International Journal of Biological Engineering and Agriculture
ISSN: 2833-5376
Volume 03 Number 03 (2024)
Impact Factor: 9.51
SJIF (2023): 3.916

www.inter-publishing.com

Article

Phytochemical Investigation for Some Species of *Galium L.* (Rubiaceae) by Utilizing Gas Chromatography-Mass Spectrometry (GC-MAS) in Iraq

Rabieuh Salman Hamid[1], Balqees Hadi Al-Musawi[2], Neepal Imtair Al-Garaawi[3]

1. Department of Biology, College of Education for Pure Science, University of Kerbala, Kerbala, Iraq
* Correspondence: e03161359@s.uokerbala.edu.iq
2. Department of Science in Biology, College of Science, University of Kerbala, Kerbala, Iraq
* Correspondence: balqees.hadi@uokerbala.edu.iq
3. Department of Biology, College of Education for Pure Science, University of Kerbala, Kerbala, Iraq
* Correspondence: neepal.i@uokerbala.edu.iq

Abstract: Ten biologically active phytochemical compounds have been recognized in the methanolic extract of the aparine plant—eleven biologically active phytochemical compounds. The phytochemical complex depends about chemical name, exaction mass, MS parts, and the structure of chemical. GC-MS analysis of plant cetassium. Detect the presence of cyclohexasiloxane, dodecamethyl-dodecamethylcyclohexasiloxane; Pentasiloxane, Dodecamethyl Dodecamethylpentasiloxane; Neovitadiene2,6,10-trimethyl-14-ethylene-14-pentadecanoic acid, methyl ester palmitic acid, methyl ester hexadecanoic acid (HexA) methyl ester methylene 2,9,12-octadecadienoic acid (Z, Z)-, phytol methyl ester, octadecanoic acid (OctA), methyl ester (MethE), 2,6,10,14,18,22-tetracosahexene, 2,6,10,15,19,23-hexamethyl-(CAS) squalene Squalene Suberin S Vitamin E dl-.alpha.-Tocopherol 2H-1-Benzopyran-6-ol,3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12)-trimethyltridecyl; 1,3-bis(trimethylsilyl)benzene; Stigmasterol, 22,23dihydro-while GC-MS analysis of plant apart was performed. Detect the presence of Pentasiloxane, dodecamethyl-Dodecamethylpentasiloxane; HexA, methyl ester (CAS) Methyl palmitate Methyl hexadecanoate Methyl N-hexadecanoate; 9,12-octadecadienoic acid, MethE; 9,12,15-Octadecatatrienoic Acid, Methyl Ester (CAS) Methyl 9,12,15-Octadecatatrienoate; 2,6,10,14,18,22-tetracosahexane, 2,6,10,15,19,23-hexamethyl-(CAS) squalene squalene suberin S; Gibberellin A3 Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methylene-,1,4a-lactone, (1.alpha, vitamin E, 13-Methyl-Z-14-nonacosine;(23S)-ethylcholest-5-en-3.beta.-ol Cholest-5-en-3-ol, 23-ethyl-, (3.beta.,23S) - (CAS 1,3-dimethyl-4-azaphenanthrene).

Keywords: phytochemical, Rubiaceae family, gas chromatography, mass spectrometry

Citation: Hamid, R. S., Al-Musawi, B. H, & Al-Garaawi, N. I. Phytochemical Investigation for Some Species of Galium L. (Rubiaceae) by Utilizing Gas Chromatography-Mass Spectrometry (GC-MAS) in Iraq. International Journal of Biological Engineering and Agriculture 2024, 3(3), 82-94.

Received: 10th March 2024
Revised: 16th March 2024
Accepted: 23rd March 2024
Published: 30th March 2024

1. Introduction

There are roughly 660 genera and 11,500 species belonging to the Rubiaceae family, the fourth-greatest angiosperms family. In addition to having a worldwide distribution, it is mainly found in locations categorized as temperate and divided into 42 different tribes. The family Rubiaceae is a member of the order Gentianales, which is classified under the Diocts class. This order comprises five families: Gentinaceae, Rubiaceae, Loganiaceae, Apocynaceae, and Gelsemiaceae [1].

A number of species belonging to the Rubiaceae family are regarded as being among the most significant. The *Galium L.* is considered one of the most extensive genera of the

Rubiaceae family. It has about 400 species, which are divided into 16 sections. These sections comprise annual and perennial herbs found in temperate and tropical parts of the globe, as stated by Friščić et al. (2018) [2].

Among its appearance characteristics are its quadrangular stems, its leaves are opposite each other, its flowers are perfect, the upper parts are epigynous, the calyx is very small or missing, the corolla is joined to the petals, gamopetalous, usually of 4 pieces. Its colors are pale white - bright yellow, yellowish green, pink, or reddish brown. The ovary is low, inferior, two-chamber. The fruit is a two-seeded schizocarp [3].

One of its genera, *G. setaceum*, is a flowering plant that grows around the world, even in temperate climates like the United States, depending on Jan et al. (2018) [4]. In traditional medicine, *G. setaceum* also known as "threkh Jeshy" in Pakistan is used to cure a variety of ailments. The herb has sedative, diuretic, antibacterial, anticancer, and antioxidant properties, according to Saavedra and Alcántara (2017) [5]. The plant was discovered to possess these qualities. An additional interesting fact is that *G. setaceum* is a perennial herbaceous set that has invaded the United States and spread across Hawaii [6]. It is also considered that some species are invasive to other species, particularly in the coastal areas of the eastern and southern parts of the Iberian Peninsula, particularly the Spanish city of Cordoba. Research has been done on this species' genetic diversity as well as its propensity for invasion.

