Nat. Volatiles & Essent. Oils, 2021; 8(5): 4560 – 4568

NVEO
Natural Volatiles & Essential Oils

Analysis of Bioactive compounds from *Stevia rebaudiana* Bertoni

R. Ramya[1] *, S. Carmel Punitha[2], G. Aruna[3]

[1]**Assistant Professor, Department of Biochemistry, J.B.A.S College for Women, Chennai- 18.*
[2]*Assistant Professor, Department of Biochemistry, J.B.A.S College for Women, Chennai- 18.*
[3]*Assistant Professor, Department of Biochemistry, J.B.A.S College for Women, Chennai- 18.*

Abstract

Stevia rebaudiana Bertoni belongs to the family Asteraceae. It is a perennial shrub and the plant is native to South America. The plant leaf has been used for centuries for its sweetness and other medicinal properties. Stevia has gained importance among the consumers and food industry because of its role in treating obesity, diabetes, and other related commodities. The phytoconstituents of the plant are non-toxic and used to cure various chronic diseases. In various food industry it is used as a low-calorie sweetener. The present study was designed to identify the phytocomponents using Gas Chromatography and Mass Spectroscopy (GC-MS) in the ethanolic leaf extract of *Stevia rebaudiana*. The results revealed the presence of 40 compounds in the chromatogram of GC-MS based on the retention time and area percentage. The major secondary metabolites identified were Oleic acid (RT- 26.431; 100%), n-Hexadecanoic acid (RT -20.999; 89%), 9,12, Octadecadienoic acid (RT-26.685;80%), Phytol (RT-25.306;11.67%), Isosteviol (RT-35.903;6.595%), Hydroxydehydrostevic acid (RT- 36.03; 20.92%), 1-Heptatriacotanol (RT-35.01;2.31%), Vitamin E (40.334; 2.34%) and Campesterol (41.25; 3.72%). The findings of the result concluded that the plant might contain the major bioactive compounds responsible for various biological and pharmacological activities.

Key Words: *Stevia rebaudiana*, Diabetes, Asteraceae, GC-MS, Bioactive compounds, Chromatogram, Retention time, Phytocomponents.

Introduction

The supreme reservoir of bioactive compounds in the field of biotechnology are medicinal plants. It is used for the production of pharmaceutical compounds. Plant-based traditional medicine is utilized globally in treating various ailments due of the presence of phytochemical constituents.[1] The world health organization has accepted the herbal medicine as they are proven to be safe and 80% of the world's population rely on traditional medicine due to their therapeutic potential and less side effect. Hence for the use of these plants in drug discovery, the active compounds had to be identified and validated for the discovery of lead compound.[2] The best technique for the identification of the bioactive compounds such as alcohols, acids, esters, phenols, alkaloids, long hydrocarbons, steroids, amino, and nitro compounds present in the crude extract is carried out by GC-MS.[3] In current years GC-MS studies have been widely applied for the examination of medicinal plants for the non-polar components, lipids, fatty acids, and alkaloid, and volatile essential oil [4,5]

The plant *stevia rebaudiana* is widely grown in northeast Paraguay, Brazil and Argentina Japan, Korea, Thailand, and Indonesia. The steviol glycosides present in stevia renders sweet taste to the leaves. The leaves of the plant are used as a source of high potential sweetener of zero-calorie in industry such as the brew and food industries. The major constituents of Stevia *rebaudiana* leaves are Stevioside and Rebaudioside.[6] In 2008 JECFA (Joint FAO/WHO Expert Committee on Food Additives) has decided to raise the ADI (Acceptable Daily Intake) for Steviol Glycoside from 2 to 4 mg/kg body weight.[7,8] The constituents of the leaf extract help to lower the rate of absorption and controls the blood glucose levels in patients with high blood pressure, and type 2 diabetes.[9] *Stevia rebaudiana* possesses different medicinal properties such as, antifungal, antitumor, anti-inflammatory, antidiabetic, antimicrobial, and immune-stimulating properties. [10] Toxicology studies have shown that the leaves of the plant are not mutagenic and carcinogenic. [11,12] Identification of the various bioactive compounds existing in the ethanolic leaf extract of *Stevia rebaudiana* promotes the importance of the plant in herbal medicine.

