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Phytochemical profiling and GC-MS analysis of Leaf Extracts of *Dendrobium anceps Sw.* (Orchidaceae)

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ABSTRACT

Orchids have been used in various traditional medicinal systems for their potential therapeutic properties. *Dendrobium anceps* Sw. is an important medicinal orchid used to treat various diseases. The primary objective of this study is to analyze the phytochemical profile and conduct GC-MS analysis on different solvent extracts derived from *D. anceps*. The shade-dried leaf powder was subjected to cold extraction using n-hexane, acetone, ethyl acetate, and methanol as solvents. Qualitative phytochemical screening was then performed on the resulting crude leaf extracts, following established standard protocols, to identify various phytocompounds. Following this, the methanol and ethyl acetate leaf extracts of *D. anceps* were subjected to GC-MS analysis. The results of the qualitative phytochemical screening indicated that the methanol leaf extract of *D. anceps* exhibited the presence of phytochemicals such as proteins, carbohydrates, alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. Further, GC-MS analysis of ethyl acetate and methanol leaf extracts showed 33 different phytochemical compounds (18 in ethyl acetate and 15 in methanol leaf extracts). The major compounds recorded in ethyl acetate leaf extract are oleic acid, phytol, hexadecanoic acid methyl ester, 2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-. Compounds such as oleic acid, flavone, thujopsene, bicyclo[7.7.0]hexadec-1[9]-ene, 1-eicosene, and 1-docosene were identified in both the leaf extracts. The findings of the present study conclude that the phytocompounds identified in these leaf extracts possess several biological activities such as antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. Further research on this plant has the potential to unveil novel bioactive compounds that can be utilized in the development of pharmaceuticals for various health conditions, thereby reducing our reliance on synthetic drugs.

Keywords: Orchid, *Dendrobium anceps*, GC-MS analysis, Bio-active compounds.

INTRODUCTION

Plants have been used in traditional medicine systems for thousands of years, serving as the cornerstone of healing practices in various cultures. This historical knowledge and experience form the basis for much of modern medicine.Many drugs and medicines used in modern healthcare are directly or indirectly derived from plants. Plant-based compounds have been the basis for developing a wide range of pharmaceuticals, including pain relievers, antibiotics, and anticancer agents [1-2]. The importance of plant-derived drugs is multifaceted, encompassing their historical efficacy, role in drug discovery, cultural significance, accessibility, and potential to address a wide spectrum of health conditions while promoting holistic well-being and sustainability [3].In light of the persistent challenges presented by the rise of new diseases, the growing threat of antibiotic resistance, and the demand for treatments that are both effective and

accessible, plant-derived drugs are gaining greater recognition as a valuable asset within the realm of healthcare and medicine. Their adaptability and capacity to address a diverse spectrum of health issues, combined with their harmonious integration with holistic healthcare approaches, underscore their heightened relevance and essential role in the contemporary global healthcare landscape.

Orchids, renowned for their exquisite beauty and enchanting blossoms, have been revered primarily for their ornamental allure. Nevertheless, beneath their aesthetic grandeur, these extraordinary plants conceal a closely guarded treasure trove – their medicinal potential. The Orchidaceae family boasts an abundance of bioactive compounds that have been utilized for centuries in diverse traditional healing practices across the globe [4-6]. Orchids have a rich history as a source of herbal remedies within traditional medicine systems [7-9]. Traditional healers and herbalists have relied on orchids for their medicinal attributes for centuries [10-11]. Various parts of the orchid plant, including the roots, leaves, and pseudobulbs, have been employed to address a wide range of health conditions and ailments [12-15].

The genus *Dendrobium* is remarkably diverse, consisting of approximately 3,160 species [16]. These species exhibit a wide range of morphological characteristics and are primarily distributed in the Sino-Himalayan regions [17]. Their habitat extends further to include areas in Australia, New Zealand, and the Pacific Islands [18]. Within India, there are around 117 species of this genus [19]. Notably, northeastern India is home to about 88 of these species [20]. The *Dendrobium*, along with various other orchid genera, holds a prominent place in the traditional medicinal practices within the regions where they thrive [21]. These orchids are believed to contain therapeutic properties and have been incorporated into remedies for addressing various health conditions [22]. *Dendrobium anceps*, like numerous orchid species, boasts a longstanding history of application in traditional medicine, especially within several Southeast Asian cultures. Although its medicinal qualities have not been as extensively studied as its ornamental attributes, it is believed to offer potential health benefits. These potential benefits encompass anti-inflammatory, analgesic, and antipyretic properties, as well as the ability to address respiratory issues such as coughs, asthma, and bronchitis.

