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Identification of bioactive compounds in ethanol extract of *Beta vulgaris* using GC-MS analysis

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Abstract
The resistance to therapeutic drugs increases the demand to discover novel chemical compounds with potential potency. Medicinal plants provide a wide window of opportunities to discover new drug leads through a screening process with scientific validation and optimization. Hyphenated techniques in drug discovery made it easier to isolate the structural information. It allows analysts to distinguish between known and unknown compounds. Advancing the research, Gas Chromatography-Mass Spectroscopy was employed in this study to identify the bioactive compound within the ethanolic extract, marking a notable progression. The spectrum confirms the presence of various medicinal components including heneicosane, n-decyl-N'-isopropyl, Octacosane, Eicosane, Hexatriacontane, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, Tetracosane, Heptacosane, 1,2-Benzenedicarboxylic acid, diundecyl ester, Di-isooctyl phthalate, Eicosane, 2-methyl, Nonacosane, Tetracosane, 11-decyl, responsible for the antimicrobial activity, anti-inflammatory, cytotoxicity, antimalarial, anti-inflammatory property of beetroot thus could play vital roles in health care programs.

Keywords: Drug discovery, medicinal plants, GC-MS, bioactive compounds, beetroot extract, antimicrobial, anti-inflammatory, cytotoxicity

Introduction
Medicinal Plants have been employed as primary sources in past decades for the development of chemical compounds, lately utilized to manufacture herbal products but also play an important role in drug discovery to expand the pharmaceutical formulations in the modern therapeutic system [1]. Different industries like chemical, pharmaceutical, cosmetics, and industrial raw materials have been utilizing plant-based chemical compounds to develop different formulations to treat human illness [2]. The US drug market recognized Different types of new drugs like vincristine, resin amine, vinblastine, and reserpine during 1950-1970 in approximately 100 different plants [3]. Apart from the complimentary use of herbal plants, the ethnopharmacological properties reveal diverse biological activity like Artemisinin from Artemisia annua, used to treat multidrug-resistant malaria [4]. Silymarin is used in liver disease, derived from the seeds of Silybum marianum [5], with the help of analytical techniques. Similarly, the contribution of beetroot in nutritional sciences for human health and diet is renowned along with its distinguished phytochemical constituents which can be used for multiple health purposes [6]. The pharmaceutical industry is creating a new revolution in modern medicine research to discover new molecules through advanced analytical methods and techniques [7]. Drug discovery always relies on the generation and innovation in analytical techniques and big data converter tools for molecule recognition and screening [8]. The major invention in analytical instrumentations includes Gas Chromatography-Mass Spectroscopy, high-performance liquid chromatography, mass spectrometry, capillary electrophoresis, vibrational spectroscopies (infrared and Raman), X-ray diffractometry, and hyperspectral imaging techniques [9].
Gas Chromatography-Mass Spectroscopy is an advanced sophisticated analytical technology with a combination of two powerful analytical tools mainly operated for Compounds identification with low detection limits based on chemical structure and potential for quantitative measures [10, 11].

GC-MS has been demonstrating and uncovering the medicinal Chemical Constituents of many plants including evolvulus alsinoides (Dwarf morning glory) [12]. Polygonum Chinense (Creeping smartweed or Chinese knotweed) [13]. Moringa oleifera (Drumstick tree) [14]. Justicia wynaadensis (Moddu Soppu) [15]. For decades, numerous beneficial chemical constituents have been waiting to be discovered. GC-MS has enormous applications not specifically in Pharmaceutical sciences but also in environmental analysis, explosives investigations, food and flavor analysis, drug testing of athletes, and forensic analysis [11]. Previous studies reported the diversity in pharmacological properties of beetroot and its therapeutic uses for human health. Some studies have confirmed the presence of many chemical compounds and phyto-components for example flavonoids, saponins, essential oils, phenols, tannins, and fatty acids. The main objective of this study is to isolate the bioactive compound in beetroot.