Another genus is named *G. aparine*; it is a species that is worldwide and a member of the Galium genus. This plant, which belongs to the Rubiaceae family, is categorized as a herbaceous plant. The plant has creeping, inflexible stalks with tiny hooks hanging on them, along with hermaphrodite blossoms with white petals. According to Ilina et al. (2019) [7], the plant may grow in common areas in North America, Europe, and Asia. Its maximum height is one meter, and its pellets are coated with many sticky hairs that may attach to animals' fur. It grows on roadsides, pastures, and uncultivated, generally moist regions.

Consequently, the plant is called "sticky" due to this characteristic. The herb *G. aparine* is utilized in traditional medicines and nutritional supplements, and it has been shown that the herbal extracts of this plant have immune-regulating properties. Additionally, they were investigated for their resistance to herbicides and their adhesive capabilities, which have also inspired the creation of industrial adhesive systems [8].

2. Materials and Methods

2.1. Production of extracts of crude chemical complex

By the methodology Markham (1982) utilized, the chemical components have been separated from the leaf powder that was the subject of the investigation [9]. However, the following adjustments were made:

1) The leaves of plants were taken from the species that was being investigated, cleaned and dried at the ambient temp, and then crushed utilizing an electric grinder to create a fine mixture. After that, the mixture was stored in a flexible container.
2) Quantum of one gram of vegetable powder was combined with ten milliliters of methanol that had an amount of ninety-nine percent, and put in a glass tube. The mixture was stirred continuously for ten minutes, then maintained at room temperature and stored in a dark location for twelve hours.
3) After that, the extract was filtered into a different glass tube utilizing a filter coupled to a medical syringe with an aperture precision of 0.45 meters.

Four hundred and one milliliters of hexane with an amount of ninety-nine percent had been added to get rid of the water and make the extract more concentrated. Hexane was utilized to separate the floating fraction from the water, and then the chemical components were assessed after the floating fraction was removed.

2.2. Utilizing Gas Chromatography/Mass Spectrometry (GC/MS) to separate and diagnose chemical components from extracting raw compounds for species leaves under investigation

2.2.1. GC-MS analysis method

Under the subsequent circumstances, a GC-MS analysis was carried out with the assistance of a GC Clarus 500 Perkin Elmer system. This system is equipped with gas chromatography, which is connected to a mass spectrometer and an AOC-20i autosampler.

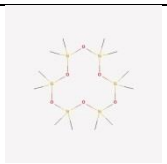
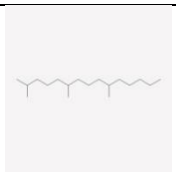
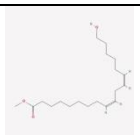
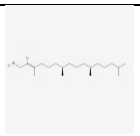
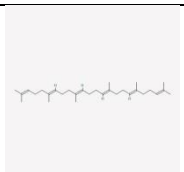
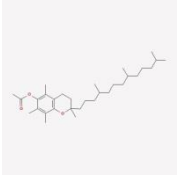
1) Injector temp of 250 degrees Celsius.
2) The ion source has a temp of 280 degrees Celsius.
3) In step three, a constant flow rate of one milliliter of helium gas (99,999 percent) is utilized as the carrier gas min.
4) The volume of the injected liquid is 0.5 microliters, and it operates in divided ratios for 1:10.
5) The oven temp is set at 110 degrees Celsius since it is planned to rise by 10 degrees Celsius automatically. Minute 1, the temperature drops to 200 degrees Celsius. Then, it rises until it reaches 280 degrees Celsius, where it remains stable for nine minutes until the completion of the experiment.
6) Once the fission rate was between 40 and 450 Dalton, the mass spectra were obtained utilizing a 70 EV basis with an inspection interval of 0.5 seconds, which was done when the fission rate changed.
7) Switching off the GC-MAS device once it has been turned on for the first time takes thirty-six min.
8) The sort of separation column utilized is an Elite-1 fused silica capillary column. This column comprises one hundred percent dimethyl polysiloxane and operates in the 70EV mode electron impact.
9) At a rate of one milliliter per second, the pressure within the device is 49.5 kilopascals—Min minus one.
10) According to Srinivasan et al. (2013), the relatively quantity of each complex was determined by comparing the mean surface area to the total areas, which was done utilizing the TurboMass Ver 5.2.0 tool, which is utilized for dealing with mass spectra and chromatograms.

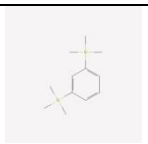
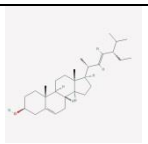
2.2.2. Determine the chemical compounds