Phytochemical studies are a crucial link between traditional herbal remedies deeply rooted in diverse cultures and modern science. They validate the therapeutic efficacy of these remedies, preserving cultural heritage while advancing evidence-based natural treatments. By revealing the active compounds in medicinal plants, these studies substantiate traditional remedies and enhance their integration into mainstream healthcare. Therefore, in view of the above background, the present study aims to screen the bioactive phyto constituents present in the *Dendrobium anceps*.

MATERIALSAND METHODS

Collection of plant materials

Plant materials of *Dendrobium anceps* Sw. (Figure 1) were collected from the forest regions of Darjeeling Himalaya and grown in the herbal garden of Acharya Nagarjuna University, Guntur India. The collected plant material was identified by consulting relevant taxonomic literature [23-25]. A voucher specimen (ANUBH 1503) was deposited in the herbarium of the Department of Botany and Microbiology for future reference. During the winter season, fresh leaves were collected from matured plants and subsequently subjected to a careful process that included rinsing, cutting into small pieces, and shade drying for a duration of 35-40 days.

Figure 1: *Dendrobium anceps* Sw.

Preparation of Plant Extract

Following the shade-drying process, the plant material was finely powdered using an electric blender. The powdered material was then sieved and carefully stored in airtight glass containers. Approximately 50 grams of the powdered leaf material was individually placed in 500 mL of n-hexane, acetone, ethyl acetate, and methanol. These extractions were conducted through the cold extraction method, with gentle stirring at room temperature, spanning 48 hours. Subsequently, the extracts were filtered using Whatman No. 41 filter paper, after which they were transferred to glass jars and preserved at a temperature of 4°C.

Qualitative Phytochemical Screening of Crude Extracts

The crude extracts derived from the plant material (leaf) were utilized to perform qualitative analyses of both primary (carbohydrates and proteins) and secondary metabolites, including alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. The preliminary phytochemical screening was carried out

by the standardized protocols [26-28].

Test for carbohydrates (Benedict's test)
About 5 ml of Benedict's reagent was mixed with a few drops of plant extract. Later the mixture was boiled and cooled. The formation of a red-coloured precipitate indicates the presence of carbohydrates.

Test for proteins (Biuret test)
A few ml of plant extract was taken and 1 ml of 40% NaOH solution and 2 ml of 1% $CuSO_4$ were added. The appearance of violet colour indicates the presence of proteins.

Test for alkaloids (Dragendorff's test)
About 5ml of 1% HCl was added to 0.5gm of extract. Later the mixture was boiled and filtered. To 1 ml of filtrate, 2-3 drops of Dragendorff's reagent were added. The formation of reddish-brown precipitate denotes the presence of alkaloids.

Test for flavonoids (1% $AlCl_3$ Test)
A few drops of 1% $AlCl_3$ were added to the plant extracts. The appearance of yellow colour indicates the presence of flavonoids.

Test for phenols (10% $FeCl_3$ test)
A few drops of 10% aqueous $FeCl_3$ solution were added to the plant extract. The appearance of blue or green colour indicates the presence of phenols.

Test for saponins (Frothing test)
About 5 ml of distilled water was added to 1 ml of plant extract and mixed well. The formation of persistent froth indicates the presence of saponins.

Test for steroids (Liebermann-Burchard test)
About 1 ml of plant extract was dissolved in 1 ml of chloroform and acetic anhydride. A few drops of concentrated HCl were added to the above extract. The appearance of a brownish-green ring at the interface indicates the presence of steroids.

Test for terpenoids (Salkowski test)
A few drops of plant extract were allowed to dissolve in 2 ml of chloroform. Concentrated H_2SO_4 was added to form a lower layer. The appearance of reddish-brown colour at the interface denotes the presence of terpenoids.

Test for tannins (Iodine Test)
A few drops of Iodine solution were added to 2 ml of plant extract. The appearance of faint bluish colour indicates the presence of tannins.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis
Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique used to identify and characterize chemical compounds present in a sample. The methanol and ethyl acetate leaf extracts of *Dendrobium anceps* Sw. were subjected to GC-MS analysis. The plant extract was injected into an HP-5 column (30 m X 0.25 mm with 0.25 μm film thickness), Agilent Technologies 6890 N JEOL GC Mate II GC-MS model. While operating the equipment following chromatographic conditions were employed: helium as the carrier gas, the flow rate of 1 mL/min; the injector was operated at 200°C and the column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following Mass Spectroscopy conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; the mass range of 50-600 mass units.

