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Research Article

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GC-MS Analysis and Antimicrobial Activity of *Cissus quadrangularis* Oil

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Abstract This study was planned to identify and quantify the oil from Sudanese *Cissus quadrangularis* and to evaluate the antimicrobial activity of the extracted oil. *Cissus quadrangularis* is a perennial climber widely used in Sudanese ethnomedicine. This plant grows in warm tropics and may propagate through stem cutting. *Cissus quadrangularis* possesses digestive, anthelmintic and tonic properties. The plant is rich in ascorbic acid, calcium and carotene. It also contains flavonoids, steroids and terpenoids. GC- MS analysis of *Cissus quadrangularis* oil revealed the presence of the following major components: E,E,Z-1,3,12-nonadecatriene-5,14-diol(22.11%); R)-(-)-14-methyl-8-hexadecyn-1-ol (15.44%); trilinolein (12.17%); 9,12-octadecadienoyl chloride, (Z,Z)- (11.02%) and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (8.67%). The oil was evaluated for its antimicrobial activity against five standard human pathogens. It showed moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Keywords *Cissus quadrangularis,* Oil, Constituents, Antimicrobail activity

Introduction

Through decades herbal medicine has been used by human beings as a natural source of therapies. Recently medicinal plants have gained considerable attention due to their tremendous contribution to the development and design of modern drugs. During the last five years more than 15000 plants have been investigated for bioactive molecules. Now scientists are focusing on this renewable source to produce a new generation of therapeutic solutions.

Cissus quadrangularis is a perennial climber widely used in Sudanese ethnomedicine. This plant grows in warm tropics and may propagate through stem cutting [1,2]. *Cissus quadrangularis* possesses digestive, anthelmintic and tonic properties [3]. The plant is rich in ascorbic acid, calcium and carotene [4,5]. It also contains flavonoids, steroids and terpenoids [5,6,7,8]. *Cissus quadrangularis* exhibited significant analgesic activity [9,10]. It also showed beneficial effect on recovery of bone mineral density in postmenopausal osteoperosis[11-15]. Some *in vitro* studies demonstrated the antioxidant activity of this plant [16,17]. It has been reported that *Cissus quadrangularis* posseses anti-inflammatory effect [18-20].

Materials and Methods

Plant material

Cissus quadrangularis seeds were collected from a forest around Damazin-Sudan. The plant was identified and authenticated by direct comparison with a 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

Test organisms used in this study are: *Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil

Powdered seeds of *Cissus quadrangularis* (300g) were macerated with n-hexane for 48h. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

(2ml) of the oil was mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving sodium (2 g) in 100 ml methanol. (7 ml). Alcoholic sulfuric acid (1ml H_2SO_4 in 100 ml methanol) was then added. The mixture was shaken for 5 minutes. The content of the test tube was left to stand overnight. Then (1ml) of supersaturated sodium chloride was added and the tube was shaken for 5 min. (2ml) of normal hexane were added and the contents were shaken thoroughly for 5 minutes. (5 µl) of the n-hexane were diluted with (5ml) of diethyl ether and dried over anhydrous sodium sulphite. (1µl) of the diluted sample was injected in the GC.MS vial.

The qualitative and quantitative analysis of the sample was carried out by using a Shimadzu machine- model (GC/MS-QP2010-Ultra). The sample was injected under the following chromatographic conditions: column oven temperature: 150.0^{o}C; injection temperature: 300.0^{o}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 (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Antimicrobial assay

The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 10^{8}cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whitman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of test sample. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and recorded as average of two replicates.

Results and Discussion

Cissus quadrangularis oil was studied by GC-MS. The analysis showed the presence of 24 consitiuents (Table 2). The total ions chromatogram is depicted in Fig. 1.

Figure 1: Total ions chromatograms

Table 2: Constituents of the oil

S. No.	Name	Ret. Time	Area%
1	Methyl tetradecanoate	13.564	0.04
2	Hexadecanoic acid, methyl ester	15.650	2.45
3	n-Hexadecanoic acid	16.034	1.55
4	Hexadecanoic acid, ethyl ester	16.310	0.19
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.300	8.67
6	9-Octadecenoic acid (Z)-, methyl ester	17.343	3.17
7	Methyl stearate	17.560	2.10
8	Linoleic acid ethyl ester	17.704	2.24
9	Oleic Acid	17.734	0.57
10	9,12-Octadecadienoic acid, ethyl ester	17.908	1.82
11	13-Hexyloxacyclotridec-10-en-2-one	18.623	4.09
12	cis-9-Hexadecenal	18.699	1.47
13	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	18.930	22.11
14	l-(+)-Ascorbic acid 2,6-dihexadecanoate	19.088	2.05
15	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	19.484	15.44
16	Heptanoic acid, tert-butyl dimethyl silanyl ester	20.094	1.68
17	9,12-Octadecadienoyl chloride, (Z,Z)-	20.552	11.02
18	Glycidol stearate	20.753	1.55
19	Z,Z-8,10-Hexadecadien-1-ol	21.046	2.30
20	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	21.601	0.70
21	cis,cis-7,10,-Hexadecadienal	21.854	2.13
22	Trilinolein	22.000	12.17
23	13-Docosenamide, (Z)-	22.892	0.33
24	Squalene	23.174	0.16

