

CHEMICAL STUDY OF THE PLANT ZIZIPHUS RUGOSA BELONGING TO THE RHAMNACEAE FAMILY USING GASS CHROMATOGRAPHY MASS

1. Salwa Hamza Hussein
2. Buthaina Abd E.Naser
3. UmAlbanin Doaim Faisal

Received 20th Nov 2023,
Accepted 21st Dec 2023,
Online 09th Jan 2023

¹ Assist. Prof, Department of Biology, College of Education for Girls, Kufa University, Iraq.

² Assist. Lecturer, Department of Biology, College of Education for Girls, Kufa University, Iraq.

³ Assist. Lecturer, Department of Biology, Jabir ibn Hayyan Medical University Faculty of Pharmacy, Iraq.

Abstract: The chemical study dealt with the chemical content of the studied leaves of the Sidr plant *Ziziphus rugosa*. The chemical compounds were identified using Gas Chromatography Mass Spectrometry (GC/MS) technology by comparing the chemical content. It was revealed that the studied sample contained the compound 3-Butyn-1-ol. The appearance of a repeat of the chemical compound was also recorded. 3-Butyn-1-ol six times in the leaves of the plant under study and with different curved areas and retention times for the same species using the GC-Mass device. Nine chemical compounds were detected accumulated in the leaves of the Sidr plant *Ziziphus rugosa*, with the highest area value being 18.79% at The peak is 15 with a holding time of 31.388 minutes for the active compound Squalene, while the lowest area is 1.55 at peak 8 with a holding time of 21.210 minutes for the compound Vaccenoylglycerol cis-1 (Figure 4-1), and the highest percentage appeared for the active compound 3-Butyn-1-ol with a value of 30.32% after collecting six different peaks (1,2,3,5,9,14)

Key words: *Ziziphus rugosa*, Gas Chromatography Mass Spectrometry (GC/MS).

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 Sidr 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 one of the multi-use medicinal plants, as all its parts are widely used in traditional and medical treatments. Its fruits are used as an antidote to poisoning, an analgesic for abdominal pain during pregnancy, and are anti-hypertension and stomach ulcers, and as compresses for wounds (Anonymous, 1989). Sidr leaves also work to strengthen hair and prevent Its shedding removes the bark and is useful for treating diseases of asthma, lungs, rheumatism, skin, and diarrhea (Nisar *et al.*, 2011). As for the sawdust and bark, it treats bleeding, intestinal ulcers, spleen diseases, dropsy, dysentery, severe diarrhea, and skin blisters. Sidr charcoal treats snake bites (Nadkarni, 1986)

Sidr leaves contain many biologically important compounds, including caclaurine alkaloids. isoboldine ; norisoboldine ; asimilobine ; iusiphine which has shown sedative effects (Ziyaev *et al.*, 1977) and the saponines jujubogenine; Ziziphin, which reduces cholesterol and is anti-cancer (Kurihara *et al.*, 1988), flavonoids and triterpenic acids such as rutinoid; coeanthic acid; maslinic acid; zizyloeranalic acid; epiceqnothic acid ; betulinic acid (Guo *et al.*, 2011).

In addition to the peptide alkaloids Sanjoinine – B, D, F, G2 and cyclic peptide alkaloids such as Melonovine – A; Franganine; Franguloine; Amphiloine – D ; Frangufoline and Daechuine - S3 and various soaps such as Jujubosides - A, B; Protojujubosides A, B, B1; acetylJajubosides A, B1, C (Matsuda *et al.*, 1999) Studies have shown that Sidr seeds possess sedative and anti-diabetic effects (Li *et al.*, 2011)

and has a role in wound healing (MacFaddin, 2000). It increases the permeability of cell membranes to some peptide drugs and Sidr roots have multiple biological activities, including sedatives, anti-cancer, anti-virus, anti-diabetes, bacteria and fungi (Elmahi *et al.*, 1997), and cytotoxicity and depression in mice. (Han *et al.*, 2011).

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 Sidr plant. The study aimed to investigate the facts of the following aspects:-

1-Study the chemical content of the leaves of the Sidr plant, *Ziziphus rugosa*, classify them, and know their concentrations and presence or absence among this type of Sidr plant.

Materials and working methods

Work methods

Preparing the powder for the leaves of the Sidr plant *Ziziphus rugosa*

Sidr 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

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: -

First: Prepare the ethanolic and acetone leaf extract according to the method of Markham (1982) with some modifications:

1-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.

2-

3-Add 5.0 ml of acetone at a concentration of 99.99% to discard the water to increase the concentration of the extract.

4-The chemical compounds in the extract were estimated after withdrawing the floating portion separated from the water with acetone.

5-After that, the separated part is ready for examination with the Gas Chromatography Mass Spectrometry (GC/Mass) device .

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:

1- Injector temperature is 280°C.

