Trop J Nat Prod Res, April 2025; 9(4): 1553 - 1558 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

GC–MS Analysis of Bioactive Compounds of Ethanol Extract of Soybean *Glycine Max* (L.) Merril.

Junaidi Junaidi[1*], Ruslan Hasani[2], Sitti S. Rowa[3], Hamsina Hamsina[4], Akhmad Rifai[5], Harliani Harliani[6], Hasifah Hasifah[7]

[1,2,6]*Departement of Nursing, Poltekkes Kemenkes Makassar, Indonesia*
[3]*Departement of Nutrition, Poltekkes Kemenkes Makassar, Indonesia*
[4]*Chemical Engineering Study Program, Faculty of Engineering, Bosowa University Makassar, Indonesia*
[5]*Department of Chemical Engineering, Ujung Pandang State Polytechnic, Makassar, Indonesia*
[7]*Nani Hasanuddin Makassar College of Health Sciences, Indonesia*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Published online

ABSTRACT

The plant species *Glycine max (L.) Merril* is commonly called soybean and originates from the Fabaceae family. Soybeans have been cultivated by humans in mainland China since 2500 BC and then spread to other countries along with the increase in trade between countries in the early 19th century. For thousands of years, soybeans have been used as food and medicine in China, Japan, and Korea. The study aimed to identify bioactive compounds in the ethanol extract of *Glycine max (L.) Merril* soybean. GC-MS Ultra QP 2010 (Shimadzu) was used to identify bioactive compounds in the ethanol extract of *Glycine max (L.) Merril* soybean. GC-MS showed that the soybeans contained 50 compounds. Retention time, molecular formula, molecular weight, peak area, structure, compound category, and activity were identified. The most abundant compounds found were Isopropyl linoleate (55.25%), n-hexadecanoic acid (24.77%), 6-octadecenoic acid, methyl ester, (Z)- (10.72%), hexadecanoic acid, methyl ester (4.03%) and Methyl stearate (1%). This study shows that the species *Glycine max (L.) Merril* contains bioactive compounds, including fatty acids, esters, terpenoids, flavonoids, steroids, etc. The presence of these phytoconstituents may justify its traditional use as a source of food and medicine.

Keywords: Bioactive compound, Ethanol extract, *Glycine max (L.) Merril*, Phytochemical, Soybean.

Introduction

The botanical kingdom has significant functions in the prophylaxis and management of various diseases.[1] Many plant species contain bioactive molecules that have been used in traditional medicine for centuries. It is estimated that two-thirds of the world's herbal species have therapeutic value,[2] and over 500,000 plant species are estimated to exist worldwide, of which over 150,000 have been studied for their medicinal benefits. There is a large reservoir of active compounds available for research and development, as many of these species contain valuable therapeutic agents. Phenols, triterpenes, flavonoids, and cinnamic acids are the most commonly known active plant compounds for their antioxidant and anti-inflammatory properties.[3,4] Glycoproteins and polysaccharides contained in plant extracts can increase lymphocyte proliferation and cytokine production which are critical for the immune response,[5] and have been shown to impact the immune system, making them an easily accessible option for those seeking alternatives for the treatment of infections.[6] Safety and ethical standards have increased as a result of the sudden increase in the practice of traditional medicine worldwide.[6,7] Traditional medicine is particularly important in developing countries, where access to modern healthcare may be limited. Traditional medicine is often seen as a more affordable and accessible option for many populations.[8] The plant species *Glycine max*

*Corresponding author; Email: junaiditdg@gmail.com
Tel.: +6285242421967

Citation: Junaidi J, Hasani R, Rowa S S, Hamsina H, Rifai A, Laubo N, Harliani H, Hasifah H . GC–MS analysis of bioactive compounds of ethanol extract of soybean *Glycine max* (L.) Merril. Trop J Nat Prod Res. 2025; 9(4): 2025; 9(4): 1553 – 1558 https://doi.org/10.26538/tjnpr/v9i4.26