Materials and Methods

Collection of Plant material

Fresh leaves of the plant *Stevia rebaudiana* were procured from the Agri Horticultural Society Garden, Chennai, India, as shown in Figure 1. The leaves were washed with water to remove the debris, shade dried at room temperature and powdered in electric blender as shown in Figure 2. The powdered leaves were stored in an air tight container for future investigation. Dried powder was subjected to Soxhlet extraction for preparing the extracts.

Figure 1 Leaves of *Stevia rebaudiana* Bertoni

Figure 2 Dry leaf powder of *Stevia rebaudiana* Bertoni

Preparation of Plant extract

About 20gms of the powdered leaf of *stevia rebaudiana* was subjected to extraction with 250 ml ethanol at a temperature range between 60C and 65C for 24 hours by using a Soxhlet extractor. The solvent was then evaporated by a rotary vacuum evaporator to attain the semisolid mass. The crude ethanolic leaf extract was exposed to GC-MS analysis.

GC-MS analysis

GC-MS analysis of the leaf extract was done using gas chromatography coupled with fame ionization detection (GC-FID, Shimadzu GC-2010 Plus, Japan). The fatty acids were separated on a FAMEWAX (USP G16) fused silica capillary column (30 m×0.32 mm×0.25 mm), and helium gas was used as carrier gas (99.995% purity) with fow rate 30 mL/min. The instrument parameter was as follows: the initial column temperature was 40 °C, and it was increased up to 150 °C at the rate of 5 °C m−1. Further, the temperature was slowly increased to 200 °C and 240 °C at the rates of 10 °C and 2 °C m−1, respectively. The injection port temperature and detector temperature were set at 250 °C. A 1µL of the derivatized oil sample was injected through AOC-

2Oi autosampler along with analytical standard. The compounds were identified based on the matching percentage of the obtained spectrum compared to the analytical standard spectrum. The percentage amount of respective compound was calculated by comparing the average peak area to the total area.

Identification of bioactive compounds

The bioactive compounds present in the ethanolic leaf extract of *Stevia rebaudiana* were identified based on the retention time. Using the database obtained from the National Institute Standard and Technology (NIST) interpretation of mass spectrum of GC-MS was performed. Figure 3 highlights the peaks in the GC-MS chromatogram showing the occurrence of bioactive compounds.

