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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *GREWIA TENAX* FORSSK (TILIACEAE) FIXED OIL

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ABSTRACT
Grewia tenax is a small tree which is prevalent in Africa and south-east Asia. *Grewia tenax* has a wide distribution in north and middle Sudan. This plant is a key species in Sudanese ethnomedicine. In this work *Grewia tenax* seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. The GC-MS analysis showed 12 components dominated by: 9,12-octadecadienoic acid methyl ester (51.69%); hexadecanoic acid methyl ester(18.83%); 9-octadecenoic acid methyl ester(16.41%) and methyl stearate(9.33%). The antimicrobial activity of the oil was evaluated via the diffusion bioassay against five standard pathogenic bacteria (Gram positive: *Staphylococcus aureus* and *Bacillus subtitlis; Gram* negative : *Esherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). The oil showed significant antibacterial activity against *Staphylococcus aureus.*It also showed significant anticandidal activity. However, it exhibited partial activity against *Pseudomonas aeruginosa* and *Escherichia coli.*

KEYWORDS: *Grewia tenax,* Fixed Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Grewia tenax(**Forssk**.) is a deciduous shrub in the family Tiliaceae. This small tree is prevalent in African and southeast Asian countries. *Grewia tenax* has a wide distribution in north and middle sudan.[1] The roots have been used traditionally against asthma, infections and jaundice.[2] The fruits are widely used in Sudan as an iron supplement for anemic children.[2] *Grewia tenax* fruits have been used traditionally as galactagogue[3] while fruit pulp is used to treat swelling in the body.[5] Fruits of *Grewia tenax* which are claimed to increase blood hemoglobin are often used in Sudan as special diet for pregnant women[6-8] Leaves and twigs are used in ethnomedicine against trachoma, tonsillitis and infections[5] beside cough, fever, diarrhea, dysentery, jaundice and rheumatism.[6] The fruits, which can be eaten ripe or stored for later use, is a rich source of carbohydrates, protein, vitamins and minerals.[4,9]

MATERIALS AND METHODS

Plant material

Fruits of *Grewia tenax* were purchased from the local market, Khartoum, Sudan. The plant was identified and authenticated by direct comparison with reference herbarium sample.

Instruments

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

Test organisms

Grewia tenax oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus*(G+ve), *Pseudomonas aeroginosa*(G-ve), *Escherichia coli*(G-ve) and the fungal species *Candida albicans.*

Methods

Extraction of oil

Powdered fruits of *Grewia tenax* (300g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure to give the oil.

GC-MS analysis

The fixed oil from *Grewia tenax* fruits was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. chromatographic conditions are displayed below.

Table 1: Oven temperature program.

Rate Temperature(^{o}C) Hold Time (min.$^{-1}$)		
-	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

The cup-plate agar diffusion bioassay was adopted, with some minor modifications, to assess the antimicrobial activity of the studied oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (6 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a test solution. The agar discs were removed, alternate cups were filled with 0.1 ml samples of test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours(for bacteria) and for 4 days at 30^{o} for the fungus. After incubation, the diameters of the resultant growth inhibition zones were measured.

RESULTS AND DISCUSSION

GC-MS analysis of *Grewia tenax* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 12 components(Table3). The typical total ion chromatograms(TIC) is depicted in Fig.1.

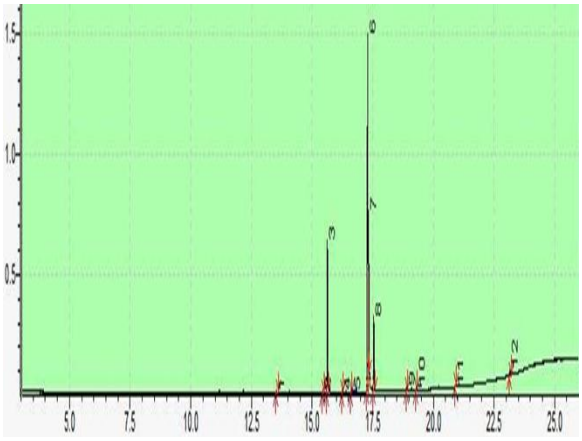

Fig. 1: Cromatograms of *Grewia tenax* oil.