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

(L.) Merril is commonly called soybean and is a plant from the Fabaceae family. Soybeans have been cultivated by humans in mainland China
since 2500 BC and then spread to other countries as trade between countries increased in the early 19th century. The destination countries for this trade included Japan, Korea, Indonesia, India, Australia, and the United States. For thousands of years, soybeans have been used as food and medicine in China, Japan, and Korea. In 1804, soybeans arrived in the United States and became very important in the South and Midwest in the mid-20th century. Soybeans (*Glycine max (L.) Merril*) contain metabolite compounds that have the potential as antioxidants,[9] such as flavonoids, isoflavones, and phenolics.[10] The quest for rich sources of natural proteins, functional foods, and medicines especially at the turn of the 20th century, has also created a need for the investigation of the functionalities of these foods and their bioactivity as medicines using modern analytical tools. Therefore, the purpose of this study was to analyze the bioactive compounds contained in the ethanol extract of *Glycine max (L.) Merril* soybeans using Gas Chromatography-Mass Spectroscopy.

Materials and Methods

Collection and identification of plant material

Glycine max (L.) Merril was obtained from the soybean plantation of Toddolimae Village, in Tompobulu District, Indonesia in July 2023, at coordinates: 5.078628,119.649016, identified by a taxonomic at the Department of Agriculture, Bosowa University, Makassar with botanical number GC.Herb.Bot.3779.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Matured soybean seeds were hand-picked and all healthy seeds were stored under shade until completely dry and ground using a mechanical mill to produce a fine powder. The powdered material was sieved to remove coarse particles and then was stored in an airtight glass container until further use.

Extraction of plant material

Approximately 500 g of dry powdered material was placed into the extractor and ethanol (96%) was added, stirred, closed tightly, and stored for 24 hours at room temperature protected from sunlight, and occasionally stirred. After 24 hours, the extract was filtered to separate the filtrate and residue. The filtrate was then evaporated using a Rotary Vacuum Evaporator at a 40ºC until a dark brown residue was obtained. 2N HCl was added to the residue and hydrolyzed in a water bath at 90ºC for 90 minutes. The residue was transferred into a separatory funnel and extracted with Hexane solvent (1/5 vol. sample) for 24 hours. The extraction results were analyzed by GCMS.

Preparation of Glycine max (L.) Merril extract for GC-MS analysis.

The crude extract was mixed with 5 mL of 96% Ethanol (p.a), and Ultrasonicated for 30 minutes at a temperature of 55°C. It was filtered using Whatman filter paper no.42 and then injected into GC-MS.

Operating GC-MS Ultra QP 2010 Shimadzu

GC-MS instrument conditions: Injector temperature 250ºC with Splitless mode, pressure 76.9 kPa and flow rate 14 mL/min and ratio 1:10. Ion source and interface temperature 200ºC and 280ºC, solvent cut time 3 minutes, 400-700 m/z. Column type SH-Rxi-5Sil MS column length 30 m with inner diameter 0.25 mm. The initial column temperature was 700ºC with a holding time of 2 minutes and the temperature increased to 200ºC with a rate of 100ºC/min and the final temperature was 280ºC with a holding time of 9 minutes with a rate 50ºC/min so the total analysis time 36 minutes. Chromatogram data obtained were read using NIST 17 and Wiley 9 libraries.[11,12]

Identification of bioactive compounds

Ethanol extract of soybean (*Glycine max (L.) Merril*) was compared for its retention index and mass spectrum fragmentation pattern with those stored in computer libraries and published literature to identify compounds. Sources from the National Institute of Standards and Technology were also used to match compounds identified from plant material.[13,14]

Results and Discussion

GC-MS is two tools combined to identify and measure the concentration of chemicals in food, used for the analysis process of volatile organic chemical compounds.[15] GC-MS is often used in various fields, such as environmental analysis, food analysis, and forensic analysis.[16]

