



GCMS Profile of Bioactive Compounds with Therapeutic Potential in *Beta vulgaris* (L.) Ethanolic Leaf Extracts

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Plants, animals, and microorganisms have all been shown to have health benefits for humans. According to World Health Organization, plant medicines continue to be used by 80 percent of the world's population in developing countries. Plant-derived secondary metabolites are macromolecules that are biosynthesized in plants and have a variety of biological properties that are beneficial to humans, including antiallergic, anti-inflammatory, anti-diabetic, and antioxidant properties. Therefore, the present investigation was done to determine the bioactive compounds present in *Beta vulgaris* (L.) leaves powder using Perkin-Elmer Gas Chromatography-Mass Spectrometry, while the mass spectra of the compounds found in the extract matched the National Institute of Standards and Technology (NIST) library. GC-MS analysis of an ethanolic extract of *Beta vulgaris* (L.) revealed the presence of 25 bioactive compounds with different area percentages and structural details. The major bioactive compounds are 1,3,5,7- Tetroxane (73.1%), Decane (83.1%), Azulene (73.8%), 4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl-(71.1%), 6-Amino-1,3,5-triazine-2,4 (1H, 3H)-dione(65.1%), Phthalic acid, 4-bromophenyl ethyl ester(83.7%), Neophtadiene(93.1%), Neophytadiene (88.2%)Hexadecanoic acid, methyl ester(84.8%), n-Hexadecanoic acid(84.3%), Phytol(86.0%), 9- octadecenoic acid, (E)(88.6%), 2-Hexadecen-1-

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ol,3,7,11,15-tetramethyl-acetate,[R-[R*,R*-(E)]-(64.0%), 1-Tricosene(72.3%) and 17-Pentatriacontene(65.6%). Most identified compounds have bioactivities such as Antimalaria, Anti-inflammatory, Antibacterial, Antifungal, Antiviral, Antidiabetic, Antioxidant, Anticancer, Analgesic, Anti-hyperlipidemic, Hypocholesterolemic, Hepatoprotective, and Anti-androgenic, so that they can be recommended as a plant of phytopharmaceutical importance. Therefore ethanol extract of *Beta vulgaris* (L.) leaves proves as a potential source of bioactive compounds of pharmacological importance.

Keywords: *Beta vulgaris* L; GC-MS; phytol; azulene; hepatoprotective; antioxidant.

1. INTRODUCTION

It is estimated that 80% of the world's population depends on medicinal plants to treat numerous human diseases. So far nearly 50,000 plant species were screened for medicinal properties [1,2]. Plant-based medicines are currently considered and used as the most common medical system in the world [3]. Plant-based medicine interacts with human biology [4]; hence, safety insurance, quality control, proper usage, observance of reference standards, and efficacy are the valuable components of herbal drug [5]. Herbal medicine, according to WHO, would be a better option for balancing therapeutic services with preventive care which can help to address the unique health challenges of the twenty-first century [6].

According to the World Health Organization, plant-based medicine supports approximately 75-80 percent of the global population, primarily in developing countries such as India, which has a diverse plant-based eco-system [7]. Because of its agro-climate zones, India has always been an opulent reservoir of medicinal plants [8]. In India, plant medicines are usually the first choice for primary healthcare of patients because of better cultural acceptability, better compatibility with the human body, and lesser side effects [9].

Most plants contain bioactive compounds that are known as phytochemicals, such as alkaloids, terpenoids, phenols, glycosides, carotenoids, flavonoids, etc [10-12]. More than 5000 individual phytochemicals have been isolated and identified in fruits, vegetables, and grains [13]. Bioactive compounds is a substance that has positive biological activity in health such as reduction of developing chronic diseases, such as cancer and diabetes [14-16]. Fruits and vegetables are related to these health benefits because they attribute to the synergistic interactions of the bioactive compounds present in the food [17].

The prospect of developing new drugs from natural plants remains appealing because

bioactive compounds have alternative and safe effects on treatment [18]. Pharmacopoeia Commission for Medicine & Homoeopathy (PCIM&H) published Pharmacopoeias and formularies for Indian medicinal plants [19]. Practitioners have been using plant medicines extensively for their antioxidant, antiviral, hepatoprotective, immunomodulatory, and thrombolytic activities for ages [20]. Knowledge of the bioactive constituents of plants would further be valuable in discovering folkloric remedies [21].

