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Research Article

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GC-MS Analysis, Antimicrobial and Antioxidant Activity of Sudanese *Merremia dissecta* (Convolvulaceae) Oil

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Abstract This study was aimed to identify and quantify the constituents of *Merremia dissecta* oil and to assess the antimicrobial and antioxidant activities of the oil. The GC-MS analysis of the oil revealed the presence of: 9 12-octadecaidenoic acid methyl ester (32.78%); hexadecanoic acid methyl ester (23.04%); 9-octadecenoic acid methyl ester 22.04%); methyl stearate (13.12%) as major constituents. The antimicrobial assay was accomplished against five standard human pathogens and good activity against *Bacillus subtilis* has been observed. The oil also showed significant antioxidant activity in the DPPH bioassay.

Keywords *Merremia dissecta*, Oil, Constituents, Antimicrobial Activity, Antioxidant Activity

Introduction

Reactive oxygen species are generated in the body as a result of the cellular metabolism and eliminated by defensive enzymes like superoxide dismutase. In the recent years, the medicinal plants have drawn interest against oxidative stress.

Merremia dissecta (Convolvulaceae) is perennial climbers, with herbaceous stems [1]. The genus *Merremia* L. is distributed in different regions of the world and especially in tropical America [2-4]. In folk medicine leaves and its juice are used as sedative and for urinary tract infections. The plant is also a remedy for skin diseases. Boiled tubers are used traditionally against chest problems, inflammation, urinary tract infections, snake bite, sprains and scabies [5-9].

Phytochemical screening of *Merremia dissecta* showed the presence of alkaloids, glycosides, tannins, saponins and sterols [10].

Materials and Methods

Plant material

Merremia dissecta was obtained from Dongola –northern Sudan. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum – Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness) was used to identify and quantify the components of *Merremia dissecta* oil. A Multiscan spectrophotometer (Thermo Scientific Co.) was used to evaluate the antioxidant activity of the oil.

Test organisms

The antimicrobial activity of *Merremia dissecta* oil was evaluated using the following standard microorganisms: *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 *Merremia dissecta* seeds

Powdered shade-dried seeds of *Merremia dissecta* (300g) were macerated with *n*-hexane at room temperature. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4 ^{o}C for further work.

GC-MS analysis

Merremia dissecta seed oil was analyzed by gas chromatography–mass spectrometry. A Shimadzo GC-MS- QP2010 Ultra instrument was used. Helium (purity; 99.99 %) was used as carrier gas. Chromatographic conditions are as follows: 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.

Antimicrobial activity

A (24) hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37º C . Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 10^8- 10^9 C.F.U/ ml. The average number of viable organisms per ml of the stock suspension was determined.

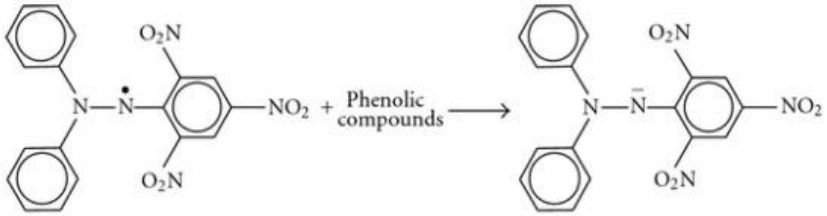
Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample were placed on the surface of the seeded agar. The inoculated plates were incubated at 37 °C for 24 h. The test was done in duplicates and the diameters (mm) of the inhibition zones were measured and averaged.

The above mentioned method was adopted for antifungal activity. Samples were used here by the same concentrations used above.

Antioxidant Activity

The DPPH assay is based [11] on the scavenging ability of antioxidant(s) towards the stable free radical of: 1,1-diphenyl-2-picrylhydrazyl (DPPH), which is deep purple in color. Upon reduction DPPH afford the corresponding hydrazine with a color change to light purple or golden yellow.



DPPH free radical DPPH reduced form

Stock solution was prepared by dissolving 1mg of the sample in 1ml of absolute ethanol (98%). Stock solution was diluted to final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.5625 µg /ml in ethanol. (0.9 ml) HCl and (1ml) of

0.1 mM DPPH in methanol were added to each concentration and the mixture was incubated at room temperature in the dark for 30 minutes. The absorbance of the resulting mixture was measured at λ_{max} 517 nm and converted to percentage antioxidant activity using the formula below:

$$\text{Antioxidant activity (\%)} = \frac{(\text{Abs. control} - \text{Abs. sample}) \times 100}{\text{Abs. control}}$$

Where:

Abs. = Absorbance

A solution of (0.9 ml) HCL+ (0.1ml) absolute ethanol was used as blank, while a solution of (0.9ml) HCl+ (0.1ml) absolute ethanol+ (1ml) DPPH was used as a positive control. Freshly prepared DPPH solution exhibits a deep purple color with a maximum absorbance at λ_{max} 517 nm. The purple colour disappears when an antioxidant is present in the medium. Hence, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger [11].

Results and discussion

GC-MS analysis of *Merremia dissecta* oil

GC-MS analysis of *Merremia dissecta* seed oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern.