Identification of Phyto-components
The Mass spectrum of GC-MS was interpreted by using the database of the National Institute of Standard and Technology (NIST) which has more than 62,000 patterns. The mass spectrum of the unknown phytocomponents was compared with the spectrum of the known components stored in the NIST library. After that, the name, molecular mass, and structure of the phytocomponents derived from the plant extract were determined.

RESULTSANDDISCUSSION

Preliminary Phytochemical Analysis
The preliminary phytochemical screening of *D. anceps* leaves in four different solvent extracts revealed the presence of various phytochemicals, encompassing primary and secondary metabolites (Table 1). The methanol leaf extract exhibited the highest number of compounds, followed by the ethyl acetate and acetone extracts. Specifically, the methanol leaf extract of *D. anceps* exhibited the presence of phytochemicals such as proteins, carbohydrates, alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. The ethyl acetate leaf extract displayed similar phytochemicals, except proteins, alkaloids saponins, and steroids. Meanwhile, the acetone extracts only showed the presence of carbohydrates and tannins. However, hexane extracts showed no phytochemicals. Differences in the occurrence of phytochemical compounds may be due to the differential solubility of the compound in the solvent used [29]. The use of different solvents for extraction has revealed variations in the phytochemical composition of *D. anceps* leaves. This indicates that the choice of solvent can significantly influence the types and quantities of compounds extracted.The presence of flavonoids, phenols, and alkaloids in the methanol and ethyl acetate extracts is noteworthy, as these compounds are often associated with potential pharmacological activities, such as antioxidant, anticancer, and antimicrobial effects [30-31].

Williams studied the chemical analysis of leaf of 142 species across 75 genera within the Orchidaceae [32]. He revealed that flavone C-glycosides were the most common constituents, present in 53% of the species. Flavonoids are well known for their antioxidant properties. They can help neutralize harmful free radicals in the body, which can reduce oxidative stress and lower the risk of chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [33-34].

The genus *Dendrobium* is rich in alkaloids [35]. The majority of orchid alkaloids are either pyrrolizidine or dendrobine

type whereas the remaining exhibit many unrelated structures [36]. *Dendrobium*alkaloids are well known for their pharmacological activities such as anti-inflammatory, antitumor, anti-diabetic, neuro-protective, and anti-viral activity [36-41].

The findings of the present investigation are in line with earlier workers, where studies have documented the existence of alkaloids, carbohydrates, coumarins, flavonoids, phenols, steroids, tannins, and terpenoids within the *Dendrobium* genus [42-45].

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of leaf extracts of *D. anceps*

The ethyl acetate leaf extract of *D. anceps* exhibited a rich phytochemical profile, with the gas chromatogram revealing the presence of approximately 18 different phytoconstituents (Figure 2). Detailed information about these compounds, including their structures, retention time, molar mass, and peak area percentage is given in Table 2. Notably, the ethyl acetate leaf extract prominently featured several major phytoconstituents, including oleic acid (Figure 3), phytol, hexadecanoic acid methyl ester, 2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-, flavone, 1-Eicosene, 3-Eicosene [E]-, and 1-Docosene.

Table 1. Phytochemical profile of different solvent extracts of *Dendrobium anceps* leaf

Phytochemical constituents	Test/ Reagents	n-hexane	acetone	ethyl acetate	methanol
Carbohydrates	Benedict's Test	-	+	+	+
Proteins	Biuret Test	-	-	-	+
Alkaloids	Dragendorff's Test	-	-	+	+
Flavonoids	1% $AlCl_3$ Test	-	-	+	+
Phenols	10% $FeCl_3$ Test	-	-	+	+
Saponins	Frothing Test	-	-	-	+
Steroids	Liebermann-Burchard Test	-	-	-	+
Tannins	Iodine Test	-	+	+	+
Terpenoids	Salkowski Test	-	-	+	+

Note: '+' = Present; '–' = Absent

The methanol leaf extract of *D. anceps*, as revealed in its gas chromatogram (Figure 4; Table 3), exhibited the presence of 15 distinct phytochemical compounds. Among these, several prominent bioactive compounds were identified, including eugenol (Figure 5), oleic acid, n-hexadecanoic acid, flavone, 1-eicosene, 1-docosene, cyclotetracosane, quinoxaline, 2-phenyl, and thujopsene-I3.

