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GC-MS Analysis of Diospyros montana (Roxb.) Leaves: A Medicinal and Poisonous Plant

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ABSTRACT

Diospyros montana (Roxb.) is a plant that contains significant levels of flavonoids, phenol, reducing sugars, terpenoids and other phytochemicals. This plant is belonging to the Ebenaceae family. This plant is known as Bistendu (Poisonous tendu) due to its dual medicinal and poisonous characteristics; hence, it was considered essential to understand its whole phytochemical profile. In the current investigation, metabolites in the methanolic extract of leaves from the D. montana (Roxb.) plant were thoroughly screened phytochemically using the GC-MS technique with an emphasis on forensics. The plant's fresh leaves methanolic extract contained 13major compounds and identified are Phenol, 4-ethenyl-2,6- dimethoxy-Sucrose, (E)-4-(3-Hydroxyprop-1- en-1-yl) -2-methoxyphenol, 4-((1E)-3-Hydroxy-1-propenyl)-2- methoxyphenol, Hexadecanoic acid, methyl ester, Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, Phytol, Linoelaidic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Octadecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester, Glycerol 1-palmitate, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 2H-1-Benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)-3, 4-dihyd.Therefore, a thorough phytochemical evaluation of metabolites may be helpful to forensic toxicologists investigating cases of plant poisoning. Moreover, because of the existence of phytochemicals and bioactive substances leaves can be utilized as a novel potential source of pharmaceutical drugs. The plant's leaves were chemically profiled using the GC-MS method.

INTRODUCTION

Diospyros montana (Roxb.) is an Ebenaceae family plant, distributed across the Malay Peninsula, China, India, Indonesia, and subtropical, tropical regions [1]. It is a tree with pubescent or glabrous young shoots, smooth bark, and slender stems. Simple petiolated leaves have an ovate-oblong shape, an acute apex, and a decurrent base, and are alternating, glabrescent or lightly pubescent [2]. When mature, the fruit is spherical, cherry-sized, and yellow. Numerous phytochemicals, including phenols, flavonoids, saponins, terpenoids, reducing sugars, and others, are present in *D. montana* [3] Furthermore, research has shown that this species phytochemicals have important pharmacological properties that are employed in the treatment of coughs, ulcers, hypersensitivity reactions, and snakebites [4,5].

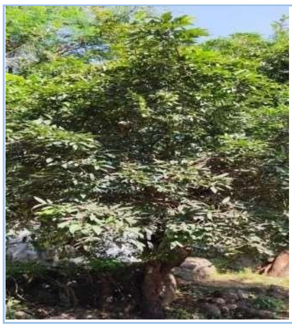

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Figure 1: *Diospyros montana* Plant

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Figure 2: Plant's Leaves

While some sources claim that the fruit is edible, others believe it to be toxic [6]. The water bearers, known as 'Bhistis', apply the fruits to the painful boils that typically develop on their hands. These fruits are reported to have been used by the Hillman of Travancore to poison fish. Crushed leaves are also used for this purpose in Chhota Nagpur, India. The relevant phytoconstituent for toxicity has to be investigated to ascertain whether fresh leaves of this plant are hazardous to fish, however, some toxicological studies havebeen reported. A preliminary range-finding test was used to determine the LC_{50} of fresh leaves using zebra fish. An LC_{50} of 1.37 g/0L was observed [7]. In contrast, studies have demonstrated the anti-leukemic, hypolipidemic, prostaglandin synthesis inhibitor, anticancer, anti-inflammatory, antimalarial and antiviral qualities of *D. montana* (Roxb.). Although its roots have been reported to be abortifacient, Indian traditional medicine uses its bark to cure jaundice and its gum to treat tuberculosis [8]. Because, this plant had both poisonous and medicinal properties, it was thought to be crucial to comprehend its entire phytochemical profile. The current work used a comprehensive phytochemical analysis of the plant's metabolites from a forensic perspective, and the results were applied to produce a chemical fingerprint for the leaves of the *Diospyros montana* (Roxb.) plant using the GC-MS technique.

2. EXPERİMENTAL SECTİON

2.1 Material &Methods

Collection and Identification of Sample

After D. montana attained maturity, fresh leaves were collected from the Patharia hill adjacent to the university campus in Sagar, Madhya Pradesh, India. The Department of Botany at Doctor Hari Singh Gour Central University (UGC-DSA/ASIST Sponsored Department) in Sagar, Madhya Pradesh, India, verified the authenticity of the plant and specimen copy of voucher number (BOT/H/04/100/04) has been deposited in the Department of Botany, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India.

