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 the 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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The prospect of developing new drugs from phytochemicals has 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 Arulandam, 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
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

Ethanolic 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 1µl; 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](image)
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(56.6%) were also obtained. The nature and uses of the phytoconstituents in Pentatriacontene(65.6%) were also obtained.

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.craspides. E-11-Hexadecanoic acid, ethyl ester act as Antifungal, Antimour, Anti-bacterial, and Hexadecanoic acid, 2-hydroxy-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 antimarial, 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, antimarial. 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$_{8}$H$_{10}$O | 120.10                   | 73.1%     |
| 2    | 7.39           | Decane               | C$_{10}$H$_{22}$ | 142.29                   | 83.1%     |
| 3    | 11.07          | Azulene              | C$_{19}$H$_{38}$ | 218.17                   | 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$_{13}$H$_{24}$O$_{2}$ | 128.09                   | 65.1%     |
| 6    | 22.00          | Phthalic acid, 4-bromophenyl ethyl ester | C$_{16}$H$_{12}$BrO$_{4}$ | 340.16                   | 83.7%     |
| 7    | 25.09          | Neophytadiene        | C$_{20}$H$_{38}$ | 278.5                    | 93.1%     |
| 8    | 25.62          | Neophytadiene        | C$_{20}$H$_{38}$ | 278.5                    | 82.8%     |
| 9    | 26.19          | Hexadecanoic acid, methyl ester | C$_{17}$H$_{34}$O$_{2}$ | 270.45                   | 84.8%     |
| 10   | 26.76          | n-Hexadceanoic acid | C$_{16}$H$_{32}$O$_{2}$ | 256.42                   | 84.3%     |
| 11   | 28.51          | Phytol               | C$_{20}$H$_{40}$ | 296.53                   | 86.0%     |
| 12   | 28.84          | 9-Octadecenoic acid, (E) | C$_{19}$H$_{38}$O | 296.48                   | 86.8%     |
| 13   | 30.51          | 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-acetate, [R-[R*,R*-(E)]] | C$_{20}$H$_{40}$ | 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 hydrocarbon | Antimalaria, non-central analgesic, antipyretic, anti-inflammatory |
| 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-6-Amino-1,3,5-triazine-2,4(1H,3H)-dione | Ketone | Antioxidant, Antimicrobial-Antibacterial |
| 5    | 18.68          |                      | 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, Antioxidant |
| 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 *S typhi*, 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*]-1,3-dimethyl-2-octanoic acid] | Alkanes | Antioxidant, Hemolytic, Hypcholesterolemic, 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’hexy10 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.

REFERENCES

1. Saslis-Lagoudakis CH, Hawkins JA, Greenhiss SJ, Pendry CA, Watson MF, Tuladhar-Douglas W et al. The evolution of traditional knowledge: Environment shapes medicinal plant use in Nepal. Proc Royal Soc B: Biol Sci. 2014;281(1780):20132768.
2. Chen S, Yu H, Luo H, Wu Q, Li CF, Steinmetz. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med. 2016;11:37.
3. Origima SAO. Web-based decision support system for prescription in herbal medicine. J Emerg Trends Eng Appl Sci. 2015;6(7):245-254.
4. Motahari Tabari N, Yousefi SS, Heydarirad G, Kardan Soraki M, Habibipour P. Exercise from the perspective of Iranian traditional medicine. J.Evid Based Complementary Altern Med. 2017;22(2):344-346.
5. Boulin AS, Wierer M. Quality standards of the European Pharmacopoeia. J. Ethnopharmacol. 2014;158:454-457.
6. WHO global report on traditional and complementary medicine. World Health Organization; 2019.
7. Evans M. A Guide of Herbal Remedies, Orient Paperbacks: Delhi, India; 1994. ISBN – 10:8122201628.
8. Mohanraj K, et al. IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics. Sci. Rep. 2018;8:4329-4346.
9. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac.J.Trop.Biomed. 2012;2:320-330.
10. Malviya N. Antidiabetic Potential of Medicinal Plants. Acta Pol. Pharma. 2010; 67:113-118.
11. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol. 2002;81:81-100.
12. Aggarwal N, Aggarwar S. A review of recent investigations on Medicinal Herbs possessing antidiabetic properties. J.Nutr. Disord. Ther.; 2011.
13. Liu RH. Dietary bioactive compounds and their health implications. J.Food Sci. 2013; 78:A18-A25.
15. Reiss R, Johnston J, Tucker K, DeSesso JM, Keen CL. Estimation of cancer risks and benefits associated with a potential increased consumption of fruits and vegetables. Food Chem. Toxicol. 2012;50:4421-4427.

16. Salvin JL, Lloyd B. Health benefits of fruits and vegetables. Adv. Nutri. 2012;3:506-516.

17. Rodríguez-Casado A. The health potential of fruits and vegetables phytochemicals: Notable examples. Crit. Rev. Food Sci. 2014;56:1097-1107.

18. Kayarohanam S, Kavimani S. Current trends of plants having antidiabetic activity: A Review. J. Bioanal. Biomed. 2015;7:55-65.

19. Pharmacopoeia commission for Indian medicine & Homoeopathy, Ministry of AYUSH. Government of India.

20. Arora R, Chawla R, Marwah R, Arora P, Sharma RK, Kaushik V, Bhardwaj JR. Potential of Complementary and Alternative Medicine in Preventive Management of Novel H1N1 Flu (Swine Flu) Pandemic: Thwarting Potential Disasters in the Bud. Evidence-Based Complementary and Alternative Medicine, eCAM. 2011;586506.

21. Mojab F, Kamalinajad M, Ghandei N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. Iranian Journal of Pharmaceutical Research. 2003;2(2):77-82.

22. Mukherjee PK. Quality Control of Herbal Drugs. An approaches to evaluation of botanicals, edition 1st Business Horizons, New Delhi. 2002;390-403.

23. Mc Lafferly FW. Registry of mass spectral data, 5ed, Wiley New York; John Wiley & Sons Inc; 1989.

24. Stein SE. National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02 Gaithersburg, Md USA; 1990.

25. Duke's Phytochemical and Ethnobotanical Databases U.S. Department of Agriculture, Agricultural Research Service 1992-1996 [online].

26. Mohmoud HO, Dardiry Amal AA, Mohamed, Eman Abdelrady. Effect of lead (Pb) on phytochemical variability of Jatropha curcas (L.): A versatile perennial of Euphorbiaceae family, 2018;1(3):133-145.

27. Pavithra KS, Annadurai J, Raganathan R. Phytochemical, antioxidant and a study of bioactive compounds from Artemisia pol lens. J. Pharm Phytochem. 2018;7:664-675.

28. Yumi Zuhanis Has-Yun Hashim, Natasha Jafar Ali, Nur Aimai Aliyah Zainurin, Phirdaous Abbas. Profiling of compounds in Hydroxol extract of Aquilaria (Agarwood) species using Gas Chromatography-Mass Spectrometry (GCMS), Biological and Natural Resources Engineering Journal. 2021;5(1):25-33.

29. Ogunlesi M, Okiei W, Ofor E, Osibote AE. Analysis of the essential oil from the dried leaves of Euphorbia hirta Linn (Euphorbiaceae), a potential medication for asthma. African J. Biotech. 2009;8:7042-7050.

30. Verma VP, SH Kumar, KV Rani, N Sehgal, O Prakash. Compound profiling in methanol extract of Kalanchoe blossfeldiana (flaming katy) leaves through GC-MS analysis and evaluation of its bioactive properties. Glob J Adv Biol Sci. 2015;1:38-49.