As a result, the current study investigated the bioactive compounds in the ethanolic extract of *Beta vulgaris* (L.) leaves. Gas Chromatography-Mass Spectroscopy, a hyphenated system, is a widely used technique for identification and quantification. The unknown organic compounds present in a complex mixture can be determined by interpretation as well as by matching the spectra with reference spectra. There are two significant advantages for using GC-MS in the analysis of plant, first, the capillary column in GC-MS has very good separation ability, which can produce a chemical fingerprint of high quality, and second with coupled mass spectral database, quantitative composition information of the plant investigated could be provided by GC-MS, which will be extremely useful for further research for elucidating the relationship between chemical constituents in plant medicine and its pharmacology in further research.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The entire parts of *Beta vulgaris* (L.) are collected from Kothagiri, Nilgiris district, Tamil Nadu, India and were authenticated by Arulanandam, Botanist, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu. The herbarium specimens are kept in The Rapinat Herbarium of PG and the Research

Department of Botany, St. Joseph's College, Tiruchirappalli (Tamil Nadu), India.

2.2 Preparation of Plant Extracts

Fresh plants were collected directly from Melvin's organic field, Nilgiris District, (Tamil Nadu), and air-dried at room temperature, and then homogenized to obtain coarse powder. The powdered samples were extracted [22] with ethanol solvent by hot extraction using the Soxhlet apparatus. The solvent-free extracts were collected and stored in a vial (-4°C) for further analysis.

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

Ethanol extract of leaves of *Beta vulgaris* (L.) was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. GC-MS analysis of some of the potent volatile constituents present in the extracts was performed at "Centre for Bioscience and Nanoscience Research (CBNR)", Coimbatore, Tamil Nadu, India. GC-MS analysis of *Beta vulgaris* (L.) leaf ethanolic extracts was performed using a GCMS (Thermo Trace GC Ultra Ver.5.0 ; Model) equipped with DB-35MS

fused silica column capillary (length 30m x outside diameter 0.25mm x internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with -70 eV ionization energy was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min and the sample injected was 1 µl; Injector temperature was 250°C; Ion source temperature was 200°C. The oven temperature was programmed from 70°C to 200°C at the rate of 10°C/min, held isothermal for 1 minute and finally raised to 250°C at 10°C/min. The interface temperature was kept at 250°C. The relative percentage of *Beta vulgaris* (L.) leaf extract constituent was expressed as a percentage showing peak area normalization.

2.4 Identification of Components

The components identified in the *Beta vulgaris* (L.) leaf ethanolic extract were assigned by their comparison of the retention time and mass spectra fragmentation patterns with those stored in the computer library and also with published literature. NIST [23,24] library sources were also used for matching the identified components from plant extract materials.

