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RESEARCH ARTICLE**GC–MS BASED CHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF
ZIZIPHUS JUJUBA SEED OIL****Mohamed Yousif Mustafa**

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Abstract

The growing problem of antimicrobial resistance has highlighted the urgent need for novel therapeutic agents. Medicinal plants and their bioactive components, particularly seed oils, have gained attention due to their rich chemical composition and diverse pharmacological activities. In this study, seed oil of *Ziziphus jujuba* was extracted and subjected to chemical profiling using gas chromatography–mass spectrometry (GC–MS), followed by evaluation of its antimicrobial potential. GC–MS analysis identified 12 major components, with heptadecanoic acid methyl ester (51.77%), tetracosanoic acid methyl ester (8.70%), and hexadecanoic acid methyl ester (7.99%) as dominant constituents. The oil exhibited strong antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*, but showed limited effectiveness against *Pseudomonas aeruginosa*. These findings suggest that *Ziziphus jujuba* seed oil is a promising source of bioactive compounds with potential applications as natural antimicrobial agents.

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Introduction:-

The exploration of natural products has attracted significant scientific attention in recent years, particularly due to the growing global concern regarding antimicrobial resistance. Conventional antimicrobial agents are gradually losing their effectiveness as pathogens evolve mechanisms of resistance, creating an urgent need for novel therapeutic alternatives. In this context, medicinal plants and their bioactive compounds have emerged as promising candidates for the development of safe, affordable, and effective antimicrobial agents. Seed oils, in particular, are widely studied for their chemical richness and potential biological activities, owing to their content of fatty acids, esters, phenolics, and other secondary metabolites. *Ziziphus jujuba*, belonging to the Rhamnaceae family, is a medicinally important plant traditionally used for treating various ailments including digestive disorders, infections, and inflammation. Its seeds are valued not only for their nutritional content but also for their pharmacological properties, including antimicrobial and antioxidant effects. Despite these recognized benefits, comprehensive chemical profiling of *Z. jujuba* seed oil and its antimicrobial activity remains relatively underexplored. Gas chromatography–mass spectrometry (GC–MS) is a powerful analytical tool widely employed for the qualitative and quantitative determination of volatile and semi-volatile compounds in plant-derived oils. This technique offers high

sensitivity and resolution, allowing the identification of individual chemical constituents and providing insight into their possible biological functions.

Materials and Methods:-

Plant material:

The seeds of *Ziziphus jujuba* were collected from around Wadmadni, Gezira state, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Test organisms:

The following standard bacterial pathogens were used to assess the antimicrobial potency of *Ziziphus jujuba* oil: *Bacillus subtilis* (Gram+ve), *Staphylococcus aureus* (Gram+ve), *Pseudomonas aeruginosa* (Gram -ve), *Escherichia coli* (Gram-ve) and the fungal species *Candida albicans*.

Methods:-

Extraction of oil from *Ziziphus jujuba* seeds Dry-powdered seeds of *Ziziphus jujuba* (500g) were extracted with n-hexane at room temperature for 48hrs. The solvent was removed under reduced pressure leaving the oil. For GC-MS analysis, a methanolic solution of sodium hydroxide and a methanolic sulphuric acid were used to esterify the oil.

GC-MS analysis:

Ziziphus jujuba oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo Ultra instrument was used with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μ m, thickness.) Analytical grade helium (purity; 99.99 %) was a carrier gas. Oven temperature program and other chromatographic conditions are displayed below.

Table 1: Oven temperature program.

Rate	Temperature(C)	Hold time (min. ⁻¹)
-	60.0	0.00
10.0	300.0	0.00

Table 2: Chromatographic conditions.

Column Oven Temperature	60.0
Injection Temperature	310.00
Injection Mode	Split
Flow Control Mode	Linear Velocity
Pressure	100.2 KPs
Total Flow	50.0 mL/min
Column Flow	1.61 mL/min
Linear Velocity	46.3 cm/sec
Purge Flow	3.0 mL/min
Spilt Ratio	-1.0
High Pressure Injection	OFF
Carrier Gas Saver	OFF
Splitter Hold	OFF

Antimicrobial assay:

Preparation of bacterial suspensions:

Diffusion method was used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were used as media for the growth of bacteria and fungi respectively. The media were prepared according to the manufacturer's instructions. One ml aliquots of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and then incubated at 37°C for 24 hours. The harvested bacterial growth was washed off using sterile normal saline, then it was suspended in (100 ml) of normal saline to give about 10⁸-10⁹ colony forming units per ml. Using the surface viable counting technique, the average number of viable organism per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline. (0.02 ml) of the appropriate dilutions were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

Fungal cultures were accomplished on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Results and Discussion:-

GC-MS analysis of Ziziphus jujuba oil:-

Ziziphus jujuba oil was analyzed by GC-MS .MS library(NIST) was checked for identification of the constituents. Furthermore, the observed fragmentation pattern was interpreted (MS library revealed about 90-95% match). The GC-MS analysis showed the presence of 12 components(Table 3). The typical total ion chromatograms (TIC) is depicted in Fig.1.