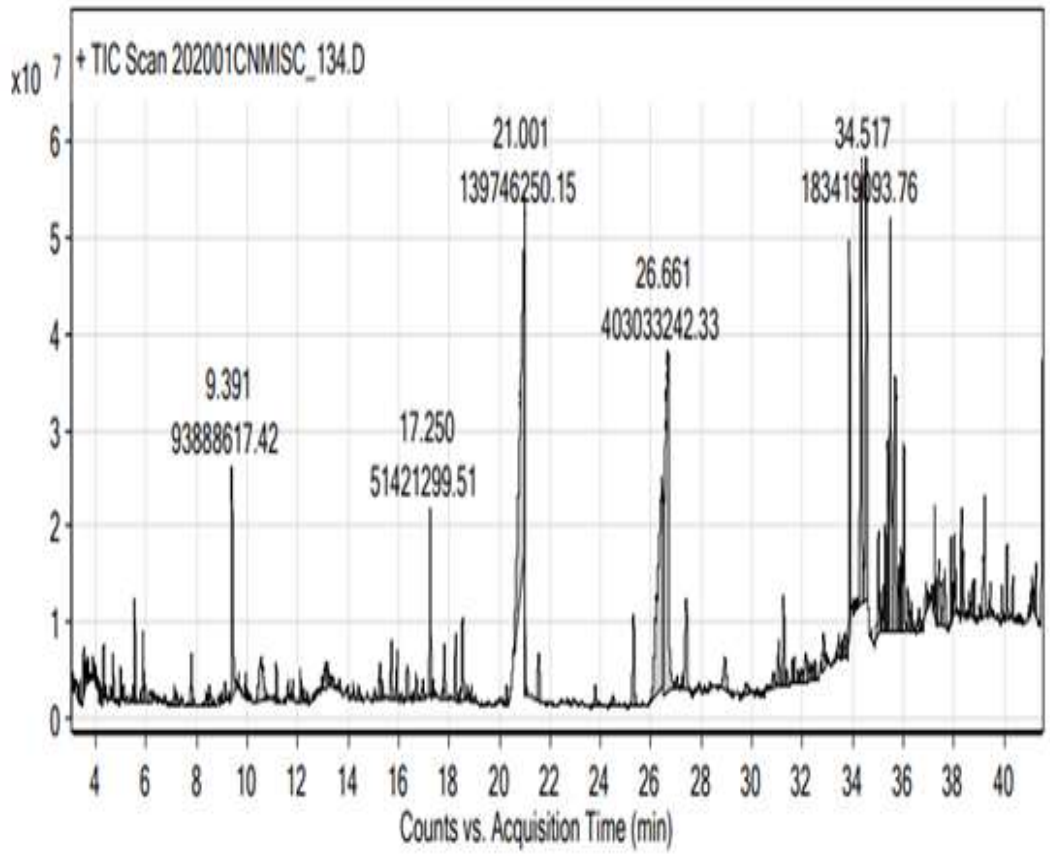

Figure 3. GC-MS Chromatogram of Ethanol leaf extract of *Stevia rebaudiana*

Results

The plant species consist of various bioactive compounds. There are many techniques employed to identify the bioactive compounds. The GC-MS technique which combines Gas chromatography (GC) and Mass spectroscopy (MS) is preferred to be the best technique for this study. The compounds such as long-chain hydrocarbons, alcohols, esters, acids, alkaloids, steroids, nitro, and amino compounds possess several commercial and biological applications.[3] The chromatogram of the GC-MS study shows the existence of 40 chemical compounds in the ethanolic leaf extract of *Stevia rebaudiana*. Table 1 displays the peak compounds with their Molecular formula, Molecular weight, Area percentage, and Retention time.

Table 1 GC-MS spectral analysis of ethanol leaf extract of *Stevia rebaudiana*

Name	**RT**	**Area %**	**Molecular weight**	**Molecular formula**
Inositol, 1-deoxy	4.681	3.45	180.16	$C_6H_{12}O_6$
Methyl salicylate	5.541	5.79	152.15	$C_8H_8O_3$
5- Hydroxymethylfurfural	5.866	5.45	126.11	$C_6H_6O_3$
Eugenol	7.778	4.04	164.2	$C_{10}H_{12}O_2$
Benzaldehyde 2 hydroxy 6- methyl	9.39	23.3	152.15	$C_8H_8O_2$
2(4H)-Benzofuranone, 5,6,7,7atetrahydro-4,4,7a-trimethyl-, (R	11.153	2.26	180.24	$C_{11}H_{16}O_2$
1H-Cycloprop[e]azulen-7-ol, decahydro1,1,7-trimethyl-4-methylene	12.107	2.06	220.35	$C_{15}H_{24}O$
4-((1E)-3-Hydroxy-1-propenyl)-2methoxyphenol	15.256	3.37	180.20	$C_{10}H_{12}O_3$
Tetradecanoic acid	15.718	4.47	228.37	$C_{14}H_{28}O_2$
5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro2H-chromene	16.316	5.27	180.29	$C_{12}H_{20}O$
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.251	12.76	296.53	$C_{20}H_{40}O$
Phytol, acetate	17.808	4.86	338.56	$C_{22}H_{42}O_2$
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.254	4.95	296.5	$C_{20}H_{40}O$
Tolycaine	18.529	9.61	278.34	$C_{15}H_{22}N_2O_3$
Phthalic acid, isobutyl 4-octyl ester	20.276	1.76	334.4	$C_{20}H_{30}O_4$
n-Hexadecanoic acid	20.999	89.09	256.42	$C_{16}H_{32}O_2$
Hexadecanoic acid, ethyl ester	21.541	5.52	284.5	$C_{18}H_{36}O_2$
Heptadecanoic acid	23.785	2.6	270.5	$C_{17}H_{34}O_2$
Phytol	25.306	11.67	296.5	$C_{20}H_{40}O$
9,12-Octadecadienoic acid (Z,Z)-	26.431	80.85	280.4	$C_{18}H_{32}O_2$
Oleic Acid	26.685	100	282.47	$C_{18}H_{34}O_2$
Octadecanoic acid	27.402	13.47	284.48	$C_{18}H_{36}O_2$
1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl	28.949	6.64	222.36	$C_{15}H_{26}O$
1,2-Benzisothiazol-3(2H)-one, 2(phenylmethyl)-, 1,1-dioxide	31.059	4.53	273.31	$C_{14}H_{11}NO_3S$
1-Naphthalenemethanol, decahydro-5-(5hydroxy-3-methyl-3-pentenyl)-1,4a-dimethyl-6methylene-	31.25	8.72	306.48	$C_{20}H_{34}O_2$
Eicosanoic acid	32.822	4.42	312.5	$C_{20}H_{40}O_2$
1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-,	33.866	28.77	222.36	$C_{15}H_{26}O$