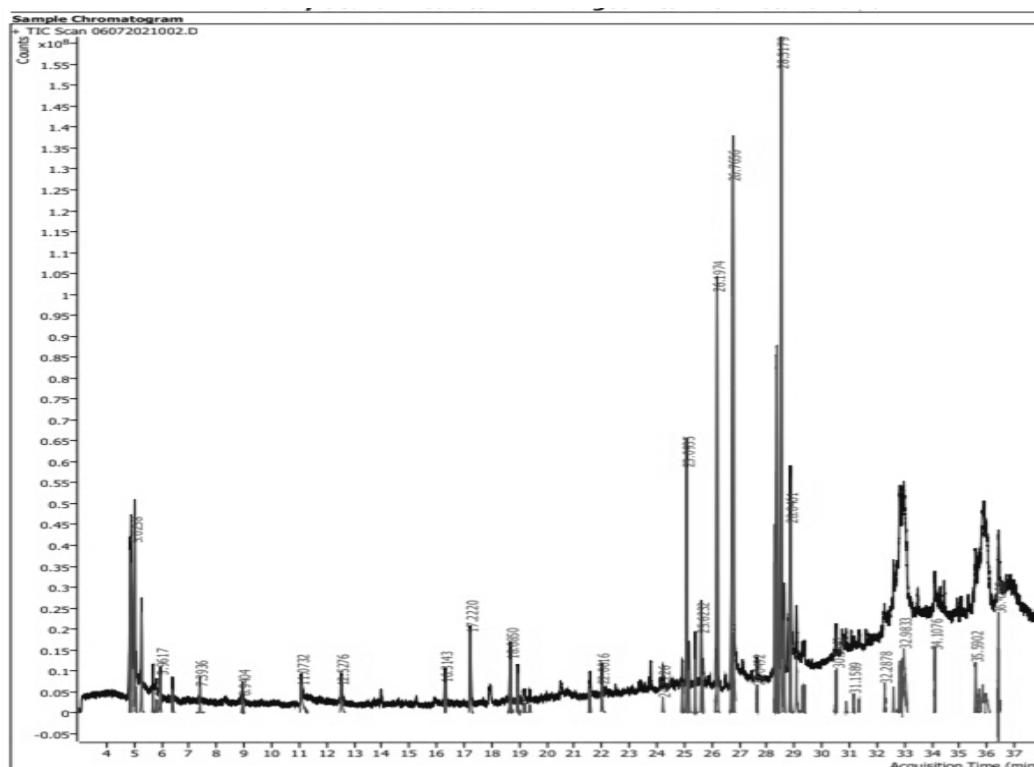


Fig. 1. Chromatogram of ethanolic extract of *Beta vulgaris* (L) leaf

3. RESULTS AND DISCUSSION

The GC-MS analysis of ethanolic extracts of leaves of *Beta vulgaris* (L.) revealed the presence of twenty-five constituents. The GC-MS running time was 37.15 minutes. The GC-MS chromatogram is presented in Fig.1. Table 1 shows the active principles along with their Retention Time (RT), Molecular Formula, Molecular Weight (MW), and peak area. The identified leaf extract compound's spectra are compared to the Wiley 9.0 and NIST libraries.

The major identified bioactive compounds and its peak area are 1,3,5,7-Tetroxane (73.1%); Decane (83.1%); Azulene (73.8%); 4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl-(71.1%); 6-Amino-1,3,5-triazine-2,4 (1H, 3H)-dione(65.1%); Phthalic acid, 4-bromophenyl ethyl ester(83.7%); Neophytadiene(93.1%); Neophytadiene (88.2%); Hexadecanoic acid, methyl ester(84.8%); n-Hexadecanoic acid(84.3%); Phytol(86.0%); 9-octadecenoic acid, (E)(88.6%); 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-acetate,[R-[R*,R*-(E)]]-(64.0%); 1-Tricosene(72.3%) and 17-Pentatriacontene(65.6%) were also obtained. The nature and uses of the phytoconstituents in ethanol extract of *Beta vulgaris*.L leaf are presented in Table 2.

Among the identified compounds, 4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl, Neophytadiene, Hexadecanoic acid, methyl ester, n-

Hexadecanoic acid, 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-acetate [R-[R*,R*-(E)]] have the property of antioxidant, antimicrobial, anti-inflammatory. n-Hexadecanoic as the common compound in the leaves of *P.stratiotes* and *E.crassipes*. E-11-Hexadecanoic acid, ethyl ester act as Antifungal, Antitumour, Anti-bacterial, and Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester found in leaf extract act as Hemolytic, pesticide, flavor, antioxidant [25]. Similarly, work on the bioactivity of n-hexadecanoic acid (also known as palmitic acid) and reported that it possesses strong antioxidant properties and pesticidal activity [26]. 1,3,5,7-Tetroxane reported having antimalarial, antipyretic, or anti-inflammatory agents. Similarly, the presence of 1,3,5,7-Tetroxane was observed in the methanolic extract of *Jatropha curcas* (L.) [27]. Azulene is reported to being effective in the treatment of Antibacterial, Antifungal, Anticancer, Analgesic, Anti-inflammatory, Anti-diabetic, Anti-hyperlipidemic, Anti-tubular activity. Neophytadiene reported antipyretic, analgesic, anti-inflammatory, anti-microbial, antioxidant. Similarly, the Azulene compound is found in GCMS hydrosol extract of *Aquilaria* (Agarwood) species [28]. Phytol showed Antimicrobial, anti-inflammatory, diuretic, anticancer, antimalarial. Phytol was found to give good, well preventive, and therapeutic results against arthritis. The results showed reactive oxygen species promoting a novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly

Table 1. GC-MS Analysis of bioactive compounds in the leaves of ethanolic extract of *Beta vulgaris* L

S.No	Retention Time	Name of the compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area
1	5.02	1,3,5,7-Tetroxane	C ₄ H ₈ O ₄	120.10	73.1%
2	7.39	Decane	C ₁₀ H ₂₂	142.29	83.1%
3	11.07	Azulene	C ₁₀ H ₈	128.17	73.8%
4	17.22	4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl-	C ₁₂ H ₂₂ O	182.30	71.7%
5	18.68	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	C ₃ H ₄ N ₄ O ₂	128.09	65.1%
6	22.00	Phthalic acid, 4-bromophenyl ethyl ester	C ₁₆ H ₁₃ BrO ₄	349.17	83.7%
7	25.09	Neophytadiene	C ₂₀ H ₃₈	278.5	93.1%
8	25.62	Neophytadiene	C ₂₀ H ₃₈	278.5	88.2%
9	26.19	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	84.8%
10	26.76	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42	84.3%
11	28.51	Phytol	C ₂₀ H ₄₀ O	296.53	86.0%
12	28.84	9-Octadecenoic acid, (E)-	C ₁₉ H ₃₆ O	296.48	88.6%
13	30.51	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,acetate, [R-[R*,R*-(E)]]-	C ₂₀ H ₄₀ O	296.53	64.0%
14	32.28	1-Tricosene	C ₂₃ H ₄₆	322.6	72.3%
15	34.10	17-Pentatriacontene	C ₃₅ H ₇₀	490.9	65.6%

Table 2. Nature and the biological activities of phytoconstituents of the leaves of ethanolic extract of *Beta vulgaris* (L.)