Table 3: Constituents of *Grewia tenax* oil.

No.	RT	Area %	Name
1	13.531	0.29	Methyl tetradecanoate
2	15.437	0.25	9-Hexadecenoic acid methyl ester
3	15.630	18.83	Hexadecanoic acid methyl ester
4	16.251	0.11	15-Methyl- Hexadecanoic acid methyl ester
5	16.606	0.18	Heptadecanoic acid methyl ester
6	17.288	51.69	9,12-Octadecenoic acid methyl ester
7	17.328	16.41	9-Octadecenoic acid methyl ester
8	17.543	9.33	Methyl stearate
9	18.906	0.89	2-(2-ethylcyclohexyl)-cyclopropaneoctanoic acid
10	19.301	0.73	Eicosenoic acid methyl ester
11	20.921	0.29	Docosanoic acid methyl ester
12	23.160	1.00	Squalene
		100.00	

Major constituents are briefly discussed below:

9,12-Octadecadienoic acid methyl ester (51.69%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2. The peak at m/z294 (R.T. 17.288) coincides with $M^{+}[C_{19}H_{34}O_2]^{+}$, while the peak at m/z263 is due to loss of a methoxyl.

9,12-Octadecadienoic exists in lipids and cell membrane. It belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human body and are available through diet.[10]

Hexadecanoic acid methyl ester(18.83%)

Figure 3 shows the mass spectrum of hexadecanoic acid methyl ester. The molecular ion: $M^{+}[C_{17}H_{34}O_2]^{+}$

appeared at m/z 270 at R.T. 15.630 in total ion chromatogram. The fragment at m/z239 is due to loss of a methoxyl function.

hexadecanoic acid(palmitic acid) is a saturated fatty acid and it is considered as the most common fatty acid in animals and humans. Palmitic acid is the precursor of long-chain fatty acids.[11] This acid is a major lipid component of human breast milk.[12,13] Palmitic acid, beside being used in soap industry, is widely used in food industry.

9-Octadecenoic acid methyl ester(16.41%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 4. The peak at m/z 296 (R.T. 17.328) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z266 is attributed to loss of a methoxyl.

9-Octadecenoic acid(oleic acid) is included in animal fats and vegatables, hence it is included in the normal human diet. Oleic acid is used as emollient.[14] It is used in small amount as excipient in pharmaceutical industries. 9-Octadecenoic acid could be responsible for the hypotensive effect of olive oil.[15] It has been claimed that the presence of oleate in olive oil is associated with decreased risk of breast cancer.[16]

Methyl stearate(9.33%)

Figure 5 displays the EI mass spectrum of methyl stearate. The molecular ion : $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T. 17.543). The peak at m/z267 corresponds to loss of a methoxyl function.