Botanical Overview

D. montana is often known as Dheki, Makrol, Bombay Ebony, and other Indian famous names include Bistendu, Jagalkanti, Manjakara, Bankini, Vakkanai, Nanchimaram, Bali, and Malayakathitholi. The Scientific classification of *D. montana* is presented in Table 1.

Table 1: Scientific classification

Kingdom	Plantae
Clade	Tracheophytes
Order	Ericales
Family	Ebenaceae
Genus	Diospyros
Species	D. montana
Botanical name	*Diospyros montana* (Roxb.)

2.2 Preparation of Methanol Extract from Plant Leaf

After cleaning the leaves with tap water, they were rinsed with distilled water and allowed to shad dry at room temperature to eliminate any remaining moisture. The dried leaves were then ground into a powder and extracted using Soxhlet apparatus with methanol AR grade. The sample of dry leaves powder was placed within filter paper to create 20cm by 4.5cm cylindrical thimbles. After being filled with the sample, the thimbles were placed in the cylindrical sample container used in the Soxhlet apparatus and filled with methanol, an organic solvent. Methanol generated a colored solution when combined with the leaf sample; the colored solvent was extracted until it turned transparent. This extraction took over twenty hours to finish. The resulting extract was collected in 100 ml screw-capped vials, and concentrated extracts were kept at 40^{0}C for later use after the solvent was evaporated in a rotary evaporator.

2.3 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

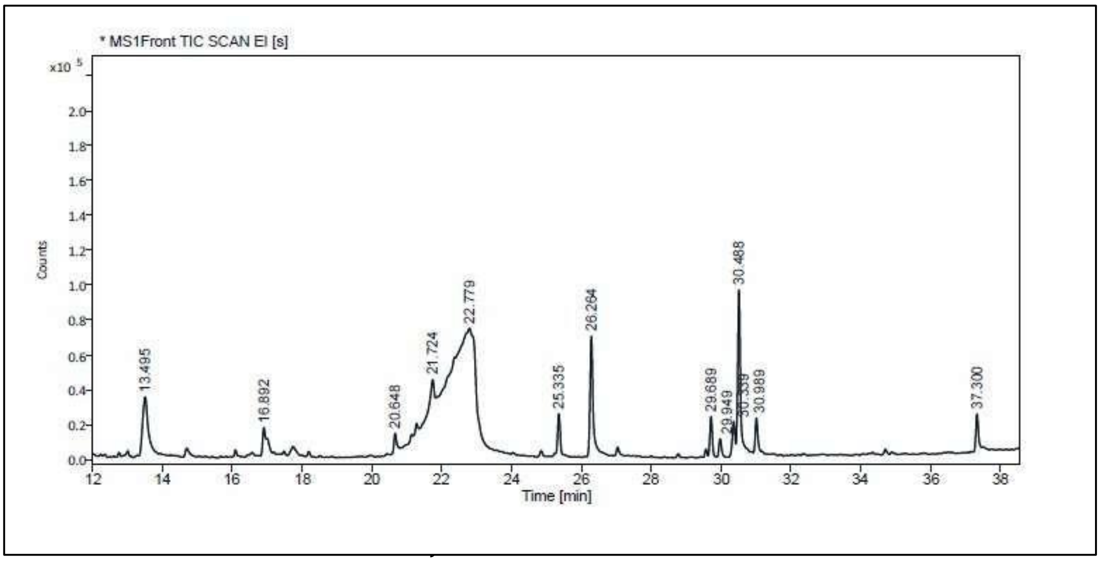
The sample for GC-MS analysis was prepared by Derivatization method [9]. The GC-MS analysis to determine the phytochemical components in the methanol extract of *D. montana* leaves was conducted using an Agilent Model 8890 GC System with Single Quadrupole Mass Spectrometer (5977B MSD) analyser. The instrument's initial temperature for GC-MS detection was set at 75°C, and the hold time was 0.5 minutes. The oven's maximum temperature was 350°C at equilibration time of one minute, temperature programming at a rate of 5°C/min with a value of 180°C, hold three minutes, and hold at a rate of 5°C/min with a value of 300°C, hold time of five minutes. The Agilent 19091S-433UI (Dimension 30 m x 250 um x 0.25 um) was used with an HP-5ms Ultra Inert, 60°C to 325°C (350°C) medium polar column. The initial temperature of the HP-5 MS UI was 75°C. A constant flow rate of 1.2 ml/min of helium gas was employed as the carrier gas. 1µl sample injected (split ratio of 15:1). Acquisition procedure for the GCMS Agilent 5977 MSD was Quad Temperature 150°C, Ion Source Temperature 230°C, Fixed Electron Energy 70 eV, 3-minute scan interval, and segment from 50 to 600 Da. The solvent delay was 3 minutes, and the overall operating time for the GC-MS was 53.5 minutes. The 50 to 600 (m/z) mass spectral scan range and 1,562 (N=2) scanning speed were chosen. A version of Open Lab CDS 2.5 was utilised to analyse the mass spectra and chromatograms, and the mass detector was a Triple-Axis Detector with a powerful dynode and a long-life electron multiplier.