1,1,6-trimethyl-3-methylene-2-(3,6,9,13tetramethyl-6-ethenye-10,14-dimethylenepentadec-4-enyl)cyclohexane	34.336	48.29	452.8	$C_{33}H_{56}$
i-Propyl 6,9,12-hexadecatrienoate	34.534	45.51	292.5	$C_{19}H_{32}O_2$
1-Heptatriacotanol	35.01	2.31	537	$C_{37}H_{76}O$
Imiprothrin	35.394	16.6	318.373	$C_{17}H_{22}N_2O_4$
Isosteviol	35.903	6.59	318.4	$C_{20}H_{30}O_3$
Hydroxydehydrostevic acid	36.03	20.92	317.4	$C_{20}H_{29}O_3$
Heptasiloxane, hexadecamethyl	37.248	5.97	533.1	$C_{16}H_{48}O_6Si_7$
2(1H)-Phenanthrenone, 3,4,4a,4b,5,6,7,8,10,10a-decahydro-1,1,4a,7,7pentamethyl	37.626	8.95	306.4	$C_{19}H_{30}O$
trans-Geranylgeraniol	37.89	5.45	290.48	$C_{20}H_{34}O$
E,E,Z-1,3,12-Nonadecatriene-5,14-diol	38.041	5.31	294.5	$C_{19}H_{34}O_2$
Heptasiloxane, hexadecamethyl	38.295	5.84	533.14	$C_{16}H_{48}O_6Si_7$
Vitamin E	40.334	2.34	430.7	$C_{29}H_{50}O_2$
Campesterol	41.252	3.72	400.68	$C_{28}H_{48}O$

The major bioactive compounds identified were Oleic acid (RT- 26.431; 100%), n-Hexadecanoic acid (RT - 20.999; 89%), 9,12, Octadecadienoic acid Z,Z (RT- 26.685; 80%), Octadecanoic acid (RT- 27.402; 13.47%), Tetradecanoic acid (RT- 15.71; 4.74%), Hexadecanoic acid ethyl ester (RT- 21.54; 5.52%), 1- Heptatriacotanol (RT- 35.01; 2.31%), Eicosanoic acid (RT-32.82; 4.42%), 5- hydroxy methylfurfural (RT-5.86; 5.45%), Euglenol (7.77; 4.04%), Isosteviol (RT-35.903; 6.595%), Hydroxydehydrostevic acid (RT- 36.03; 20.92%), trans - Geranylgeraniol (RT-37.89; 5.74%), Vitamin E (RT- 40.334; 2.34%) and Campesterol (RT- 41.25; 3.72%).

Discussion

The bioactive compounds present in the medicinal plants could serve as novel primes for modern drug design. 40 chemical compounds have been analysed in the ethanolic extract of *Stevia rebaudiana* by the GC-MS study, out of which 18 major secondary metabolites are identified to possess biological activity. The pharmacological importance of the identified bioactive compounds was recorded in Table 2. Among the identified compounds n-Hexadecanoic acid exhibited various biological activities and was reported to contain antioxidant, anti-inflammatory, anthelmintic, antibacterial, antiallergic, and hypocholesterolemic activity.[1,2] Octadecadienoic acid has the property of 5-alpha reductase inhibitor, anti-inflammatory, cancer preventive, hypocholesterolemic, and antiandrogenic. The results obtained in the present work are in agreement with earlier studies.[12,13] Oleic acid and Octadecanoic acid are fatty acid compounds that showed hypocholesterolemic activity because of the presence of 5- alpha-reductase inhibitor. The results are in accordance with the GC-MS analysis reported by Igwe *et al*., in 2015.[14,15]

Table 2 Biological activities of bioactive compounds identified in the ethanol leaf extract of *Stevia rebaudiana.*

Name of the compound	Biological activity
Eugenol	Anti-inflammatory agents and antioxidants. [19]

