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Gas Chromatography/Mass Spectrometry (GC/MS) analysis of *Heliotropium ovalifolium* Forssk. root and aerial part extracts

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Abstract. In the history of folk medicine *Heliotropium ovalifolium* plant used to cure for inflammation, poison bites, skin and nervous disorders. The roots and aerial parts extracted by n-hexane and subjected to analysis using Gas Chromatography/Mass Spectrometry (GC/MS). Various constituents identified. Root extract was identified by abundance of hexadecanoic acid, methyl ester (24.50%), methyl stearate (15.61%), oxiraneoctanoic acid, 3-octyl, methyl ester (12.29%), 9-octadecenoic acid (Z)-, methyl ester (10.17%) and 9,12-Octadecadienoic acid (Z, Z)-, methyl ester (7.29%). While aerial parts extract showed eight alkane compounds which were identified as major constituents (62.89%) out of these compounds was heneicosane which showed high percentage (34.14%) and consider as a bioactive molecule. The present study revealed that the fatty acids in *H. ovalifolium* may be explored for manufacturing industrial products and pharmaceutical agents.

Keywords. *Heliotropium ovalifolium*, Boraginaceae, chemical composition, GC/MS, hexadecanoic acid, methyl ester and heneicosane.

1. Introduction

The plant Heliotropium ovalifolium (family Boraginaceae) is a perennial herb and widespread in Sudan. The family Boraginaceae consists of hundreds of genera and about two thousand species. The family is wide spread in tropical and temperate regions, especially the Mediterranean. Heliotropium, Arnebia, Martensia, Cordia, and Trichodesma are the main genera of the Boraginaceae family. Heliotropium is a large genus of the Boraginaceae family, consisting of about 250–300 species worldwide. The name "heliotrope" originates from the ancient ideas that these plants turned their leaves towards the sun. 'Helios' meaning in Greek languages is 'sun,' and the 'tropium' word comes from another Greek word 'tropein' and the meaning of this word is 'to turn' [1]. In folk medicines the Heliotropium species used to cure for gout, skin and nervous disorders, inflammation, and poison bites [2]. Mohanraj et. Al. [3] have given account on helifoline, a pyrrolizidine alkaloid, these alkaloids are a common constituent of the Botaginaceae family, which exhibited pronounced hepatotoxic effects, also mutagenic and carcinogenic activities have been reported [4]. Heliotropamide, an alkaloid with oxopyrrolidine -3- carboxamide central moiety, has been isolated as the major product of the dichloromethane extract of H. ovalifolium aerial parts [5]. Also, benzoquinones, heliotropinone A and B, have been isolated from the aerial parts of H. ovalifolium, they have antifungal activities against Cladosporum cucumerinum and Candida albicans as well as antibacterial activity against Bacillus subtilis [6]. Tiwari and Masood [7] carried out analysis of chemical constituents of H. ovalifoliums. Kulkarni [8] reported Novel leads from Heliotropium ovalifolium, 4,7,8-trimethoxynaphthalene-2-carboxylic acid and 6-hydroxy-5,7-dimethoxynaphthalene-2-carbaldehyde which showed specific IL-6 inhibitory activity in THP-1 cells and primary human monocytes.

2. Materials and Methods

2.1. Plant materials

The plant under investigation (H. ovalifolium) was authenticated at the Department of Botany by Prof. Hatil, H. ELKamali, Omdurman Islamic University.

2.2. Preparation of crude plant extracts

The plant material was air dried and ground into coarse powder using mortar and pestle. One hundred and fifty grams from the powder were soaked in n hexane for three days in a shaker and then filtered using Whatman No. 3 filter paper. The filtrates evaporated to dryness using a rotatory evaporator then weighed and stored.

2.3. GC/MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japan's Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30mX0.25 mmX0.25um). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 5 minutes hold time, the injection port temperature was 300c, the ion source temperature was 200℃ and the interface temperature was 250℃. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 29 minutes. Identification of the sample components was achieved by computer searches in commercial library, the National Institute of Standards and Technology (NIST).