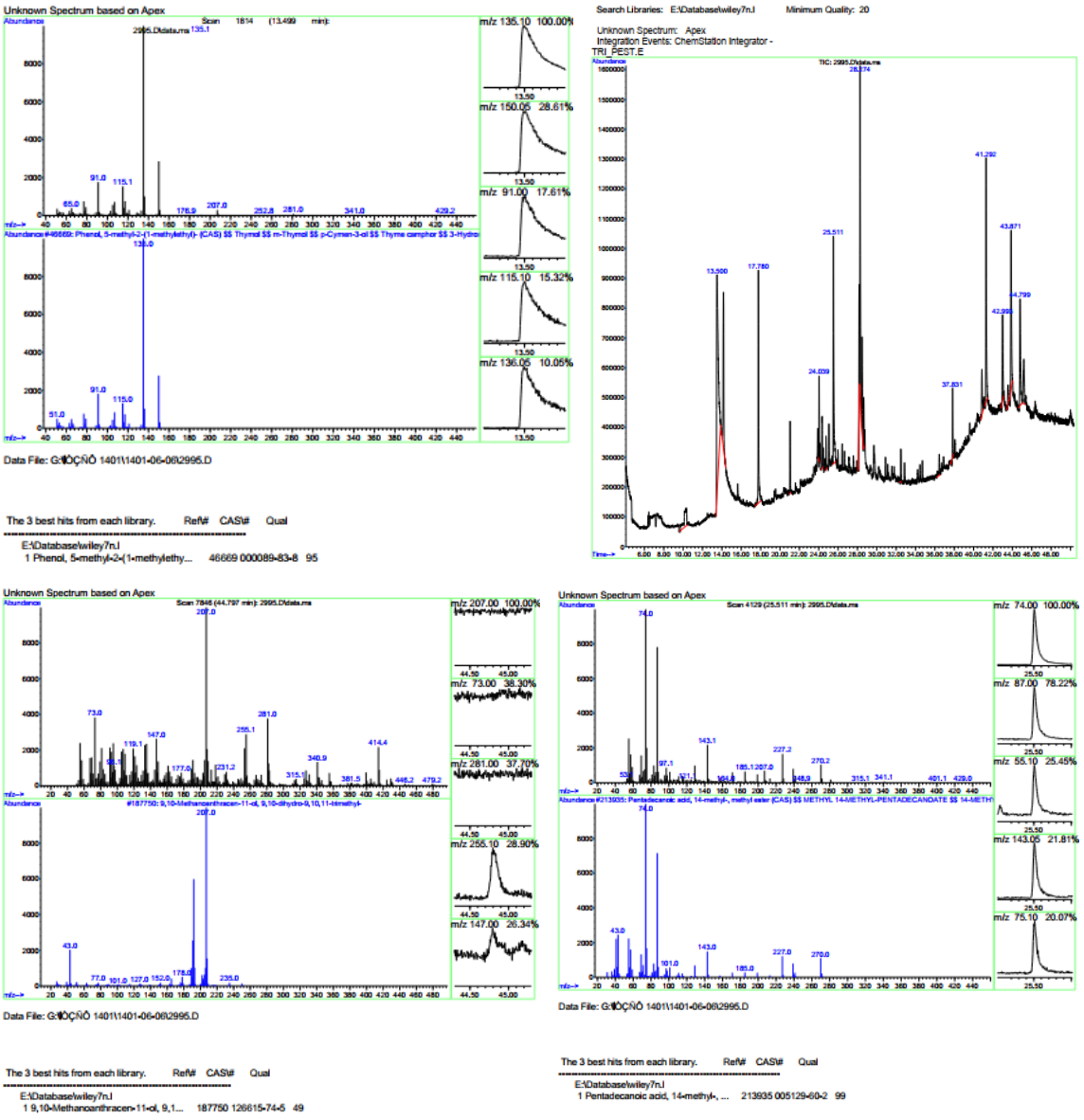
The GC-MAS Unit, Al-Amin Laboratory were the participants in this test. In order to determine the components, the performance of the mass spectrum of the GC-MAS was utilized, and the database of the National Institute of Standards and Technology, which included at least 62,000 specific patterns, was utilized. It was determined that the structure, label, and molecular weight of the sample's complex could be determined by comparing the unknown complex spectrum produced with a variety of previously identified complex kept in the NIST library.

3. Results

Table 1. Active substances isolated from *G. setaceum* plant extract

No	Chemical name	Retention time	The structure of chemical	Molecular formula	Molecular weight	Composite type
1	Cyclohexasiloxane, dodecamethyl- Dodecamethylcyclohexasiloxane	14.163		C12H36O6Si6	444.92	
2	Pentasiloxane, dodecamethyl- Dodecamethylpentasiloxane	17.780		C12H36O4Si5	384.84	
3	NEOPHYTADIENE 2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	24.042		C18H38	254.5	
4	HexA, methyl ester Palmitic acid, methyl ester n-HexA methyl ester Metholene 2	25.505		C17H32O2	270.5	
5	Octadecadienoic acid (Z,Z)-, -9,12 MethE	28.167		C19H34O3	310.5	
6	Phytol	28.463		C20H40O	296.5	
7	OctA, MethE	28.655		C19H34O3	296.5	
8	Tetracosahexaene, -2,6,10,14,18,22 2,6,10,15,19,23-hexamethyl- (CAS) Squalene Skvalen Supraene S	37.834		C30H50	326.6	
9	Vitamin e dl-.alpha.-Tocopherol 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridec	41.295		C31H52O3	472.7	

