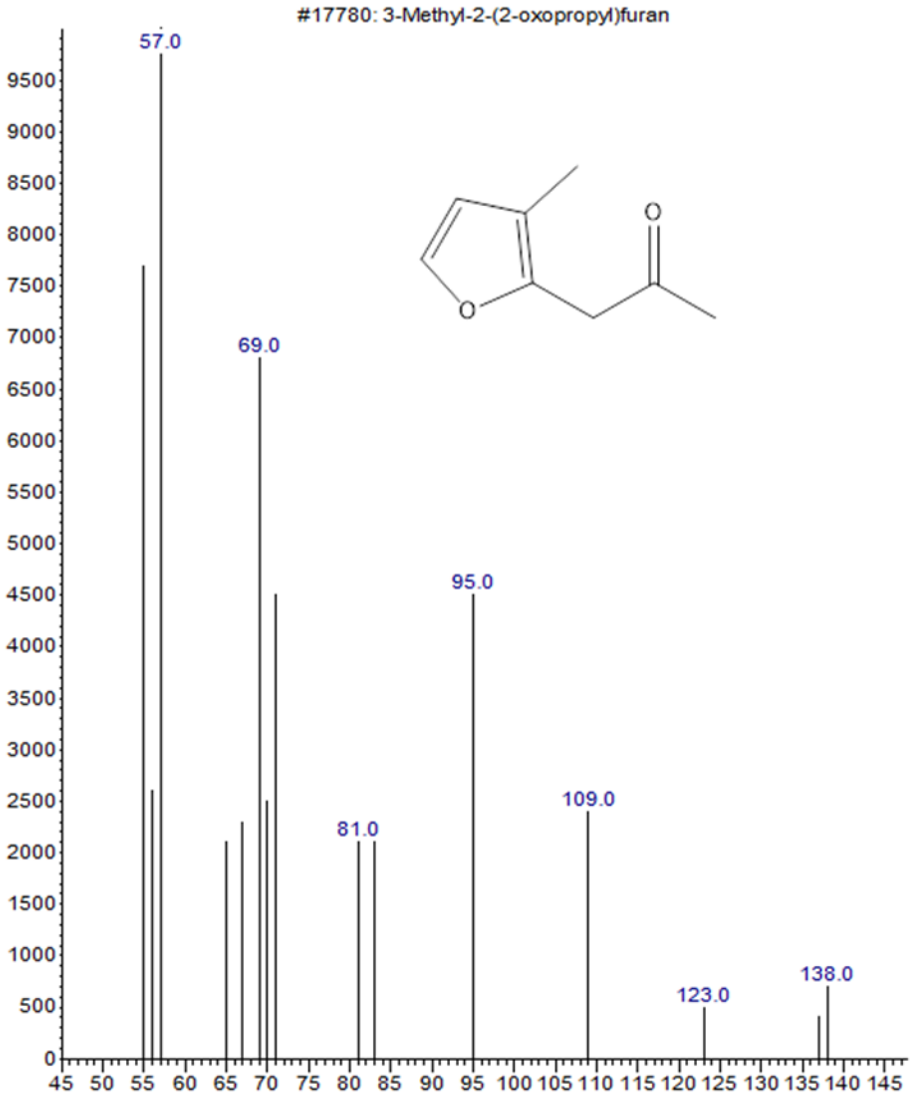

Figure 10. Spectral of the 3-methyl-2-(2-oxopropylfuran) in the Ethanol Bark Extract of Detarium senegalense

