

Chemical Insights into *Zosima absinthifolia* Essential Oil: Exploring A Natural Source of Antioxidant and Antimicrobial Agents

Ifrar Ahmed

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Ashif Sajjad*

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta, P.C 87300, Pakistan.

Tel: +92-81-9211261; Fax; +92-81-9211277;

Email; ashif.sajjad@um.uob.edu.pk

Abdul Manan Kakar

Hi tech lab, Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Kulsoom Baloch

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Rukhsar Bhatti

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Qurat ul Ain

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Aiman Gul

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Muhammad Nadeem Khan

Institute of Biochemistry, University of Balochistan, Sariab Road Quetta P.C 87300, Pakistan.

Jamila Yousaf

Government Girls Post Graduate college Cantt Quetta, Share-e-Gulistan, Quetta, P.C 87300, Pakistan.

ABSTRACT

Zosima absinthifolia (Vent.) Link, a medicinally important yet underexplored plant collected from the Nushki district of Balochistan, was investigated for its essential oil composition using hydro-distillation followed by GC-MS analysis. A total of 26 chemical constituents were identified, representing 99.99% of the total oil. The most abundant constituents included 9,12,15-octadecatrienoic acid (29.36%), n-hexadecanoic acid (14.80%), 2-hydroxy-5-methylbenzaldehyde (8.48%), methyl α -D-glucopyranoside (5.97%), and phytol (5.03%). These compounds span diverse chemical classes such as fatty acids, aldehydes, sugar derivatives, and diterpenes,

each known for distinct biological activities. Notably, palmitic acid and α -linolenic acid have established antioxidant and antimicrobial properties, while phytol and aromatic aldehydes contribute additional anti-inflammatory and therapeutic potential. Comparisons with related studies highlight the unique phytochemical profile of *Z. absinthifolia*, particularly its high α -linolenic acid content, which has implications for nutraceutical and pharmaceutical development. The presence of multiple bioactive compounds suggests synergistic effects that could enhance the plant's pharmacological efficacy. This study provides the first comprehensive chemical characterization of *Z. absinthifolia* essential oil from Nushki district and underscores its promise as a source of natural antioxidants and antimicrobial agents. Further research on isolation, biological evaluation, and formulation could pave the way for its application in health care and herbal product industries.

Key words: *Zosima absinthifolia*, Essential oil, GC-MS analysis, Chemical composition

Introduction

Balochistan, Pakistan is rich in medicinal plant diversity, with numerous species used for traditional healthcare by local communities. Studies have documented a wide range of plants utilized for treating various ailments across Balochistan (Ayoub et al., 2023; Bibi et al., 2014; Zaidi & Crow, 2005). At present, there is a great global interest in natural medicines, with a growing demand for treatments derived from local medicinal plants instead of synthetic sources. Because, traditional medicines are considered safer as compare to synthetic drugs, which are often associated with various adverse effects and toxicity which gives attention to the researchers for production of new antibiotics (Abdul et al., 2020).

Traditional knowledge of medicinal plants serves as a significant foundation for modern scientific therapeutics. Today, numerous plants that have been traditionally used for medicinal purposes are being investigated for their antimicrobial properties and chemical constituents. There are still thousands of plant species that remain to be explored in the future. The broad-spectrum activities of green plants for new and effective antimicrobial agents have increased. The rising antibiotic resistance of microorganisms, are caused by the widespread and unaware use of modern antibiotics. The antibacterial activities of various extracts are being investigated worldwide (Kakar et al., 2012). Screening and evaluation of plant extracts and phytochemicals for antimicrobial activities contributes as initial step in the discovery of new antimicrobial drugs. Phytochemical researches have engaged the interest of plant scientists, as it plays a crucial role in identifying new sources of raw materials for the pharmaceutical industry (Ryan, 2018). Plant extracts as well as phytochemicals with testified antimicrobial properties can be highly valuable in therapeutic treatments. In few past years, numerous research works are done in various countries to demonstrate this gap. Many plants are utilized for their antimicrobial properties, which arise from compounds are synthesized during the plant's secondary metabolism (Amenu, 2014).

Many medicinal plant extracts contain various types of chemicals, which have the ability to regulate a range of biological and pharmacological activities against

microbes (Ahmed et al., 2020). The medicinal plants and their products are used for various diseases all over the world, especially in developing countries, where 80% of the population relies on plants or their derivatives (Ullah et al., 2024). Consequently, it is important to formulate novel antimicrobial agents derived by utilizing locally available plants (Dabur et al., 2007; Sieradzki et al., 1999).