3. Results and Discussion

Table 1 represented the chemical classes and constituents of the H. ovalifolium root n-hexane extract. Twenty fatty acids were identified by GC/MS mainly consists of saturated fatty acids (70.69%) followed by polyunsaturated fatty acids (11.23%) and monounsaturated fatty acids (10.65%). The main components were palmitic acid (24.50%) while stearic acid exhibited 15.61%. Palmitic acid is used as an emollient, perfuming agent, detergent making, wetting agent, stabilizer, lubricating agent and plasticizer in various industries [9]. The fatty acids mostly palmitic and stearic acid are used in pharmaceutical and cosmetic industry [10] and skin care products. Also, oxiraneoctanoic acid methyl ester and oleic acid represented 12.29% and 10.17% respectively. The derivatives of unsaturated fatty acids oleate and linoleate are known to display some toxicity [11], moreover palmitate derivatives represented strong toxicity. Guntern [6] isolated Heliotropinones A, heliotropinones B and quinones from H. ovalifolium. Also, hexadecenoic acid, which was reported by Isidrov et al. [12] in the royal jelly, of the nurse bees, represented bactericide, anti- inflammatory and anticancer activities.

Table 2 showed the relative percentages and chemical classes of the constituents of the n-hexane extract of the H. ovalifolium aerial parts. Eight alkane compounds were identified as major constituents (62.89%), out of these compounds was Heneicosane which showed high percentage 34.14%. Moreover, it considers as bioactive molecule used for measuring the antimicrobial activity. Depending on the results obtained by Vanitha et. al. [13] it was verified that heneicosane exhibited excellent antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus*. Hence heneicosane is the bioactive molecule with pronounced antimicrobial activity. The fatty acids were mainly consisting of polyunsaturated fatty acids (20.44%). The lipophilic nature of polyunsaturated fatty acids (PUFAs) can be used as an applicable carrier to increase therapeutic efficacy of anticancer drugs [9]. Also saturated and monounsaturated fatty acids showed 8.56% and 2.13% respectively. Furthermore, the result indicated 9,12,15-Octadecatrienoic acid, methyl ester (10.52%), linoleic acid (9.92%) and tetrapentacontane (9.01%).

Table 1. Chemical compounds identified using n-hexane extract of the *H. ovalifolium* roots

Chemical class	Compound	%	R.Time
Saturated Fatty Acids (SFA)	Lauric acid (Dodecanoic acid, methyl ester)	0.24	11.263
	Tridecanoic acid 4,8,12-trimethyl-,methyl ester	0.18	14.348
	Methyl pentadecanoate (Pentadecanoic acid, methyl ester)	0.23	14.487
	Palmitic acid (Hexadecanoic acid, methyl ester)	24.50	15.673
	Hexadecanoic acid, 15-methyl,methyl ester	0.11	16.295
	Hexadecanoic acid , 14-methyl,methyl ester	0.16	16.377
	Magaric acid (Heptasecanoic acid, methyl ester)	0.51	16.649
	Stearic acid (Methyl stearate)	15.61	17.587
	Oxiraneoctanoic acid, 3-octyl-,methyl ester	12.29	19.106
	Arachidic acid (Eicosanoic acid, methyl ester)	1.81	19.341
	Octanosecanoic acid, 9,10-dihydroxy-, methyl ester	2.26	20.096
	Behenic acid (Docosanoic acid , methyl ester)	2.84	20.962
	Methyl tetradecanoate	1.53	
	Lignoceric acid (Tetracosanoic acid, methyl ester)	1.55	22.464
	Ethyl stearate – 9,12-diepoxy	6.87	20.328
Monounsaturated Fatty Acids (MUFA)	Cis-5-Dodecenoic acid, methyl ester	0.23	14.487
	Palmitoleic acid (9-Hexadecenoic acid, methyl ester, (Z)-	0.25	15.479
	Oleic acid (9-Octadecenoic acid (Z)-, methyl ester	10.17	17.369
Polyunsaturated Fatty Acids (PUFA)	Linoleic acid (9,12-Octadecadienoic acid, (Z,Z)- methyl ester	7.29	17.323
	8,11,14-Docosatrienoic acid, methyl ester	3.94	20.571
Alcoholic derivatives	1-Hexanol,2-ethyl-	3.69	4.642
	1-Heptacosanol	1.70	20.681
Ketone	2-Pentadecanone – 6,10,14-trimethyl-	0.15	16.649
Amide derivatives	13-Docosenamide , (Z)-	1.91	22.924