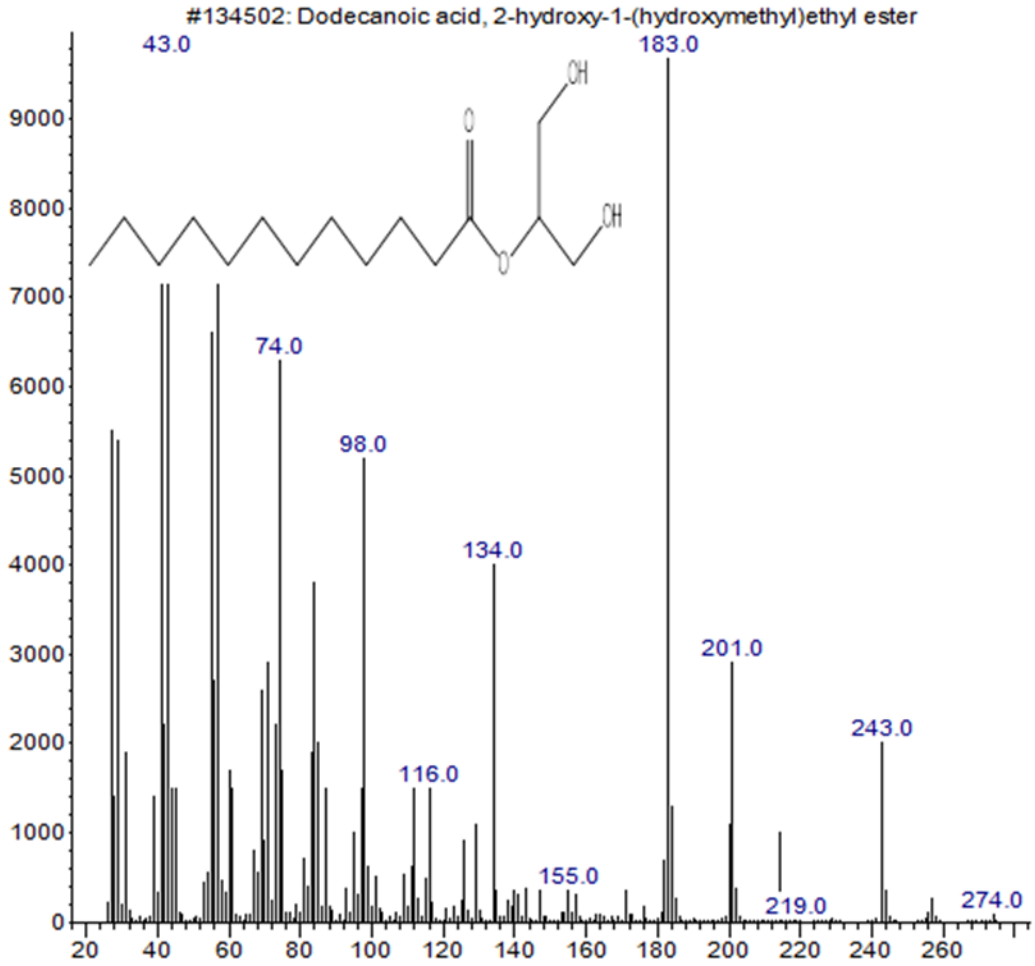

Figure 11. Spectral of Dodecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester in the Ethanol Bark Extract of Detarium senegalense