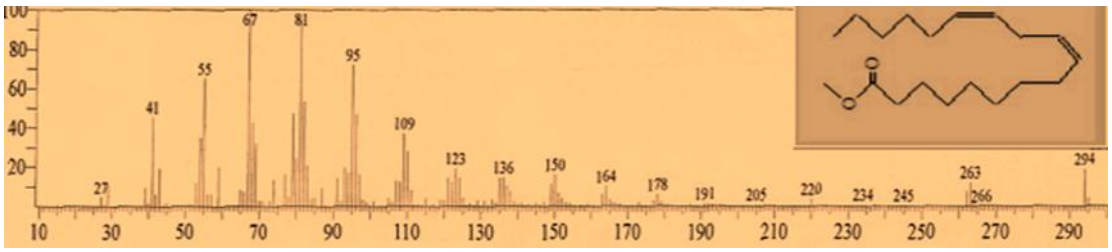

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

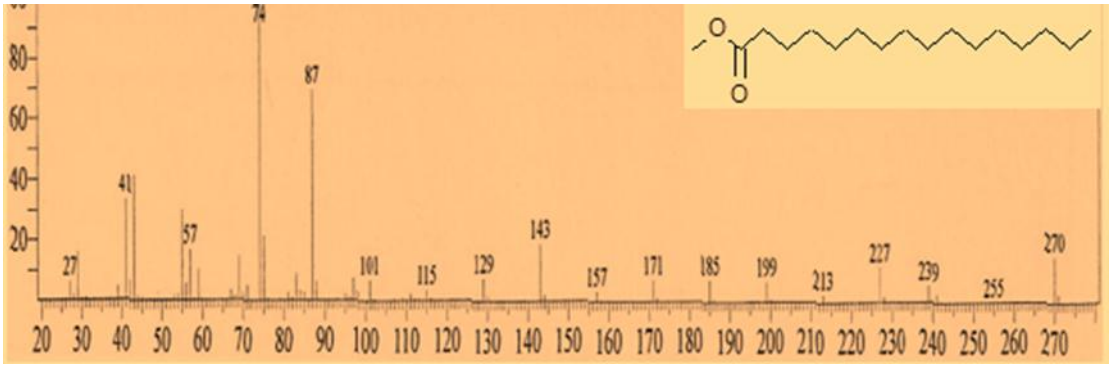

Fig. 3: Mass spectrum of hexadecanoic acid methyl ester.

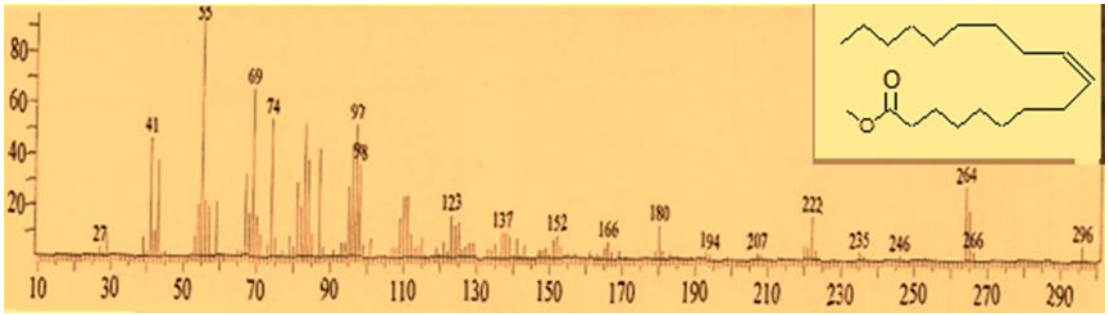

Fig. 4: Mass spectrum of 9-octadecenoic acid methyl ester.

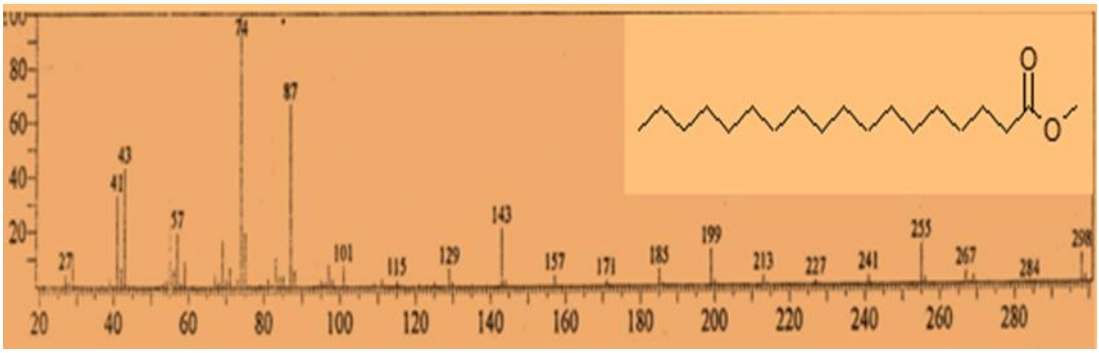

Fig. 5: Mass spectrum of methyl stearate.

Antimicrobial activity

In cup plate agar diffusion assay, the oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table (4). The results were interpreted in terms of the commonly used terms; <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 chemotherapeutics respectively.

The oil showed significant antibacterial activity against *Staphylococcus aureus.* It also showed significant anticandidal activity. However, it exhibited partial activity against *Pseudomonas aeruginosa* and *Escherichia coli.*

Table 4: Antimicrobial activity of the oil.

Sample	Ec	Pa	Sa	Bs	Ca
Grewia tenax oil (100mg/ml)	10	12	20	-	20

Table 5: Antibacterial activity of standard drugs.

Drug	Conc. (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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 drug.

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

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

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