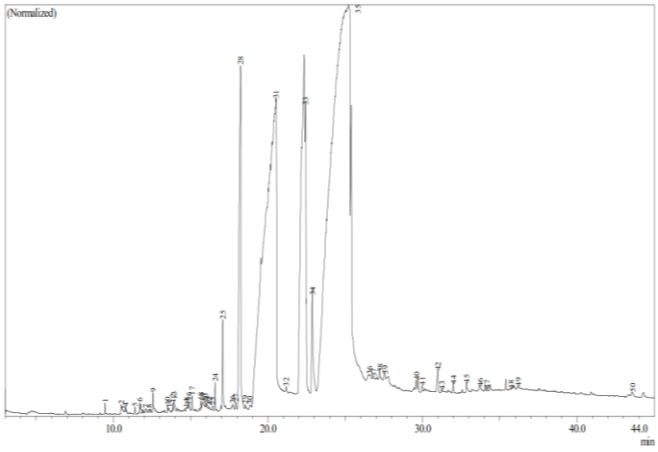


Figure 1: Chromatogram of ethanol extract of soybean (*Glycine max (L.) Merril*)

Fifty compounds were identified in the extract of soybean (*Glycine max* (L.) Merril) presented in Table 1, and the total running time was 44.0 minutes. The spectrum of the compounds was matched with Wiley 9.0 and the National Institute of Standards and Technology library. Five main active compounds are most commonly found, namely Isopropyl linoleate (55.25%), n-Hexadecanoic acid (24.77%), 6-Octadecenoic acid, methyl ester, (Z)- (10.72%), Hexadecanoic Acid, Methyl Ester (4.03%) and Methyl stearate (1%).