10	Bis(trimethylsilyl)benzene-1,3	42.991		C12H22Si2	222.47
11	Stigmasterol, 22,23-dihydro-	43.873		C29H48O	412.7



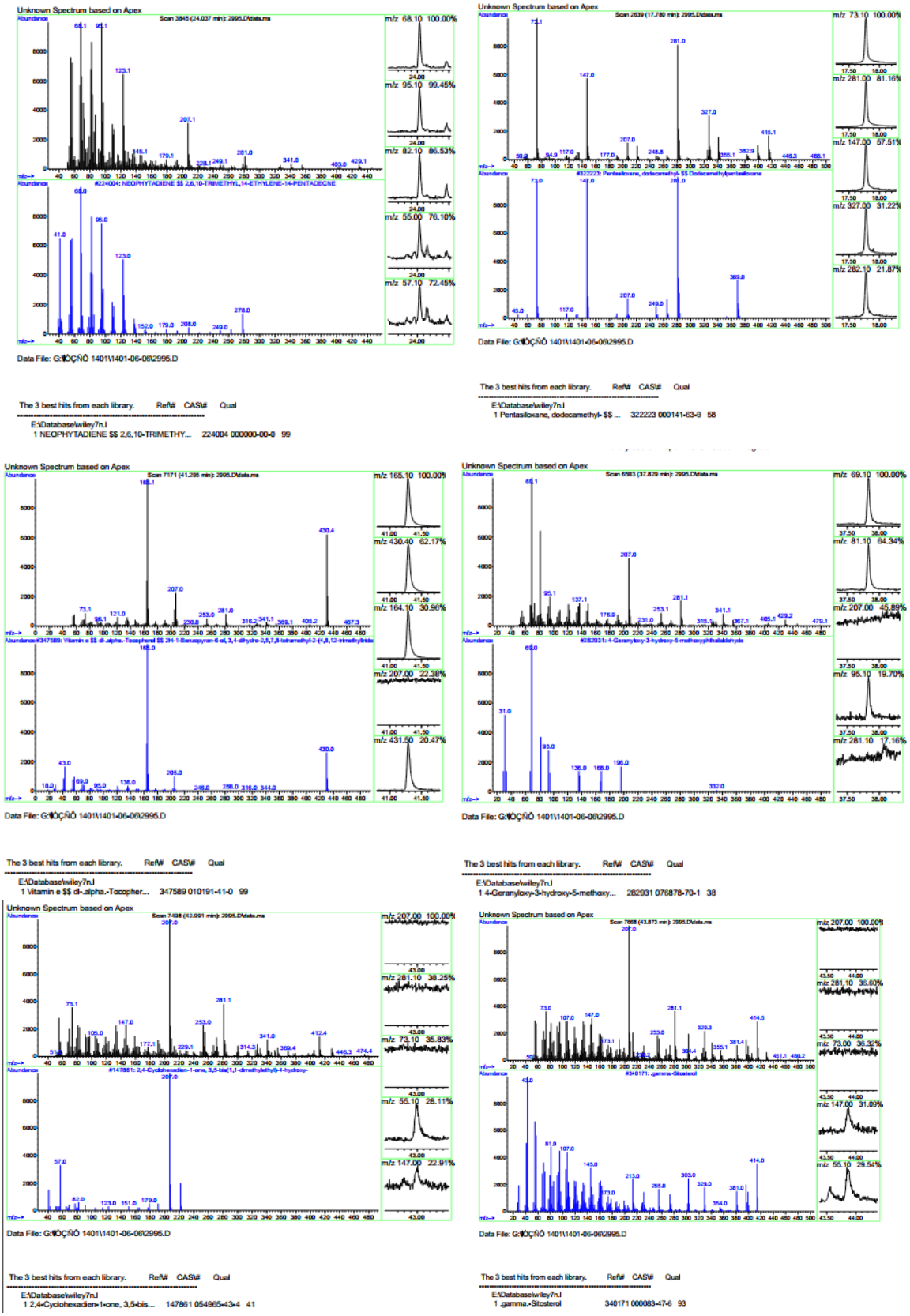
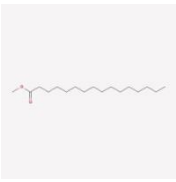
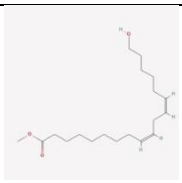
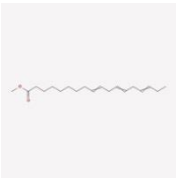
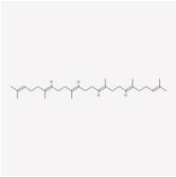
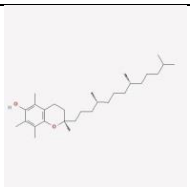
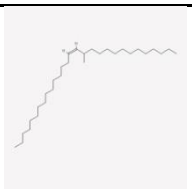
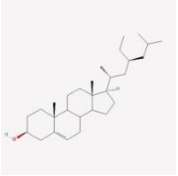
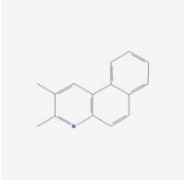
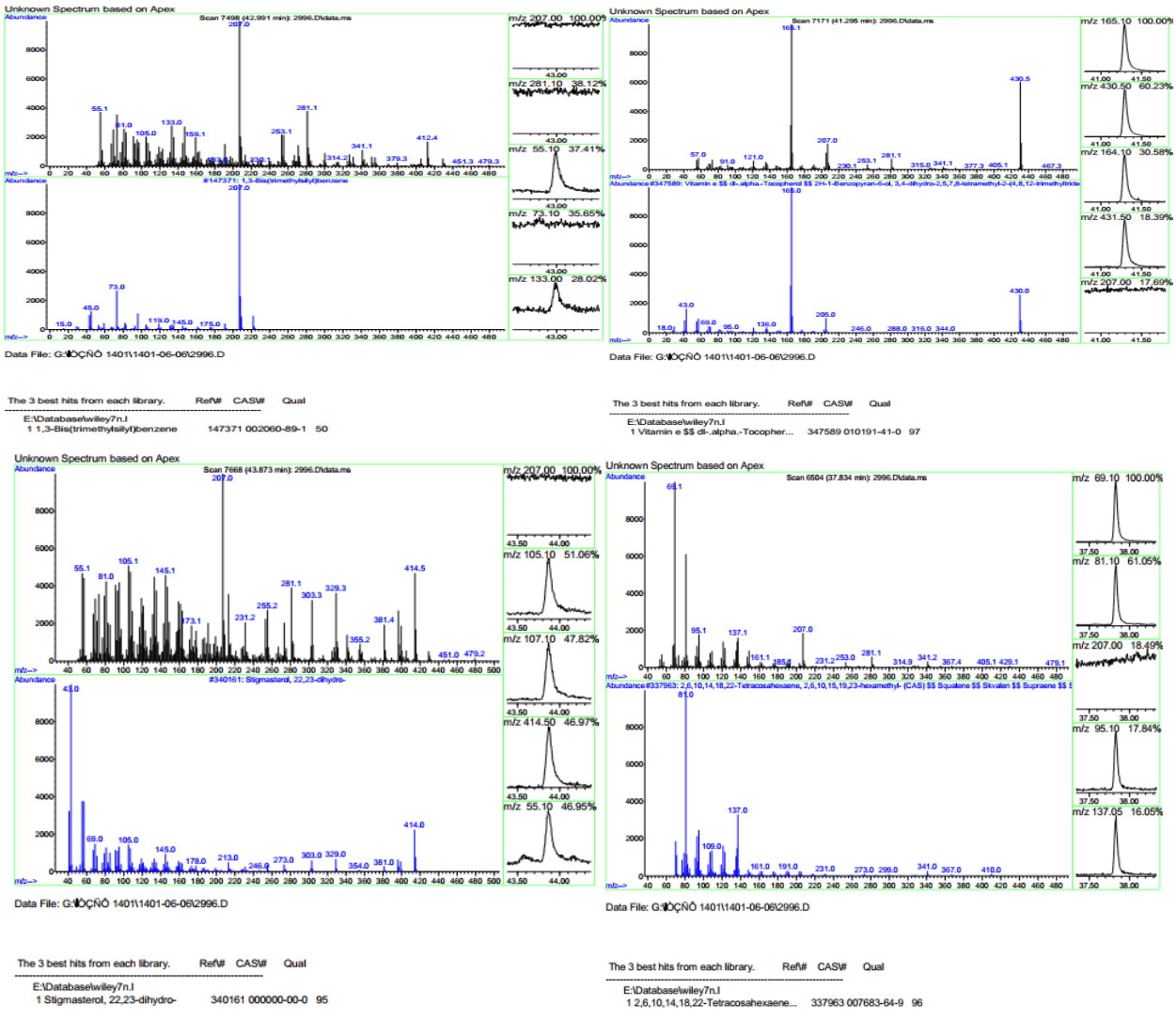