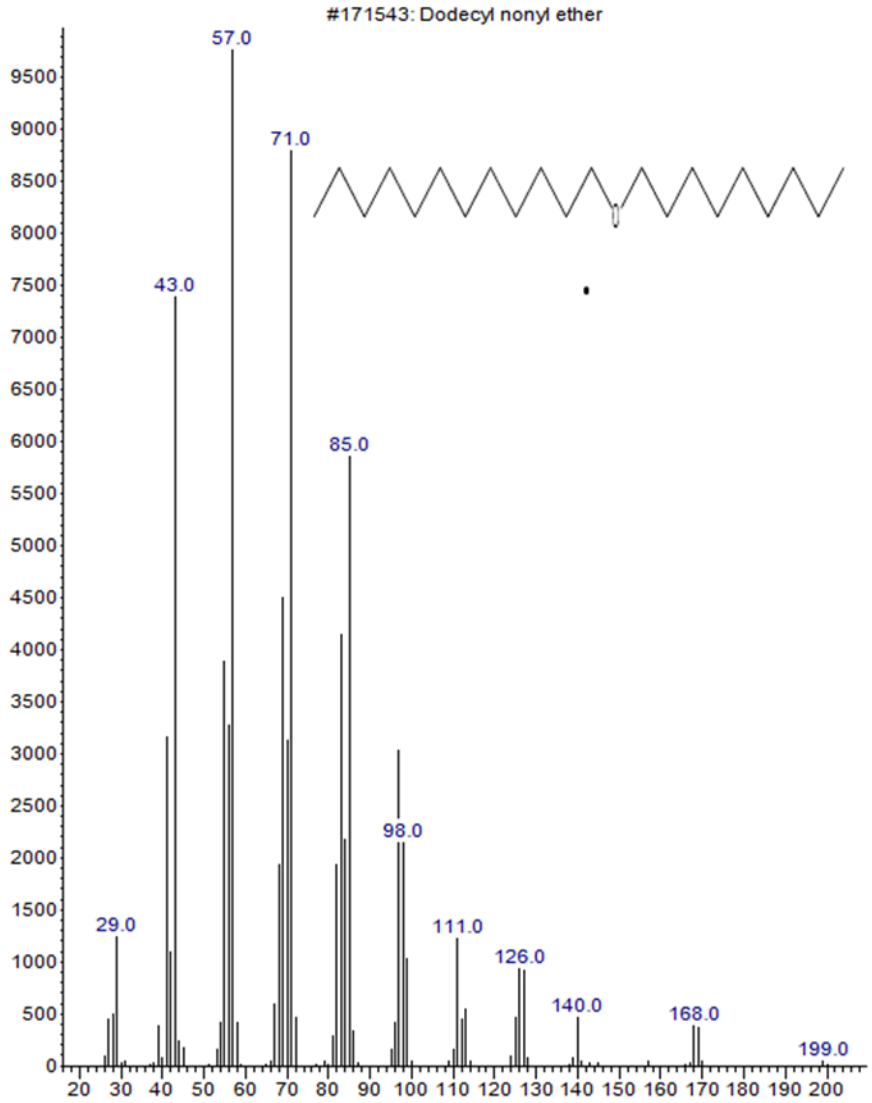

Figure 12. Spectral of Dodecyl nonyl ether in the Ethanol Bark Extract of Detarium senegalense

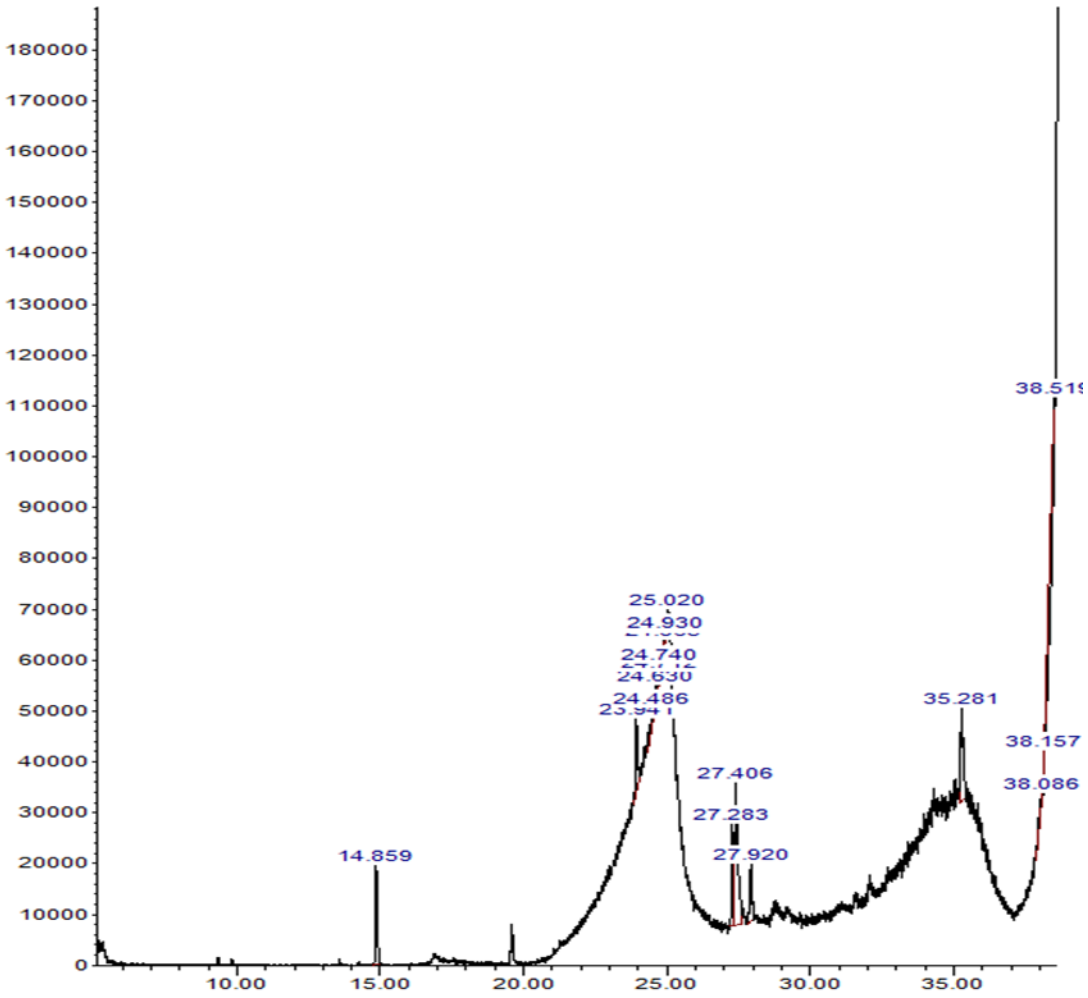

Figure 13. Different Spectral of the Bioactive Compounds in the Ethanol extract of Detarium senegalense