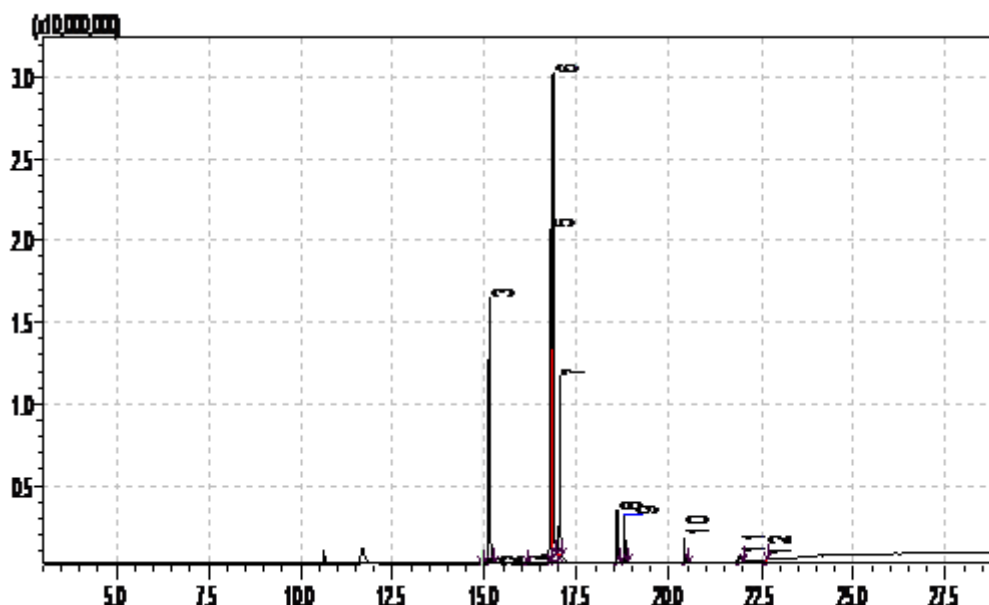


Fig. 1; Total ion chromatograms.

ID#	Name	Ret.Time	Area	Area%
	7-Hexadecenoic acid, methyl ester, (Z)-	3.135	522964	6.70
	9-Hexadecenoic acid, methyl ester, (Z)-	3.310	492173	6.31
	Hexadecanoic acid, methyl ester	3.624	623496	7.99
	Heptadecanoic acid, methyl ester	4.277	4038920	51.77
	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9.493	88632	1.14
	9-Octadecenoic acid (Z)-, methyl ester	9.548	306174	3.92
	Methyl stearate	9.680	456801	5.85
	cis-11-Eicosenoic acid, methyl ester	9.744	66016	0.85
	Eicosanoic acid, methyl ester	9.902	83786	1.07
	Docosanoic acid, methyl ester	9.987	91210	1.17
	Tetracosanoic acid, methyl ester	10.725	679251	8.70
	Squalene	10.812	150511	1.93

(1) Heptadecanoic acid, methyl ester(51.77%):

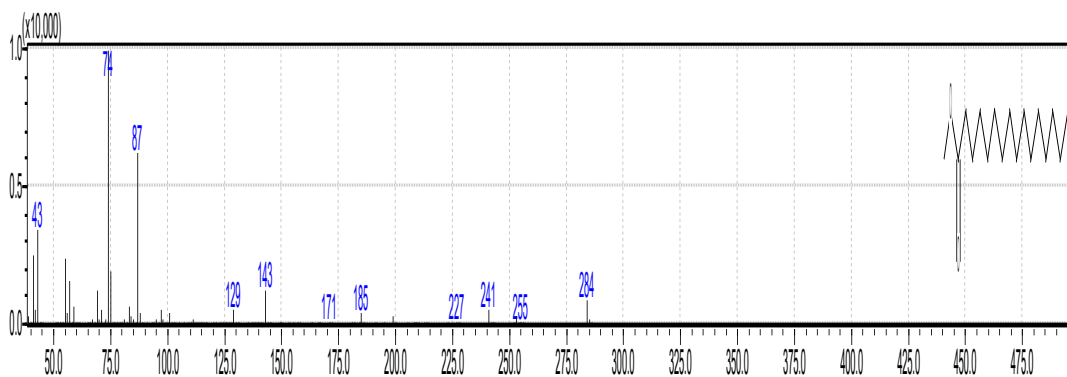


Fig. 2: Mass spectrum of Heptadecanoic acid, methyl ester

Fig. 2 shows the EI mass spectrum of Heptadecanoic acid, methyl ester. The peak at m/z 284, which appeared at R.T. 4.277 in total ion chromatogram, corresponds to $M^+[C_{18}H_{36}O_2]^+$. The peak at m/z 255 corresponds to loss of a methoxyl function.