The GC-MS analysis showed the presence of steroids, aldehydes and ketones as minor constituents. The oil was dominated by:

i- E,E,Z-1,3,12-Nonadecatriene-5,14-diol (22.11%)
ii- (R)-(-)-14-Methyl-8-hexadecyn-1-ol (15.44%)
iii- Trilinolein(12.17%)
iv- 9,12-Octadecadienoyl chloride, (Z,Z)- (11.02%)
v- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (8.67%).

The mass spectrum of E,E,Z-1,3,12-nonadecatriene-5,14-diol [$C_{19}H_{34}O_2$] is shown in Figure 2. The peak at m/z 292 (R.T. 18.930 in total ion chromatogram), corresponds: M^+ - 2H. The mass spectrum of (R)-(-)-14-Methyl-8-

hexadecyn-1-ol is illustrated in Figure 3. The signal at m/z 252 (RT, 19.484) accounts for the molecular ion: $M^+[C_{17}H_{32}O]^+$. Fig. 4 shows the mass spectrum of trilinolein. The peak at m/z 878 (RT, 22.000) accounts for: $M^+[C_{57}H_{98}O_6]^+$. The mass spectrum of 9,12-Octadecadienoyl chloride, (Z,Z)- is illustrated in Figure 5. The molecular ion: $M^+[C_{18}H_{31}ClO]^+$ appeared at m/z 298.

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Figure 6. The peak at m/z 294 (R.T.17.300 in total ion chromatogram), corresponds: $M^+[C_{19}H_{34}O_2]^+$. The peak at m/z 263 is due to loss of a methoxyl function.

