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RESEARCH ARTICLE

GC–MS BASED CHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF ZIZIPHUS JUJUBA SEEDS OIL

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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.

Introduction:-

The exploration of natural products has attracted significant scientific attention in recent years, particularly due to the growing global concern regarding antimicrobial resistance **[1,2]**. Conventional antimicrobial agents are gradually losing their effectiveness as pathogens evolve mechanisms of resistance, creating an urgent need for novel therapeutic alternatives **[3]**. In this context, medicinal plants and their bioactive compounds have emerged as promising candidates for the development of safe, affordable, and effective antimicrobial agents **[4]**. 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 **[5]**. **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 **[6]**. 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 **[7]**.

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 aeroginosa (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 µm, 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:

Antimicrobial screening of the oil was performed using the diffusion method. Bacterial inocula were prepared from 24-hour broth cultures, adjusted to 10^8–10^9 CFU/ml, serially diluted, plated on nutrient agar, and incubated at 37°C for 24 hours. Fungal cultures were grown on Sabouraud dextrose agar at 25°C for four days, washed, and stored in saline until testing.

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.

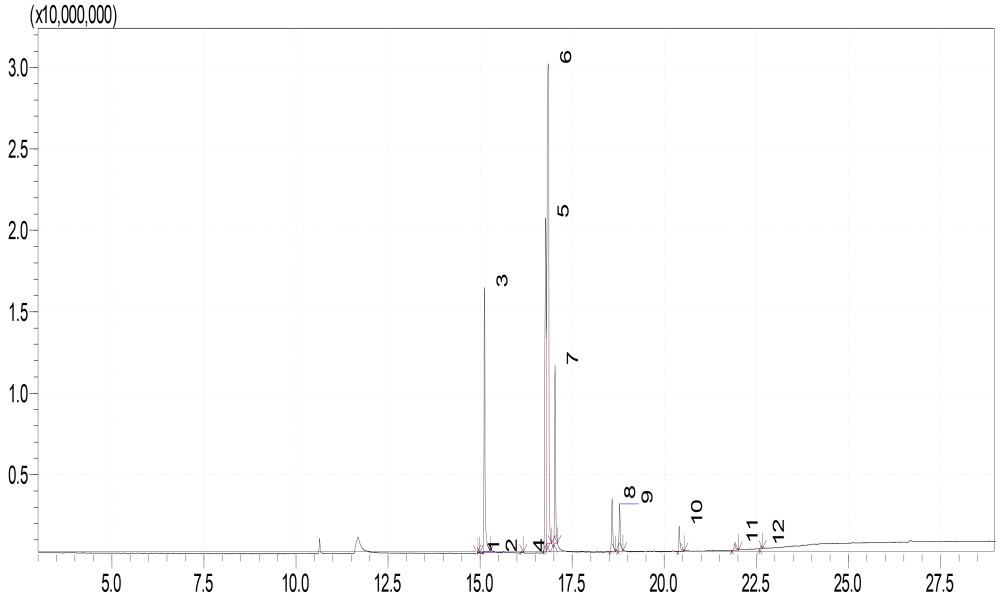

Fig. 1; Total ion chromatograms.

Table3: Constituent of Ziziphus Jujuba seeds oil

ID#	Name	Ret.Time	Area	Area%
1	7-Hexadecenoic acid, methyl ester, (Z)-	3.135	522964	6.7
2	9-Hexadecenoic acid, methyl ester, (Z)-	3.31	492173	6.31
3	Hexadecanoic acid, methyl ester	3.624	623496	7.99
4	Heptadecanoic acid, methyl ester	4.277	4038920	51.77
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9.493	88632	1.14
6	9-Octadecenoic acid (Z)-, methyl ester	9.548	306174	3.92
7	Methyl stearate	9.68	456801	5.85
8	cis-11-Eicosenoic acid, methyl ester	9.744	66016	0.85
9	Eicosanoic acid, methyl ester	9.902	83786	1.07
10	Docosanoic acid, methyl ester	9.987	91210	1.17
11	Tetracosanoic acid, methyl ester	10.725	679251	8.7
12	Squalene	10.812	150511	1.93

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

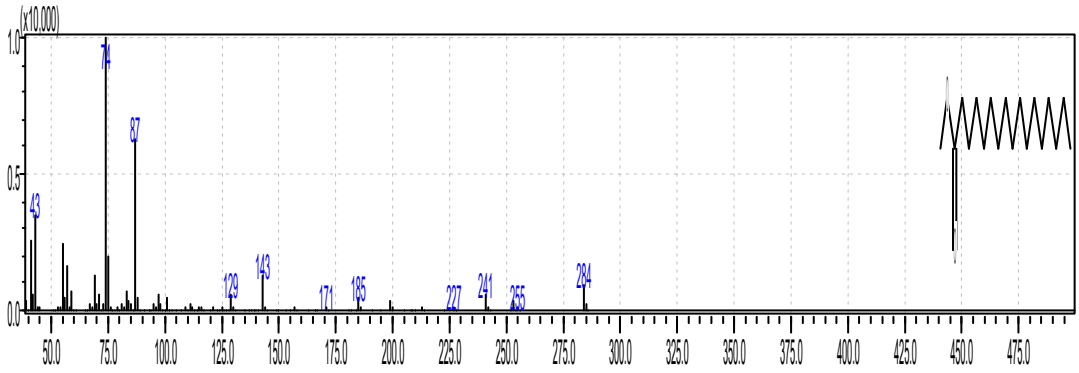

Fig. 2: Mass spectrum of Heptadecanoic acid, methyl ester

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 $M^{+}[C18H36O2]^{+}$.The peak at m/z 255 corresponds to loss of a methoxyl function.