(2) Tetracosanoic acid, methyl ester (8.70%)

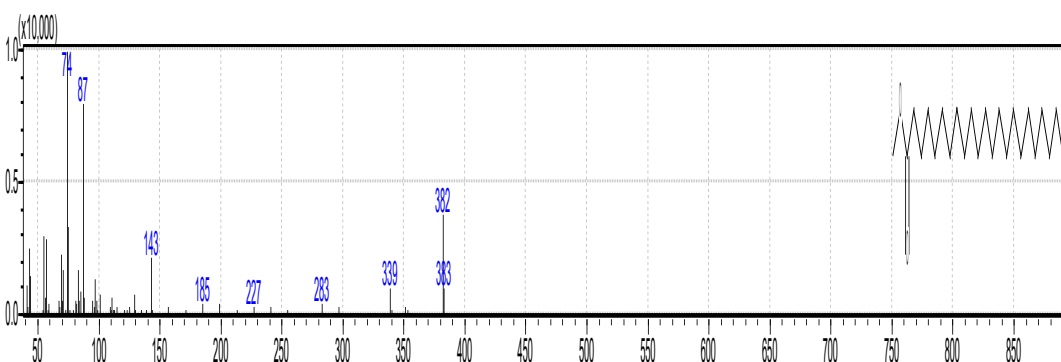


Fig. 3: Mass spectrum of Tetracosanoic acid, methyl ester

Fig. 3 shows the EI mass spectrum of **Tetracosanoic acid, methyl ester**. The peak at m/z 383, which appeared at R.T. 10.725 in total ion chromatogram, corresponds to $M^+[C_{25}H_{50}O_2]^+$, while the peak at m/z 339 accounts for loss of a methoxyl function.

(3) Hexadecanoic acid, methyl ester (7.99%)

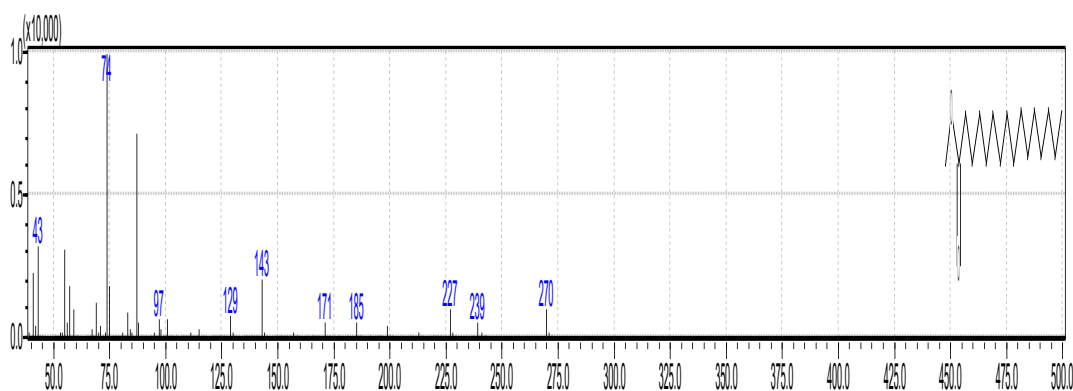


Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester

Mass spectrum of hexadecanoic acid, methyl ester depicted in Fig.4. The peak at m/z 270, which appeared at R.T. 3.624 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 is attributed to loss of a methyl function.

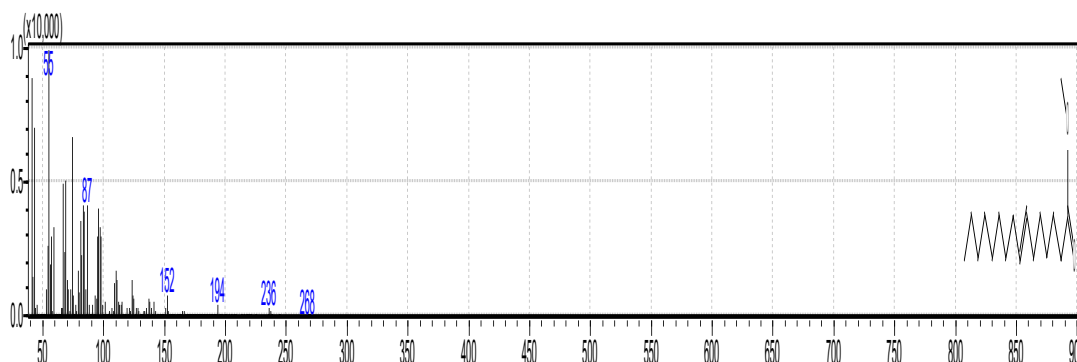
(4) 7-Hexadecenoic acid, methyl ester, (Z) (6.70%):**Fig. 5: Mass spectrum of 7- hexadecanoic acid, methyl ester**