This perennial plant *Z. absinthifolia* (Vent.) Link member of Apiaceae family, founds across various regions including Iran, Afghanistan, Pakistan, Turkey, and Central Asia. It typically survives in plains, steppes, and limestone slopes at altitudes ranging from 400 to 2000 meters (Başer et al., 2000). The height of plant is around 50 cm and stems are mostly straight. Its elongated and lanceolate shaped basal leaves are 3-pinnately distributed two or three times. The umbels are multi-radiant, comprises of uneven rays. The size of flowers is small and petals are yellow in color. Fruits are about 7 to 11 mm in length, elliptical and flattened. The flowering season is mostly from April to May, while June and July are peak fruiting period (Yusifov, 2021). The aerial parts of *Z. absinthifolia*, mostly fruits, are consumed as a food spice, also cooked as an edible item, and traditional medicine (Razavi et al., 2013).

The traditional medicinal plant *Z. absinthifolia* locally known as Gwathak from Balochistan, Pakistan, used to cure bowel disorders, treating coughs, as well as stomach gas and indigestion (Haq et al., 2018). The plant extracts and essential oils shows various biological activities, including antimicrobial (both antibacterial and antifungal), anti-inflammatory, and cytotoxic properties. The major components of essential oil composition in fruits of *Z. absinthifolia* primarily includes octyl hexanoate, octyl octanoate, octyl acetate, and 1-octanol. For this reason, all parts of medicinal plants have significant biological activity because of their phytochemical compounds. Ecological and phonological conditions, harvesting time, and hereditary differences are key factors that can change the chemical constituents and their compositions and biological activity of medicinal plants (Mollaei et al., 2020).

The plant also contains many bioactive compounds, such as flavonoids, coumarins, pyranocoumarins, furocoumarins, monoterpenes, and sesquiterpenes (Alikhanova & Novruzov, 2022). Its diverse range of bioactive compounds have a lot of medicinal applications and is used in neighboring countries (Alikhanova & Novruzov, 2022; Razavi et al., 2009).

Pakistan's weather conditions are diverse and the country is rich in medicinal plants biodiversity. In general, medicinal plants are produced in larger quantities in mountainous areas compared to grasslands, primarily due to more favorable habitats and suitable climatic conditions and out of six thousand plants, 600 plants have been classified as medicinally important plants (Jan et al., 2021; Rehman et al., 2017). Numerous researches have documented the medicinal uses of plants, with knowledge of traditional herbal remedies typically transfers orally from generation to generation (Aziz et al., 2018; Aziz et al., 2017).

In Pakistan, the Balochistan province almost covers 44% area of land, the geographical region features an arid to semi-arid landscape, ranging from cool temperate zones to coastal tropical areas. Diverse ecological conditions of the Balochistan influence the diversity of its flora in the region. Local communities extensively use wild plants for traditional medicinal purposes in huge quantity. Many of the semi-arid regions of the province Balochistan, such as the district Nushki is is

rich in botanical diversity (Ahmed et al., 2020).

District Nushki is located 144 kilometers southwest of the provincial capital city the Quetta, at a latitude of 29.5514° N and a longitude of 66.0107° E. This covers a total area of 7,597 square kilometers (172,000 hectares) and sits at an altitude of 2,900 feet. The weather condition of the area is arid to semi-arid, characterized by unpredictable and limited rainfall during winter. The weather is predominantly hot, with occasional cold spells. District Nushki has a population of 178,796. The region features dry tropical forests, encompassing both sand dunes desert forest sub-zones and tropical thorn forest. The local communities in the area have great traditional knowledge of plants, with their primary healthcare system consists heavily on their ethnobotanical expertise.

This present study aimed to explore chemical composition of essential oil from *Zosima absinthifolia*, and to highlight the bioactive compounds that could be incorporated into herbal formulation with potential application in nutraceuticals and pharmaceuticals.

Materials and Methods

Plant collection

The *Z. absinthifolia* plants were collected from union council Kashangi of district Nushki, Balochistan. The collected plant samples were washed and dried at room temperature. Taxonomy of *Z. absinthifolia* was submitted to Eco-taxonomy lab Herbarium, Department of Botany, University of Balochistan Quetta for identification. Plant samples were stored for further extraction purposes.

Essential oil extraction

50 grams of plant samples of *Z. absinthifolia* were hydro-distilled in 500 mL of water by a Clevenger apparatus for 2-3 hours. The resulting essential oils were dehydrated in a desiccator containing anhydrous potassium sulphate for overnight and stored at 4 °C in a freezer prior to GC-MS analysis.

