

Article

A Comparative Chemical Study of Two Species of the Genus *Ziziphus* in Central and Southern Iraq

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Abstract: Arterial The chemical study dealt with the chemical content of the studied leaves of the *Ziziphus* plant, as the chemical compounds were identified using GasChromatography Mass Spectrometry (GC/MS) technology by comparing the chemical content. The two studied samples showed that they contained six compounds: Octadecenoic acid (Z), oxiranylmethyl ester and Bis(2-ethylhexyl) phthalate, Hexadecanoic acid, ethyl ester, Neophytadiene, and n-Hexadecanoic acid. This article explains the differences in the phytochemical composition of species of the genus *Ziziphus* in Iraq using GC-MS technology, as 49 phytochemical compounds were recorded in the ethanolic extracts of the plant's leaves under review. The study and study using GC-MS technology. As for the rest of the chemical compounds, their presence varied according to the type. Some of them were limited to one type, some were present in two types, and some were more than 25.506. The highest value of the retention time was 25.506 at peak 22 for the active compound in Myristic acid glycidyl ester of type *Z. mauritius* (Tofahy Var.), while the lowest retention time is 2.242 at peak (1) for the compound Methoxyacetaldehyde diethyl acetal in *Z. jujuba* species.

Keywords: Ethanolic acetone extracts, *Ziziphus*, GC-MS technology

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1. Introduction

The genus *Ziziphus* belongs to the Sidr family, Rhamnaceae. This family is one of the large plant families, as it includes approximately 55 genera and approximately 950 species. The genus *Ziziphus* is one of the most prominent genera of the Sidridae family, as the total number of species belonging to this genus is approximately 170 species, which are widespread in the tropical, subtropical, and warm temperate regions of the world (Nisar *et al.*, 2011).

The Sidr plant is considered a multi-use plant. Sidr leaves are used to strengthen hair, prevent hair loss, remove dandruff, and are useful for treating diseases of asthma, lung, rheumatism, skin, and diarrhea (Nisar *et al.*, 2011). Its leaf extract is also known as an anti-fungal and an inhibitor of many types of antibacterial bacteria. In addition to containing antioxidants and anti-hyperglycemia, the substances found in Sidr extract are used in drug

development and drug effectiveness in the countries of the Middle East and South and East Asia (Asgarpanah and Hahighat, 2012).

The extract is also used Its leaves are used to treat skin diseases, anemia, diarrhea, fever, pharyngitis, bronchitis, and insomnia, in addition to using it to relieve toothache, as an astringent compress, mouth wash and antiseptic, and an emollient and skin softener. Its toxic effectiveness against cells has also been proven. Cancer in the colon and breast (Latifa *et al.*, 2019). For microorganisms, bacteria, fungi, and viruses (Elhachimi *et al.*, 2019).

Studies have shown that leaf extracts are effective in stimulating the immune system, anti-allergic, anti-inflammatory and stomach ulcer (Slimani *et al.*, 2017) and anti-fungal (Soel *et al.*, 2007).

Due to the importance of the Sidr plant from a medical standpoint, I proposed studying it chemically in an attempt to cover more precise details in terms of its chemical composition and to be one of the links in an integrated series to study everything related to the *Ziziphius* plant. The study aimed to investigate the facts of the following aspects; Studying the chemical content of *Ziziphius* leaves, classifying them, and knowing their concentrations and their presence or absence among these types of *Ziziphius* plants.

2. Materials and Method

2.1. Work Methods

2.1.1. Preparing The Powder For The Leaves Of The Plant

Ziziphius leaf powder was prepared by collecting a quantity of Sidr tree leaves found in the gardens of the College of Education for Girls on Friday, then dried the paper by placing it on filter paper in the laboratory atmosphere, and after the leaves dried completely, they were ground using an electric blender until the leaf powder was obtained, which was stored in Refrigerate until use.

