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CONSTITUENTS OF SUDANESE *ZIZIPHUS JUJUBE* MILL. OIL AND ANTIMICROBIAL ACTIVITY OF THE OIL

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ABSTRACT

Ziziphus jujube is used traditionally against rectal and intestinal ulcers. The plant is considered as a natural remedy for liver diseases. In this study *Ziziphus jujube* seed oil was analyzed by GC-MS. The analysis revealed the presence of 30 components. The fatty acids constituted 51.89% of the oil; alcohols constituted 47.20%, the rest are hydrocarbon, ketones, vitamin E and a phenol. The following major constituents have been detected by GC-MS analysis: i)-cis-13-eicosenoic acid methyl ester(28.40%) ; (ii)-9-octadecen-1-ol(Z)-(21.56%).; (iii)- 9-octadecen-1-ol(E)-(20.27%) and (iv)-13-docosenoic acid methyl ester(10.83%). In the well diffusion bioassay The oil showed significant activity against *Bacillus subtilis*, *Pseudomonas aeroginosa* and the yeast *Candida albicans.* It also exhibited weak activity against *Staphylococcus aureus*. However, the oil did not show any inhibitory effect against *Escherichia coli*.

KEYWORDS: *Ziziphus jujube*, Seed Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Jugube (*Ziziphus jujube* Mill) is a plant in the family Rhamnaceae. This family comprises 45 genera and 550 species distributed in tropical and sub-tropical regions.[1,2] This plant can tolerate conditions of extreme draught. The edible fruit of *Ziziphus jujube* is used medicinally.[3] The fruit is used in ethnomedicine to improve digestion. It is also used against rectal and intestinal ulcers. The plant is considered as a natural remedy for liver diseases,[4] The fruit contains, among others, proteins, minerals, vitamins, caretonoids, carbohydrates and organic acids.[5,6]

Bark is used by local healers against dysentery, cough, headache and diarrhea.[7-9] Leaves possess antipyretic properties and are used traditionally to reduce obesity. Seeds are used for eye diseases vomiting and leucorrhoea. Kernels are tonic and are used in Chinese system of medicine as sedative.[10]

Several alkaloids have been isolated from different parts of Ziziphus jujube.[7,11-16] Also some biologically active flavonoids and saponins have been reported from seeds.[17-20]

MATERIALS AND METHODS

Materials

Plant material

Seeds of *Ziziphus jujube* were collected from a forest reserve around Damazin, Sudan. The plant was authenticated by The Medicinal and Aromatic Plants Research Institute-Khartoum (Sudan).

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness).

Test organisms

The studied oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and *Candida albicans*.

Methods

Extraction of oil

Powdered plant material (300g) was exhaustively macerated with n-hexane. The solvent was removed under reduced pressure to afford the oil.

GC-MS analysis

The target oil was analyzed by the hyphenated technique gas chromatography-mass spectrometry. A Shimadzo

GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (99% pure) was used as carrier gas. Oven temperature program and other chromatographic conditions are presented below:

Table 1: Oven temperature program.

Rate	Hold Time (min.$^{-1}$)	Temperature(^{o}C)
-	150.0	1.00
4.00	300.0	0.00

Table 2: Chromatographic conditions.

Column oven temperature 150.0^{o}C
Injection temperature 300.0^{o}C
Injection mode Split
Flow control mode Linear velocity
Pressure 139.3KPa
Total flow 50.0ml/ min
Column flow 1.54ml/sec.
Linear velocity 47.2cm/sec.
Purge flow 3.0ml/min.
Spilt ratio - 1.0

Antimicrobial assay

Antimicrobial activity was performed by the well diffusion method.[21,22] Four strains of bacteria(*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa* and *Escherichia coli*) and one yeast(*Candida albicans*) were used in the antimicrobial assay. The inoculum size of each test strain was standardized according to the National Committee for Clinical Laboratory Standards,[23] The bacterial and yeast strains were inoculated into Mueller Hinton broth - MHb agar plates. A volume of (20 µL) of the test sample was applied into 6.0 mm diameter wells. After holding the plates at room temperature for 2 hours to allow diffusion of test-drug into the agar, they were incubated at 37 °C for 24 hours. Tests were performed in duplicates. After incubation the diameters the inhibition zones were measured in millimeters (mm) and averaged as indicator of activity.

RESULTS AND DISCUSSION

Ziziphus jujube seed oil was analyzed by GC-MS. Figure 1 presents the total ions chromatograms, while Table 3 displays the different constituents of the oil.