Ethical Considerations

The study's ethics approval was sought from the Ethical Committee of the Institute of Pharmaceutical Science of Peoples University of Medical and Health Sciences for Women (PUMHSW) (Reference #: PUMHS-032-2024

Material

The fresh beetroot (Beta vulgaris) was purchased from the local market in March 2024, Shaheed Benazirabad, Sindh, Pakistan, identified by Dr Tehseen for research at Peoples University of Medical and Health Sciences for Women (PUMHSW), Shaheed Benazirabad, Sindh, Pakistan.

Method

Preparation of Extract

The Fresh beetroot was cleaned, dried, decorticated, and ground into coarser powder using a mechanical grinder. The powder was weighed about 5 gm, dissolved in 100 ml of ethanol, and then macerated for 72 hours at room temperature. The extract was filtered using cellulose filter paper. A 10 µg/ml sample was submitted for GC-MS analysis at the Institute of Pharmaceutical Science Liaquat University of Medical and Health Sciences Jamshoro Sindh Pakistan.

GC-MS Identification

The GC-MS analysis of ethanol extract of Beta Vulgaris was performed by using Shimadzu GC 2010 comprising AOC-20i+s autosampler, a Shimadzu single quadrupole GCMS-QP2020 NX gas chromatograph interfaced with a mass spectrometer (GC-MS) equipped with an Elite 5-MS capillary column 30 m x 0.25 mm I.D. x 0.25 µm thick film with a composition of (5% 1,4-bis (Dimethylsiloxy) phenylene, 95% dimethyl polysiloxane). The injection mode was normal with 0.3 sec injection port dwell time and 3 times pumping. Column Temperature was 80 ºC for 2 min., rising to 300 °C then held for 4mins. Linear Flow Control Mode was maintained throughout the process, 95.8 KPa Column Pressure, GCMS-QP 2020 with TCD detector, Injector and detector temperature 200 ºC and 300 ºC respectively. Helium utilized as a carrier gas with a constant Flow rate at 1.40 ml/min and Injection Volume 1µl was employed with Injection split mode (Ratio of 10:0). The total flow was 18.4 ml/min. Mass spectra were taken at 1.10 kv+0.20 kv, with a scan time of 0.30 sec, and fragments from 42 to 550 in ACQ mode with a scan speed of 2000 cm. The solvent cut time was 3min and the total GC-MS running time was 27 min.

Result

GC-MS Chromatogram of ethanolic extract of *Beta vulgaris* showed different peaks (figure1) which indicated the presence of phytochemicals constituents. The mass spectra are presented in (Figure 2). The Molecular formula, retention time, molecular weight, and bioactivities of *beta vulgaris* are shown in Table 1.