2.2. Chemical Study

The chemical study was based on the powder of Sidr plant leaves studied for dry samples. This test was conducted in the GC-Mass Unit / Department of Environment and Water / Ministry of Science and Technology / Iraq, and the method was carried out as follows:

2.2.1. Methods

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1. First: Prepare the ethanolic and acetone leaf extract according to the method of Markham (1982) with some modifications:
 - Take 0.5 grams of dry leaf powder and place it tightly on a filter paper, then place the latter with the powder inside a glass box with 5 ml of 99.99% ethanolic alcohol in a test tube and tightly close it while continuing to stir for 10 hours.
 - then leave it at room temperature. Room temperature in a dark place for 12 hours. I was then filtered with 102 Filter paper clata sheet medium.
 - Add 5.0 ml of acetone at a concentration of 99.99% to discard the water to increase the concentration of the extract.
 - The chemical compounds in the extract were estimated after withdrawing the floating portion separated from the water with acetone.
 - After that, the separated part is ready for examination with the Gas Chromatography Mass Spectrometry (GC/Mass) device.
2. Second: Analysis using the Gas Chromatography Mass Spectrometry (GC-Mass) device according to the method of (Ingole, 2017). The alcoholic extract of the leaves

was analyzed using a GC device (GC Clarus 500 Perkin Elmer) and a mass spectrometer according to the following analysis conditions:

- Injector temperature is 280°C.
- The temperature of the ion source is 200 degrees Celsius.
- Helium gas 99.999% was used as a carrier gas at a continuous flow rate of one ml/min.
- The injected liquid was 8 microliters in volume and worked with a division ratio of 0.5.
- The oven temperature is 110 Celsius, as it was automatically programmed in increments of 10 Celsius. One minute, and the temperature continues to increase until it reaches 280 Celsius and remains there for 8 minutes until the end.
- Mass spectra were taken at 70 EV with an interval of 0.5 seconds .
- The total time is 32 minutes from the start of the device until it turns off .
- Elite-1 fused silica capillary column, which consists of 100% dimethyl polysiloxane, which operates in 70 EV (electron sniping detector) mode
- The pressure inside the device is 100.0 Kpa at a rate of one ml. minute .

3. Third: Identifying chemical compounds

The chemical components were identified according to the GC-Mass mass spectrometer, which used the database of the National Institute of Standard and Technology, with 62,000 or more known patterns.

The resulting spectrum of the unknown component was then compared with a set of known components stored in the NIST library to confirm the molecular weight, name, and structure of the components of the test materials.

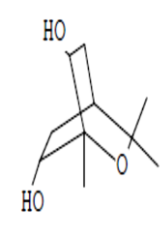
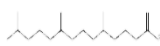
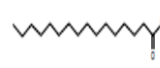
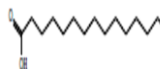
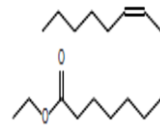
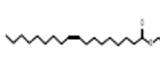
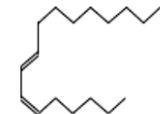
3. Results and Discussion

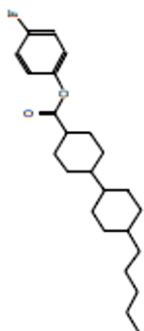
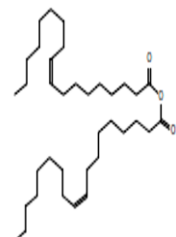
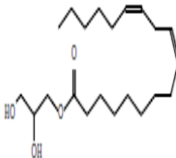
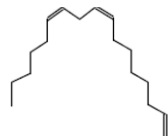
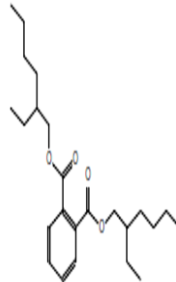
The results of the analysis using the GC-Mass technique showed that there were prominent differences in the distribution of chemical compounds between the leaves of the Sidr plant under study. Six chemical compounds were detected collected in the leaves of the *Ziziphius* plant, with the highest retention time value reaching 25.506 at peak 22 for the active compound in Myristic acid. glycidyl ester of *Z. mauritius* (Tofahy Var.), while the lowest retention time is 2.242 at peak (1) for the compound Methoxyacetaldehyde diethyl acetal in *Z. jujube*.

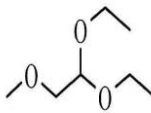
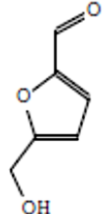
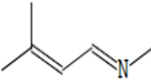
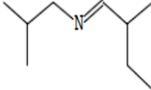
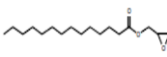
Chemical classification has effectively contributed to solving taxonomic problems at the taxonomic level (Iserin, 2001). Many studies and chemical methods have been conducted to identify and diagnose these different types of compounds in various sciences due to their great role in identifying and classifying different living organisms, and because they are among the most Secondary metabolites are widespread. In nature, according to a study by (Castellino *et al.*, 2012)., in general

Accordingly, this study led to the discovery of the extraction and identification of the chemical compounds of the studied sample for the purpose of determining its content of chemical compounds and their concentration for use as chemical properties to help isolate, diagnose and compare their chemical composition. . By comparing the chemical content, it was noted that the studied sample was characterized by containing many different compounds, which gave appropriate taxonomic importance to the isolate. The difference is due to genetic differences, environmental conditions, soil type, and the amount of mineral elements in the soil (Ahmad *et al.*, 2006).