Identification of Chemical Compounds through GC-MS

GC-MS (5977B-Agilent USA) instrument having DB-1 with silica column (25m × 0.250 mm. film thickness 0.25 µm) to perform the GC-MS of essential oil. GC oven temperature was kept at 70°C for 3 min and programmed to 270°C at a rate of 10°C/min for 13 min, and kept constant at 270°C for 2 min and then programmed to 300°C at a rate of 1°C/min, and total held time was 36 minutes. Split ratio was adjusted at 20:1 and transporter gas (Helium) was used with a flow rate of 36.796 cm/sec. Injector temperature was set at 250°C, and auto injection was carried triplicate with same operational parameters. Mass spectra were recorded at ionization energy of 70 eV. Mass range was from m/z 30 to 650. The chemical compounds were identified and quantified through data analysis software on the basis of area percentage peak.

Results and Discussion

Z. absinthifolia essential oil was extracted from dried plants by hydro-distillation, and the chemical composition was analyzed using Gas chromatography Mass Spectrometry (GC-MS). 26 compounds were identified, accounting 99.99 % of the oil content shown is (Table 1) and the spectra resulted by GC-MC obtained after analysis (Fig. 1). The major constituents were n-Hexadecanoic acid (14.80%), 2-Hydroxy-5-methylbenzaldehyde (8.48%), methyl α -D-Glucopyranoside, (5.97%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (29.36%), and Phytol 5.03%). Among all the above components the major component with the highest abundance with respect to the mass/charge and retention time shown in (Table 1 & Fig. 1).

Table-1: Chemical Composition of *Z. absinthifolia* essential oil

Peak No.	Retention Time (min)	Identified Compound	CAS No.	Area % (Approx.)	Remarks
1	5.175	Benzeneacetaldehyde	122-78-1	0.88%	Aromatic aldehyde
2	7.058	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	28564-83-2	2.31%	Known antioxidant (maltol derivative)
3	9.684	2-Methoxy-4-vinylphenol	7786-61-0	0.90%	Phenolic compound; flavor compound
4	11.961	2-Hydroxy-5-methylbenzaldehyde	613-84-3	8.48%	Aromatic aldehyde
5	12.104	Imidazole, 2-trifluoromethyl-	66675-22-7	0.88%	Nitrogen heterocycle
6	12.131	3,5-Dibromo-4-(4-nitro-benzyloxy)-benzaldehyde	1000295-77-6	2.92%	Substituted aromatic compound
7	12.450	Phenol, 2,4-bis(1,1-dimethylethyl)-	96-76-4	1.88%	Common antioxidant (BHT-related)
8	13.142	α -D-Glucopyranoside, methyl	97-30-3	5.97%	Sugar derivative
9	13.267	β -D-Glucopyranoside, methyl	709-50-2	1.31%	Sugar derivative
10	13.498	D-Glucuronic acid, γ -lactone	32449-92-6	0.70%	Lactone, metabolic intermediate
11	13.648	α -Aminooxy-propionic acid, ethyl ester	5766-86-9	2.22%	Nitrogen-containing compound

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12	13.711	Lactose	63-42-3	3.35%	Disaccharide sugar
13	15.020	Octanal, 2-(phenylmethylene)-	101-86-0	1.39%	Fragrance-related aldehyde
14	16.694	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3	0.94%	Spiro compound; antioxidant
15	16.981	Hexadecanoic acid, methyl ester	112-39-0	0.71%	Common fatty acid methyl ester (palmitic acid)
16	17.373	n-Hexadecanoic acid (Palmitic acid)	57-10-3	14.80%	Most abundant compound; saturated fatty acid
17	18.532	9,12-Octadecadienoic acid, methyl ester (linoleic acid)	112-63-0	1.06%	Unsaturated fatty acid methyl ester
18	18.571	9,12,15-Octadecatrienoic acid, methyl ester (linolenic acid)	301-00-8	2.48%	Polyunsaturated fatty acid methyl ester
19	18.791	Phytol	7541-49-3	5.03%	Diterpene
20	18.956	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (α -linolenic acid)	463-40-1	29.36%	Polyunsaturated fatty acid
21	19.231	Methyl 9,12-heptadecadienoate	14101-91-8	3.50%	Unsaturated fatty acid methyl ester
22	20.293	Cyclohexane, 3,4-bis(1-methylethenyl)-1,1dimethyl-	61142-74-3	0.87%	Sesquiterpene hydrocarbon
23	21.948	Cyclopropaneoctanal, 2-octyl-	56196-06-6	2.00%	Aliphatic aldehyde
24	22.196	Bis(2-ethylhexyl) phthalate	117-81-7	2.23%	Phthalate ester
25	23.253	1,3,12-Nonadecatriene	16356-11-9	0.75%	Polyunsaturated hydrocarbon
26	23.308	7,10,13-Hexadecatrienoic	56554-30-4	3.07%	Polyunsaturated fatty acid