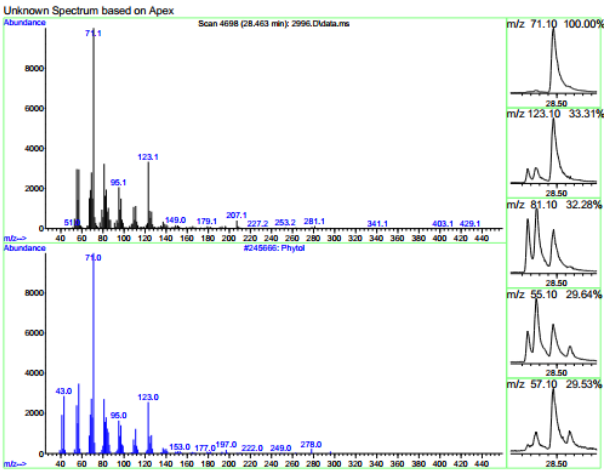
Figure 1. Gas spectrometer reading of the active compounds in the *G. setaceum* plant extract

Table 2. Active substances isolated from *G. aparine* plant extract

No	Chemical label	Retention duration	The Structure of Chemical	The formula of molecular	Molecular weight	Composite kind
1	Pentasiloxane, dodecamethyl- Dodecamethylpentasiloxane	17.780		C12H36O4Si5	384.84	
2	HexA, methyl ester (CAS) Methyl palmitate Methyl hexadecanoate Methyl n-hexadecanoate	25.511		C17H34O2	270.5	
3	9,12-Octadecadienoic acid, methyl ester	28.167		C19H34O3	310.5	
4	9,12,15-Octadecatrienoic acid, methyl ester (CAS) Methyl 9,12,15-octadecatrienoate	28.271		C19H32O2	292.5	
5	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene Skvalen Supraene S	37.828		C30H50	410.7	
6	Gibberellin A3 Gibb-3-ene-1,10-dicarboxylic acid, 2,4a,7-trihydroxy-1-methyl-8-methylene-, 1,4a-lactone, (1.alpha	40.859		C19H22O6	346.4	
7	Vitamin e	41.289		C29H50O2	430.7	

8	13-Methyl-Z-14-nonconsent	43.546		C30H60	420.8
9	(23S)-ethylcholest-5-en-3.beta.-ol Cholest-5-en-3-ol, 23-ethyl-, (3.beta.,23S)- (CAS	43.863		C29H50O	414.7
10	1,3-dimethyl-4-azaphenanthrene	45.181		C15H13N	207.27

