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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *GREWIA VILLOSA* (TILIACEAE) OIL

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ABSTRACT

Grewia villosa is a shrub which is distributed in Africa and India usually in arid areas. This shrub is often growing on river banks liable to flooding or on shade of larger trees. In Sudan the fruits are marketed as a fermented drink. In its native range leaves are considered as a good livestock fodder. *Grewia villosa* root is antidiarrhoeal. It is also used against cough. A mixture of root and bark is used traditionally against syphilis, In this study, *Grewia villosa* oil has been analyzed by GC-MS. The analysis showed 21 components. Major constituents are: i)9, 12-octadecadienoic acid (Z, Z)-, methyl ester(48.65%) ii)- hexadecanoic acid, methyl ester(18.33%). iii)9-octadecenoic acid (Z)-, methyl ester (16.15%). iv)- methyl stearate (9.45%) The antmicrobial activity of the oil has been assessed. The studied oil showed significant activity against *Klebsiella pneumoniae*. However, it failed to exhibit activity against other test organisms.

KEYWORDS: *Grewia villosa*, Oil, Constituents, Antimirobial Activity.

INTRODUCTION

Grewia is a genus in the family Tiliaceae. This genus includes shrubs and trees mainly distributed in the warmer regions of the world. The genus *Grewia* comprises around 40 species distributed throughout the globe. *Grewia* species are used in traditional medicine. The species: *Grewia carpinifolia* has been shown to possess antiparasitic and antioxidant activities.[1] Members of this genus are known to elicit various CNS activities. *Grewia bicolor* is used as tranquilizer. It is also used against skin lesions.[2] The CNS depressant activity of *Grewia elastic*, *Grewia tenax* and *G.tiliaefolia* has been documented,[3,4] The hypotensive propertiy of aerial parts of *G. umbellifera* has been reported.[3]

Grewia villosa is a shrub which is distributed in Africa and India usually in arid areas.[4] This shrub is often growing on river banks liable to flooding or on shade of larger trees.[5] In Sudan it is commonly encountered on sandy or clay soils in the savanna zone of central Sudan, Darfur, Kordofan and Red Sea Hills.[6] Fruit of *Grewia villosa* are edible, and may be found in some local markets as substitute for the more valuable species-*G.tenax*.[7] The plant is often fed to lactating mothers to improve their lactating abilities. In Sudan the fruits are marketed as a fermented drink.[8] In its native range leaves are considered as a good livestock fodder.[7] *Grewia villosa* root is antidiarrhoeal It is also used against cough and tuberculosis.[3] A mixture of root and bark is used traditionally against syphilis, genitourinary infections, and smallpox. It has been reported that the root methanol extract contains beta-carboline alkaloids.[4]

MATERIALS AND METHODS

Plant Material

Grewia villosa seeds were collected from Damazin (Sudan) and authenticated by direct comparison with a reference herbarium sample.

Instruments

Grewia villosa oil was studied by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness).

Microorganisms

The antimicrobial assay was performed by using the following standard microorganisms: *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeroginosa Escherichia coli* and *Candida albicans.*

Extraction of oil

Dry powdered *Grewia villosa* seeds (350g) were macerated with n-hexane at room temperature for

72hr.The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further work.

GC-MS analysis

Constituents of *Grewia villosa* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are as follows: column oven temperature150.0°C; injection temperature:300.0°C;injection mode: split; flow mode: linear velocity; pressure:139KPa; total flow: 50.0ml/min; column flow:1.54ml/sec.; linear velocity: 47.2cm/sec. purge flow:3.0 ml/min.; split ratio: -1.0. Oven temperature program is presented Table 1:

Table 1: Oven temperature program.

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

Antimicrobial activity

The antimicrobial assay was accomplished using the cup plate agar diffusion bioassay Briefly ·Bacterial culture was maintained in nutrient agar while fungal culture was accomplished on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer (No. 4). Test samples were added into wells of the seeded medium and then incubated for 24 hrs.(at 37°C)-for bacteria and for 72 hrs at 25°C for fungal species. The diameters of inhibition zones were measured as average of two replicates.

RESULTS AND DISCUSSION

GC-MS analysis

Grewia villosa oil has been analyzed by GC-MS. The analysis revealed the presence of 21 components (Table 1). The typical total ion chromatograms (TIC) is presented in Fig. 1. Major constituents of the oil are:i)9, 12-octadecadienoic acid (Z, Z)-, methyl ester (48.65%) ii)- hexadecanoic acid, methyl ester(18.33%). iii)9-octadecenoic acid (Z)-, methyl ester (16.15%) and iv)-methyl stearate (9.45%).

Table 1: Contituents of *Grewia villosa* oil.