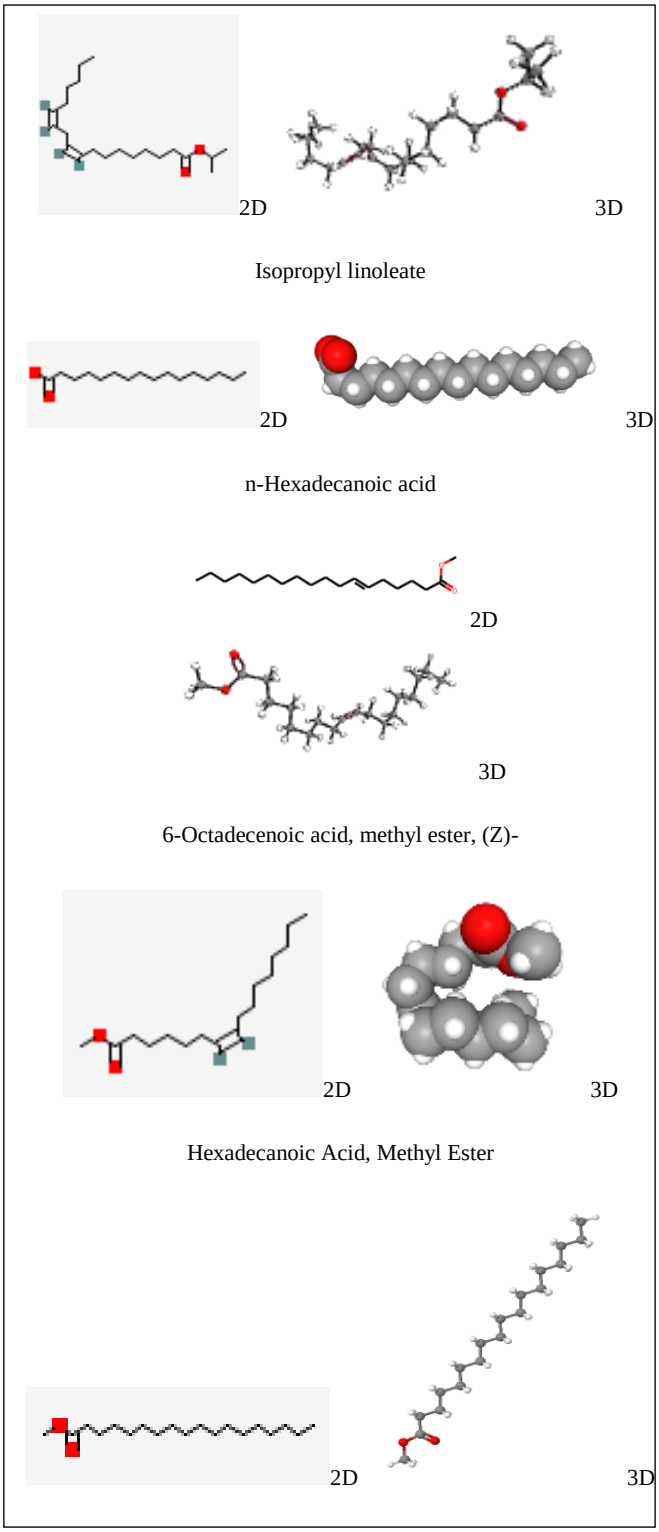


Figure 2: 2D and 3D chemical structures of the main bioactive compounds identified in the ethanol extract of soybean *Glycine max (L.) Merril.*

Table 1: Phytochemical compounds identified in the ethanol extract of soybean Glycine max (L.) Merril

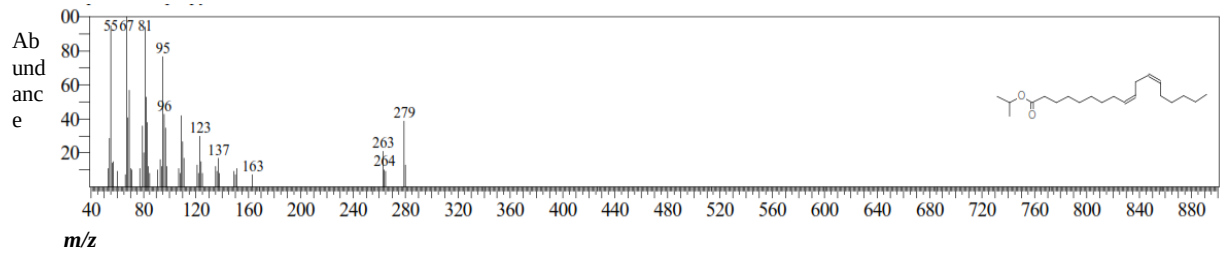
RT	NAME OF THE COMPOUND	MOLECULAR FORMULA	MW	PA (%)
9.452	Amodiaquine, 2TMS derivative	$C_{26}H_{38}ClN_3OSi_2$	499	0.04
10.515	3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164	0.08
10.667	2(3H)-Furanone, dihydro-5-pentyl-	$C_9H_{16}O_2$	156	0,02
10.783	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	$C_{13}H_{18}O$	190	0.04
11.393	Caryophyllene	$C_{15}H_{24}$	204	0.03
11.712	1,6,10-DODECATRIENE, 7,11-DIMETHYL-3-METHYLENE-, (E)-	$C_{15}H_{24}$	204	0.07
12.095	1-Dodecanol	$C_{12}H_{26}O$	186	0.04
12.308	Ethanone, 1-(2,5-diethyl-4-methyl-1,3,2-dioxaborolan-4-yl)-	$C_9H_{17}BO_3$	184	0.03
12.56	3-Buten-2-one, 1-(2,3,6-trimethylphenyl)- (CAS)	$C_{13}H_{16}O$	188	0.12
13.508	cis-1-Chloro-9-octadecene	$C_{18}H_{35}Cl$	286	0.03
13.592	1,2-Benzenedicarboxylic acid, diisononyl ester	$C_{26}H_{42}O_4$	418	0.03
13.842	Benzoic acid, 4-amino-2-hydroxy-, tris(trimethylsilyl) deriv. (CAS)	$C_{16}H_{31}NO_3Si_3$	369	0.03
13.934	3-Buten-2-one, 1-(2,3,6-trimethylphenyl)-	$C_{13}H_{16}O$	188	0.09
14.717	Acetic acid, 6,7-diacetyl-5,5-dimethylbicyclo[4.1.0]hept-2-yl ester	$C_{15}H_{22}O_4$	266	0.02
14.794	Heneicosane (CAS)	$C_{21}H_{44}$	296	0.02