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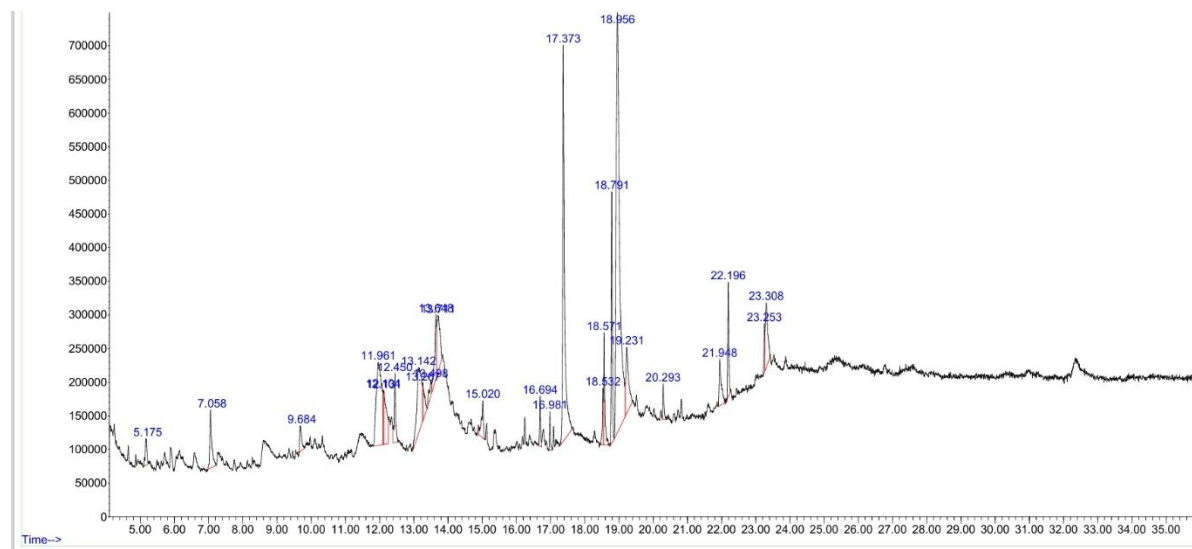
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Figure 1: GC-MS Spectra of *Z. absinthifolia* Essential Oil
n-Hexadecanoic acid (Retention Time: 17.373 Area: 14.80%)



The above chemical constituent is also commonly known as Palmitic acid has formula $C_{16}H_{32}O_2$ a well known long-chain saturated fatty acid commonly present in many plants and animals. Palmitic acid displays antioxidant properties and also shows antibacterial properties against certain organisms such as gram positive and negative bacterial strains, for more than two decades (Huang et al., 2011). n-Hexadecanoic acid was identified from the GC-MS results of the *Z. absinthifolia* plant with a peak of 14.80 % of essential oil among the identified components (Fig. 2)

n-Hexadecanoic is reported as the major component isolated from leaves of *E. agallocha* that exhibited 30.74 % in the GC-MS results (Purushothaman et al., 2024), which is higher percentage of n-Hexadecanoic as compared to obtained results of presented study. Purushothaman et al. (2024), also concluded that n-Hexadecanoic resulted with high percentage of inhibition and potential antibacterial activity against *E. coli* was 11.02 ± 0.03 mm, *B. subtilis* was 12.10 ± 0.01 mm, *A. hydrophilia* was 13.2 ± 0.02 mm, *V. harveyi* was 13.1 ± 0.10 mm, and *K. pneumonia* was 11.30 ± 0.02 mm respectively, at the maximum concentration of 150 $\mu\text{g/ml}$.