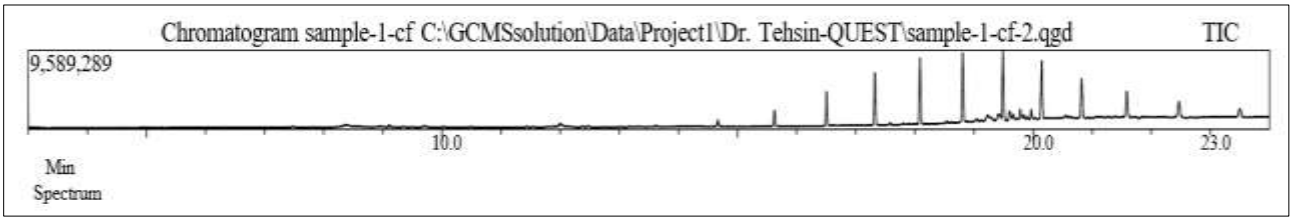
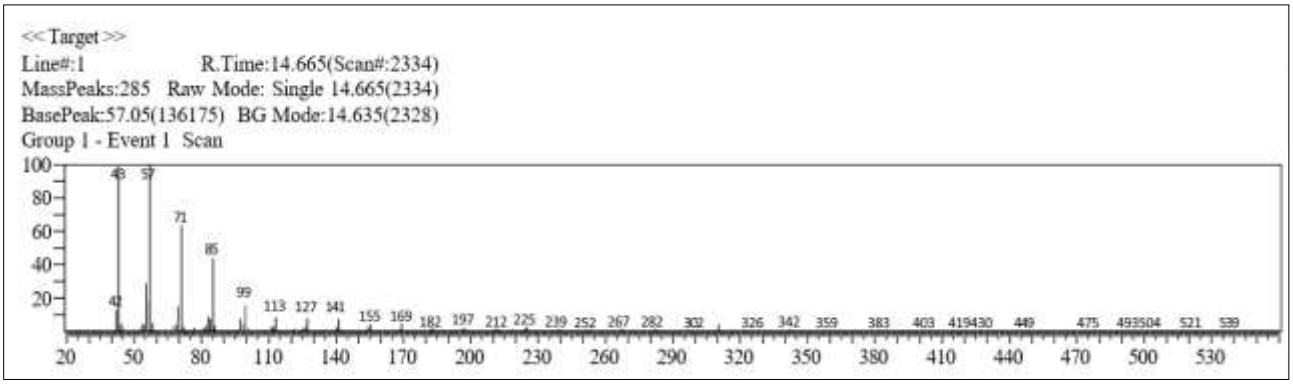
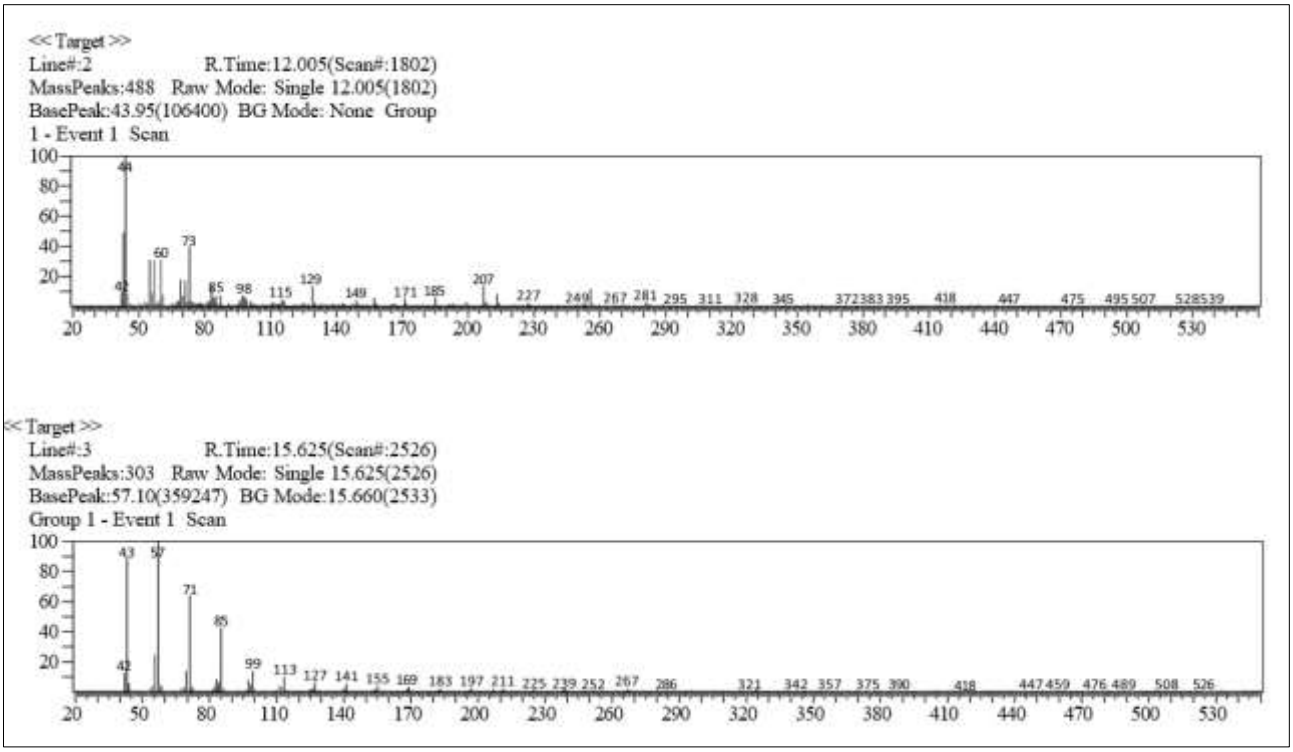
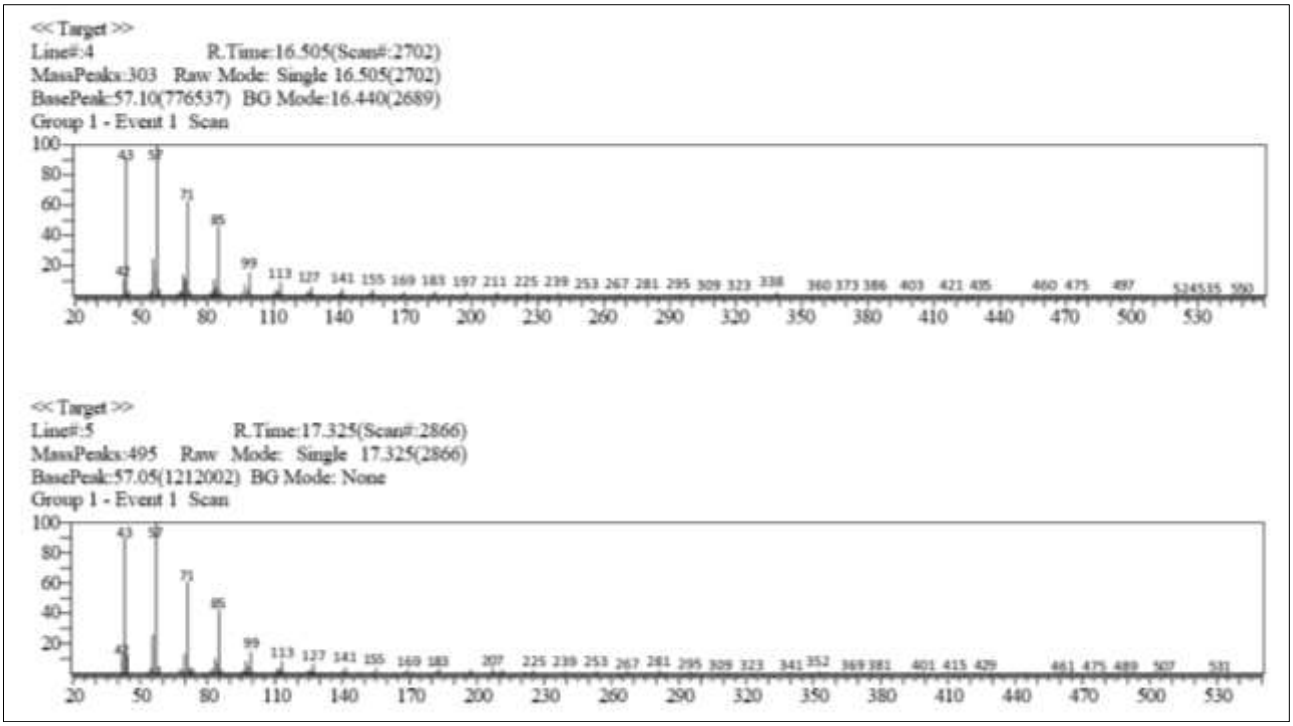