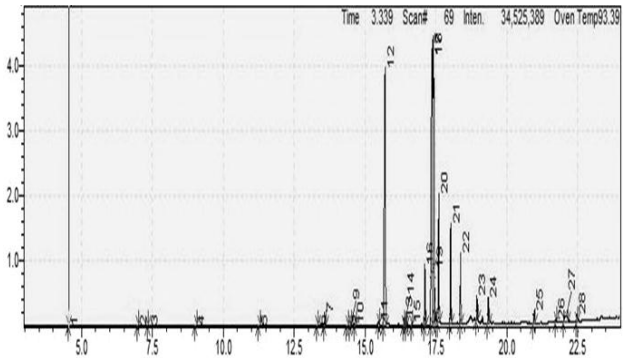

Fig. 1: Total ions chromatograms.

Table 3: Consituents of the oil.

Peak#	R.Time	Area	Area%	Name
1	6.987	91615	0.02	L-.alpha.-Terpineol
2	7.334	28678	0.01	Nonanoic acid, methyl ester
3	8.700	33158	0.01	Decanoic acid, methyl ester
4	13.299	34668	0.01	cis-5-Dodecenoic acid, methyl ester
5	13.575	111307	0.02	Methyl tetradecanoate
6	14.648	55456	0.01	Pentadecanoic acid, methyl ester
7	15.230	454688	0.10	1-Hexadecanol
8	15.436	883223	0.19	9-Hexadecenoic acid, methyl ester, (Z)-
9	15.480	134933	0.03	7-Hexadecenoic acid, methyl ester, (Z)-
10	15.672	4825556	1.05	Hexadecanoic acid, methyl ester
11	16.443	195593	0.04	cis-10-Heptadecenoic acid, methyl ester
12	16.998	1066254	0.23	9-Hexadecen-1-ol, (Z)-
13	17.037	1888220	0.41	1,15-Hexadecadiene
14	17.324	1352011	0.29	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
15	17.375	30261123	6.57	9-Octadecenoic acid (Z)-, methyl ester
16	17.417	2202096	0.48	9-Octadecenoic acid, methyl ester, (E)-
17	17.585	315664	0.07	Methyl stearate
18	18.848	93354074	20.27	9-Octadecen-1-ol, (E)-
19	19.186	130847730	28.40	cis-13-Eicosenoic acid, methyl ester
20	19.346	419885	0.09	Eicosanoic acid, methyl ester
21	19.673	323392	0.07	2-Dodecylcyclohexanone
22	20.264	234320	0.05	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl ester
23	20.533	99332190	21.56	9-Octadecen-1-ol, (Z)-
24	20.684	6484857	1.41	Tetracosyl pentafluoropropionate
25	20.800	49865839	10.83	13-Docosenoic acid, methyl ester, (Z)-
26	20.838	2116772	0.46	17-Octadecynoic acid, methyl ester
27	20.964	861436	0.19	Docosanoic acid, methyl ester
28	21.765	1680959	0.36	Vitamin E
29	22.071	23218499	5.04	13-Docosen-1-ol, (Z)-
30	22.312	7977337	1.73	15-Tetracosenoic acid, methyl ester, (Z)-
		460651533	100.00	

The fatty acids constituted 51.89% of the oil; alcohols constituted 47.20%, the rest are: hydrocarbon, ketone, vitamin E and a phenol. The following major constituents have been detected by GC-MS analysis:
(i)-cis-13-Eicosenoic acid methyl ester(28.40%)
(ii)-9-Octadecen-1-ol(Z)-(21.56%).
(iii)- 9-Octadecen-1-ol(E)-(20.27%).
(iv)-13-Docosenoic acid methyl ester (10.83%).

In Fig. 2 (the mass spectrum of cis-13-eicosenoic acid methyl ester), the molecular ion $[C_{21}H_{40}O_2]^+$ corresponds m/z 324(RT 19.186). The signal at m/z293 is attributed to loss of a methoxyl. The mass spectrum of 9-Octadecen-1-ol(Z)- is shown in Fig.3. The molecular ion $M^+[C_{18}H_{36}O]^+$ corresponds m/z 268 (RT. 20.533), while the signal at m/z250 accounts for loss of a hydroxyl. Fig.4 displays the mass spectrum of 9-octadecen-1-ol(E)- the peak at m/z 268(R.T. 18.848) is due to the molecular ion: $M^+[C_{18}H_{36}O]^+$, while the signal at m/z 250 accounts for loss of a hydroxyl function. The mass spectrum of 13-docosenoic acid methyl ester is presented in Fig.5. The peak at m/z 352, which appeared at R.T. 20.800 in total ion chromatogram, corresponds M^+ $[C_{23}H_{44}O_2]^+$, while the signal at m/z 321 corresponds to loss of a methoxyl function.