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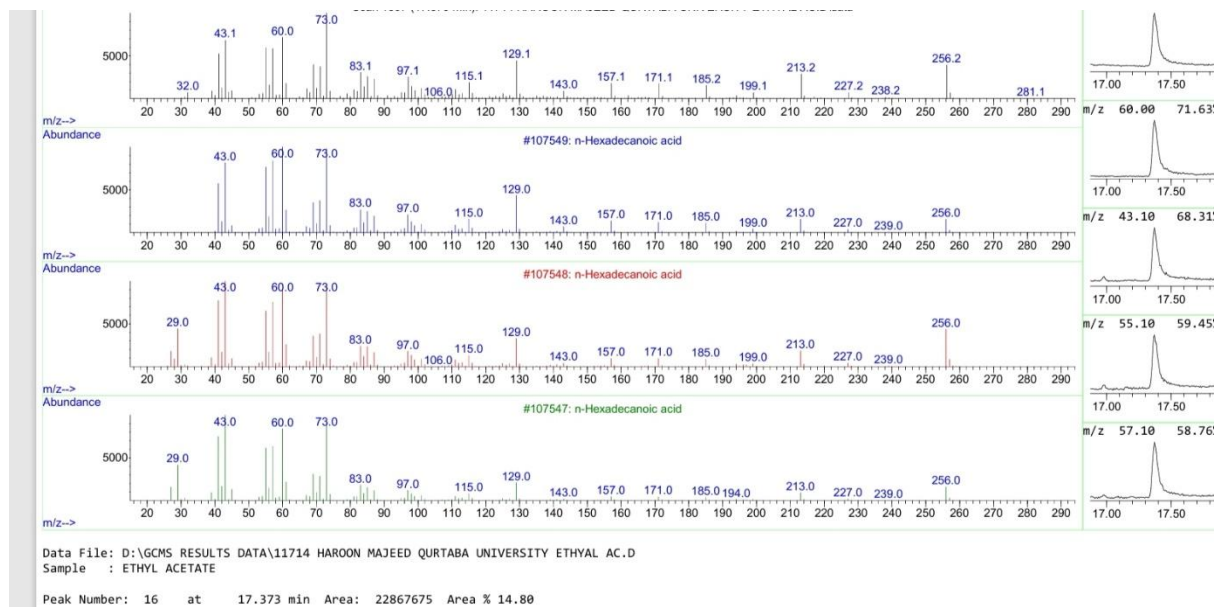
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Figure 2: GC-MS spectra of essential oil of *Z. absinthifolia* for n-Hexadecanoic Area: 14.80%

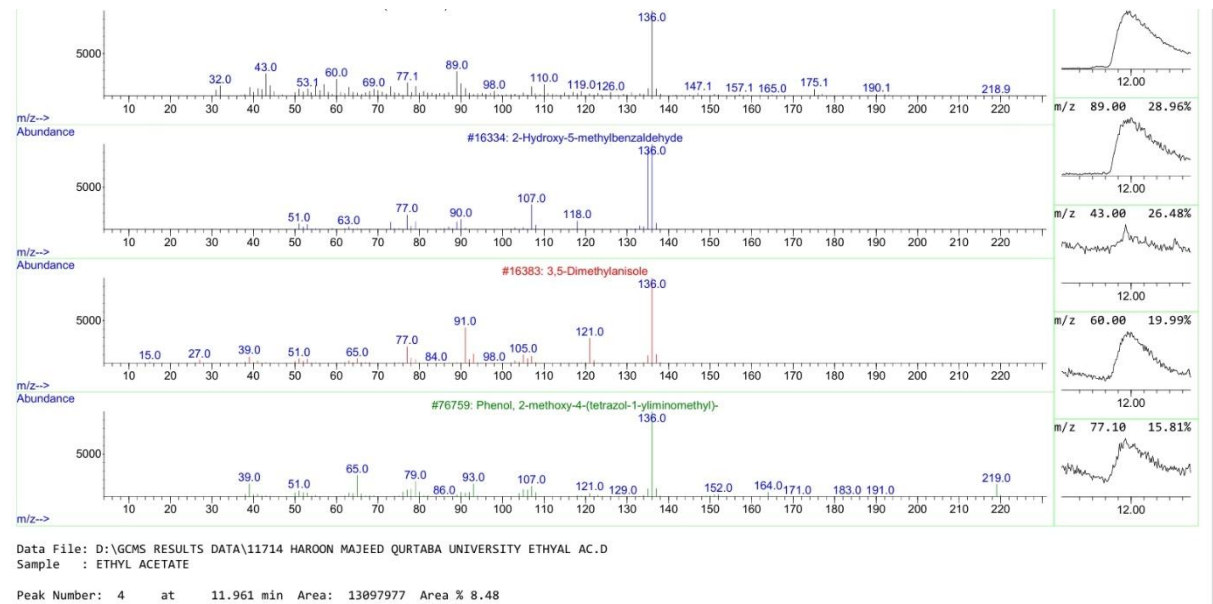
In a study it was found that n-Hexadecanoic constitutes 12.34 % in GC-MS results of the methanol crude extract of *Vernonia amygdalina* root (Igbidun & Nimenibo, 2019). That shows a percentage near to the obtained results. Another experiment was carried out in which the n-Hexadecanoic was identified from the leaves extract of *Ipomoea eriocarpa* was structurally characterized and its antioxidant and antibacterial activities and it showed reasonable antibacterial activities against studied organisms such as *S. aureus* was 7.96 ± 0.05 mm, *B. subtilis* 10.96 ± 0.15 mm was, *E. coli* 11.10 ± 0.17 mm was, and *K. pneumonia* was 11.93 ± 0.11 mm, at the maximum concentration of 50 µg/ml (Ganesan et al., 2024). Therefore, the reported biological activity of n-hexadecanoic made it a potential candidate to be used as a natural antioxidant and antibacterial agent in the pharmaceutical setting.