5- Hydroxymethylfurfural	Antioxidant, Cancer chemoprevention. [34]
Tetradecanoic acid	Antioxidant, cancer preventive, nematocide, hypercholesterolemic, lubricant [35]
n-Hexadecanoic acid	Antioxidant, Anti-inflammatory, Hemolytic, Anthelmintic, Antibacterial, Antiallergic, Hypocholesterolemic, Hemolytic, Pesticide, Nematicide, Lubricant, Flavour, 5 alpha-reductase inhibitor.[1,2]
Hexadecanoic acid, ethyl ester	Antioxidant, Anti-inflammatory, Anthelmintic, Antibacterial, Antiallergic, Hypocholesterolemic, Hemolytic, Pesticide, Nematicide, Lubricant, Flavour, 5 alpha-reductase inhibitor, Antiandrogenic.[13,16]
Heptadecanoic acid	Antioxidant. [28]
Phytol	Antimicrobial, Anticancer, Anti-inflammatory, Diuretic. [3,18]
9,12-Octadecadienoic acid (Z,Z)-	Cancer preventive, Insectifuge, treatment of Psoriasis, Rheumatoid arthritis, anti-inflammatory.[26,27,28,29]
Oleic Acid	Prevents metabolic syndrome and cardiovascular disease .[36]
Octadecanoic acid	Anti-inflammatory, Anti-androgenic, Prevents cancer, Hypocholesterolemic,5-Alpha reductase inhibitor, Flavour, insectifuge.[14,15]
Eicosanoic acid	Anti-inflammatory, anti- therogenic. [35]
1-Heptatriacotanol	Antioxidant, anticancer, anti-inflammatory, and sex hormone activity. [19]
Isosteviol	Anti-hyperglycaemic, Anti-hypertensive, Anti-inflammatory, Anti-tumor, Anti-diarrheal, diuretic, and immunomodulatory actions. [21]
Hydroxydehydrostevic acid is	Anti-diabetic, Anti-microbial, Anti-viral, Anti-fungal, Anti-tumor, Anti-hypertensive, anti-inflammatory. [20]
trans-Geranylgeraniol	Anticancer, Antimicrobial. [24]
Vitamin E	Antioxidant, Anti-inflammatory, Antimicrobial.[22,23]
Campesterol	Inhibits the absorption of cholesterol in the intestine, Antioxidant.[33]

Phytol is a diterpene compound that showed antimicrobial and anticancer activity.[16,17] Phytol also proves to be effective in treating rheumatoid arthritis and other inflammatory diseases.[3,18] 1-Heptatriacotanol exhibits a wide spectrum of activities such as sex hormone activity, anti-inflammatory, anticancer, and. Antioxidant. [19] Euglenol is an aromatic essential oil that acts as an antioxidant and anti-inflammatory agent. Similar results are reported by Ghaidaa *et al.,* in 2016. Hexadecanoic acid ethyl ester was found to possess antioxidant, anti-inflammatory, anthelmintic, antibacterial, antiallergic, and hemolytic activity. [13,16] Literature studies indicates steviol and hydroxydehydrostevic acid identified in *Stevia rebaudiana* were found to be effective in the treatment of health complications such as diabetes, some type of cancer, and inflammatory conditions.[20] This study confirms the inhibition of human DNA topoisomerase II and DNA polymerases by Isosteviol.[21] Vitamin E was identified as the major bioactive compound and has many biological activities such as antioxidant, anti-inflammatory, immune-enhancing activity and inhibition of platelet aggregation [22,23,24,25]

A potential cancer preventive, anti-arthritic activities, and anti-inflammatory were observed by 9, 12 octadecadienoic acid methyl ester.[26,27,28,29] Similar studies were carried out in *Mollugo oppositifolia* by Nagannawar and Jayaraj in 2020. Campesterol is a phytosterol similar to cholesterol structure possesses antioxidant property and inhibits the rate of absorption of cholesterol in the human intestine. [30, 31] A recent *in vitro* study of ethanolic leaf extract of *Stevia rebaudiana* specifies that it acts as a natural antioxidant. [32] Several studies also confirm that intake of plant sterols can lower the LDL-cholesterol and total serum cholesterol in humans. [33] The present study also confirms the presence of Campesterol which might be responsible for reduced absorption of cholesterol in humans. The presence of the above-mentioned phytocompounds approves the use of the plant in various pharmaceutical and industrial applications.

Conclusion

There are many countries in the world that uses Stevia as a sugar substitute and sweetener because of its low gastrointestinal absorption and zero calories. The ethanolic leaf extract of *Stevia rebaudiana* Bertoni showed the presence of 18 bioactive compounds of medicinal value. The results of the present study provide an insight to medicinal properties of the plant as a potential source of a lead compound in drug development. The presence of these bioactive compounds justifies the therapeutic use of *Stevia rebaudiana* for treating various ailments. Further studies are required in the separation and isolation of distinct phytocomponents to explore their pharmacological activity.