The temperature of the ion source is 200 degrees Celsius.2-

3-Helium gas 99.999% was used as a carrier gas at a continuous flow rate of one ml/min.

4-The injected liquid was 8 microliters in volume and worked with a division ratio of 0.5.

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.

6- Mass spectra were taken at 70 EV with an interval of 0.5 seconds .

7- The total time is 32 minutes from the start of the device until it turns off .

8- Elite-1 fused silica capillary column, which consists of 100% dimethyl polysiloxane, which operates in 70 EV (electron sniping detector) mode .

10- The pressure inside the device is 100.0 Kpa at a rate of one ml. minute .

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.

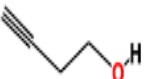
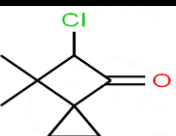
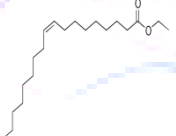
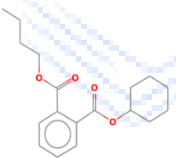
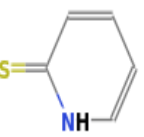
The resulting spectrum of the unknown component was then compared with a set of known components stored in the NIST library to confirm the molecular weight, name, and structure of the components of the test materials.

Results and discussion

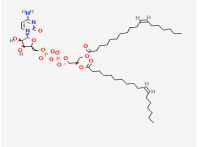
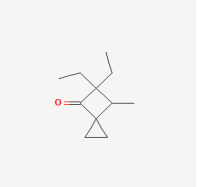
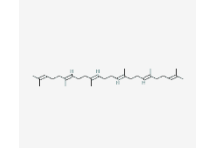
Chemical Study

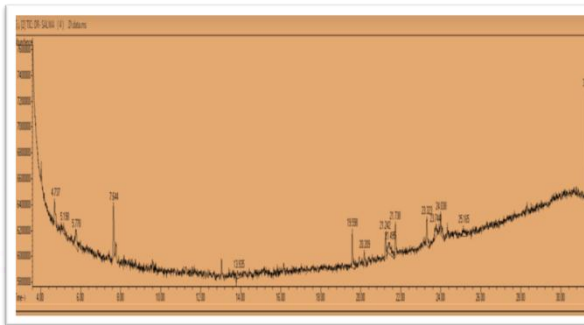
The results of the analysis of the GC-Mass technique showed the presence of prominent variations in the distribution of chemical compounds among the leaves of the Sidr plant under study, as 9 chemical compounds were detected collected in the leaves of the Sidr plant *Ziziphius rugosa*, where the highest area value was 18.79% at the apex15 with a retention time of 13.388 minutes for the compound. The effective Squalene, while the lowest area was 3.40 at peak 5 with a holding time of 13.939 minutes for the compound 3-Butyn-1-ol, Figure (4-1), and the highest percentage of the active compound 3-Butyn-1-ol, with a value of 30.32%, appeared after collecting six different peaks. It is (1,2,3,5,9,14) (4-1).

Table: (1) GC-Mass analysis of chemical compounds extracted from *Ziziphius rugosa* leaves.

Chemical Structure	Molecular weight	Molecular Formula	R.time	Area%	Peak	Chemical Names	NO.
	70.0898	C ₄ H ₆ O	4.738	4.86	1	3-Butyn-1-ol	1-
			5.197	3.79	2		
			5.777	7.14	3		
			13.939	3.40	5		
			21.495	7.43	9		
			25.164	3.70	14		
				30.32			
	152.23	C ₁₀ H ₁₆ O	7.646	10.97	4	Spiro[2.3]hexan-4-one, 5-chloro-6-methyl-	2-
	158.62	C ₈ H ₁₁ ClO	19.599	7.75	6	Spirohexan-4-one, 5-chloro-6,6-dimethyl-	-3
	310	C ₂₀ H ₃₈ O ₂	18.352	1.86	7	Ethyl Oleate	-4
	304.3808	C ₁₈ H ₂₄ O ₄	21.244	4.69	8	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	-5
	111.165	C ₅ H ₅ NS	21.737	6.49	10	2-Pyrrolidinethione	6-
	352	C ₂₃ H ₄₄ O ₂	21.189	1.64	11	Trans-9-Octadecenoic acid	-7

retention times and curve areas for the same type

	356	C21H40O4	21.210	1.55	12	-cis-1 Vaccenoylglycerol	-8
	166.26	C11H18O	24.039	6.35	13	Spiro[2.3]hexan-4- one, 5,5-dichloro-6- methyl-	--9
	410.7	C30H50	31.388	18.79	15	Squalene	-10



using the

(Figure 1)Scheme of chemical compounds in plant leaves *Ziziphius rugose*.