14.881	2-Hydroxy-2-phenyl-2-[(2-hydroxyethyl)phenyl]acetic acid, 3-methyl-	$C_{32}H_{38}O_4$	486	0.07
15.101	Cyclopentanetridecanoic acid, methyl ester (CAS)	$C_{19}H_{36}O_2$	296	0.08
15.663	Amodiaquine, 2TMS derivative	$C_{26}H_{38}ClN_3OSi2$	499	0.03
15.771	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	206	0.13
15.963	1-Nonadecene	$C_{19}H_{38}$	266	0.05
16.047	Eicosane	$C_{20}H_{42}$	282	0.04
16.195	5-Octadecenoic acid, methyl ester (CAS)	$C_{19}H_{36}O_2$	296	0.05
16.416	Pentadecanoic acid, methyl ester (CAS)	$C_{16}H_{32}O_2$	256	0.05
16.579	Neophytadiene	$C_{20}H_{38}$	278	0.11
17.072	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	0.49
17.708	9-Hexadecenoic acid, methyl ester, (Z)- (CAS)	$C_{17}H_{32}O_2$	268	0.03
17.922	EICOSAMETHYLCYCLODECASILOXANE	$C_{20}H_{60}O_{10}Si_{10}$	740	0.04
18.233	HEXADECANOIC ACID, METHYL ESTER	$C_{17}H_{34}O_2$	270	4.03
18.536	Isophytol	$C_{20}H_{40}O$	296	0.03
18,833	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.03
20.514	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	24.77
21.185	Benzoic acid, 4-amino-2-hydroxy-, tris(trimethylsilyl) deriv. (CAS)	$C_{16}H_{31}NO_3Si_3$	369	0.04
22.436	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	10.72
22.884	Methyl stearate	$C_{19}H_{38}O_2$	298	1
25.227	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	55.25
26.585	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	$C_{19}H_{32}O_2$	292	0.15
26.858	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.11