3. Result and Discussion

The chemical constituents in the methanolic extract of the leaves of the *Diospyros montana* plant were identified by GC-MS analysis as shown in Table-1 Compounds were identified by comparing sample mass spectra to mass spectra of the NIST data library. People are reluctant to learn more about current synthetic and chemical treatments because of their negative side effects [10]. However, because to its all-natural components, environmental friendliness, and absence of side effects, traditional herbal medicines are growing in popularity [11]. Even with all the advantages of modern synthetic pharmaceuticals, medicinal plants are unique in their ability to provide treatment for a range of human ailments because of the many beneficial phytoconstituents present in varied plant parts [12,13,14]

Historically, India has used around 80,000 different types of medicinal plants to cure a wide range of diseases using various traditional medical systems [15]. *Diospyros montana* is also a well-known medicinal herb (10,11). Previous review reports (12,13) on phytochemical investigations of plants indicated that *Diospyros montana* contains phenols, saponins terpenoids, flavonoids, reducing sugars and some other phyto-constituents that are dispersed in various parts of this plant. Chemical analysis of this plant revealed that it contains both unsaturated and saturated fatty acids and has antibacterial properties (14) There have also been reports of antibacterial action in this plant's leaves and seeds [16]. Several fatty acids are known to have antibacterial and antifungal properties, including oleic acids, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, and octadecanoic acid. It is also recognized that oleic, linoleic, lauric, linolenic, myristic acids, stearic, and palmitic possess possible antibacterial and antifungal properties [17,18,19]. The present study's findings demonstrated that plant's leaves exhibited a significant quantity of phenol, 4-ethenyl-2,6-dimethoxyphenol, and fatty acids. According to reports, phenolic compounds have anti-inflammatory and antioxidant properties [20]. This plant's leaves are also used to stuff fish (18) thus more investigation is needed to figure out how the component that was found relates to this leaf activity. Thus, scientists looking for medications from natural sources can find value in the results.

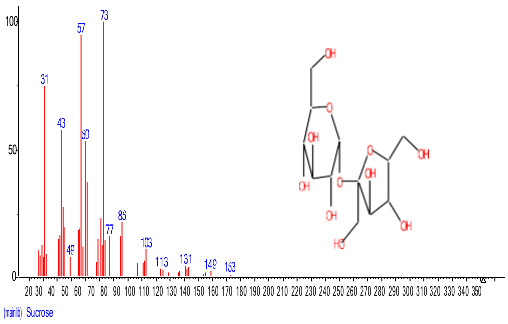
Figure 3: GC-MS TIC Chromatogram of *Diospyros montana* leaf extract



S. No.	Molecular Formula	Compound Name	RT	Score	Rev. Score	Area	Area %	Molecular weight	CAS #	Library Id	Phyto-chemical Class
1	$C_{12}H_{22}O_{11}$	Sucrose	13.495	719	813	301894.635	12.75	342.30	57-50-1	44727	Carbohydrate
2	$C_{10}H_{12}O_3$	Phenol, 4-ethenyl-2,6-dimethoxy	16.892	753	823	79601.264	3.36	182.21	28343-22-8	184115	Phenols
3	$C_{10}H_{12}O_3$	(E)-4-(3 Hydroxyprop-1-en-1-yl) -2-methoxyphenol	20.648	703	773	51839.390	2.19	180.20	32811-40-8	136081	Phenols
4	$C_6H_{12}O_6$	L-Glucose	21.724	763	773	73004.320	3.08	180.16	921-60-8	44368	Carbohydrate
5	$C_6H_{14}O_6$	Sorbitol	22.779	775	807	410763.956	17.35	182.17	50-70-4	44932	Sugar alcohol
6	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	25.335	820	827	107205.424	4.53	*270.45*	112-39-0	48957	Fatty acids