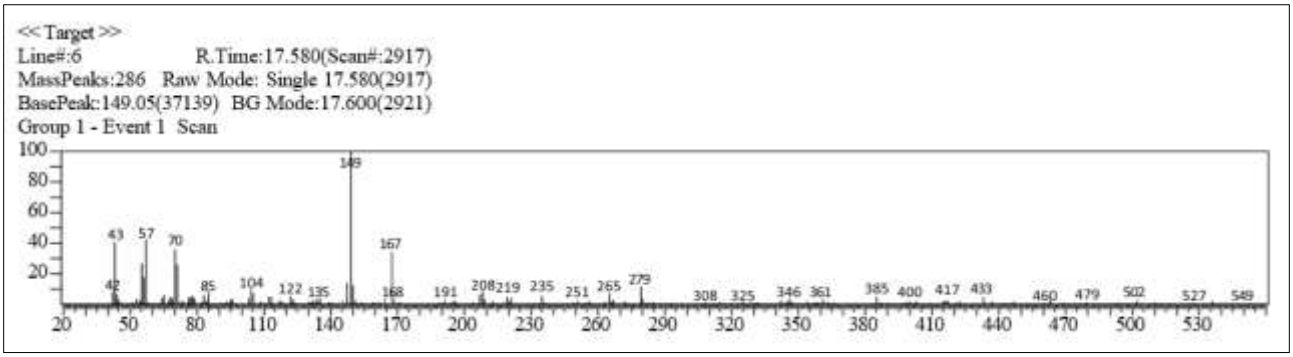
Fig 1: Shows the Chromatogram of Beta vulgaris