Hydroxy-5-methylbenzaldehyde (Retention Time: 11.961 Area: 8.48%)

2-Hydroxy-5-methylbenzaldehyde, also known as 5-methylsalicylaldehyde or 2, 5-Cresotaldehyde, is an Aromatic aldehyde with the molecular formula $C_8H_8O_2$. It is a benzaldehyde derivative featuring a hydroxyl group and a methyl group at specific positions on the benzene ring. This compound and its derivatives have applications in numerous areas, such as medicinal chemistry, materials science, and catalysis (Jayareshmi et al., 2021). The reported chemical was with a peak of 8.48% in GC-MS results of present study (Fig. 3). Janakiraman, N. (2016), analyzed the ethanolic extracts of *Cyathea nilgirensis*, *C. gigantea*, and *C. crinita* through GC-MS and resulted that *Cyathea crinita* accounts 55.45 % of 2-hydroxy-5-methylbenzaldehyde this indicates *C. crinita* has more 2-hydroxy-5-methylbenzaldehyde percentage as compared to carried out study. The same study also investigated that *Cythea gigantean* ethanolic extract constitutes 16.26 % for 2-hydroxy-5-methylbenzaldehyde

while *Cyathea nilgirensis* ethanolic extracts not contain this compound.

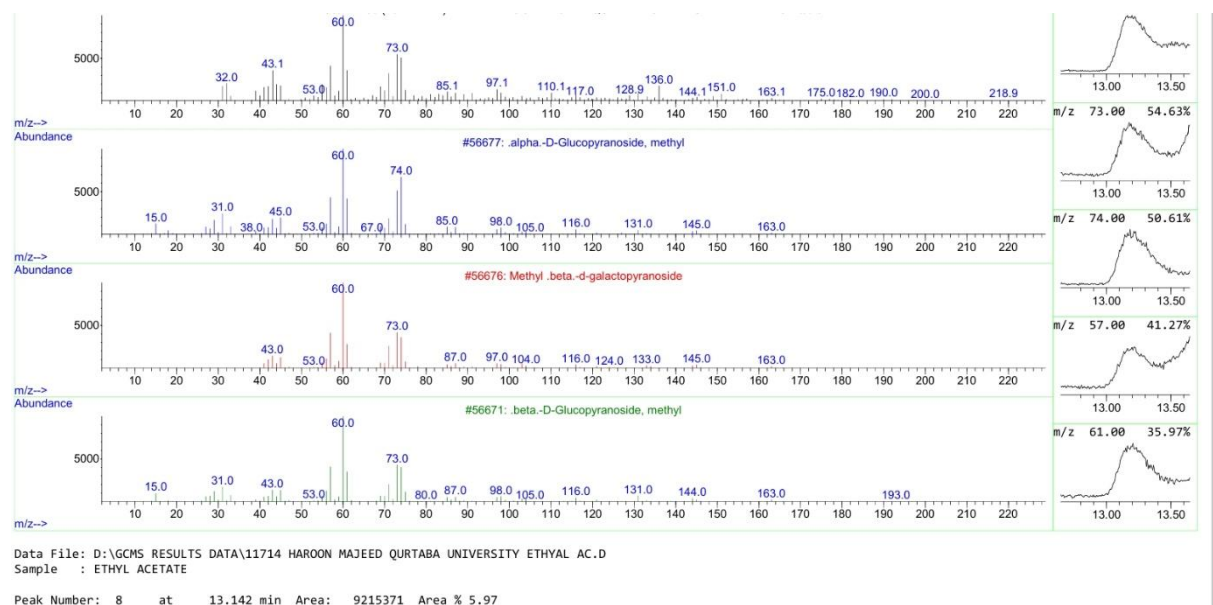
Figure 3: GC-MS Spectra of essential oil of *Z. absinthifolia* for 2-Hydroxy-5-



methylbenzaldehyde Area: 8.48%)

Methyl α -D-Glucopyranoside (MDPG) (Retention Time: 13.142 Area: 5.97%)

The above naturally occurring derivative of carbohydrates attract the medicinal chemists' interest due to its possible medical applications, particularly as an antibacterial and antifungal agent. Methyl α -D-Glucopyranoside, is identified (5.97%) in extracted essential oil of *Z. absinthifolia* plant (Fig. 4). A study reported that methyl α -D-Glucopyranoside and its derivatives have a broad range of antiviral, antifungal, antibacterial and anticancer effects. Owing to their widespread interest as possible drug design compounds, methyl α -D-Glucopyranoside and its derivatives has been chosen for assessment of their biological importance against different infections through computational techniques (Arabi et al., 2024). This indicates the presence of methyl α -D-Glucopyranoside in present study may have profound impacts in herbal products.