Compounds such as oleic acid, flavone, thujopsene, bicyclo[7.7.0]hexadec-1[9]-ene, 1-eicosene, and 1-docosene were identified in both extracts. The chemical compounds recorded in the *D. anceps* leaf extracts possess a variety of biological activities. Oleic acid was identified in both the ethyl acetate and methanol leaf extracts of *D. anceps*. It is a monounsaturated omega-9 fatty acid (MUFA). The consumption of MUFAs has been linked to several health benefits, including the reduction of low-density lipoprotein (LDL) cholesterol levels and potentially an increase in high-density lipoprotein (HDL) cholesterol [46]. This fatty acid is also known for its hypotensive effects, contributing to a decrease in blood pressure [47]. Furthermore, it has been associated with a reduced risk of breast cancer [48].

Thujopsene, which was found in both ethyl acetate and methanol leaf extracts, is a natural sesquiterpene known for its notable antibacterial and antifungal properties. Several studies [49-51] have provided evidence of its potential to effectively counter bacterial and fungal infections. This makes thujopsene a compelling candidate for various applications related to antimicrobial treatments.

Hexadecanoic acid, methyl ester, has been identified in the ethyl acetate leaf extract. This compound possesses both antioxidant and antifungal properties [52].

The n-Hexadecanoic acid, commonly known as palmitic acid, has been detected in the ethyl acetate leaf extract of *D. anceps*. This 16-carbon saturated long-chain fatty acid was also previously reported in *D. aphyllum*, *D. amoenum*, and *D. moschatum*[44-45, 53]. Furthermore, n-hexadecanoic acid exhibits selective inhibition of DNA topoisomerase-I, thereby hindering the proliferation of human fibroblast cells [54]. This compound also demonstrates anti-inflammatory properties and is recognized for its antioxidant, hypocholesterolemic, and potent mosquito larvicidal activities [55-56].

Cyclotetracosane and eugenol, both identified in the methanol leaf extract of *D. anceps*, exhibit antioxidant and anticancer properties. Tetracosane, in particular, demonstrated significant cytotoxic activity against AGS, MDA-MB-231, HT29, and NIH3T3 cancer cell lines [57]. The antioxidant properties of these compounds are attributed to their ability to scavenge free radicals, thus impeding or suppressing the chain reaction of oxidation, ultimately delaying or inhibiting the oxidation process. The antioxidant potential of phenols and their derivatives is closely linked to their anticancer attributes [58-59]. Furthermore, Carrasco et al. provided additional evidence for the anticancer effects of eugenol (4-allyl-2-methoxyphenol) and its synthetic analogues, which were found to hinder the proliferation of cancer cells [60].

Table 2. Phytocompounds identified in ethyl acetate leaf extract of *D. anceps*

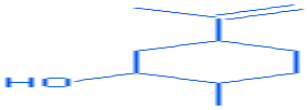
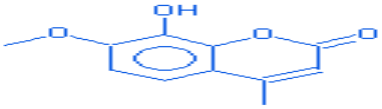
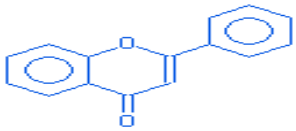
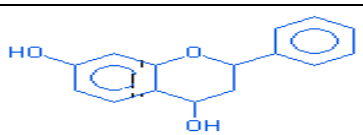
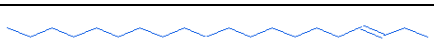
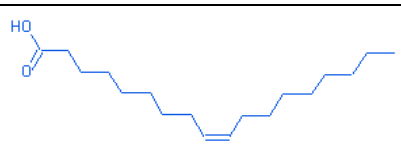
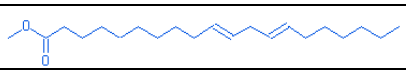
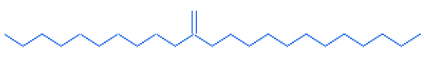
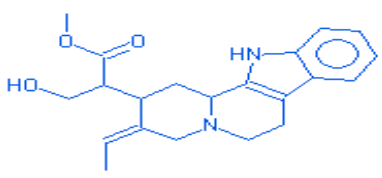
Structure	Compound	RT	Mass	Peak Area %
	a-Pinene	10.92	136.0000	1.8067
	Cyclohexanol,2-methyl-5-[1-methylethenyl]-	11.57	154.0000	1.7753
	Thujopsene-[I2]	13.13	204.0000	1.8685
	Coumarin-8-ol,7-methoxy-4-methyl-	13.82	206.0000	2.6288
	Longipinane,[E]-	14.42	206.0000	2.5631
	Bicyclo[7.7.0]hexadec-1[9]-ene	14.63	220.0000	3.5765
	Flavone	14.88	222.0000	6.3732
	2H-1-Benzopyran-4,7-diol, 3,4-dihydro-2-phenyl-	15.07	242.0000	8.6228
	Hexadecanoic acid, methyl ester	15.28	270.0000	8.7092
	1-Eicosene	15.92	280.0000	4.9204
	3-Eicosene, [E]-	16.22	280.0000	4.4665
	Oleic Acid	16.65	282.0000	31.1550
	Phytol	17.02	296.0000	8.9179
	1-Docosene	17.85	308.0000	4.0703
	10,13-Eicosadienoic acid, methyl ester	19.38	322.0000	3.0766
	1-Tetradecene, 2-decyl-	19.68	336.0000	3.3524
	Z-12-Pentacosene	22.02	350.0000	2.5970
	Corynan-16-Carboxylic acid, 19,20-didehydro-17-hydroxy-methyl ester [16R,19E]-	25.53	354.0000	2.0819