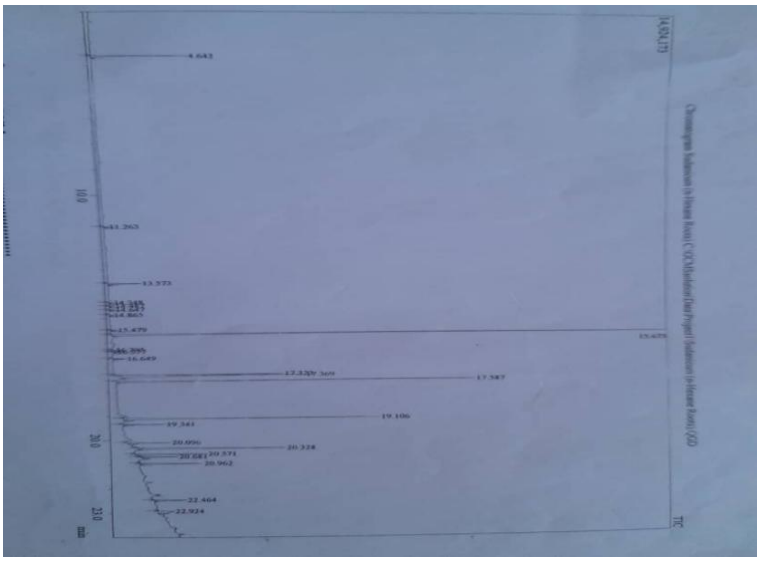

Figure 1. Chromatogram of n- hexane root extract

Table 2. Chemical compounds identified using n-hexane extract of the *H. ovalifolium* aerial parts

Chemical class	Compound	%	R. Time
Saturated Fatty Acids (SFA)	Methyl tetradecanoate	0.66	13.582
	Methyl stearate	1.27	17.592
	Palmitic acid (Hexadecanoic acid, methyl ester)	6.63	15.679
Monounsaturated Fatty Acids (MUFA)	Oleic acid (9-Octadecenoic acid (Z)-, methyl ester	2.13	17.330
Polyunsaturated Fatty Acids (PUFA)	Linoleic acid (9,12-Octadecadienoic acid, (Z,Z)- methyl ester	9.92	17.385
	9,12,15-Octadecatrienoic acid, methyl ester	10.52	17.401
Diterpene	Phytol	0.78	17.497
Ketone	2-Pentadecanone – 6,10,14-trimethyl-	0.78	14.872
Alkane	Pentatriacontane	1.48	20.701
	Hexatriacontane	1.38	21.189
	Octadecane,3-methyl-	4.41	22.020
	Dotriacontane	5.61	22.212
	Tetratriacontane	4.03	22.662
	Tetratetracontane2	2.83	22.926
	Tetrapentacontane	9.01	23.443
	Heneicosane	34.14	23.618
Steroid	Squalene	2.84	23.212
Phenol derivatives	Phenol,2,2-methylenebis[6-(1,1-dimethyl-	0.80	20,266
Benzonitrile	Benzonitrile,m-phenethyl-	0.77	20.564