7	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	26.264	872	874	350762.646	14.81	*256.42*	57-10-3	9946	Fatty acids
8	$C_{19}H_{32}O_2$	9,12,15Octadecatrienoicacid, methyl ester (Z, Z, Z)-	29.689	771	771	104181.812	4.40	*292.45*	301-00-8	53064	Fatty Acyls
9	$C_{20}H_{40}O$	Phytol	29.949	750	767	54353.632	2.30	*296.53*	150-86-7	41598	Diterpen-oids
10	$C_{18}H_{32}O_2$	Linoelaidic acid	30.339	821	848	105693.030	4.46	*280.44*	506-21-8	35442	Fatty Acyls
11	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	30.488	881	886	520594.024	21.99	*278.429*	463-40-1	53045	Fatty Acyls
12	$C_{18}H_{36}O_2$	Octadecanoic acid	30.989	772	799	107708.339	4.55	284.48	57-11-4	9948	Fatty acids
13	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyls)ethylester	37.300	691	761	100258.420	4.23	*330.50*	23470-00-0	8214	Glycerolipids

Figure 4: Mass Spectra of Sucrose



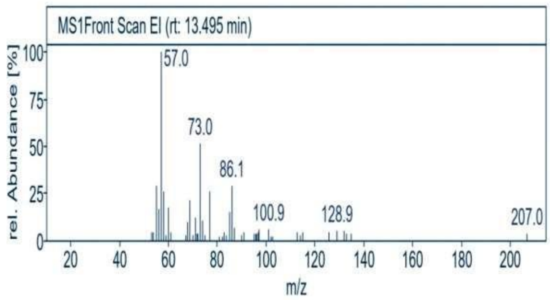
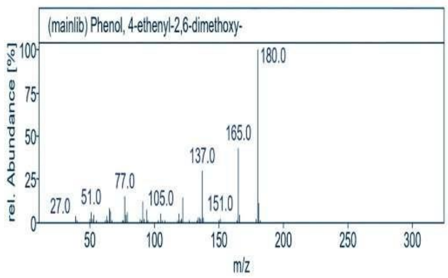


Figure 5: Mass Spectra of Phenol, 4-ethenyl-2,6- dimethoxy-



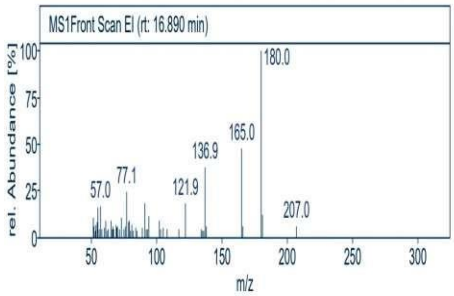
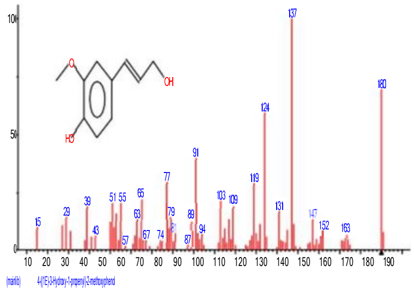


Figure 6: Mass Spectra of (E)-4-(3-Hydroxyprop-1- en-1-yl)-2-methoxyphenol



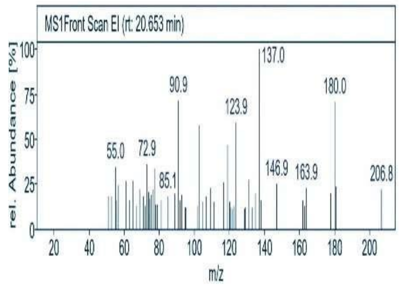
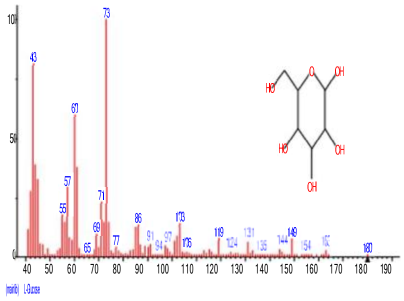