Table 1. shows the chemical compounds in the acetone ethanolic alcoholic extract (99.99%) of the leaves of the plant *mauritius* Z (Tofahy Var.) with GC- Mass.

NO	Name	Peak No.	SI	R.T.	Area %	Classification	Mol. Weight	Mol. Formula	Structure
1	-1,3,3Trimethyl-2-oxabicyclo[2.2.2]octane-6,7-endo,endo-diol	1	83	2.338	0.46	Monoterpenoid	186	C ₁₀ H ₁₈ O ₃	
2	Neophytadiene	2	91	6.139	0.97	Hydrocarbon	278	C ₂₀ H ₃₈	
3	Hexadecanoic acid, ethyl ester	3	93	9.482	0.39	Fatty Acid	284	C ₁₈ H ₃₆ O ₂	
4	n-Hexadecanoc acid	4	96	10.568	1.02	Fatty Acid	256	C ₁₆ H ₃₂ O ₂	
5	Ethyl Oleate	5	89	12.577	0.46	Hydrocarbon	310	C ₂₀ H ₃₈ O ₂	
6	Linoleic acid ethyl ester	6	91	14.378	1.21	fatty acid ethyl ester	308	C ₂₀ H ₃₆ O ₂	
7	Oleic acid, butyl ester	7	81	16.540	4.20	fatty acid ester	338	C ₂₂ H ₄₂ O ₂	
8	10E,12Z-Octadecadienoic acid	8 21	87 85	16.659 23.680	9.05 3.33	Fatty Acids	280	C ₁₈ H ₃₂ O ₂	

9	[1,1'-Bicyclohexyl]-4-carboxylic acid, 4'-pentyl-, 4-fluorophenyl ester	9	83	18.355	2.46	aromatic carboxylic acids	374	C24H35FO2	
10	Oleic anhydride	10	86	18.455	6.87	Fatty Acids	546	C36H66O3	
11	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	11	90	18.775	47.11	Fatty Acids	354	C21H38O4	
12	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	12	88	18.840	6.96	Fatty Acids	338	C21H38O3	
13	1,8,11-Heptadecatriene, (Z,Z,Z)-	13	87	19.214	2.74	Alkatrienes	234	C17H30	
14	Bis(2-ethylhexyl) phthalate	14	95	19.548	0.39	Carboxylic Acids	390	C24H38O4	

15	Squalene	15	95	19.981	0.86	Triterpenoid	410	C ₃₀ H ₅₀	
16	No compounds	16		21.197	3.17				
17	Methoxyacetaldehyde diethyl acetal	17	84	21.326	0.95	Acetaldehyde	148	C ₇ H ₁₆ O ₃	
18	5-Hydroxymethylfurfural	18	95	21.579	3.07	Alcohol	126	C ₆ H ₆ O ₃	
19	Methanamine, N-[3-methyl-2-butenylidene]	19	85	21.712	0.98	Methanamine	97	C ₆ H ₁₁ N	
20	N-(2-Methylbutylidene)isobutylamine	20	81	22.345	1.47	Amine	141	C ₉ H ₁₉ N	
22	Myristic acid glycidyl ester	22	90	25.506	1.87		284	C ₁₇ H ₃₂ O ₃	