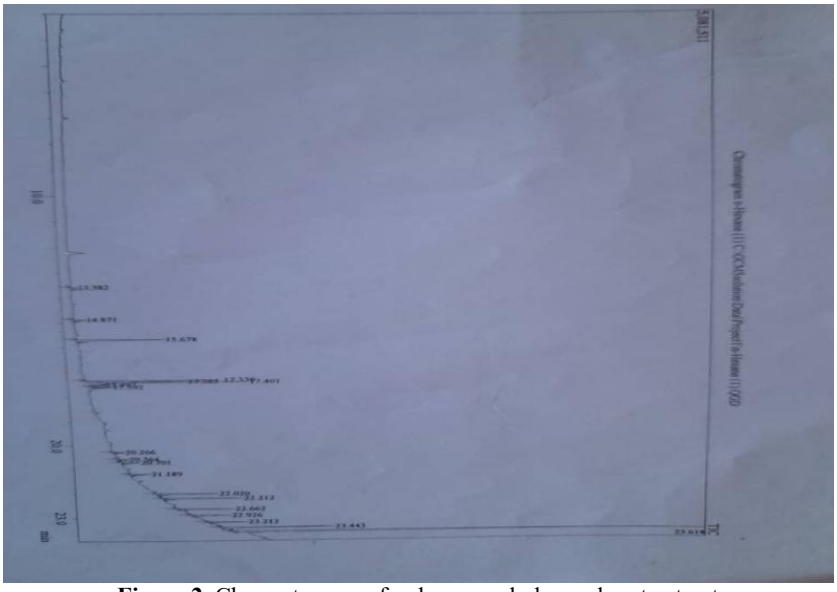

Figure 2. Chromatogram of n- hexane whole areal part extract

4. Conclusion

The plant H ovalifolium which contains bioactive compounds as revealed by GC/MS analysis, represented many constituents which have pharmacological and industrial uses, as well as heneicosane and palmitic acid. Hence, the plant might be used as a new and promising natural source in pharmacology and industrial products.

References

[1] Selvi, F., Bigazzi, M., (2001). Leaf surface and anatomy in Boraginaceae tribe Boragineae with respect to ecology and taxonomy. Flora 196, 269–285.

[2] Kumar, P, Ayyanar,M., Lgnacimuthu, S. (2007). Medicinal plants used by Malasar tribes of Coimbatore district,Tamil Nadu, Indian J. of traditional knowledge. 6(4),579-582.

[3] Mohanraj S, Kulanthaivel P, Subramanian PS, Herz W(1981). Helifoline, a pyrrolizidine alkaloid from Heliotropium ovalifolium, Phytochemistry 20(8), - 1991-1995.

[4] Gurib-Fakim, A. (2006). Heliotropium ovalifolium Forssk. [Internet] Record from PROTA 4U. Schmelzer, G.H. and Gurib-Fakim , A. (Editors). PROTA (Plant Resources of Tropical Africa/, Wageningen, Netherlands.

[5] Guntern A. et al., (2003). Heliotropamide, a novel oxopyrrolidine -3-carboxamide from Heliotropium ovalifolium. J.Nat.Prod. PMID: 14695794.

[6] Guntern A, Ioset J-R, Queiroz EF, Foggin CM, Hostettmann K(2001). Quinones from Heliotropium ovalifolium. Phytochemistry,58(4), 631-635.

[7] Tiwari KP and Masood M. (1977) “Chemical constituents of Heliotropium ovalifolium”. Proceedings of the National Academy of Science (India), Sect. A 47: 72

[8] Kulkarni-Almeida A., et al. (2008) “Novel leads from Heliotropium ovalifolium, 4, 7, 8-trimethoxy-naphthalene-2-carboxylic acid and 6-hydroxy-5, 7-dimethoxy-naphthalene-2-carbaldehyde show specific IL-6 inhibitory activity in THP-1 cells and primary human monocytes”. Phytomedicine 15: 1079-1086

[9] Wang *et al.* (2012). The powerful applications of polyunsaturated fatty acids in improving the therapeutic efficacy of anticancer drugs. Expert Opin. Drug Deliv.

[10] Ahmad M.S. (1984). An 8-hydroxyoctadeca-cis-11, 14-dienoic acid from Mirabilis jalapa seed oil. Phytochem.J.

[11] Ortsater H (2011). Arachidonic acid fights palmitate: New insights into fatty acid toxicity in β-cells. J. Clin. Sci. 120:179-181.

[12] Isidrov VA, Czyzewska V, Jankowsk M, Bakier S (2011). Determination of royal jelly acid in honey. J. Food Chem. 124:387-391.

[13] Vanitha, V., Vijayakumar S., Nilavukkarasi M, Punitha V. N.,Vidhya E. (2020). Heneicosane—A novel microbicidal bioactive alkane identified from Plumbago zeylanica L. J. Industrial crops and products 154.