Acknowledgment

I would like to thank Dr. Kumar, Sathyabama Institute of Science and Technology, Chennai. for providing all the facilities and support to carry out the work.

Conflict of Interest

The authors declared that there is no conflict of interest.

Funding

Nil

References

1. Anju G, Nagannawar, Jayaraj M. GC-MS analysis of bioactive compounds from ethanolic extract of whole plant of *Mollugo oppositifolia* L. and their pharmacological activities. International Journal of Pharmaceutical Science and Research. 2020; 11(5): 2504-2509. DOI: 10.13040/IJPSR.0975-8232.11(5).2504-09.
2. Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol. 2015; 52(2):1212–1217. DOI: 10.1007/s13197-013-1105-9.
3. Velmurugan G, Anand SP. GC-MS Analysis of Bioactive Compounds on Ethanolic Leaf Extract of *Phyllodium pulchellum* L. Desv. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(1); 114-118. DOI:10.25258ijpaprv9il.8051
4. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2016; 7(2): 1843- 1868.
5. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). Journal of Pharmacognosy and Phytotherapy. 2016; 8(3): 49-59.

6. Chaturvedula VSP, Zamora J. Reversed- Phase HPLC Analysis of Steviol Glycosides isolated from *Stevia rebaudiana* Bertoni. Food and Nutrition Sciences. 2014:5:1711-1716. doi: 10.4236/fns.2014.517184.
7. JECFA (2008) Steviol glycoside. In: Combined compendium of food additive specifications, 69th meeting of the joint FAO/WHO expert committee on food additives (JECFA). Rome. Accessed 13 Jan 2010.
8. Ursula WR, Christa L, Andreas W, Matthias W. Improved HPLC method for the evaluation of the major steviol glycosides in leaves of *Stevia rebaudiana*. Eur Food RES. Techno. 2010; 231: 581-588.
9. Gregersen S, Jeppesen PB, Holst JJ, Hermansen K. Antihyperglycemic effects of stevioside in type 2 diabetic subjects. Metabolism. 2004; 53:73-6. DOI: 10.1016/j.metabol.2003.07.013.
10. Gupta E, Purwar S, Sundaram S, Rai GK. Nutritional and therapeutic values of *Stevia rebaudiana:* A review. Journal of Medicinal Plants Research. 2013;7(46): 3343-3353. DOI: 10.5897/JMPR2013.5276
11. Pol J, Hohnová B, Hyötyläinen T. Characterization of Stevia rebaudiana by comprehensive two-dimensional liquid chromatography time-of-flight mass spectrometry. J. Chromatogr. A. 2007; 1150(1-2):85–92. DOI: 10.1016/j.chroma.2006.09.008.
12. Joint FAO/WHO Expert committee on Food Additives. Evaluation of certain food additives. World Health Organ Tech Rep Ser. 2009 (952):1-208,1p following 208.
13. Dukes, James A (2016). Dr. Duke's Phytochemical and Ethnobotanical Databases.https://phytochem.nal.usda.gov/phytochem/plants/show/1909?et=
14. Igwe KK, Nwankwo PO, Otuokere IE, Ijioma SN, Amaku FJ. GCMS analysis of Phytocomponents in the Methanolic Extract of *Moringa oleifera* Leave. Journal of Research in Pharmaceutical Science. 2015; 2 (11): 01-06.
15. Gopalakrishnan S. GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* linn. International Journal of Pharma and Bio Sciences. 2011; 2(1): 313 – 320.
16. Casuga FP, Castillo AL, Tolentino Corpuz MJ. GC–MS analysis of bioactive compounds present in different extracts of an endemic plant *Broussonetia luzonica* (Blanco) (Moraceae) leaves. Asian Pac J Trop Biomed. 2016; 6(11): 957–961.
17. Satyal P, Dosoky NS, Poudol A, Setzer WN. Essential oil constituents and their biological activities from the leaves of *Cassia fistula* growing in Nepal. Open Access J Med Aromat Plants. 2012; 3(2): 1-4.
18. Sudha T, Chidambarampillai S, Mohan VR. GC-MS Analysis of Bioactive Components of Aerial parts of *Fluggea leucopyrus* Willd. (Euphorbiaceae). Journal of Applied Pharmaceutical Science. 2013; 3 (05): 126-130. DOI: 10.7324/JAPS.2013.3524
19. Ghaidaa Jihadi Mohammed, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography – Mass Spectrum and Fourier-Transform Infrared Spectroscopy. International Journal of Pharmacognosy and Phytochemical Research. 2016; 8(6): 977-996.
20. Amir Abbas MB, Esmaeili SA, Abdollahi E, Sahebkar A. A Review on the pharmacology and toxicology of steviol glycosides extracted from *Stevia rebaudiana*. Current Pharmaceutical Design. 2016; 22(46):1-23.
21. Ullah A, Munir S, Mabkhot Y, Lal Badshah S. Bioactivity Profile of the Diterpene Isosteviol and its Derivatives. Molecules. 2019; 24(4): 678.
1. **https://doi.org/10.3390/molecules24040678**
22. Chin, KY, Ima-Nirwana, S. The Role of Vitamin E in Preventing and Treating Osteoarthritis - A Review of the Current Evidence. Front. Pharmacol. 2018;9: 946.
2. DOI: 10.3389/fphar.2018.00946
23. Naguib, MM, Valvano MA. Vitamin E Increases Antimicrobial Sensitivity by Inhibiting Bacterial Lipocalin Antibiotic Binding. MSphere. 2018; 3, e00564-18.
3. DOI: 10.1128/mSphere.00564-18