Peak#	R.Time	Area	Area%	Name
1	6.996	154487	0.03	.alpha.-Terpineol
2	13.577	1213232	0.27	Methyl tetradecanoate
3	14.653	325511	0.07	Pentadecanoic acid, methyl ester
4	15.487	1338256	0.30	9-Hexadecenoic acid, methyl ester, (Z)-
5	15.703	82651608	18.33	Hexadecanoic acid, methyl ester
6	16.449	952046	0.21	9,12-Octadecadienoyl chloride, (Z,Z)-
7	16.660	1239715	0.27	Heptadecanoic acid, methyl ester
8	17.409	219346746	48.65	9,12-Octadecadienoic acid (Z,Z)-, methyl e
9	17.439	72819289	16.15	9-Octadecenoic acid (Z)-, methyl ester
10	17.613	42592056	9.45	Methyl stearate
11	17.761	1910015	0.42	Oleic Acid
12	17.957	2883814	0.64	Octadecanoic acid
13	18.955	4121498	0.91	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl
14	19.155	2438674	0.54	cis-11-Eicosenoic acid, methyl ester
15	19.353	6131987	1.36	Eicosanoic acid, methyl ester
16	19.409	1113427	0.25	PGHI, methyl ester
17	19.521	1048707	0.23	1-Naphthalenol, decahydro-4a-methyl-
18	20.973	3660165	0.81	Docosanoic acid, methyl ester
19	21.737	592599	0.13	Tricosanoic acid, methyl ester
20	22.475	2133610	0.47	Tetracosanoic acid, methyl ester
21	23.213	2232953	0.50	Squalene
		450900395	100.00	

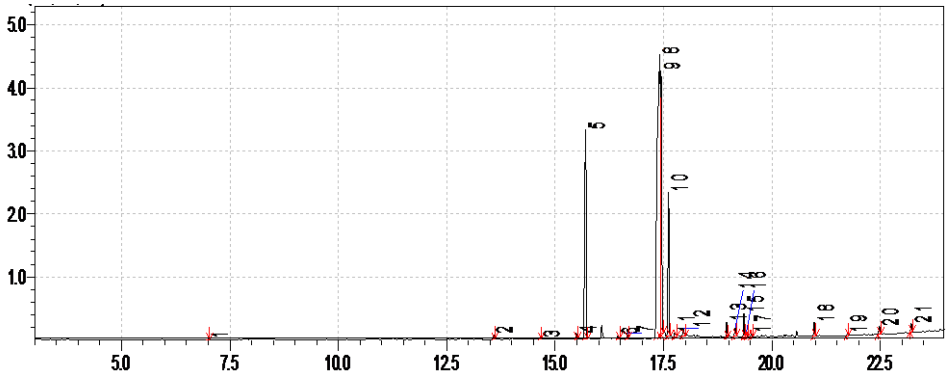
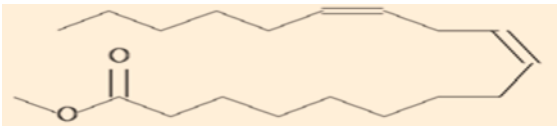


Fig. 1: Typical total ion chromatogram.

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.2. The peak at m/z294 with retention time 17.409 corresponds to the molecular ion M^+ $[C_{19}H_{34}O_2]^+$ while the signal at m/z263 is due to loss of a methoxyl group. Fig. 3 represents the mass spectrum of hexadecanoic acid, methyl ester. The peak at m/z270 with retention time 15.703 is due to the molecular ion M^+ $[C_{17}H_{34}O_2]^+$. The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.4. The peak at m/z 296 with retention time 17.439 accounts for the molecular ion M^+ $[C_{19}H_{36}O_2]^+$. Fig.5 shows the mass spectrum of methyl stearate. The signal at m/z298 (retention time:17.613) is due to the molecular ion M^+ $[C_{19}H_{38}O_2]^+$. The peak at m/z267 is due to loss of a methoxyl.



9, 12-octadecadienoic acid (Z, Z)-, methyl ester.

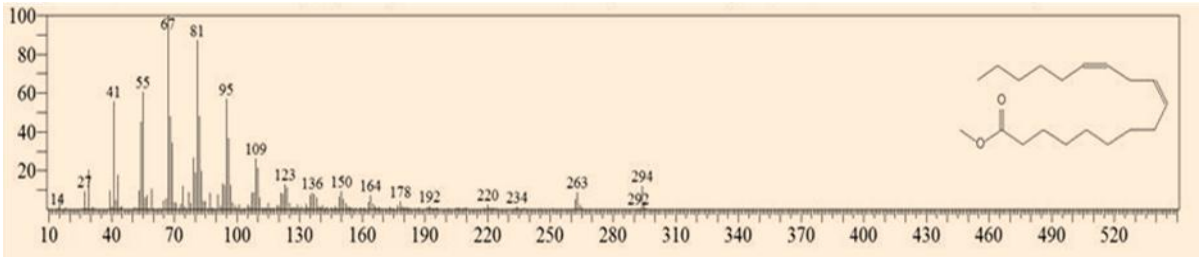
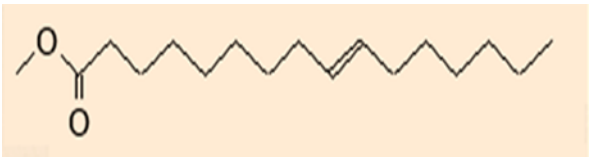


Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester.



hexadecanoic acid, methyl ester.