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Figure 4; GC-MS Spectra of essential oil of *Z. absinthifolia* for Methyl α -D-Glucopyranoside Area: 5.97%)

9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Retention Time: 18.956 Area: 29.36 %)

This compound constitutes the major peak (29.36 %) and it accounts for one third composition of the essential oil of *Z. absinthifolia* (Fig. 5). The 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- is known as Alpha-lonolenic acid has molecular formula $C_{18}H_{30}O_2$ and it is a polyunsaturated fatty acid. Such structure is likely to have high biological activity and could be an essential active component for herbal products due to presence of phytochemicals contained in it. The phytochemical screening of the methanol crude extract of *Vernonia amygdalina* root was investigated through GC-MS by Igbinidu & Nimenibo (2019) concluded that chemical constituent 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- was found in small quantity (1.11%) with comparison to the obtained percent (29.36 %) of presented study.

While in another study, Krishnaveni et al., (2014) carried out the analysis of phytochemicals, profiling of fatty acid, and antimicrobial activity of *Gossypium* seeds and the results obtained by GC-MS for phytochemical analysis, the constituent 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- was also found with highest peak area 70.10 % in *Gossypium* Seeds. The *Gossypium* Seeds powder showed antimicrobial activity in 50 and 100 μ g/ml against *Aspergillus niger* 11 - 16 mm (Zone of inhibition), *Aspergillus flavus* 12 - 13 mm, *Escherichia coli* 13 - 20 mm, and *Staphylococcus aureus* 20 - 20 mm accordingly. It was found to be more active in killing bacteria like *E. coli*, and *S. aureus*. The obtained result of this study supports that *Z. absinthifolia* is also a best source for the chemical constituent 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)- and have potential for health care products.

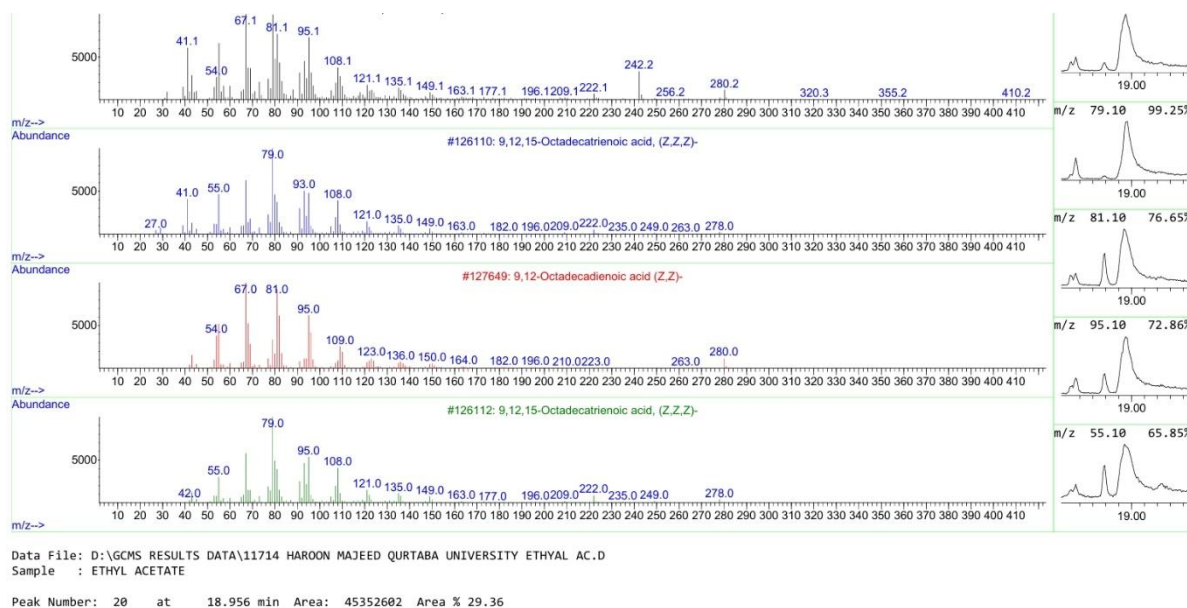
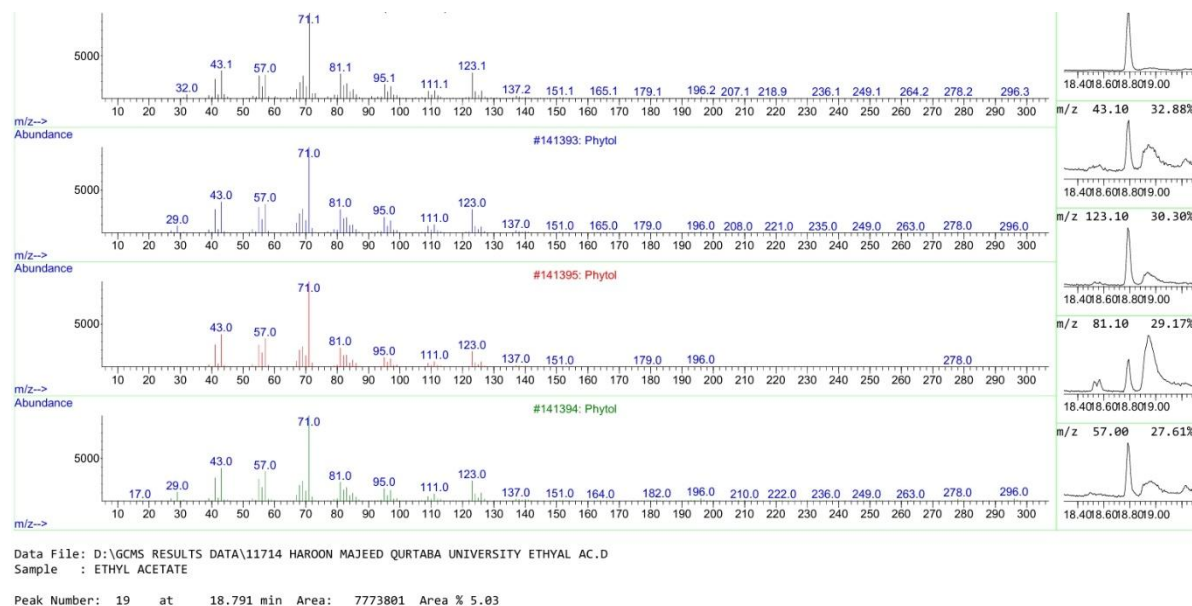