24. Nguyen NH, Nguyen TT, Ma PC, Ta QTH, Duong TH, Vo VG. Potential Antimicrobial and Anticancer activities of an ethanol extract from *Bouea macrophylla*. Molecules, 2020; 25(8):1996. https://doi.org/10.3390/molecules25081996.
25. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The Role of Vitamin E in Human Health and Some Diseases. Sultan Qaboos University Medical Journal, 2014; 14 (2): e157-e165.
26. Rani LS, Mohan VR, Regini GS, Kalidass C. GC-MS analysis of ethanolic extract of *Pothos scandens* leaf. J Herb Medi Toxicol. 2009; 3:159–160.
27. Uma B, Prabhakar K, Rajendran S, Sarayu LY. Studies on GC/MS spectroscopic analysis of some bioactive antimicrobial compounds from *Cinnamomum zeylanicum*. J Med Plants. 2009; 8(31):125–131.
28. Ponnamma SU, Manjunath K. GC-MS Analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (nees) T. anders. Int J Pharm Bio Sci, 2012; 3(3):570–576.
29. Parthipan B, Suky MGT, VR. Mohan. GC-MS Analysis of Phytocomponent in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle, (Rutaceae). Journal of Pharmacognosy and Phytochemistry. 2015; 4(1): 216-222.
30. Choudhary SP, Tran LS. Phytosterols: Perspectives in human nutrition and clinical therapy, Current medicinal chemistry. 2011; 18(29): 4557–67. DOI: 10.2174/092986711797287593
31. Premlata S, Mourya KK, Padma kumar. Identification of some bio-active compounds of iso-propyl alcohol extract of *Motha dhaman* grass by gas chromatography-mass spectrometric analysis. 2016; 72:122-135.
32. Shuklaa S, Mehta A, Mehta P, Bajpai VK. Antioxidant ability and total phenolic content of aqueous leaf extract of *Stevia rebaudiana* Bert. Experimental and Toxicologic Pathology. 2012; 64:807-811. DOI: 10.1016/j.etp.2011.02.002
33. Raphael J. Ogbe1, Dickson O. Ochalefu, Simon G. Mafulul, Olumide B. Olaniru. A review on dietary phytosterols: Their occurrence, metabolism and health benefits. Asian Journal of Plant Science and Research. 2015; 5(4):10-21
34. Neeranjna M, Dr. Navin Kumar J. Comparative phytochemical screening of *Curcuma angustifolia*, *Curcuma decipiens* and *Curcuma longa* by using GC-MS. Journal of Pharmacognosy and Phytochemistry. 2019; 8(2): 1227-1234.
35. Achi NK, Ohaeri OC. GC-MS Determination of Bioactive Constituents of the Methanolic Fractions of *Cnidoscolus aconitifolius*. British Journal of Pharmaceutical Research, 2015; 5(3): 163-172. https://doi.org/10.9734/BJPR/2015/13893
36. Gillingham L. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids, 2011; 46(3):209–228.DOI: 10.1007/s11745-010-3524-y.