S.No	Retention Time	Name of the Compound	Compound Nature	Uses
1	5.02	1,3,5,7-Tetroxane	Hetero compound, Oxane	Antimalaria, non-central analgesic, antipyretic, antiinflammatory
2	7.39	Decane	Alkanes hydrocarbon	Antibacterial, neurotropic
3	11.07	Azulene	Aromatic hydrocarbon	Anti-inflammatory, antineoplastic, antidiabetes, antiretroviral, antimicrobial, antifungal
4	17.22	4-Hepten-2-one, 5-ethyl-3,3,4-trimethyl-	Ketone	Antioxidant, Antimicrobial-Antibacterial
5	18.68	6-Amino-1,3,5-triazine-2,4(1H,3H)-dione	Amino diol	Antibacterial, Antifungal, Anticancer, Analgesic, Anti-inflammatory, Antidiabetic, Antihyperlipidemic, Anti tubular activity
6	22.00	Phthalic acid, 4-bromophenyl ethyl ester	Acid	Antibacterial activity, Antifungal activity
7	25.09	Neophytadiene	Hydrocarbons	Antipyretic, Analgesic, antimicrobial, Antioxidant, Anti-inflammatory,
8	25.62	Neophytadiene	Hydrocarbons	Antipyretic, Analgesic, Anti-inflammatory, Anti-microbial. Antioxidant
9	26.19	Hexadecanoic acid, methyl ester	Amino compound	Antioxidant, nematicide, flavoring agent, pesticide, anti-androgenic, hypocholesterolemic, lubricant
10	26.76	n-Hexadecanoic acid	Palmitic acid ester	Antioxidant, hypocholesterolemic, antiandrogenic, hemolytic, lubricant
11	28.51	Phytol	Diterpene	Antimicrobial, anti-inflammatory, Antifungal against <i>S. typhi</i> , resistant gonorrhea, diuretic, headache, hernia, anticancer, resistant gonorrhea, joint dislocation, stimulant, and antimalarial
12	28.84	9-Octadecenoic acid, (E)-	Polyenoic fatty acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antiviral, anti-eczemic
13	30.51	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,acetate, [R-[R*,R*-(E)]-	Alkanes	Antioxidant, Hemolytic, Hypocholesterolemic, flavor, nematicide, anti-androgenic, antibacterial, antifungal
14	32.28	1-Tricosene	Alkene	Anticancer, Anti-inflammatory
15	34.10	17-Pentatriacontene	Alkene	Antimicrobial, Anti-inflammatory, Anticancer

other chronic inflammatory diseases [29]. 1-Tricosene and 17-Pentatriacontene showed anticancer, anti-inflammatory, and anti-cancer

activity. The Pesticidal potential of 1-tricosene, (Z)-and [1,1' – bicyclopropyl]-2-octanoic acid,

2'hexylo methyl ester was reported by Verma et al. [30].

Several other compounds with notable medicinal properties were also detected using the GCMS chromatogram. The aforementioned compounds found in the ethanol extract of *Beta vulgaris* (L.) leaf can be used in pharmacological research. Thus, GC-MS analysis of plant extracts is the first step toward understanding the nature of active components found in medicinal plants. This type of research will be useful for future research on plant medicinal active constituents. Separating individual secondary metabolites and subjecting them to biological activity, on the other hand, will yield fruitful results in the future. It could be concluded that *Beta vulgaris* (L.) leaf contains various bioactive compounds. So it is recommended as a leaf of pharmaceutical importance. However, further studies are needed to be done to undertake its bioactivity and toxicity profile.

4. CONCLUSION

GC-MS analysis of an ethanol extract of *Beta vulgaris* (L.) leaf revealed the presence of secondary metabolites with anticancer, antimicrobial, antioxidant, analgesic, anti-androgenic, and anti-inflammatory activities, suggesting a potential industrial application. We concluded that the biological values of *Beta vulgaris* (L.) contain pharmacologically active compounds that may improve its use of modern plant-based drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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