27.261	1H-PURIN-6-AMINE, [(2-FLUOROPHENYL)METHYL]-	$C_{12}H_{10}FN_5$	243	0.28
27.584	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	$C_{19}H_{32}O_2$	292	0.45
29.609	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	$C_{33}H_{56}$	452	0.22
29.992	Hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl ester	$C_{22}H_{42}O_4$	370	0.13
30.992	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.2
31.258	n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322	0.03
31.992	SILICONE OIL	CAS:0-00-0	9999	0.12
32.858	9,12,15-Octadecatrienoic acid, 2,2-dimethyl-1,3-dioxolan-4-ylmethyl ester, (Z,Z,Z)-	$C_{24}H_{40}O_4$	392	0.09
33,73	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262	0.14
34,192	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	0.04
35,725	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	0.08
36,199	Silane, chlorodiethylpentadecyloxy-	$C_{19}H_{41}ClOSi$	348	0.12
43,594	23-R-METHYLCHOLESTEROL	C28H48O	400	0.11

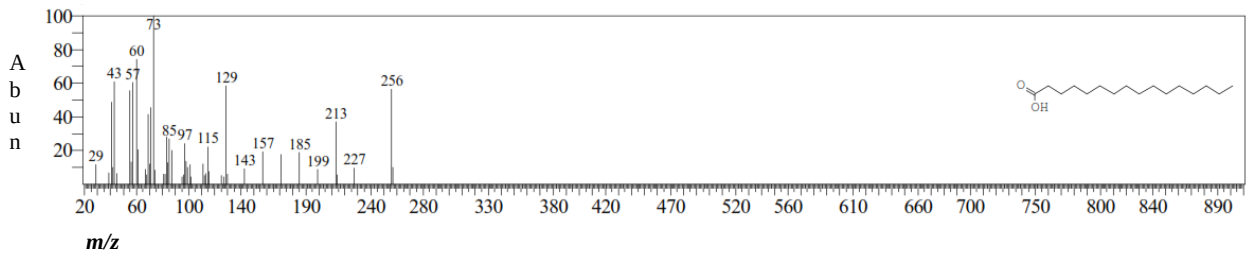
RT: Retention time; PA: Peak area; MW: MolWeight