Fig. 5 shows the EI mass spectrum of 7-Hexadecenoic acid, methyl ester. The peak at m/z 286, which appeared at R.T. 3.135 in total ion chromatogram, corresponds to $M^+[C_{17}H_{32}O_2]^+$, while the peak at m/z 236 accounts for loss of a methoxyl function.

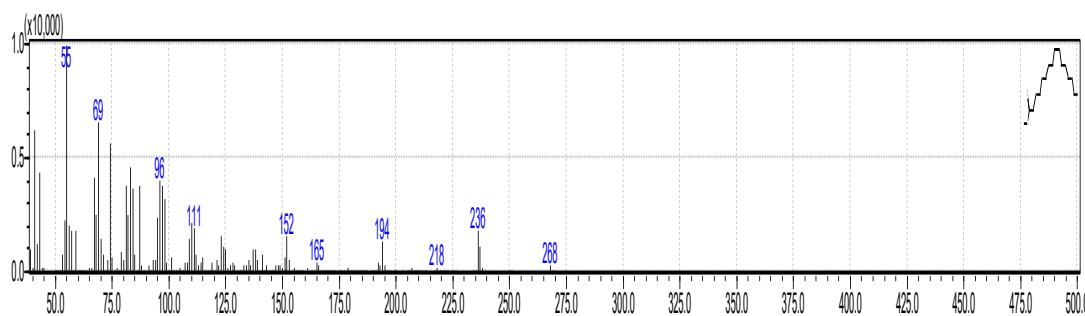
(5) 9-Hexadecenoic acid, methyl ester, (Z) (6.31%):**Fig. 6: Mass spectrum of 9-hexadecanoic acid, methyl ester**

Fig. 3 shows the EI mass spectrum of 9-hexadecanoic acid, methyl ester. The peak at m/z 268, which appeared at R.T. 3.310 in total ion chromatogram, corresponds to $M^+[C_{17}H_{32}O_2]^+$, while the peak at m/z 236 accounts for loss of a methoxyl function.

Antibacterial activity:

Ziziphus jujuba oil was screened for antimicrobial activity against five standard human pathogens. The results are depicted in Table (4). The results were interpreted as follows: (>9mm: inactive; 9-12mm: partially active; 13-18mm: active; <18mm: very active). Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4: Antibacterial activity of Ziziphus jujuba oil;

Type	Concentration (mg/ml)	Sa	Bs	Es	Ps	Ca
Oil	100	19	18	20	10	18
	50	16	15	18	8	15
	25	15	10	15	-	13
	12.5	10		12	-	9
	6.25	-	-	10	-	-

Table 4:-Antibacterial activity of standard chemotherapeutic agents

Drug	Concentration(mg/ml)	Bs	Sa	Ec	Ps
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table5:-Antifungal activity of standard chemotherapeutic agent:

Drug	Con.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: Staphylococcus aureus

Ec.: Escherichia coli

Pa.: Pseudomonas aeruginosa

An.: Aspergillus niger

Ca.: Candida albicans

Bs.: Bacillus subtilis

The oil showed excellent activity against the bacterial strain Staphylococcus aureus in the concentration range 25-100 mg/ml, as well as against Bacillus subtilis in the concentration range 50-100 mg/ml, and against Escherichia coli in the concentration range 50 – 100 mg/ml. The oil also showed excellent activity against the yeast Candida albicans at the concentration range 50- 100mg/ml., but did not show effective activity against Pseudomonas aeruginosa.

Conclusion:-

This study demonstrates that Ziziphus jujuba seed oil contains a rich profile of bioactive fatty acid esters, as revealed by GC–MS analysis. The oil exhibited promising antimicrobial activity against a range of bacterial and fungal pathogens, with notable effectiveness against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Candida albicans. These findings highlight the potential of Z. jujuba seed oil as a natural source of antimicrobial agents. Future work should focus on isolating the active constituents, evaluating their mechanisms of action, and testing their efficacy in vivo to further validate their therapeutic potential.