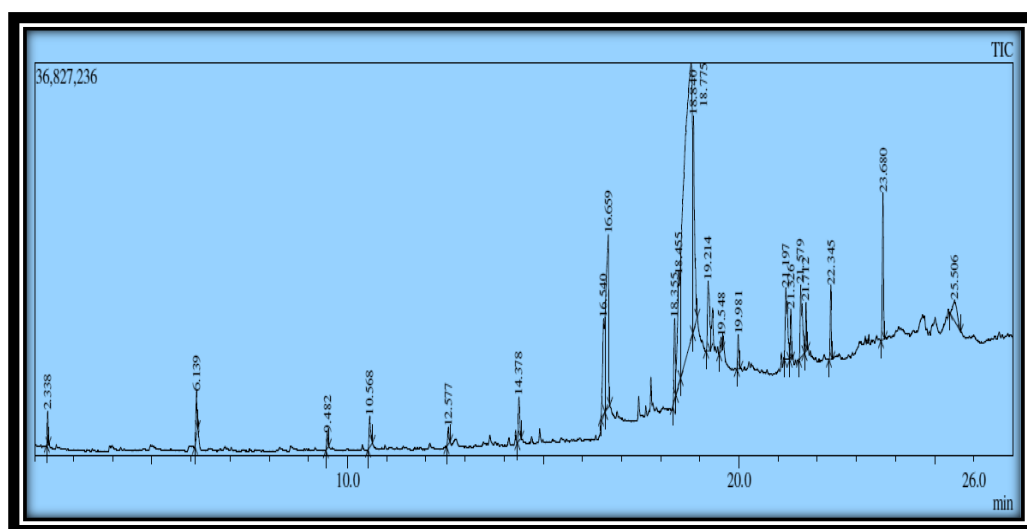
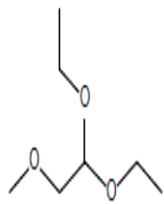
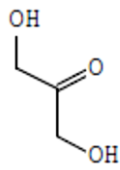
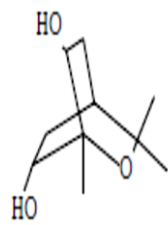
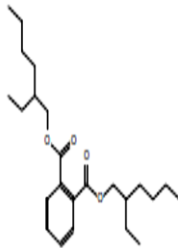
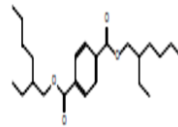
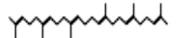
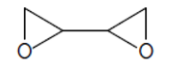
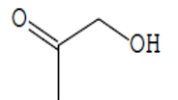
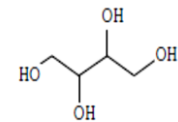
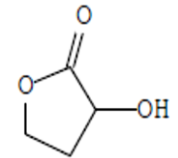
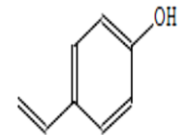
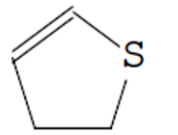


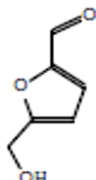
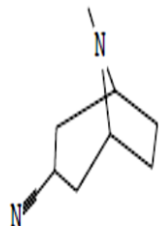
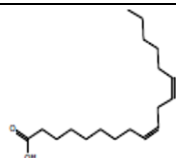
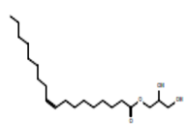
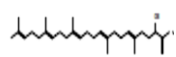
Figure 1. Curve of chemical compounds of the ethanolic acetone extract (99.99%) of *Z. mauritius* (Tofahy Var.) leaves using the GC-Mass technique.

Table 2. Shows the chemical compounds in the acetone ethanolic alcoholic extract (99.99%) of the leaves with GC- Mass of the plant *Z.jujuba*

NO	Name	Peak No.	SI	R.T.	Area %	Classification	Mol. Weight	Mol. Formula	Structure
1	Methoxyacetaldehyde diethyl acetal	1	83	2.242	0.06	Ethers	148	C ₇ H ₁₆ O ₃	
2	Dihydroxyacetone	2	94	2.381	2.77	Monosaccharides	90	C ₃ H ₆ O ₃	
3	-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane-6,7-endo,endo-diol	3	85	2.597	0.45	Terpenoid	186	C ₁₀ H ₁₈ O ₃	

4	Neophytadiene	4	96	4.005	1.93	Hydrocarbon	278	C ₂₀ H ₃₈	
5	Hexadecanoic acid, ethyl ester	5	93	5.216	1.32	fatty acid ester	284	C ₁₈ H ₃₆ O ₂	
6	n-Hexadecanoic acid	6	96	6.011	0.39	Fatty Acid	256	C ₁₆ H ₃₂ O ₂	
7	Phytol	7	96	6.162	2.21	Diterpenes	296	C ₂₀ H ₄₀ O	
8	-9,12-Octadecadienoic acid, ethyl ester	8	88	8.586	0.83	Fatty Acids	308	C ₂₀ H ₃₆ O ₂	
9	6-Octadecenoic acid, (Z)-	9	88	9.319	0.11	Fatty Acids	282	C ₁₈ H ₃₄ O ₂	
10	i-Propyl 9-octadecenoate (Z)	10	84	9.509	0.24	Fatty Acids	324	C ₂₁ H ₄₀ O ₂	
11	Oleic anhydride	11	87	10.575	0.79	Fatty Acids	546	C ₃₆ H ₆₆ O ₃	
12	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	12	86	14.381	1.07	Ethers	338	C ₂₁ H ₃₈ O ₃	