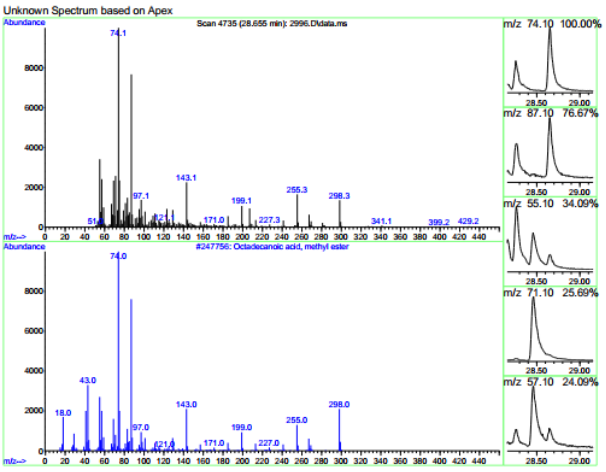




Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

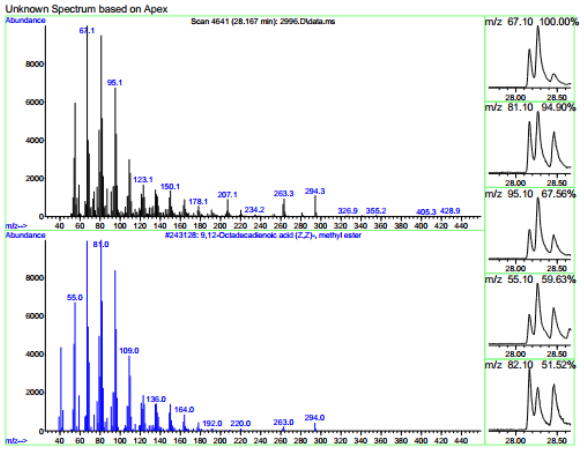
E:\Database\wiley7n.l
1 Phytol 245666 000150-86-7 92



Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

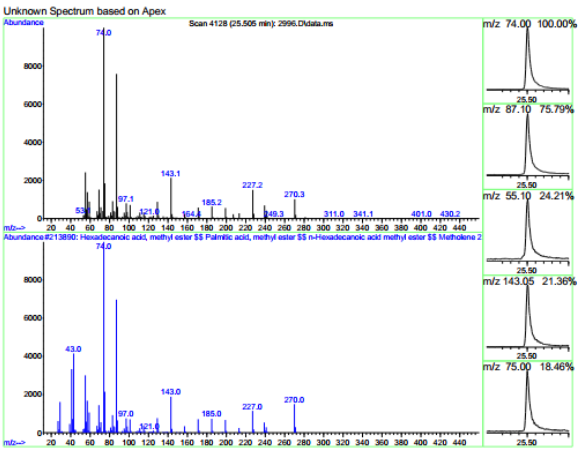
E:\Database\wiley7n.l
1 Octadecanoic acid, methyl ester 247756 000112-61-8 98



Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

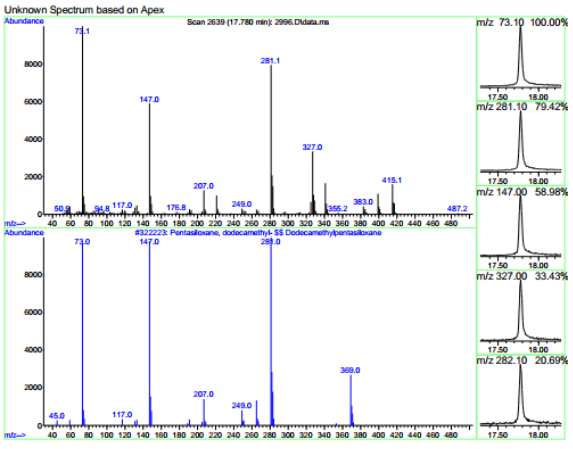
E:\Database\wiley7n.l
1 9,12-Octadecadienoic acid (Z,Z)-... 243128 000112-63-0 99



Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

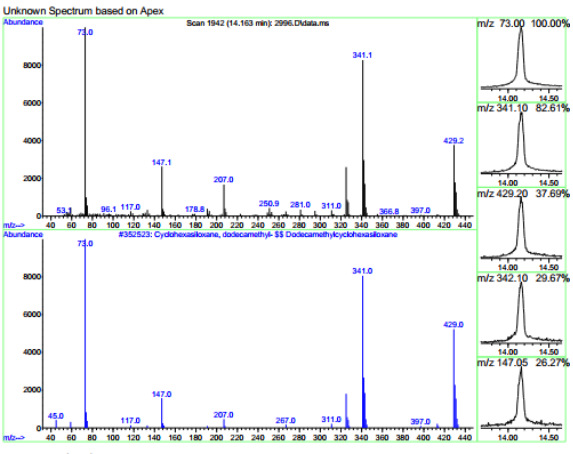
E:\Database\wiley7n.l
1 Hexadecanoic acid, methyl ester ... 213890 000112-39-0 98



Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

E:\Database\wiley7n.l
1 Pentasiloxane, dodecamethyl- $$ ... 322223 000141-63-9 52



Data File: G:\ÐÒÇÑÖ 1401\1401-06-06\2996.D

The 3 best hits from each library. Ref\# CAS\# Qual

E:\Database\wiley7n.l
1 Cyclohexasiloxane, dodecamethyl-... 352523 000540-97-6 90