The GC-MS spectrum of the studied oil revealed the presence of 20 components as depicted in Table 1.

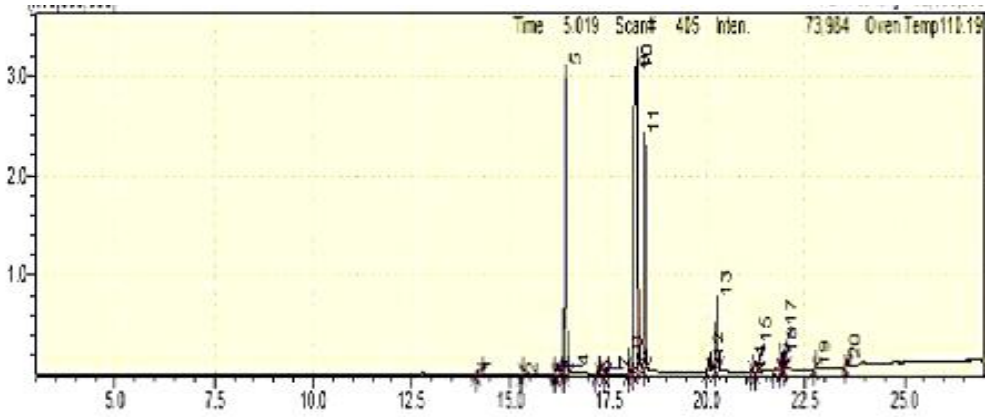


Figure 1: Total ions chromatograms of Merremia dissecter oil

The following constituents were found as major components of the oil:

i) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (32.78 %)

ii) Hexadecanoic acid, methyl ester (23.04 %)

iii) 9-Octadecenoic acid (Z)-, methyl ester (22.04 %)

iv) Methyl stearate (13.12%).

The mass spectrum of 9,12-octadecadienoic acid (Z,Z) methyl ester is shown in Fig. 2. The peak at m/z 294 (RT.18189) is due to the ion: $M^+[C_{19}H_{34}O_2]^+$. Fig. 3 presents the mass spectrum of hexadecanoic acid methyl ester. The peak at m/z 270 (R.T.16.420) corresponds: $M^+[C_{17}H_{34}O_2]^+$. Mass spectrum of 9-octadecenoic acid (Z) methyl ester is illustrated in Fig. 4. The peak at m/z 296, which appeared at R.T. 17.605 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$. Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T. 17.763) accounts for the molecular ion: $M^+[C_{19}H_{38}O_2]^+$.

Table 1: Constituents of *Merremia dissecta* oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	14.187	1001016	0.26
2.	Pentadecanoic acid, methyl ester	15.317	148225	0.04
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.165	138376	0.04
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.194	1616242	0.42
5.	Hexadecanoic acid, methyl ester	16.420	88746045	23.04
6.	cis-10-Heptadecenoic acid, methyl ester	17.211	317146	0.08
7.	Heptadecanoic acid, methyl ester	17.424	1014215	0.26
8.	.gamma.-Linolenic acid, methyl ester	18.007	5222197	1.36
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.189	126215622	32.78
10	9-Octadecenoic acid (Z)-, methyl ester	18.240	84908512	22.04
11	Methyl stearate	18.428	50559962	13.12
12	cis-11-Eicosenoic acid, methyl ester	20.055	2682726	0.70
13	Eicosanoic acid, methyl ester	20.259	13537822	3.51
14	Heneicosanoic acid, methyl ester	21.126	282233	0.07
15	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.290	205759	0.05
16	cis-9-Hexadecenal	21.754	1039371	0.27
17	10,13-Eicosadienoic acid, methyl ester	21.912	1352185	0.35
18	Docosanoic acid, methyl ester	21.962	3397232	0.88
19	Tricosanoic acid, methyl ester	22.766	547438	0.14
20	Tetracosanoic acid, methyl ester	23.540	2289571	0.59
			385221895	100.00