Figure 2: GC-MS chromatogram of ethyl acetate leaf extract of *D. anceps*

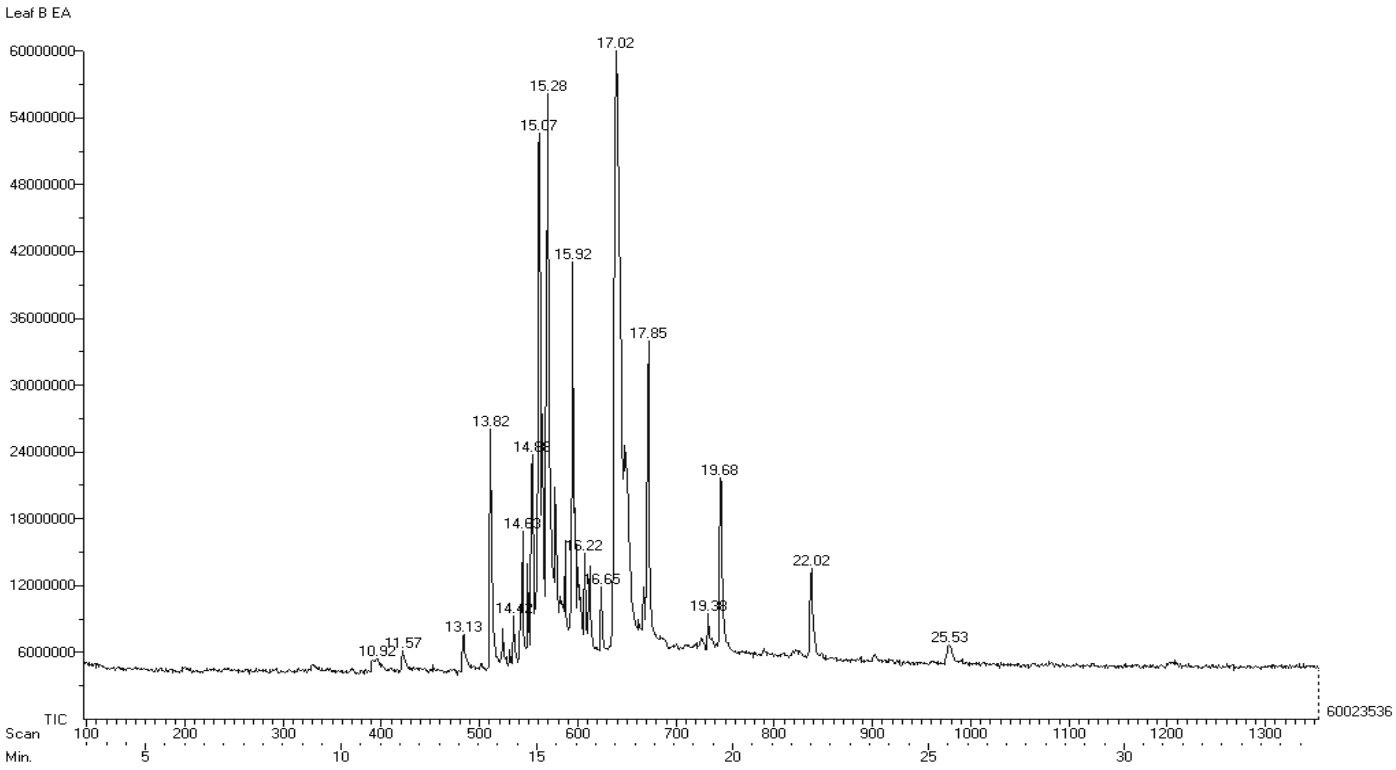


Figure 3: Major phytocompound oleic acid recorded in the ethyl acetate leaf extract of *D. anceps*