<< Target >>
Line#:2 R.Time:12.005(Scan#:1802)
MassPeaks:488 Raw Mode: Single 12.005(1802)
BasePeak:43.95(106400) BG Mode: None Group
1 - Event 1 Scan
<< Target >>
Line#:3 R.Time:15.625(Scan#:2526)
MassPeaks:303 Raw Mode: Single 15.625(2526)
BasePeak:57.10(359247) BG Mode:15.660(2533)
Group 1 - Event 1 Scan

Target >>


<< Target >>
Line#:4 R.Time:16.505(Scan#:2702)
MassPeaks:303 Raw Mode: Single 16.505(2702)
BasePeak:57.10(776537) BG Mode:16.440(2689)
Group 1 - Event 1 Scan
<< Target >>
Line#:5 R.Time:17.325(Scan#:2866)
MassPeaks:495 Raw Mode: Single 17.325(2866)
BasePeak:57.05(1212002) BG Mode: None
Group 1 - Event 1 Scan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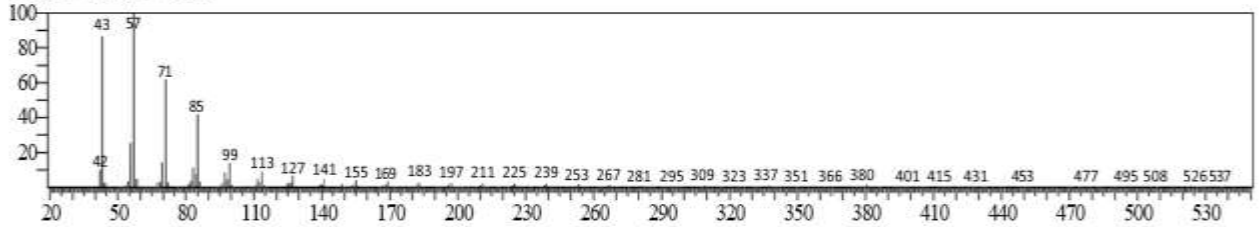
Line#:6 R.Time:17.580(Scan#:2917)
MassPeaks:286 Raw Mode: Single 17.580(2917)
BasePeak:149.05(37139) BG Mode:17.600(2921)
Group 1 - Event 1 Scan

<<Target>>
Line#:7 R.Time:18.810(Scan#:3163)
MassPeaks:334 Raw Mode: Single 18.810(3163)
BasePeak:57.05(1574130) BG Mode:18.765(3154)
Group 1 - Event 1 Scan