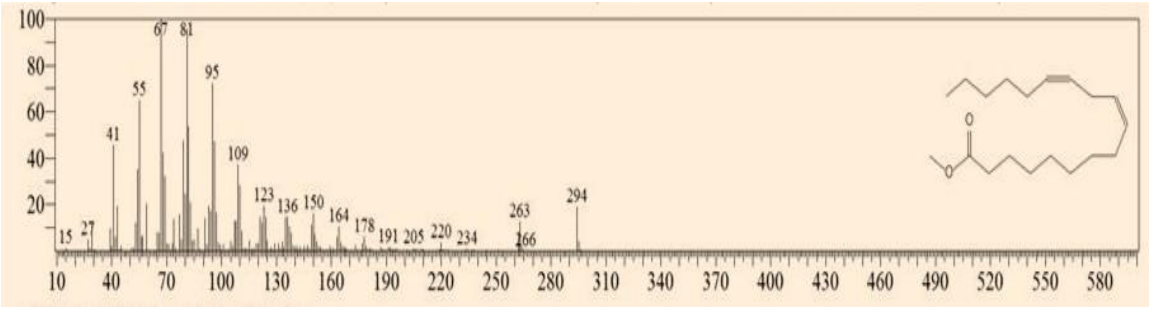


Figure 2: Mass spectrum of 9 12-octadecadienoic acid methyl ester

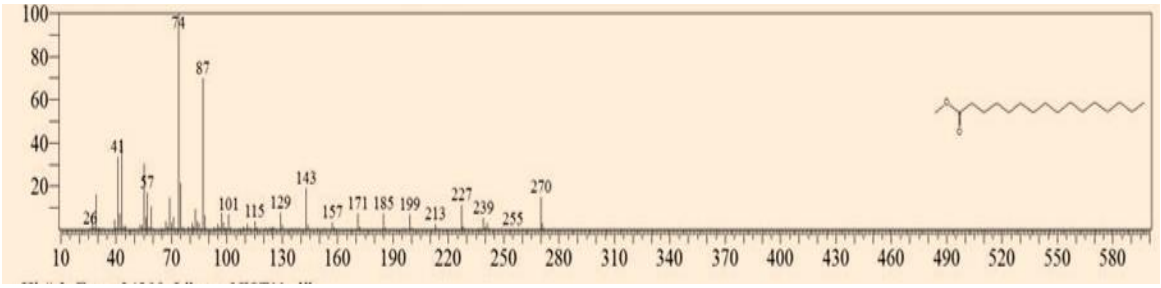


Figure 3: Mass spectrum of hexadecanoic acid methyl ester

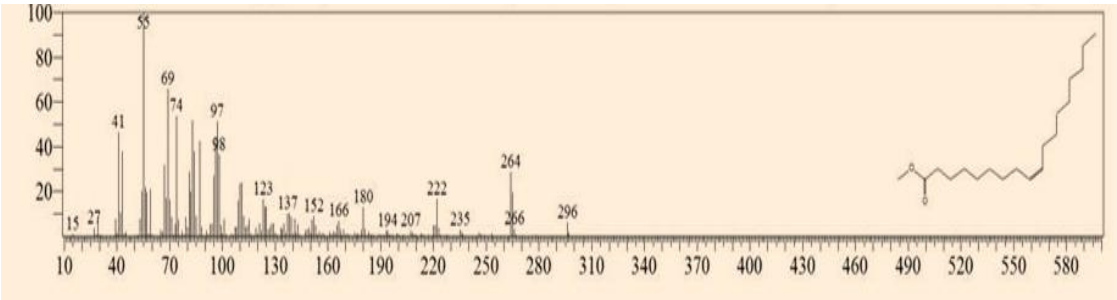


Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

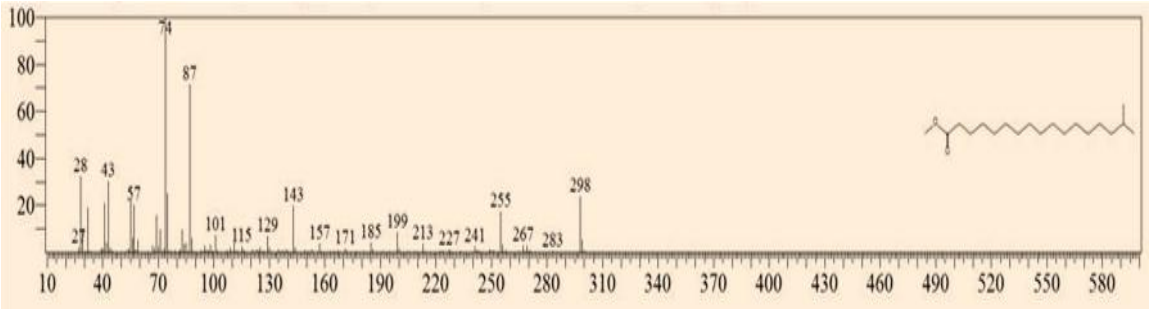


Figure 5: Mass spectrum of methyl stearate

Antimicrobial and antioxidant activity

The oil was evaluated for antimicrobial activity. The averages of the diameters of the growth of inhibition zones are shown in Table (2) .The results were interpreted in terms of the commonly used terms; < 9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (3) and (4) represent the activity of standard antibacterial and antifungal chemotherapeutic agents standard bacteria and fungi respectively. The oil showed good antibacterial activity against *Bacillus subtilis.* The oil also exhibited significant antioxidant activity (Table 5).

Table 2: Inhibition zones (mm) of *Merremia dissecta* oil

Sample	Bs	Sa	Ps	Ec	Ca
Oil (100mg/ml)	15	-	-	-	-

Table 3: Inhibition zones (mm) of standard antibacterial agent

Drug	Conc. 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 4: Inhibition zones (mm) of standard antifungal agent

Drug	Conc. mg/ml	Ca.
Clotrimazole	30	38
	15	31
	7.5	29

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

Table 5: Antioxidant activity of *Merremia dissecta* oil

Sample	Activity % ± SD
M. dissecta oil	68.08 ± 0.23
Propyl Gallate (Positive control)	93 ± 0.01

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