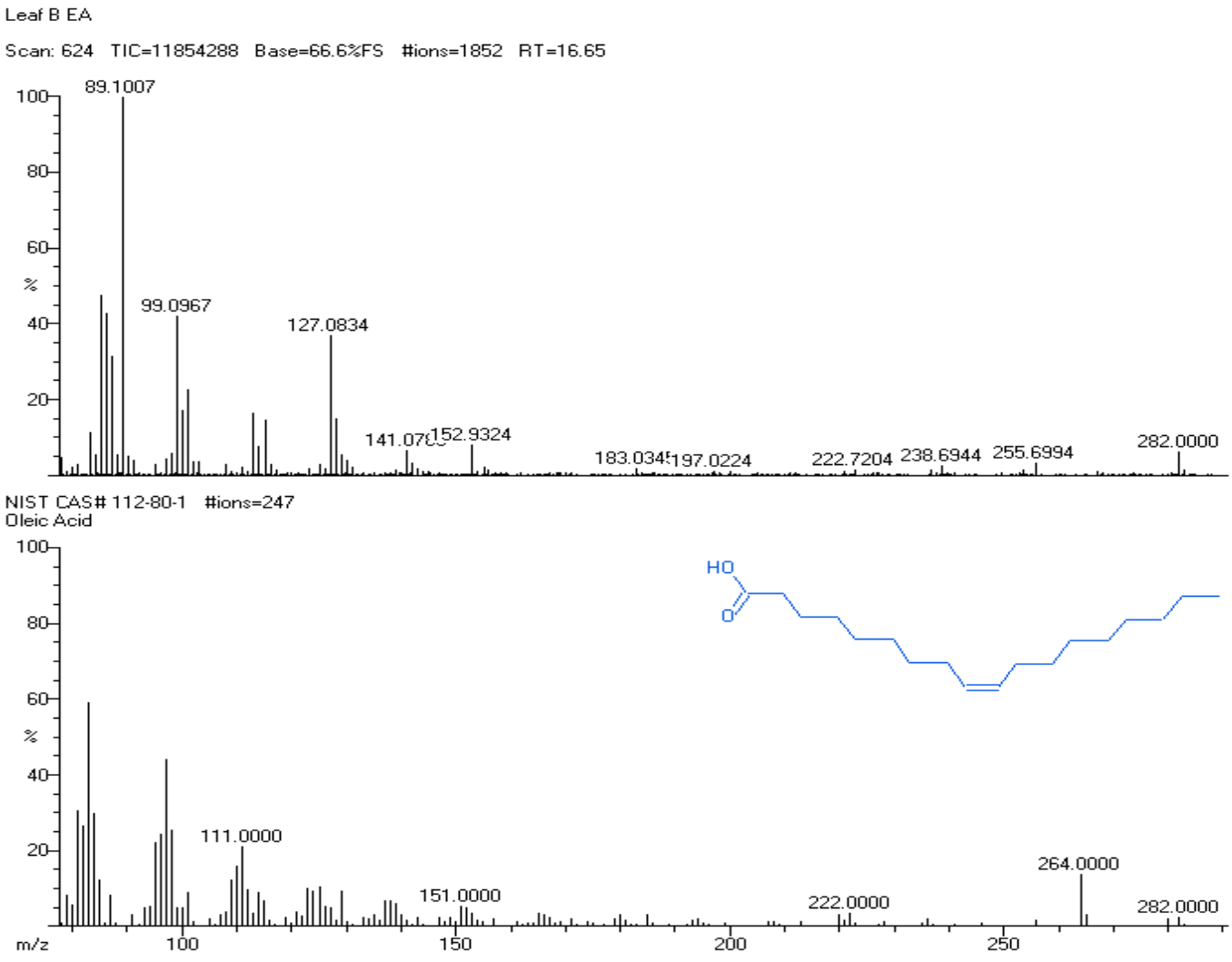


Figure 4: GC-MS chromatogram of methanol leaf extract of *D. anceps*

