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Research Article

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Constituents and Attempted Antimicrobial Activity of Oil from *Croton cordofana* Grown in Sudan

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Abstract This study was designed to investigate the constituents and antimicrobial activity of *Croton cordofana* oil. The genus *Croton* comprise a diverse group of plants including trees, herbs and shrubs. *Croton* species are used against a wide array of diseases including cancer, diabetes, dysentery, worms, ulcers, inflammations and weight loss. GC- MS analysis of *Croton cordofana* oil revealed the presence of four major constituents: 9,12-octadecenoic acid methyl ester (44.32%); 9-octadecenoic acid methyl ester (23.42%); hexdecanoic acid (15.18%) and methyl stearate (12.99%). In the cup plate agar diffusion assay, the oil was screened for antimicrobial activity against five standard human pathogens. However, the oil failed to show any activity.

Keywords *Croton cordofana,* Oil, Constituents, Antimicrobial Activity

Introduction

Since antiquity herbal medicine played an important role in fighting diseases. Medicinal plants are now involved in drug design and drug development. Recently extensive research has been initiated to investigate the pharmacological activity of plant bioactive molecules. In contrast with modern drugs herbal medicine is more affordable and is usually associated with less side effects. According to the WHO about 80% of the world population is relying on traditional medicine.

The genus *Croton* comprise a diverse group of plants including trees, herbs and shrubs. *Croton* species are used against a wide array of diseases including cancer,diabetes, dysentery , worms ,ulcers, inflammations and weight loss [1-6]. The medicinally important species: *Croton sylvaticas* is used traditionally against tuberculosis, inflammatory conditions and infections [7]. The acetylcholinesterase inhibitory action of this species has been reported [8]. Also the antiplasmodial effect has been demonstrated [9]. Another *Croton* species, namely: *Croton gratissirnas* showed anticancer, antioxidant and anti-inflammatory properties [10]. The medicinally important *Croton menyherthi* possesses antimicrobial and selected enzyme inhibitory effect [11]. Another Croton species: *Croton limae* exhibited antimicrobial activity [12].

Materials and Methods

Materials

Plant material

Seeds of *Croton cordofana* were collected from Kordofan-western Sudan . The plant was authenticated by direct comparison with reference herbarium sample.

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 targeted oil was assessed for antimicrobial activity using the standard microorganisms shown in Table (1).

Table 1: Test organisms

S. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
6	*Candida albicans*	fungi

Methods

Extraction of oil

Powdered seeds of *Croton cordofana* (400g) were macerated with n-hexane. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

The oil (2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight. (2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaked for five minutes. The hexane layer was then separated. (5μl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate(1μl) was injected in the GC-MS vial.

GC-MS analysis

The extracted oil was analyzed by 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 (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 Assay

Preparation of Bacterial Suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9} colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of Fungal Suspensions

Fungal cultures were maintained on Saboraud dextrose agar incubated at 25°C for 72h. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antimicrobial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the 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 (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for the same test sample.

The agar discs were removed, alternate cup were filled with 0.1 ml of sample 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.

The above procedure was repeated for antifungal assay but Saboraud dextrose agar was used instead of nutrient agar. After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

Results and Discussion

GC-MS analysis

Croton cordofana oil was studied by GC-MS. The analysis revealed the presence of 19 components as shown in Table 2. The total ions chromatogram is presented in Fig. 1.

The following compounds were detected in the chromatogram as major constituents:

i) 9 12-Octadecenoic acid methyl ester (44.32%)
ii) 9-Octadecenoic acid methyl ester (23.42%).
iii) Hexdecanoic acid (15.18%)
iv) Methyl stearate (12.99%).