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BasePeak:57.05(1563929) BG Mode: None
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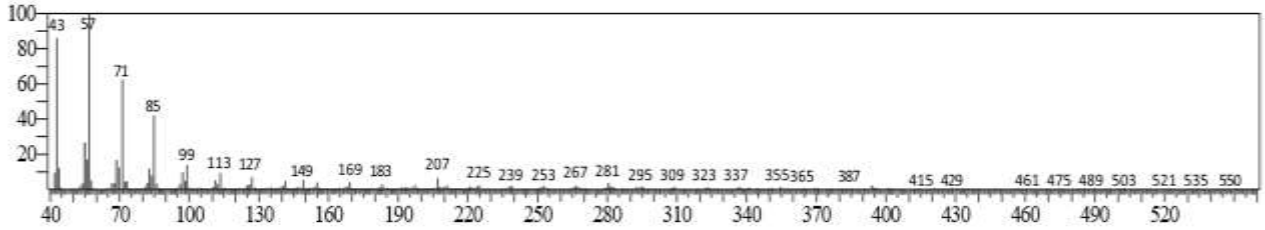

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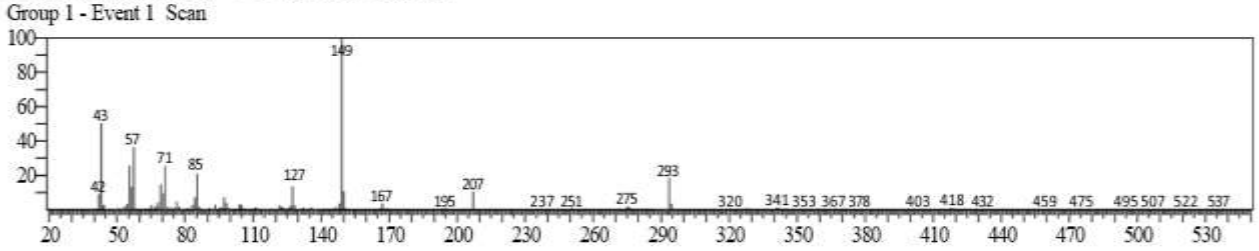
Line#:9 R.Time:19.785(Scan#:3358)
MassPeaks:293 Raw Mode: Single 19.785(3358)
BasePeak:149.05(254521) BG Mode:19.740(3349)
Group 1 - Event 1 Scan



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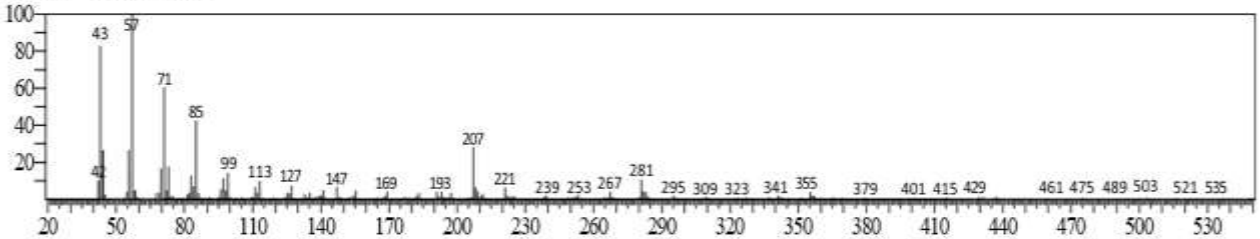
Group 1 - Event 1 Scan