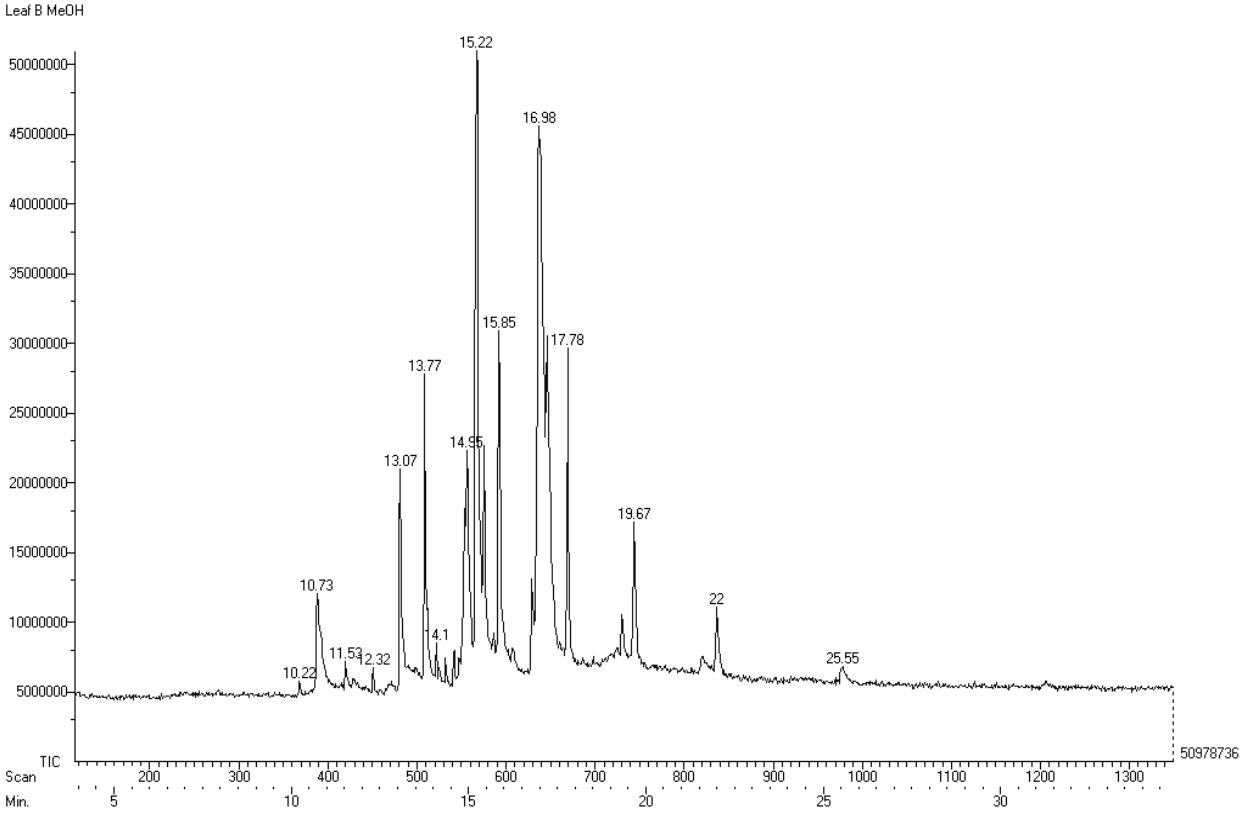


Figure 5: Major phytocompound eugenol recorded in the methanol leaf extract of *D. anceps*