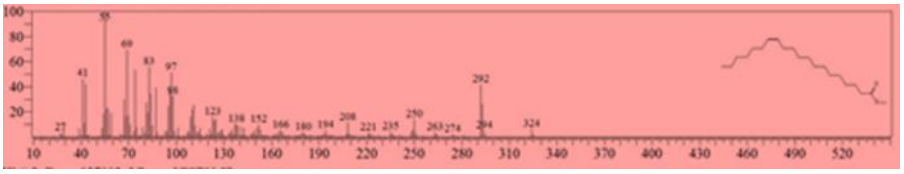

Fig. 2: Mass spectrum of cis-13-eicosenoic acid methyl ester.

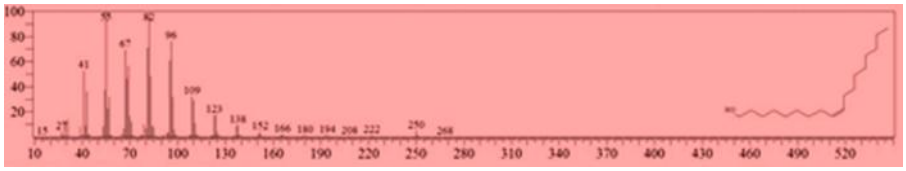

Fig. 3: Mass spectrum of 9-octadecen-1-ol (Z).

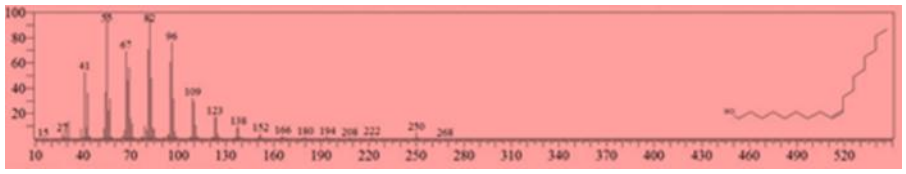

Fig. 4: Mass spectrum of 9-octadecen-1-ol €.

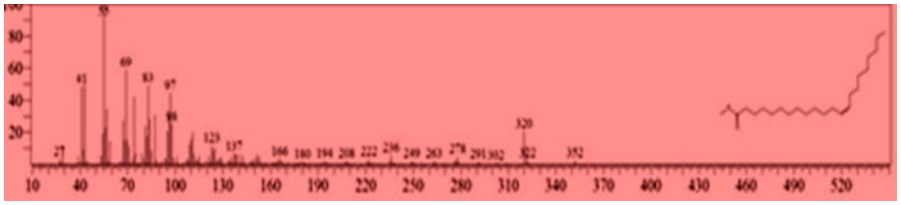

Fig. 5: Mass spectrum of 13-docosenoic acid methyl ester.

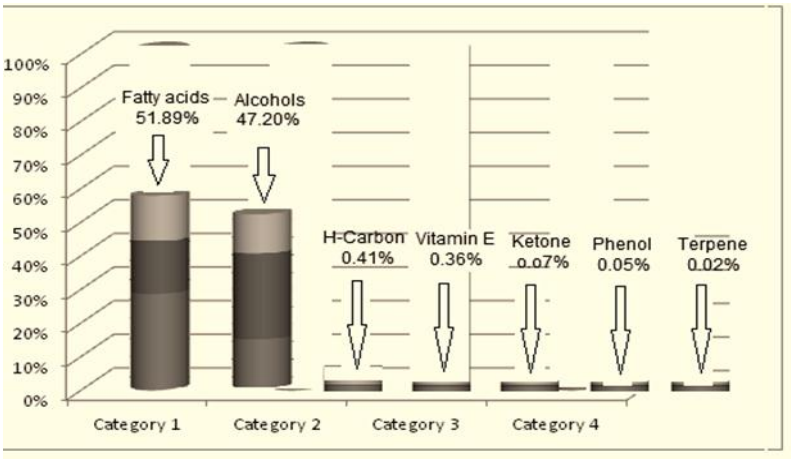

Fig. 6: Constituents of *Ziziphus jujube* oil.

Antimicrobial activity

Z. jujube oil was screened for antimicrobial activity against five standard human pathogens. The inhibition zones are displayed in Table 4. The oil showed significant activity against *Bacillus subtilis*, *Pseudomonas aeroginosa* and the yeast *Candida albicans.* It also exhibited weak activity against *Staphylococcus aureus*. However, the oil did not show any inhibitory effect against *Escherichia coli*.

Table 4: Inhibtion zones of *Z. jujube* oil.

Sample	Sa	Bs	Ec	Pa.	Ca
Oil 100mg/ml	13	18	-	17	17

Sa.: *Staphylococcus aureus*.
Bs.: *Bacillus subtilis*.
Ec.: *Escherichia coli*.
Pa.: *Pseudomonas aeroginosa*.
Ca.: *Candida albicans*.

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