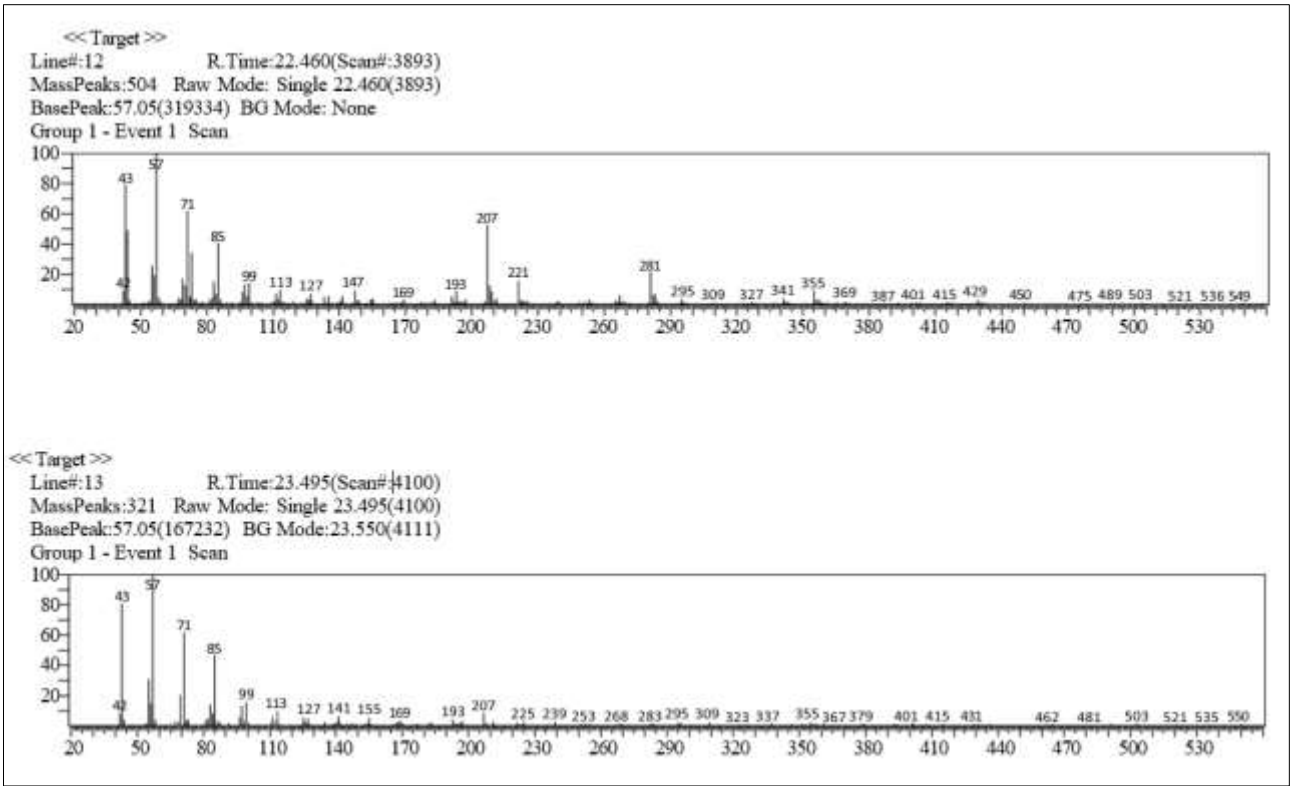


Fig 2: shows the mass spectra of different compounds by GC-MS analysis

Table 1: Shows the name, Retention time, Molecular formula, Molecular weight, bioactivity, and Molecular structure of compounds identified in Beta vulgaris by GCMS analysis.

S. No	Name of Compound	Retention time	Molecular formula	Molecular weight	Molecular Structure	Bioactivity
1	Heneicosane	14.665	C21H44	296		Antibacterial
2	n-decyl-N'-isopropyl	12.005	C14H30N2O	242		Unknown
3	Octacosane	15.625	C28H58	394		Antimalarial Anti-inflammatory
4	Eicosane	16.505	C20H42	282		Anti-inflammatory
5	Hexatriacontane	17.325	C36H74	506		Unknown
6	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	17.580	C24H38O4	390		Unknown
7	Tetracosane	18.810	C24H50	338		Antibacterial antioxidant
8	Heptacosane	19.490	C27H56	380		Cytotoxic, and antibacterial activity
9	1,2-Benzenedicarboxylic acid, diundecyl ester	19.785	C30H50O4	474		Unknown
10	Di-isooctyl phthalate	19.970	C24H38O4	390		Unknown
11	Eicosane, 2-methyl	21.585	C21H44	296		Carrier compound
12	Nonacosane	22.460	C29H60	408		Antibacterial
13	Tetracosane, 11-decyl	23.495	C34H70	478		Antibacterial

Bioactivity source: PubChem, Molecular Structure source: Chemdraw

Discussion

Interpretation on mass-spectrum GC-MS was conducted using the database of the National Institute of Standard and Technology (NIST) having more than 60,000 patterns of different components. GC-MS chromatogram of ethanolic extract of beetroot demonstrated mass peaks at different retention times The unknown components were analyzed by comparing their spectra with the known components stored in the NIST library. After collecting, assembling, and screening the results, 13 mass peaks were found and resembled the compound present in the NIST library. The identified compounds and chromatogram details are mentioned above in Table 1.