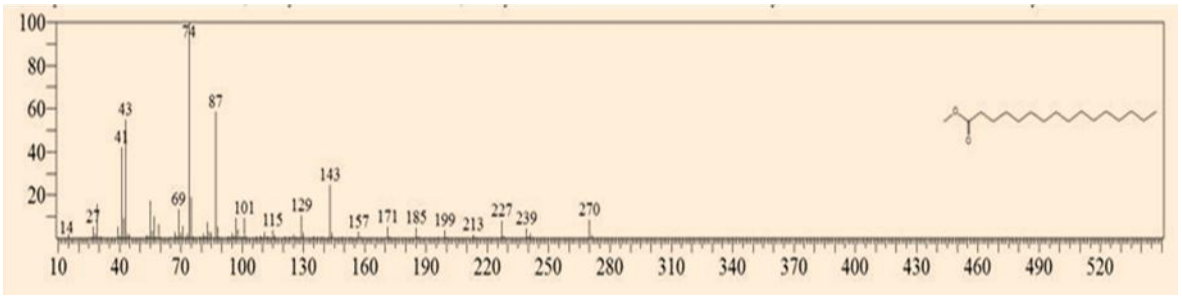


Fig. 3: Mass spectrum of hexadecanoic acid, methyl ester.

9-octadecenoic acid (Z)-, methyl ester

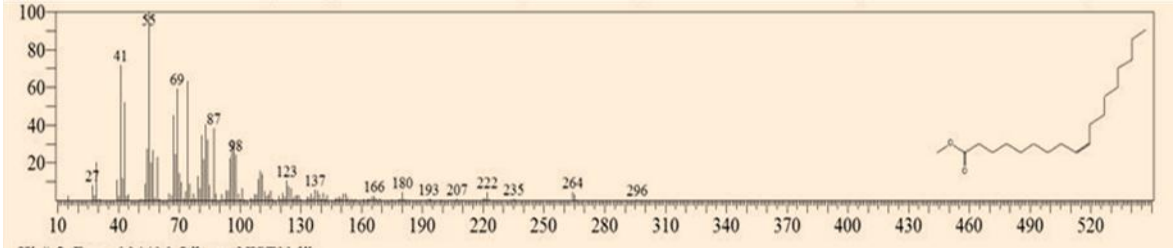
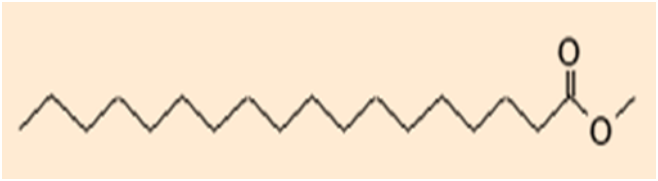


Fig. 4: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.



Methyl stearate

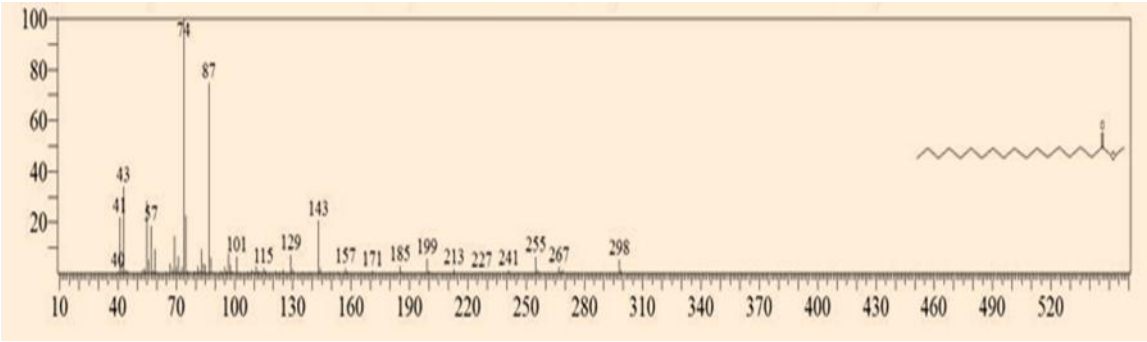


Fig. 5: Mass spectrum of methyl stearate.

Antimicrobial activity

In the cup plate agar diffusion assay the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 2. The studied oil showed significant activity against *Klebsiella pneumoniae*. However it failed to exhibit activity against other test organisms.

Table 2: Inhibition zones of the oil (mm).

Type	Ec.	Ps.	Sa.	Kp.	Ca.
Oil 100mg/ml	--	--	--	16	--

Kp. =Klebsiella pneumoniae
Sa: Staphylococcus aureus.
Ec: Escherichia coli.
Ps: Pseudomonas aeruginosa.
Ca: Candida albicans.

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