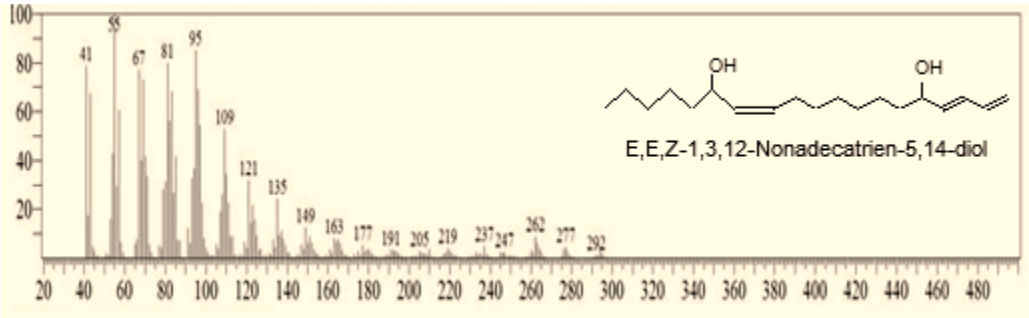

Figure 2: Mass spectrum of E,E,Z,1,3,12-Nonadecatrien-5,14-diol

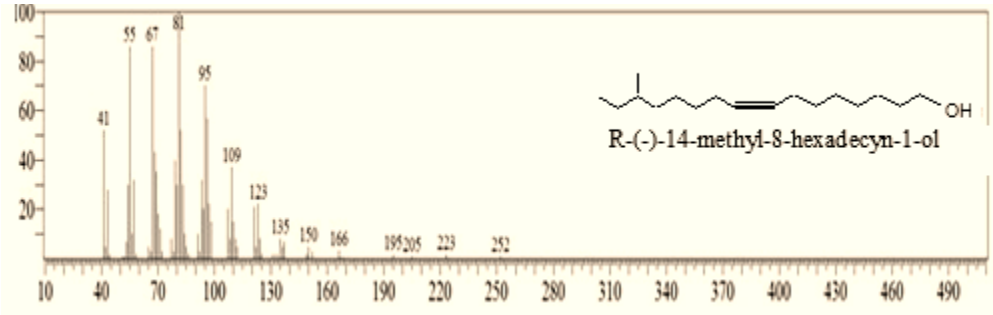

Figure 3: Mass spectrum of R-(-)-14-methyl-8-hexadecyn-1-ol

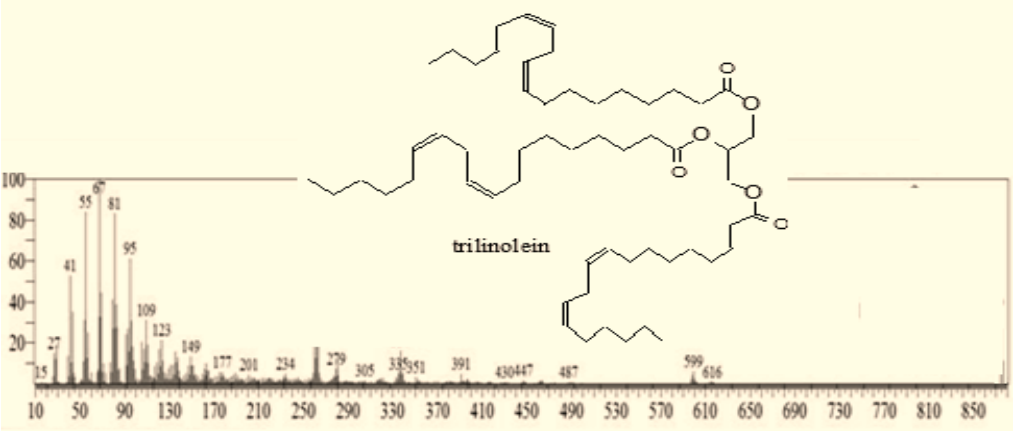

Figure 4: Mass spectrum of trilinolein

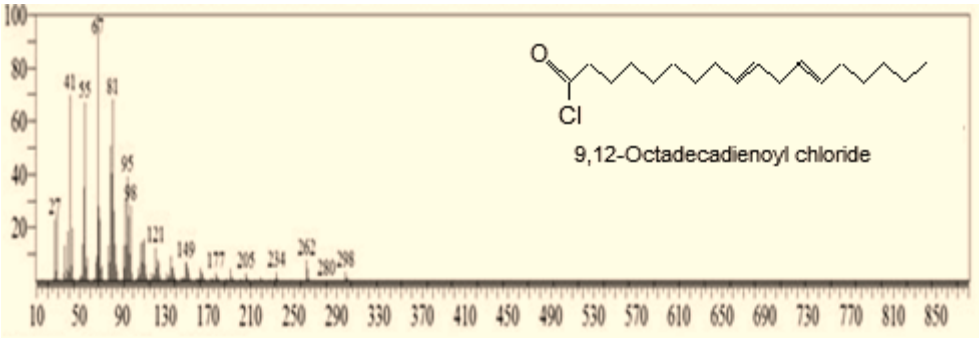

Figure 5: Mass spectrum of 9,12-octadecadienoyl chloride

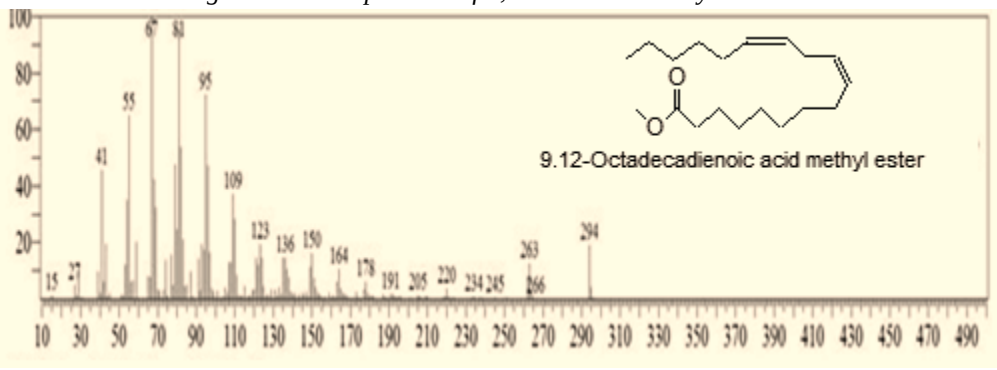

Figure 6: Mass spectrum of 9,12-octadecadienoic acid methyl ester

Antimicrobial assay

Cissus quadrangularis oil was evaluated for its antimicrobial activity against five pathogenic microbes (*Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* -fungus). The oil showed moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Table 2: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	14	--	--	16	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentacycin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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