Figure 7: Mass Spectra of L-Glucose



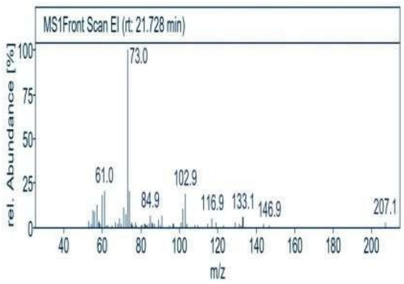
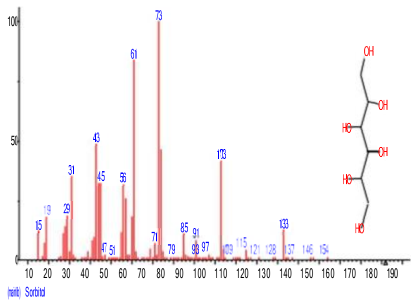


Figure 8: Mass Spectra of Sorbitol



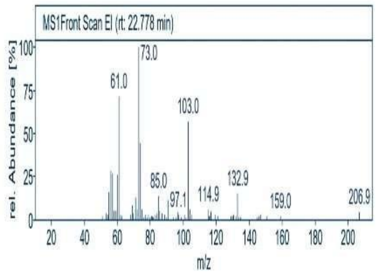
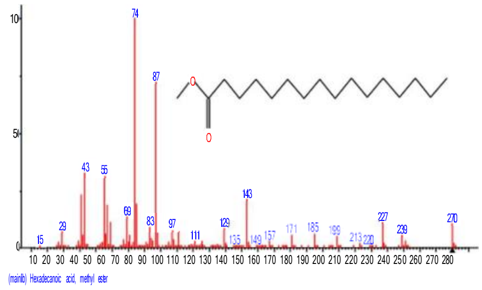


Figure 9: Mass Spectra of Hexadecanoic acid, methyl ester



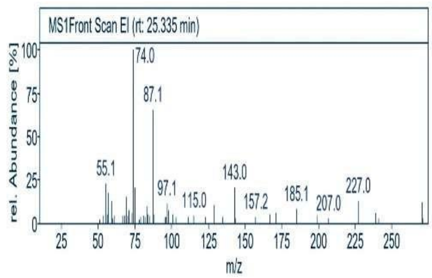
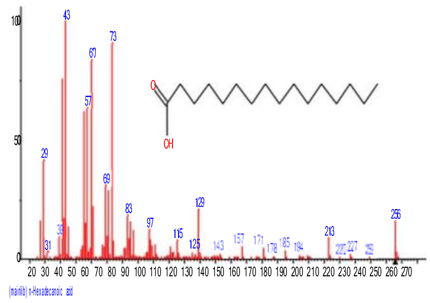


Figure 10: Mass Spectra of n-Hexadecanoic acid



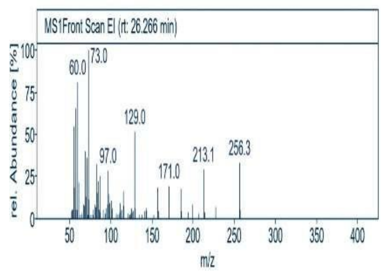
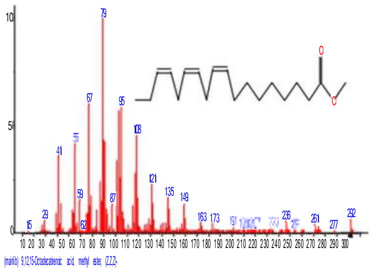


Figure 11: Mass Spectra of 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-



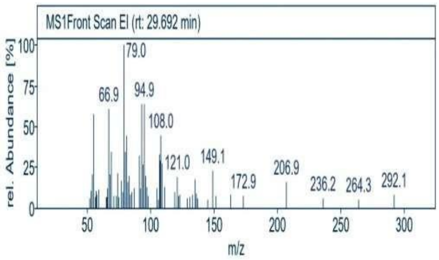
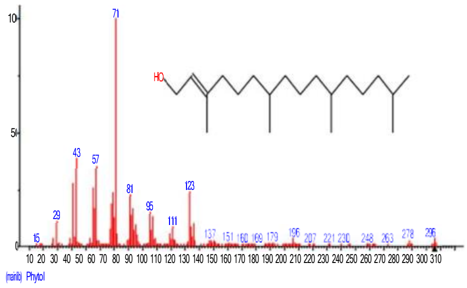