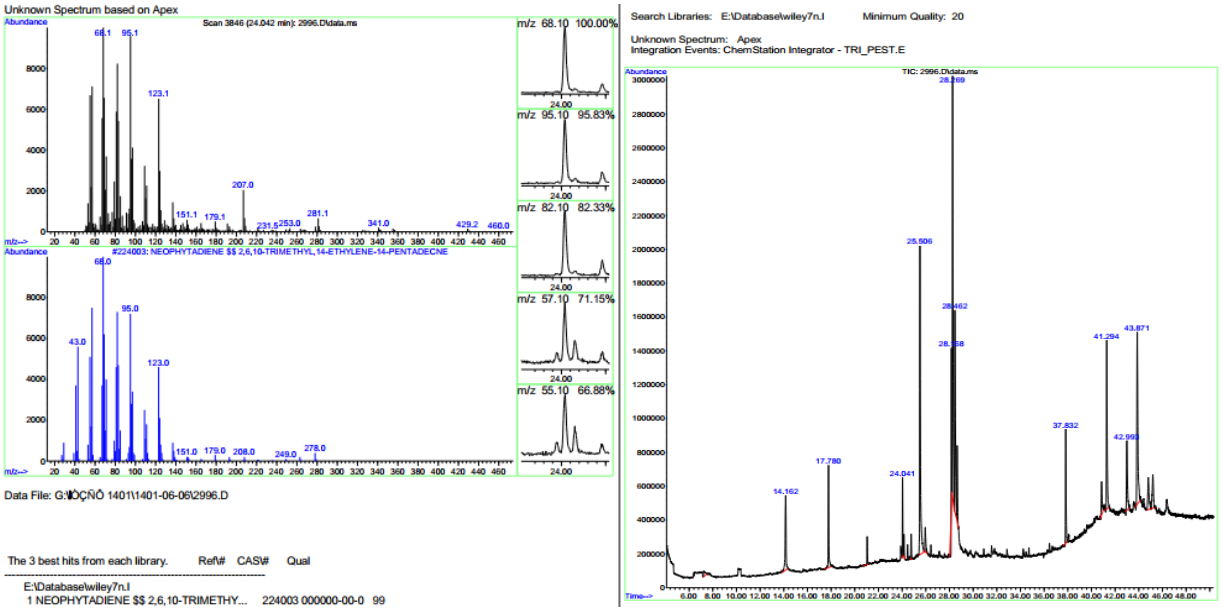


Figure 2. Gas spectrometer reading of the active compounds in the *G. aparine* plant extract

Table 3. Active substances shared between *G. aparine* and *G. setaceum*

أنواع / مركبات	G. aparine	G. setaceum
Pentasiloxane, dodecamethyl- Dodecamethylpentasiloxane	+	+
HexA, methyl ester (CAS) Methyl palmitate Methyl hexadecanoate Methyl n-hexadecanoate	+	+
2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl- (CAS) Squalene Skvalen Supraene S	+	+

4. Discussion

There are many biologically active compounds in the current study. By comparing the composites in the present investigation between the two species (*G. setaceum* and *G. aparin*), there were some compounds related between them and the compounds were as follows; (2,6,10,15,19,23-hexamethyl-(CAS) Squalene Skvalen Supraene, 2,6,10,14,18,22-Tetracosahexaene, methyl ester (CAS) Methyl palmitate Methyl hexadecenoic Methyl n-hexadecanoic, HexA, dodecamethyl-Dodecamethylpentasiloxane, and Pentasiloxane), and when comparing the current results with Jan et al. (2018), a related compound was

obtained, which is HexA, in addition to other compounds such as 2-hydroxy acid, HexA, and 5-methyl -3-Heptanone, 2-Isopropyl-1-methoxy In addition, three new taraxestane triterpenoids and five known taraxestane triterpenoids were obtained from the ethanol extract [4]. The Gallium family has been shown to have biomedical potential in antibacterial, antitumor, anti-inflammatory, and bone regeneration therapies [10].

5. Conclusion

In the present investigation, eleven chemical compounds have been separated from the Galium plant's leaves, and ten chemical compounds have been recognized from the *G. aparin* plant's leaves utilizing gas chromatography-mass spectrometry. It has been shown that plants possess a highly diverse profile that may be advantageous in various ways, including antioxidant, anticancer, anti-microbial, and anti-inflammatory activities. Therefore, it is recommended for chemical classification, phytopharmacology, properties, bioactivity, and toxicity profile.

REFERENCES

[1] M. G. Simpson, *Plant systematics. 2nd*. Burlington, MA: Academic Press. xii, 2010.

[2] M. Friščić, M. Š. Baglama, M. Milović, and ..., "Content of bioactive constituents and antioxidant potential of Galium L. species," *Croatica chemica …*, 2018, [Online]. Available: https://hrcak.srce.hr/clanak/300045

[3] R. H. Keijzer and M. de Keijzer, "Plantae tinctoriae: The 1759 Dissertation on Dye Plants by Engelbert Jörlin," *Heritage*, 2023, [Online]. Available: https://www.mdpi.com/2571-9408/6/2/81

[4] A. K. Jan, A. Khan, U. Farooq, N. Rehman, M. Tariq, and ..., "Composition of the Essential Oil of Galium setaceum," *Chemistry of Natural …*, 2018, doi: 10.1007/s10600-018-2416-x.

[5] J. Saavedra, *Estrategia customer relationship management y fidelización de clientes en la empresa América SAC Trujillo, 2017*. Tesis Licenciatura). Universidad …, 2017.

[6] M. V Sogonov, L. A. Castlebury, A. Y. Rossman, and ..., "The type species of the genus Gnomonia, G. gnomon, and the closely related G. setacea," *SYDOWIA …*, 2005, [Online]. Available: https://www.researchgate.net/profile/James-White-31/publication/43264098_The_type_species_of_the_genus_Gnomonia_G_gnomon_and_the_closely_related_G_setacea/links/0fcfd50e9aafc3ae0b000000/The-type-species-of-the-genus-Gnomonia-G-gnomon-and-the-closely-related-G-setacea.pdf?origin=journalDetail&_tp=eyJwYWdlIjoiam91cm5hbERldGFpbCJ9

[7] T. Ilina, N. Kashpur, S. Granica, A. Bazylko, I. Shinkovenko, and ..., "Phytochemical Profiles and In Vitro Immunomodulatory Activity of Ethanolic Extracts from Galium aparine L.," *Plants*, 2019, [Online]. Available: https://www.mdpi.com/2223-7747/8/12/541

[8] I. Nosratti and A. Muhammadyari, "First Report of Multiple Resistance in Galium aparine to ALS-Inhibiting and Auxin Analog Herbicides in Kermanshah, Iran," *Planta Daninha*, 2019, [Online]. Available: https://www.scielo.br/j/pd/a/gkXj3L8NCkLzY3hQZ4rKRjD/

[9] K. R. Markham, *Techniques of flavonoid identification.* cabidigitallibrary.org, 1982. doi: 10.5555/19850773265.