Tables (2): Chemical compounds with the highest and lowest retention times.				
Highest holding time	Compound name	Minimum holding time	Compound name	Species
31.388	Squalene	4.738	3-Butyn-1-ol	leaves <i>Ziziphius rugose</i> .

The occurrence of recurrence of some chemical compounds in the leaves of the Sidr plant under study was recorded at different

GC-Mass device. By extracting and counting the numbers of chemical compounds that frequently occur in the leaves of the Sidr plant under study, it was noted that the highest number of recurrent chemical compounds was Six chemical compounds in the leaves of the Sidr plant, the chemical compound 3-Butyn-1-ol .

In general, the study detected 7 chemical compounds collected in the sample under study. The lowest number of chemical compounds, 7 compounds, was identified in the alcoholic extract of the leaves. The lowest retention time was also recorded at 4,738 minutes for the compound 3-Butyn-1-ol, while the highest retention time was 31,388 minutes for the compound. Squalene in the leaves.

Chemotaxonomy has effectively contributed to solving taxonomic problems at the level of taxonomic ranks (Singh, 2008), and there have been many studies and chemical methods 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 widespread secondary metabolic substances. In nature, according to the study of (Castellano *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 characteristics to help in isolating, diagnosing and comparing their chemical composition. By comparing the chemical content it was noted that the studied sample was distinguished by containing many different compounds, which was given importance Classification that is fine for isolation.

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.

References

1. **Anonymous, K.(1989).**The Wealth of India (Raw material), Vol XI: X-Z, (Council of Industrial and Scientific Research, New Delhi,) 111-124.
2. **Castellano, G.; Tena, J. and Torrens, F.(2012).** Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia Oceanica* (L.) Delile. Match . Communications in Mathematical and in Computer Chemistry. 67:231-250pp.
3. **Elmahi, M. Essassi, E.M. Hamamouchi, M. and Hamamouchi, J. (1997).** Study on the antimicrobial and antibilharzia activity of *Ziziphus vulgaris*. Fitoterapia. ,68: 34-36.
4. **Guo,S.; Duan,J.; Tang,Y.; Qian,D.; Zhu,Z.; Qian,Y.; Yang ,N.; Shang,E-X.; Su,S and Key,J.(2011). Characterization of nucleosides and nucleobases in fruits of Ziziphus jujuba by UPLC-DAD-MS. J .Agric. Food. Chem., 13;58 (19):10774-10780.**
5. **Han,J. Ji,C-J. He,W-J. Shen,Y. Leng,Y. Xu,W-Y. Fan,J-T. Zeng,G-Z. Ling-Dong Kong,L-D. and Ning-Hua Tan,N-H.(2011). Cyclopeptide Alkaloids from Ziziphus apetala.J.Nat.Prod.,74(12):2571-2575.**
6. **Ingole ,S.N. (2017) .**Morphotaxonomic study and phytochemical analysis by GCMS method of underestimated medicinal grass: *Cynodon dactylon* (L.) Pers. (Poaceae). International Journal Applied Research . 3(3)3-20pp.

7. **Kurihara, Y. Oohubo, K. Tasaki, H. Kodama, H. Akiyama, Y. Yagi, A. and Halperm, B. (1988).** Studies on taste modifiers. I. Purification and structure in leaves of *Ziziphus jujuba*. *Tetrahedron*, 44 (1): 61-66.
8. **Li,J.; Shan,L.; Liu,Y.; Fan,L. and Ai,L.(2011).** Screening of a functional polysaccharide from *Zizyphus Jujuba* cv. Jinsixiaozao and its property. *Int J Biol Macromol.*, 1;49 (3):255-259.
9. **MacFaddin, J.F. (2000).**Biochemical test for of Identification medical bacteria,3 rd ed. Lippincott Williams and Wilkins, USA : PP. 912.
10. **Markham, K. R. (1982).** Techniques of Flavonoid Identification ,Acad.press London. 400pp.Metchalfe,C.R.and Chalk,L.(1950) Anatomy of Dicotyledons.Vol.2 Clarendon press.Oxford.1500pp.
11. **Nadkarni, K.M. (1986).** Indian Materia Medica. (Popular Prakashan, Bombay): 1315-1319.
12. **Nisar,M. Kaleem,W.A. Khan,I. Adhikari, A. Khan, N. Muhammad Shah,R.; Khan,I.A. Qayum,M. Samiullah,L. Muhammad,I. and Aman,A.(2011). Molecular simulations probing Kushecarpin A as a new lipooxygenase inhibitor. *Fitoterapia*, 82 (7):1008-1011 .**
13. **Ziyaev, R. Irgashev, T. Israilov, I.A. Abdullaev, N.D. Yunusov, M.S. and Yunusov, S.Y.(1977).** Alkaloids of *Ziziphus jujuba*. Structure of iusiphine and iusirine. *Khimiya Prirodnikh Soedinenii*. 2: 239-243.