Carboxylic Acids									
13	Bis(2-ethylhexyl) phthalate	13	96	16.540	3.45		390	C ₂₄ H ₃₈ O ₄	
14	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	14	95	16.669	9.52	Carboxylic Acids	390	C ₂₄ H ₃₈ O ₄	
15	Squalene	15	94	17.767	3.50	Triterpenes	410	C ₃₀ H ₅₀	
16	No compound	16	-	18.460	3.20	-	-	-	-
17	2,2'-Bioxirane	17	83	18.752	48.56	Ethers	86	C ₄ H ₆ O ₂	
18	2-Propanone, 1-hydroxy-	18	91	19.207	2.00	Ketones	74	C ₃ H ₆ O ₂	
19	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	19	88	19.563	0.16	Monosaccharides	122	C ₄ H ₁₀ O ₄	
20	2-Hydroxy-gamma-butyrolactone	20	83	19.978	0.53	Lactones	102	C ₄ H ₆ O ₃	
Phenols									
21	4-Vinylphenol	21	94	21.195	1.89		120	C ₈ H ₈ O	
22	Thiophene, 2,3-dihydro-	22	82	21.575	0.90	organic heterocyclic compound	86	C ₄ H ₆ S	

23	5-Hydroxymethylfurfural	23	94	22.348	1.56	Aldehydes	126	C ₆ H ₆ O ₃	
24	8-Azabicyclo[3.2.1]octane-3-carbonitrile, 8-methyl-, endo-	24	85	23.564	1.07	Alkaloids	150	C ₉ H ₁₄ N ₂	
25	9,12-Octadecadienoic acid (Z,Z)-	25	75	23.712	10.69	Fatty Acids	280	C ₁₈ H ₃₂ O ₂	
26	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	26	66	24.010	0.29	Glycerides	356	C ₂₁ H ₄₀ O ₄	
27	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-, (all-E)-(.+/-)-	27	90	24.809	0.50	Triterpenoid	426	C ₃₀ H ₅₀ O	

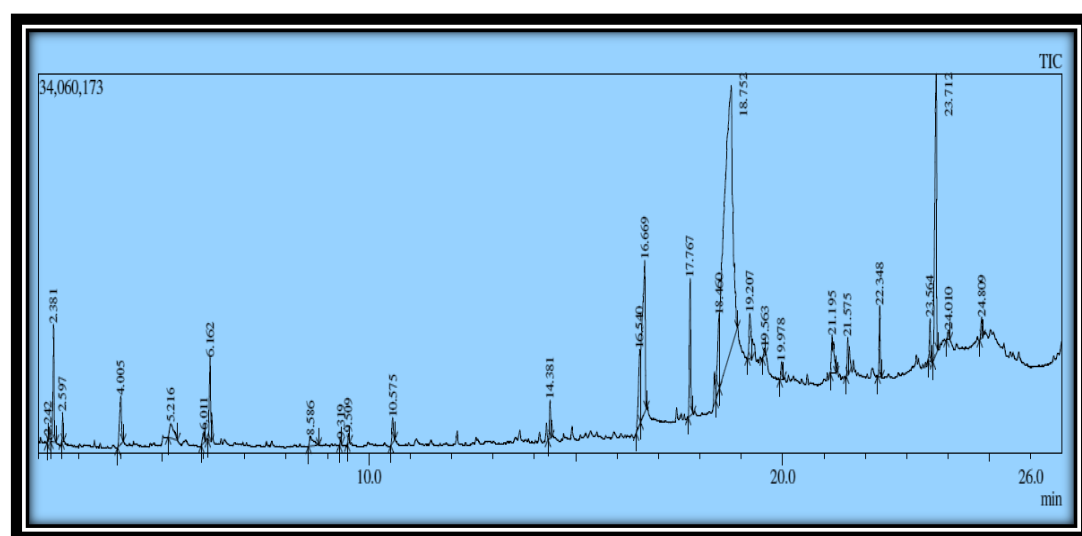


Figure 2. Curve of chemical compounds of the ethanolic acetone extract (99.99%) of *Z.jujuba* leaves using the GC-Mass technique.

4. Conclusion

The present study has proven its assisting in the classification of species studied using chemotaxonomic techniques, especially the GC-MS technique, which represent a direct and fast analytical approach for the identification of phytoconstituents.

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