[10] W. Sun, M. Qi, S. Cheng, C. Li, B. Dong, and L. Wang, "Gallium and gallium compounds: New insights into the 'Trojan horse' strategy in medical applications," *Materials &Design*, 2023, [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0264127523001193

[11] A. F. Al-Rubaye, M. J. Kadhim, and ..., "Determination of bioactive chemical composition of methanolic leaves extract of Sinapis arvensis using GC-MS technique," *Int J Toxicol Pharmacol …*, 2017, [Online]. Available: https://www.researchgate.net/profile/Imad-Hameed/publication/319418977_Determination_of_Bioactive_Chemical_Composition_of_Methanolic_Leaves_Extract_of_Sinapis_arvensis_Using_GC-MS_Technique/links/59b8cd15458515bb9c4468b3/Determination-of-Bioactive-Chemical-Composition-of-Methanolic-Leaves-Extract-of-Sinapis-arvensis-Using-GC-MS-Technique.pdf

[12] A. H. Marzoqi, M. Y. Hadi, and I. H. Hameed, "Determination of metabolites products by Cassia angustifolia and evaluate antimicrobial activity," *Journal of Pharmacognosy and Phytotherapy*, 2015.

[13] H. L. Chakravarty, "Plant wealth of Iraq," *… of Agriculture and Agrarian Reform, Baghdad, Iraq*, 1976.

[14] G. G. Belz, K. Breithaupt-Grögler, and ..., "Treatment of congestive heart failure–current status of use of digitoxin," *European journal of …*, 2001, doi: 10.1111/j.1365-2362.2001.00012.x.

[15] A. Ghannadi, M. Rabbani, L. Ghaemmaghami, and ..., "Phytochemical screening and essential oil analysis of one of the Persian sedges; Cyperus rotundus L.," *International Journal of …*, 2012, [Online]. Available: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=2005c58d43038e0c387893973feb5c3e1924ceba

[16] H. A. Elbaz, T. A. Stueckle, W. Tse, Y. Rojanasakul, and ..., "Digitoxin and its analogs as novel cancer therapeutics," *… hematology &oncology*, 2012, doi: 10.1186/2162-3619-1-4.

[17] I. H. Hameed, I. A. Ibraheam, and ..., "Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of Rosmarinus oficinalis leaves," *… of Pharmacognosy and …*, 2015, [Online]. Available: https://academicjournals.org/journal/JPP/article-full-text-pdf/C17730553452.pdf

[18] A. K. Jan, M. R. Shah, I. Anis, and I. K. Marwat, "In vitro antifungal and antibacterial activities of extracts of Galium tricornutum subsp. longipedunculatum," *Journal of Enzyme Inhibition …*, 2009, doi: 10.1080/14756360802051255.

[19] J. de Moraes, R. N. de Oliveira, J. P. Costa, and ..., "Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected tropical disease Schistosomiasis mansoni," *PLoS neglected …*, 2014, [Online]. Available: https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002617

[20] A. D. Kawarase and S. G. Kunjalwar, "Pollen morphological studies in five species of Cyperusfrom Cyperaceae of Wardha district, Maharashtra, India," *Int J Sci Res*, 2016.

[21] M. T. Ghaneian, M. H. Ehrampoush, A. Jebali, and ..., "Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant," *Environmental Health …*, 2015, [Online]. Available: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=2610015

[22] R. E. Mokni, S. Hammami, S. Dall'Acqua, and ..., "Chemical Composition, Antioxidant and Cytotoxic Activities of Essential Oil of the Inflorescence of Anacamptis coriophora subsp. fragrans (Orchidaceae) from Tunisia," *Natural Product …*, 2016, doi: 10.1177/1934578X1601100640.

[23] C. Santos, M. S. Salvadori, V. G. Mota, L. M. Costa, and ..., "Antinociceptive and antioxidant activities of phytol in vivo and in vitro models," *Neuroscience …*, 2013, [Online]. Available: https://www.hindawi.com/journals/archive/2013/949452/

[24] A. S. Clive and C. Stace, "Plant taxonomy and Biosystematics," *Paper back*, 1980.

[25] C. C. Townsend, E. Guest, S. A. Omar, and A. H. Al-Khayat, *Flora of Iraq: Monocotyledones Excluding Gramineae (Hutchinson, 1959)*. Ministry of Agriculture and Agrarian …, 1985.

[26] H. A. Leslie, "Discovery and quantification of plastic particle pollution in human blood," *Environ Int*, vol. 163, 2022, doi: 10.1016/j.envint.2022.107199.

[27] A. H. Emwas, "Nmr spectroscopy for metabolomics research," *Metabolites*, vol. 9, no. 7, 2019, doi: 10.3390/metabo9070123.

[28] A. B. Patel, "Polycyclic Aromatic Hydrocarbons: Sources, Toxicity, and Remediation Approaches," *Front Microbiol*, vol. 11, 2020, doi: 10.3389/fmicb.2020.562813.

[29] A. R. Abubakar, "Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes," *J Pharm Bioallied Sci*, vol. 12, no. 1, pp. 1–10, 2020, doi: 10.4103/jpbs.JPBS_175_19.

[30] S. Alseekh, "Mass spectrometry-based metabolomics: a guide for annotation, quantification and best reporting practices," *Nat Methods*, vol. 18, no. 7, pp. 747–756, 2021, doi: 10.1038/s41592-021-01197-1.