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

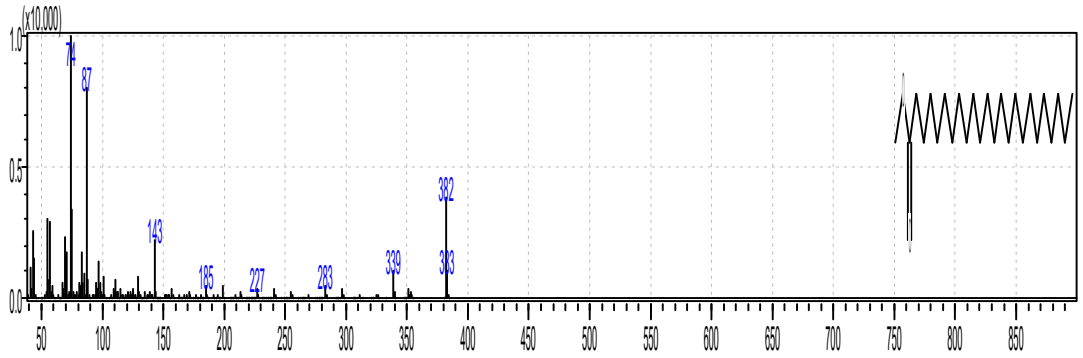

Fig. 3: Mass spectrum of Tetracosanoic acid, methyl ester

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/z339 accounts for loss of a methoxyl function.

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

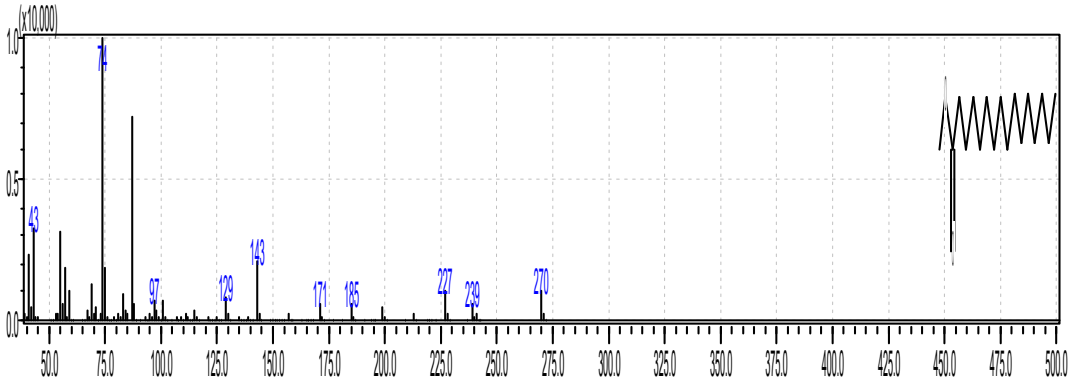

Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester

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 $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 is attributed to loss of a methyl function.

(4) **7-Hexadecenoic acid, methyl ester, (Z) (6.70%):**

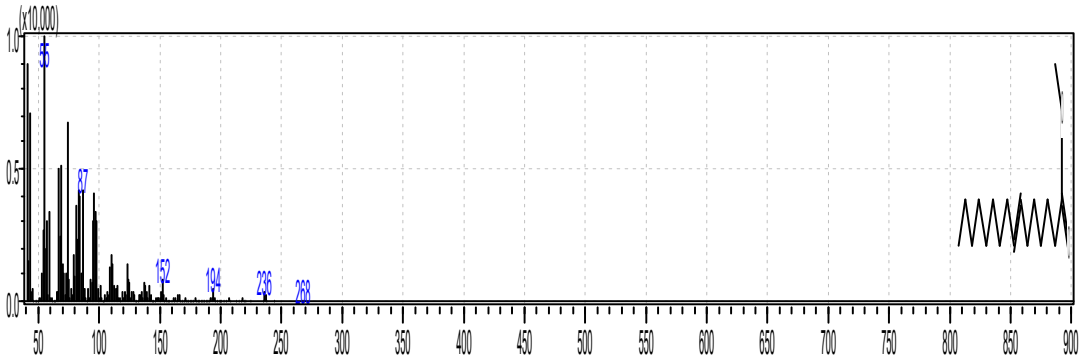

Fig. 5: Mass spectrum of 7- hexadecanoic acid, methyl ester

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/z236 accounts for loss of a methoxyl function.

(5) **9-Hexadecenoic acid, methyl ester, (Z) (6.31%):**

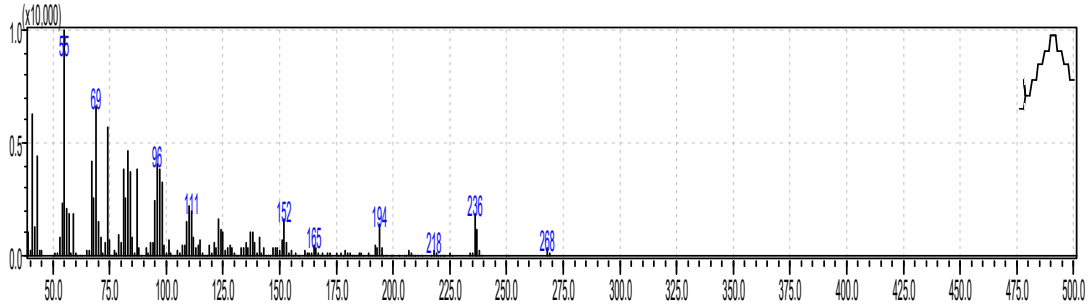

Fig. 6: Mass spectrum of 9-hexadecanoic acid, methyl ester

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/z236 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: inative;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 5:-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

Table 6:-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.

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