The article's studies focus on utilizing Gas Chromatography-Mass Spectroscopy (GC-MS) to investigate the bioactive compounds observed in the ethanolic extract of Beta vulgaris (Beetroot). This examination addresses the escalating demand for novel chemical entities with potent pharmacological properties due to the increasing resistance to conventional therapeutic drugs. The observation confirms the existence of many bioactive components and also sheds light on its role in the major activity associated with beetroot i.e. antibacterial activity. The employment of hyphenated techniques for example GC-MS enables the accurate identification and isolation of compounds and presents a significant advancement inside the realm of drug discovery within medicinal plants.

The compounds heneicosane [16] tetracosane [17], heptacosane [18], nonacosane [19], Tetracosane, 11-decyl [20] with retention time 14.665, 18.810, 19.490, 22.460, 23.495 found to possess antibacterial activity because of the presence of long chain alkane compound. Most compounds have microbial activity, this plant is mainly beneficial as herbal medicine against antibacterial resistance. Additionally, tetracosane [21] and heptacosane also showed the ability of anti-oxidant and cytotoxic [22]. The compound octacosane with a retention time of 15.625 was found to possess antimalarial [23] and anti-inflammatory activity [24], while eicosane at 16.505 exhibits only anti-inflammatory activity [24]. therefore, this plant could be beneficial in the management of inflammation. The eicosane-2methyl at 21.585 retention time is not a bioactive compound but could work as a carrier compound [25] and serve as a reservoir in a well-controlled environment for bioactive compounds in drug formulation for industrial purposes.

The biological activity of Compound n-decyl-N'-isopropyl, Hexatriacontane, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 1,2-Benzenedicarboxylic acid, diundecyl ester, Di-isooctyl phthalate is unknown.

The Importance of this research extends beyond the mere identification of many bioactive compounds in beetroot. It underscores the extensive capacity of natural sources in drug development and the pivotal role of advanced analytical methodologies in uncovering novel bioactive compounds. The GC-MS evaluation was carried out to exemplify how cutting-edge scientific tools can unveil hidden therapeutic agents inside natural sources. Employing elucidating the presence of many compounds and linking them to the antimicrobial properties of beetroot, this examination not only contributes to the understanding of the pharmacological and therapeutic properties of this plant but, additionally, exemplifies the strength of analytical technique in unlocking the pharmaceutical potential of natural substances.

Conclusions

In the last decades' medicinal plants have been the subject of drug discovery. It caught the interest of researchers worldwide to discover new lead compounds with the help of analytical techniques. GC-MS performs a vital role in assisting the investigations and Preliminary information which provides a reliable source for the researcher. This research article highlights the presence of bioactive alkanes and different compounds in Beta vulgaris. Figuring out the compounds contributes to understanding beetroot's medicinal chemistry diversity and promotes its potential use in antimicrobial, anti-inflammatory, antioxidant, and cytotoxicity applications. Furthermore, this work provides a foundation for future investigations aimed at isolating and characterizing other extensive compounds present in beetroot.

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Conflict of interest

None declared.

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List of abbreviations

- **GC-MS:** Gas Chromatography-Mass Spectroscopy
- **AOC-20i+s:** Automatic operation controller (autosampler)
- **GCMS-QP2020 NX:** Equipment number
- **Elite 5-MS:** Column
- **TCD:** Thermal conductivity detector
- **ACQ mode:** Acquisition mode
- **NIST:** National Institute of Standard and Technology

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