DISCUSSION

The bioactive components of several plant products have been studied worldwide (Mukherjee et al., 2001). These products have been extracted and evaluated for their physiological and biological properties (Kusuma et al., 2011). Many of these biologically active compounds which are of pharmaceutical importance do not act alone but with other bioactive substances that are diverse in nature [Reddy et al., 2020]. These compounds include phenols, steroids, tannins and alkaloids. They have various roles in the physiology of plants (Reddy et al., 2020). An important aspect of such studies is that they have been found to act as chemical leads in the manufacture of medicines for several ailments (Cragg et al., 2001). There are several methods for their extraction and characterization. Gas chromatography-mass spectrometry (GC-MS) consists of two main techniques which involves, first, separation of volatile compounds and second, identification and determination of molecular weights of separated components. The Gas chromatography technique separates multiple components of the mixture into distinct ones to allow for one component at a time to be analyzed. Mass spectrometry measures the mass-to-charge ratio (m/z) of charged particles. It is used to determine the molecular weight and chemical structures of molecules (Abdurrahmana, 2023; Cheriyedath, 2019). The data obtained from GC-MS contain mass spectra and the chromatogram which are used for the identification of the unknown compounds through qualitative and quantitative analysis. GC-MS has been used for bioanalysis of human and animal body fluids and for the detection of other substances such as drugs, barbiturates, alcohols and narcotics. It has also been used for the extraction of sunflower oil (Selvaraju et al., 2022). Species of the plant in genus Detarium have proven to be promising sources of plants which contain compounds with pharmacological properties. The GC–MS analysis of the ethanol extract of stem bark of Detarium senegalense revealed that it contains 1,2,3,4-Decanetetrol, D-Fucose, alpha-L-Galactopyranoside, methyl 6-deoxy, 2-deoxy-D-glucose, 3-methyl-2-(2-oxopropyl) furan, docosanoic acid, nonyl ester, 6-Hepten-3-ol, 4-Heptenoic acid, methyl ester, 3-hexanol,1,5-dimethoxy-2,4-dimethyl, 3-methyl-2-(2-oxopropylfuran), dodecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl and dodecyl nonyl ether. Plant extracts have been used extensively in medical (Avwioro, 2010; Gamde et al., 2022; Mokogwu et al., 2022; Muhammad et al., 2021) and pharmaceutical sciences (Onyije et al., 2012; Fowotade et al., 2017) as well as in the laboratory for histological diagnosis of diseases (Avwioro et al., 2006; Okorie et al., 2019). The latex of the stem of Jatropha gossypifolia has also been used as a haemostatic agent (Oduola et al., 2005; Oduola et al., 2007). Substances isolated from plants are useful in several ways (Okorie et al., 2020) and have used for therapeutic purposes particularly in the low income countries (Gideon, 2015). D. senegalense is rich in alkaloids (Akah et al., 2012). Its stem decoction is a useful remedy for the treatment of gonorrhoea and for the treatment of snakebite (Afolabi, and Afolabi, 2013). Studies have shown that it contains substances of nutritional value (Cisse et al., 2010). The leaves have been used in the treatment of trypanosomiasis (Cisse et al., 2010; Wang et al., et al 1996). The data obtained from the GC–MS analysis of the stem bark of D. senegalense may be a good source for the production of therapeutic drugs for some ailments. The industrial applications of some of the compounds isolated from D. senegalense have been reported in previous studies.

CONCLUSION

In the present study, D. senegalense have shown to have various secondary metabolites which possess many pharmacological properties. The GC-MS analysis showed the presence of 16 phytochemical constituents which contribute to the activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory, and other activities. Hence, the presence of these phytochemicals are responsible for their therapeutic effects.

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CONFLICT OF INTEREST: The authors report no conflict of interest.

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