Figure 12: Mass Spectra of Phytol



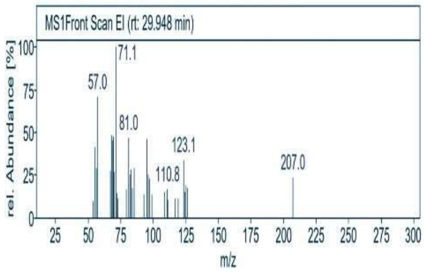
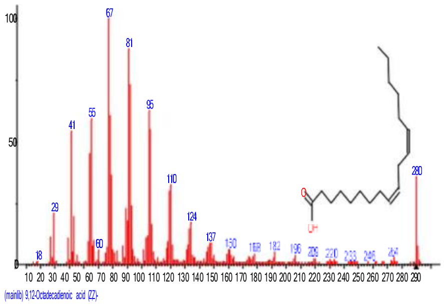


Figure 13: Mass Spectra of Linoelaidic acid



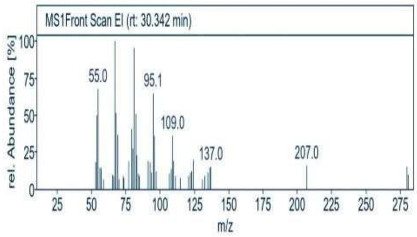
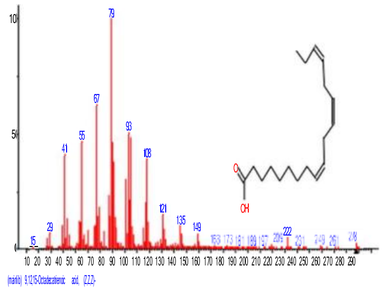


Figure 14: Mass Spectra of 9,12,15-Octadecatrienoic acid, (Z, Z, Z)-



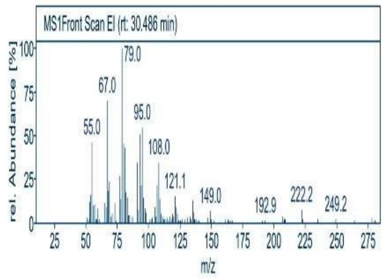
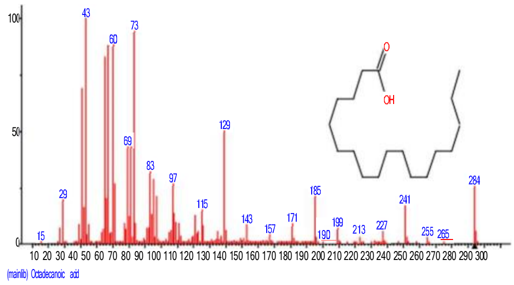


Figure 15: Mass Spectra of Octadecanoic acid



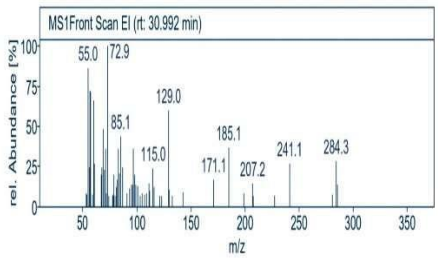
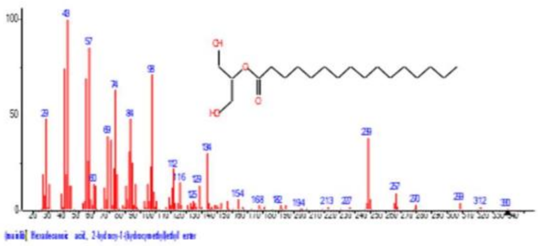
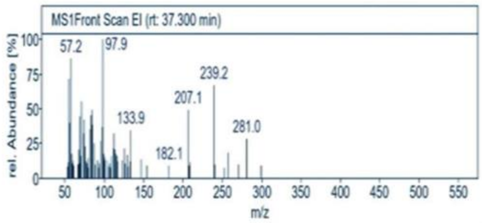


Figure 16: Mass Spectra of Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl)ethyl ester

100





CONCLUSION

The GC-MS analysis's findings indicated that *Diospyros montana* (Roxb.) methanolic extract includes many bioactive chemicals with potential use in pharmacology. On the other hand, *Diospyros montana* is a valuable herb that is used in traditional medicine for the treatment of a variety of diseases. Therefore, further investigation is needed to isolate and pinpoint the specific phytochemical compounds involved in disease prevention, which may eventually lead to the creation of new drugs. Additionally, because this plant has been thought to have both medicinal and toxic properties, it is important to understand the specific mechanism of action of each constituent.

5. Conflict of Interest

The authors declare there is no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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