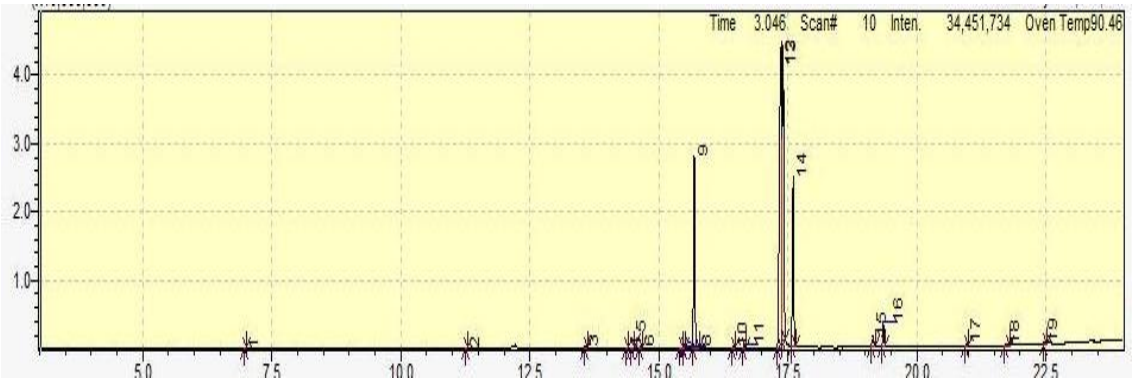

Figure 1: Total ions chromatograms

Table 2: Constituents of *Croton cordofana* oil

No.	Name	Ret. Time	Area%
1.	L-.alpha.-Terpineol	6.988	0.04
2.	Dodecanoic acid, methyl ester	11.263	0.01
3.	Methyl tetradecanoate	13.579	0.15
4.	6-Octadecenoic acid, methyl ester, (Z)-	14.386	0.02
5.	5-Octadecenoic acid, methyl ester	14.492	0.03
6.	Pentadecanoic acid, methyl ester	14.652	0.09
7.	7-Hexadecenoic acid, methyl ester, (Z)-	15.443	0.05
8.	9-Hexadecenoic acid, methyl ester, (Z)-	15.486	0.05
9.	Hexadecanoic acid, methyl ester	15.687	15.18
10.	cis-10-Heptadecenoic acid, methyl ester	16.448	0.08

11. Heptadecanoic acid, methyl ester	16.656	0.24
12. 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.371	44.32
13. 9-Octadecenoic acid (Z)-, methyl ester	17.410	23.42
14. Methyl stearate	17.602	12.99
15. cis-11-Eicosenoic acid, methyl ester	19.150	0.93
16. Eicosanoic acid, methyl ester	19.349	1.74
17. Docosanoic acid, methyl ester	20.972	0.33
18. Tricosanoic acid, methyl ester	21.736	0.08
19. Tetracosanoic acid, methyl ester	22.477	0.25

Fig. 2 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 17.371) corresponds M^+ $[C_{19}H_{34}O_2]^+$. The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3. The signal at m/z296 (RT.17.410) corresponds M^+ $[C_{19}H_{36}O_2]^+$.

The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 4.The peak at m/z 270 (RT.15.687) is due to M^+ $[C_{17}H_{32}O_2]^+$. Fig. 5 shows the mass spectrum of methyl stereate. The signal at m/z 298 (R.T.17.602) corresponds M+$[C_{19}H_{38}O_2]$+ , while the peak at m/z 267 accounts for loss of a methoxyl.