Figure 5: GC-MS Spectra of essential oil of *Z. absinthifolia* for 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Area: 29.36 %)

Phytol (Retention Time: 18.791 Area: 5.03 %)

Phytol ($C_{20}H_{40}O$) is a diterpene component found in plants. It has antioxidant, anti-inflammatory and antimicrobial properties. Phytol was found (5.03 %) in extracted essential oil of *Z. absinthifolia* plant (Fig. 6). A study in Turkey analyzed the essential oil of *Z. absinthifolia* investigated that flowers of *Z. absinthifolia* contains 0.5 % of phytol (Karakaya et al., 2019). According to Idu et al. (2021) GC-MS analysis and phytochemical screening of Poly herbal aqueous leaves (PALE) were examined. The GC-MS results showed only 1.07 % of phytol in PALE extract. Karki et al. (2020) reported that methanolic extract of *Euphorbia hirta* exhibited 4.78 % of phytol. These plants have less amount of phytol as compared with obtained essential oil results. In contrast, the GC-MS spectrum of chloroform fraction of *Adhatoda vasica* leaf constitute more phytol percentage of 27.39 (Saha & Bandyopadhyay, 2020).

Figure 6: GC-MS spectra of essential oil of *Z. absinthifolia* for Phytol Area: 5.03 %



Phytochemicals and fatty acids are the most important sources which is essential for the medicinal properties to be used as antimicrobial, antifungal agents.

Conclusion and Recommendation

The chemical insight of *Z. absinthifolia* essential oil showed a total of 26 bioactive compounds were identified through GC-MS. The major constituents include n-Hexadecanoic acid (14.80%), 2-Hydroxy-5-methylbenzaldehyde (8.48%), α -D-Glucopyranoside, methyl (5.97%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (29.36 %), and Phytol (5.03 %) accordingly. Detected compounds represent diverse chemical classes, such as aromatic aldehydes, phenolic compounds, sugar derivatives, fatty acid esters, and heterocycles. The presence of fatty acids in the extracted essential oil like linoleic acid, palmitic acid, and linolenic acid suggests potential natural source of antioxidant, anti-inflammatory, and antimicrobial properties. The findings support the pharmacological potential of the extract and its relevance in nutraceutical or

medicinal applications. Further studies are recommended to isolate these compounds and evaluate their individual therapeutic effects.

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Conflict of Interest

Author declares no conflict of interest

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