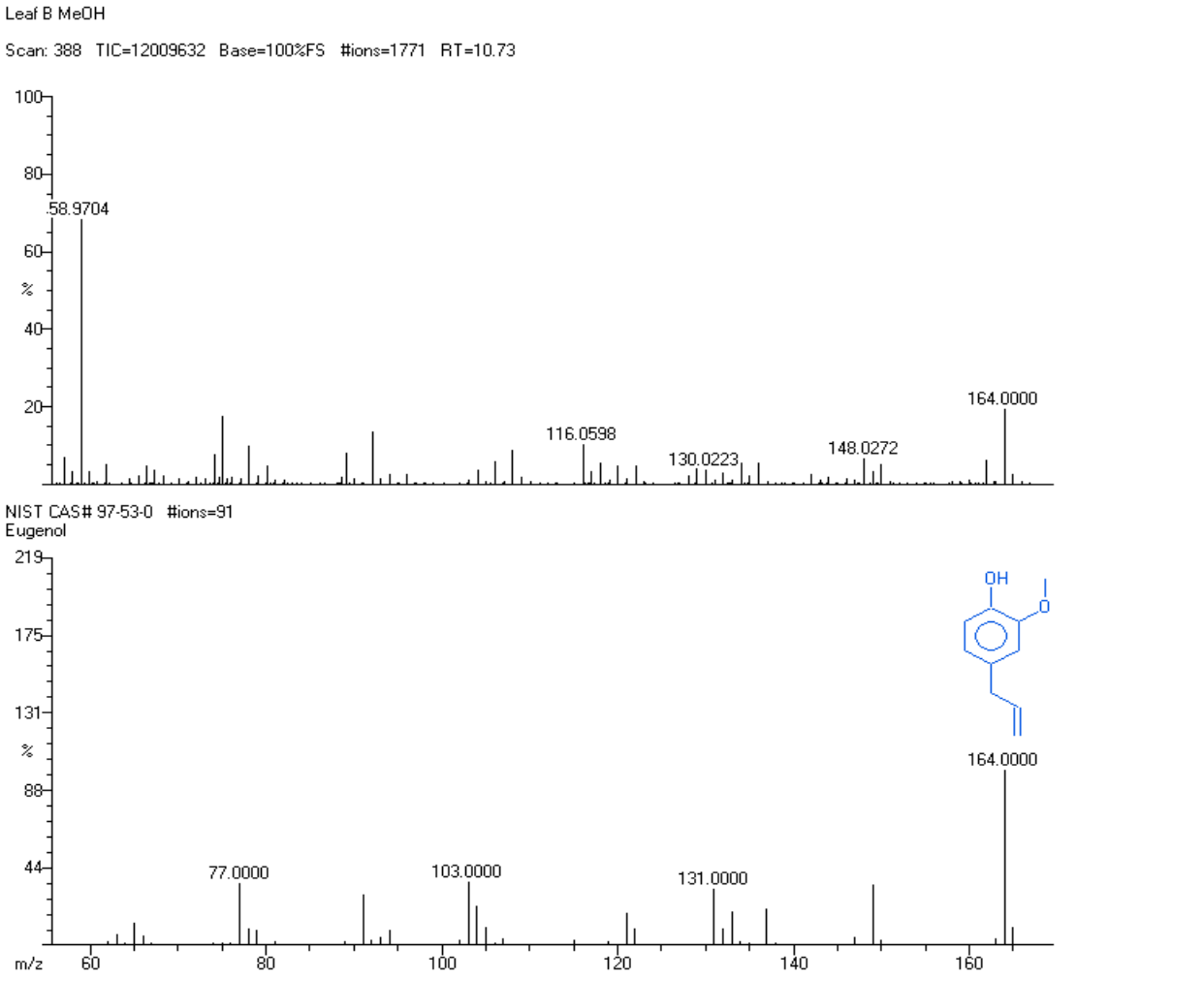
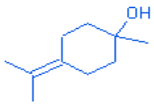
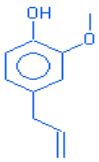
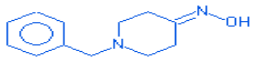
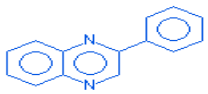
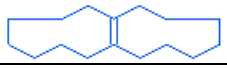
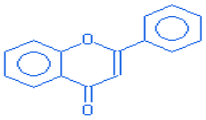


Table 3. Phytocompounds identified in methanol leaf extract of *D. anceps*

Structure	Compound	RT	Mass	Peak Area %
	c-Terpineol	10.22	154.0000	2.407
	Eugenol	10.73	164.0000	32.857
	2,2'-Bipyridine, 6,6'-dimethyl-	11.53	184.0000	2.781
	4-Piperidinone, 1-[phenylmethyl]-, oxime	12.32	204.0000	2.550
	Thujopsene-I3	13.07	204.0000	3.675
	Quinoxaline, 2-phenyl-	13.77	206.0000	4.096
	Bicyclo[7.7.0]hexadec-1[9]-ene	14.1	220.0000	3.954
	Flavone	14.95	222.0000	7.810
	n-Hexadecanoic acid	15.22	256.0000	8.809
	1-Eicosene	15.85	280.0000	5.106
	Oleic acid	16.98	282.0000	10.614
	1-Docosene	17.78	308.0000	4.633
	Cyclotetracosane	19.67	336.0000	4.272
	1-Hexacosene	22	364.0000	3.5068
	Docosanoic acid, 2-hydroxy-, methyl ester	25.55	369.0000	2.923

The results of the present study confirm the presence of a diverse array of bioactive compounds in the ethyl acetate and methanol leaf extracts of *D. anceps*. These findings provide compelling scientific validation for the traditional practices of tribal healers who have been utilizing these leaves as a source of treatment for various ailments. The identification of bioactive compounds in these leaves paves the way for further research and potential drug development. These natural compounds could serve as the foundation for new pharmaceuticals, leading to innovative treatments.

CONCLUSION

The presence of bioactive compounds in the ethyl acetate and methanol leaf extracts of *D. anceps* underscores the rich potential of these natural resources. These compounds exhibit a wide array of valuable biological properties, including antibacterial, antifungal, antiviral, antioxidant, and anticancer activities. These findings open up avenues for further research and exploration, offering the prospect of developing novel pharmaceuticals, dietary supplements, or other applications with potential benefits for human health and the natural world. However, it is essential to conduct additional studies to isolate and characterize these compounds in greater detail, uncover their mechanisms of action, and evaluate their therapeutic potential in specific contexts.

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