Table 1 above shows that Isopropyl linoleate is the first main compound with a peak area percentage of 55.25%, Isopropyl linoleate has antioxidant, antimicrobial, and anticancer properties[20]. The second main compound is n-Hexadecanoic acid with a peak percentage of 24.77% has biological properties as Hypocholesterolemic, antioxidant, nematicide, and pesticide[21]. The third main compound is 6-Octadecenoic acid, methyl ester, (Z)- with a peak area percentage of 10.72% has biological activity as an apoptosis inducer, antineoplastic agent, anti-inflammatory agent, and antiatherogenic agent. The fourth main compound is Hexadecanoic Acid, Methyl Ester with a peak area percentage of 4.03%. There are many biological activities of hexadecanoic acid, including antioxidant, hypocholesterolemic, nematicide, and pesticide[22]. The fifth main compound is Methyl stearate with a peak area percentage of 1% has anti-inflammatory, regulation of intestinal lipid metabolism, nematicide, antinociceptive, antioxidant, and antifungal activities[23,24].

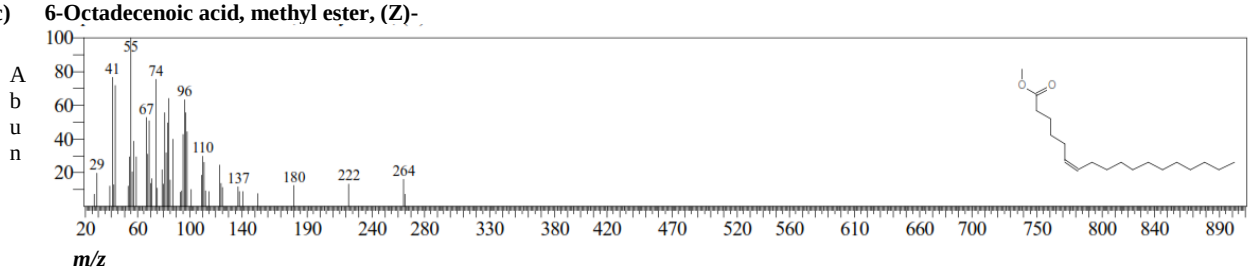
(a) Isopropyl linoleate



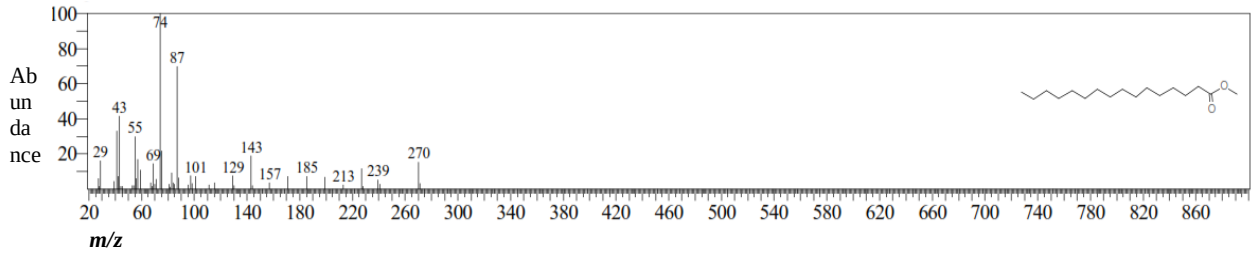
(b) n-Hexadecanoic acid



(c) 6-Octadecenoic acid, methyl ester, (Z)-



(d) Hexadecanoic Acid, Methyl Ester



(e) Methyl stearate

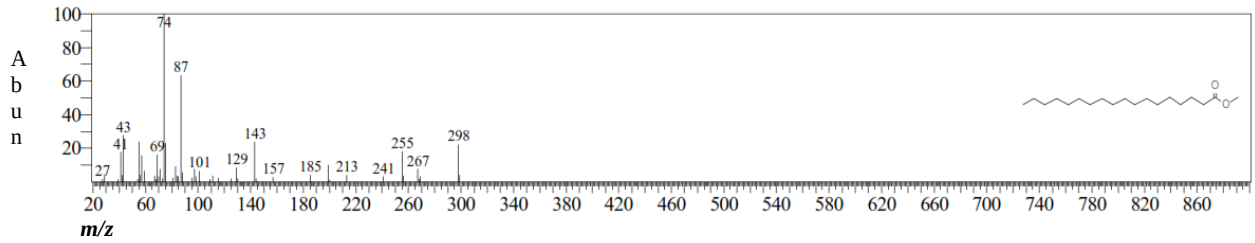


Figure 3: Mass spectrum of the main bioactive compounds identified in the ethanol extract of soybean *Glycine max (L.) Merril*

This is because the combination of GC and MS allows the separation of very complex compounds and it is a very sensitive analytical tool.[17] The advantage of GC-MS is its accuracy in determining the molecular weight of a compound and using mass spectroscopy, the molecular formula can be identified without performing elemental analysis.[18] Characterisation and profiling of phytochemical compounds are essential to expand our knowledge of medicinal plants and optimise their use in contemporary medicine. Researchers can systematically find and measure bioactive components in plant extracts using advanced analytical techniques such as mass spectrometry (MS). This careful analysis helps to gain an understanding of the specific compounds responsible for the therapeutic effects. This facilitates the creation of efficacious and standardised formulations.[19] GC-MS analysis of the ethanol extract of *Glycine max (L.) Merril* soybean revealed 50 compounds, which are listed in Table 1, along with their chemical composition, while the total ionic chromatogram is shown in Figure 1. The 2D and 3D chemical structures of the main bioactive compounds identified in the ethanol extract of *Glycine max (L.) Merril* is presented in Figure 2 and the mass spectrum of the main bioactive compounds identified in the ethanol extract of soybean *Glycine max (L.) Merril* in Figure 3.

Conclusion

This study shows that the species *Glycine max (L.) Merril* can provide many bioactive compounds, such as fatty acids, esters, terpenoids, flavonoids, steroids, and so on. This justifies the traditional use of this species as a functional food and a major source of medicines.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

We like to express our gratitude for the Poltekkes Kemenkes Makassar Grant's financial assistance in 2023. The Maritime Laboratory of the Ujung Pandang State Polytechnic provided the facilities for sample preparation and GCMS analysis of the bioactive content of research samples, for which the authors are also grateful.

References

1. Srivastava JN, Singh AK. Prevention and Management of Plant Diseases through Plant Products. In: Diseases of Horticultural Crops [Internet]. Boca Raton: Apple Academic Press; 2022. p. 251–268.
2. Sharma P, Manchanda R, Goswami R, Chawla S. Biodiversity and Therapeutic Potential of Medicinal Plants. In: Environmental Concerns and Sustainable Development. Singapore: Springer Singapore; 2020. p. 27–44.
3. Nunes C dos R, Barreto Arantes M, Menezes de Faria Pereira S, Leandro da Cruz L, de Souza Passos M, Pereira de Moraes L, Curvicino Vieira IJ, Barros de Oliveira D. Plants as Sources of Anti-Inflammatory Agents. Molecules. 2020;25(16):3726.
4. Tariq L, Bhat BA, Hamdani SS, Mir RA. Phytochemistry, Pharmacology and Toxicity of Medicinal Plants. In: Medicinal and Aromatic Plants [Internet]. Cham: Springer International Publishing; 2021. p. 217–240.
5. Son YO, Lee JC. Glycoproteins and Polysaccharides are the Main Class of Active Constituents Required for Lymphocyte Stimulation and Antigen-Specific Immune Response Induction by Traditional Medicinal Herbal Plants. J Med Food. 2017; 20(10): 1011-1021.
6. Geszke-Moritz M, Nowak G, Moritz M. Pharmacological Properties and Safe Use of 12 Medicinal Plant Species and Their Bioactive Compounds Affecting the Immune System. Appl Sci. 2023;13(11):6477.
7. Monalisa, Fakih M, Perbawati C. Relevance of WHO traditional medicine strategy (2014-2023) with traditional health care policy in the perspective of national law and international law. Asian J Leg Stud. 2022;1(1):25–34.
8. W.H.O. Traditional Medicine Strategy (2002–2005). WHO/EDM/TRM/2002.1. 2002;