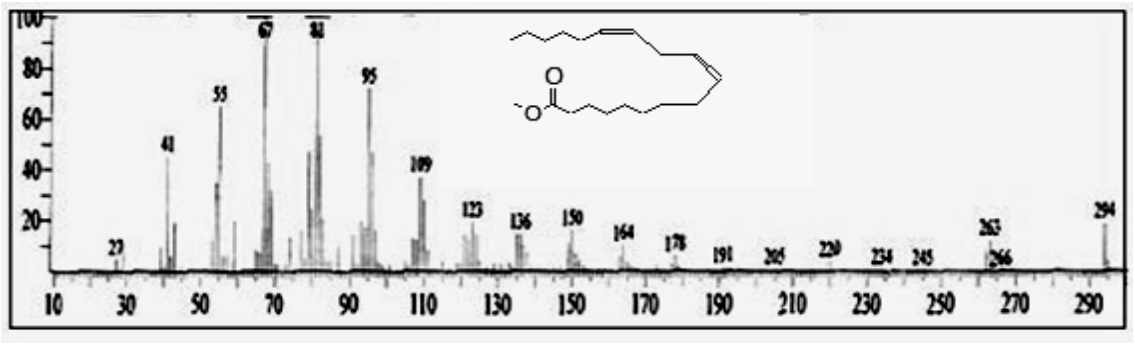

Figure 2: Mass spectrum of 9,12-octadecadienoic acid

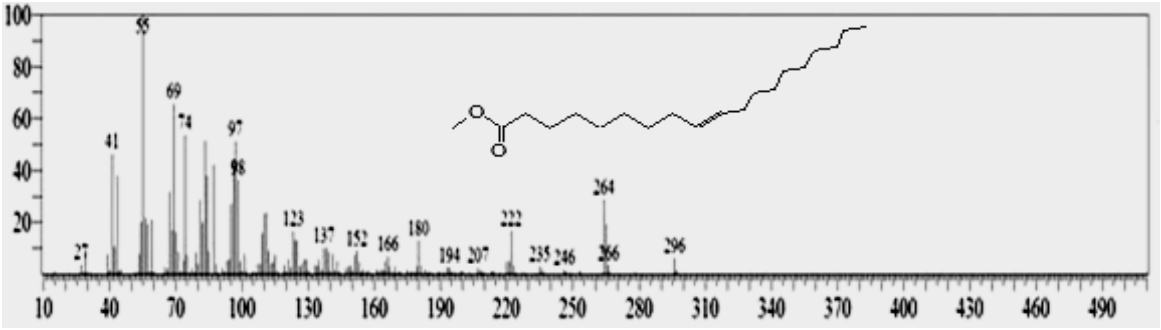

Figure 3: Mass spectrum for 9-octadecenoic acid[z]-, methyl ester

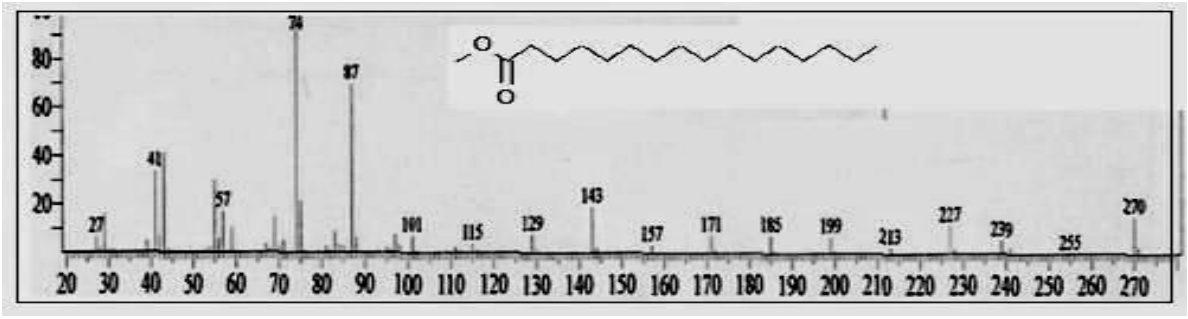

Figure 4: Mass spectrum of hexadecanoic acid methyl ester

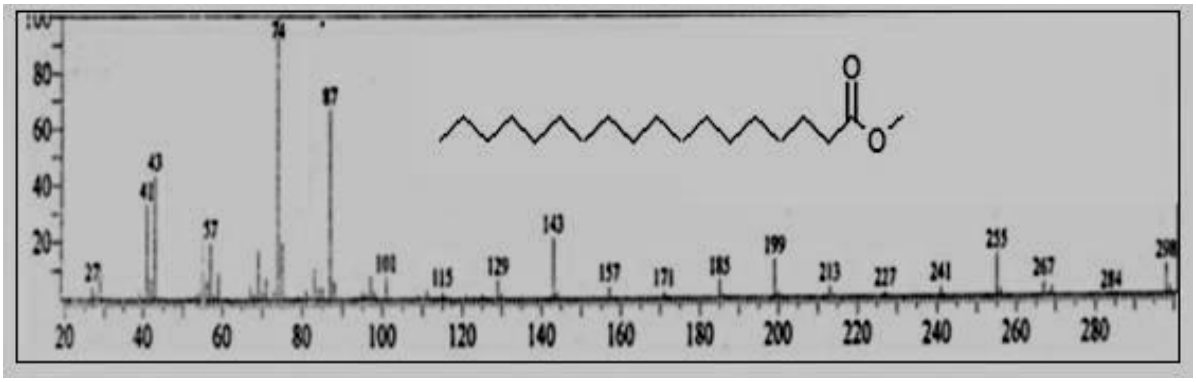

Figure 5: Mass spectrum of methyl stearate

Antimicrobial Assay

Croton cordofana oil was assessed for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are presented in Table (3).Results were interpreted in conventional terms: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil failed to show activity against any of the test organisms.

Table 3: Inhibition zones (mm/mg sample)

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

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

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