9. Malenčić D, Popović M, Miladinović J. Phenolic Content and Antioxidant Properties of Soybean (*Glycine max (L.) Merr*.) Seeds. Molecules. 2007;12(3):576–581.
10. Giradkar P, Kulkarni D., More D. Understanding the antioxidant mechanism of Soybean (*Glycine max L. Merril*). Int J Res Indian Med. 2020;04(4):1–11.
11. Fridayanti A, Purwanto DA, Hendradi E, Preliminary Phytochemical Screening and GC-MS Analysis of Ethanol Extract of Bulbs of Eleutherine sp. Trop J Nat Prod Res. 2022; 6(3):361-364.
12. Otuokere IE, Akoh OU, Echeme JO, Nwadire FC, Nwankwo CI, Egbucha JN, Ammasai, K. GC-MS Analysis and Molecular Docking Studies to Identify Potential SARS-CoV-2 Nonstructural Protein Inhibitors from Icacina trichantha Oliv Tubers. Trop J Nat Prod Res. 2022; 6(8):1336-1342
13 McLafferty, Stauffer. The Wiley/NBS Registry of Mass Spectral Data. New York: J Wiley and Sons; 1989.
14. Stein. Mass Spectral Database and Software. Gaithersburg USA: National Institute of Standards and Technology (NIST); 1990.
15. Vazquez-Roig P, Pico Y. Gas chromatography and mass spectroscopy techniques for the detection of chemical contaminants and residues in foods. In: Chemical Contaminants and Residues in Food. Elsevier; 2012. p. 17–61.
16. Song H, Liu J. GC-O-MS technique and its applications in food flavor analysis. Food Res Int. 2018;114:187–198.
17. Đurović S. Application of the GC/MS technique in environmental analytics: Case of the essential oils. In: Green Sustainable Process for Chemical and Environmental Engineering and Science. Elsevier; 2021. p. 197–208.
18. Feroz S. GC-MS Analysis of Fixed Oils of *Nigella Sativa* Seeds. Univers J Pharm Res. 2018;3(2):38–42.
19. Rozirwan R, Hananda H, Nugroho RY, Apri R, Khotimah NN,Fauziyah F, Putri WA, Aryawati R. Antioxidant Activity, Total Phenolic, Phytochemical Content, and HPLC Profile of Selected Mangrove Species from Tanjung Api-Api Port Area, South Sumatra, Indonesia. Trop J Nat Prod Res. 2023; 7(7):3482-3489. Doi.org/10.26538/tjnpr/v7i7.29
20. Rajendran P, Bharathidasan R, Sureka. Phytochemical Screening GC-MS and FT-IR Analysis of Sugarcane Juice. Int J Pharma Res Health Sci. 2017;5(6):1962-7.
21. Aftab T, Hakeem KR. Medicinal and Aromatic Plants Healthcare and Industrial Applications [Internet]. Aftab T, Hakeem KR, editors. Cham: Springer International Publishing; 2021.
22. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol Reports. 2019;24: 1-10.
23. Pinto ME, Araújo SG, Morais MI, Sa NP, Lima CM, Rosa CA, Siqueira EP, Johann S, Lima. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. An Acad Bras Cienc. 2017;89(3):1671–1681.
24. Adnan M, Nazim Uddin Chy M, Mostafa Kamal ATM, Azad MOK, Paul A, Uddin SB, Barlow JW, Faruque MO, Park CH, Cho DH. Investigation of the Biological Activities and Characterization of Bioactive Constituents of *Ophiorrhiza rugosa var. prostrata* (D.Don) & Mondal Leaves through *In Vivo*, *In Vitro*, and *In Silico* Approaches. Molecules. 2019;24(7):1-24.