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Research Article

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GC-MS analysis and Antimicrobial Activity of Sudanese *Jatropha curcas* L. (Eupharbiaceae) Fixed Oil

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Abstract *Jatropha curcas* seed oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. Twenty two components were detected by GC-MS analysis. Main constituents are: Z,Z-9,12-octadecadienoic acid methyl ester (37.56%); Z-9-octadecenoic acid methyl ester (26.03%%); hexadecanoic acid methyl ester (19.09%); methyl stearate (13.20%).

The antibacterial activity of the oil was evaluated via the cup plate agar 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 excellent activity against *Escherichia coli* and excellent anticandidal activity.

Keywords GC-MS analysis, Antimicrobial Activity, *Jatropha curcas*

1. Introduction

Jatropha curcas L. (also known as physic nut) is a small tree up to 5m in height in the family Eupharbiaceae. The plant is native to tropical America but now distributed in tropical and sub-tropical Africa

Jatropha curcas is a medicinal plant of many attributes. This multipurpose plant has been used traditionally against a wide range of diseases. It is mainly used against sexual diseases, inflammations, diabetes, jaundice, dysentery, fever, gastric problems and skin diseases. Bark aqueous extract has been used in ethnomedicine to control blood sugar levels [1-3].

The antihyperglycemic effect of leave ethanolic extract has been reported [4]. Uche and Aprioku [5] demonstrated the analgesic activity of leave methanolic extract in model animals. The methanol extract also rduced liver lesions, lymphocytic infiltration and hepatic necrosis induced by aflatoxin B1. Different parts of *Jatropha curcas* have been used traditionally for wound healing [6-9].

Shetty *et al*., [10] reported that the bark extract accelerated wound healing in experimental models. The plant possesses anticoagulant activity. Latex from *Jatropha curcas* significantly reduced the clotting time in human blood [10]. The antifertility activity of different fractions of *Jatropha curcas* has been testified [11].

Jatropha curcas which contains diterpenes as major secondary metabolites is used against cancer [12]. The cytototoxic and tumor inhibiting properties of the diterpenes have been documented [13]. Root aqueous extract showed significant cytotoxic activity suggesting a lead for anticancer therapeutic agent [14]. The antiviral activity of different fractions of *Jatropha curcas* has been studied [15-16].

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

Seeds of *Jatropha curcas* were purchased from the local market-Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2. 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)**.**

2.1.3. Test organisms

Jatropha curcas oil was screened for antibacterial and antifungal activities 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
5.	*Candida albicans*	fungi

2.2. Methods

2.2.1. Extraction of oil

Powdered seeds of *Jatropha curcas* (500g) were exhaustively extracted 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 manipulation.

The oil (2 ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7 ml of alcoholic sulphuric acid. The tube was shaked vigorously for five minutes and then left overnight. (2 ml) 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.

2.2.2. GC-MS analysis

The oil of *Jatropha curcas* was analyzed by gas chromatography–mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are given in Tables (2) and (3).

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

Table 3: Oven temperature program

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

2.2.3. Antimicrobial test

2.2.3.1. 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 10^8-10^9colony 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.

2.2.3.2. Preparation of fungal suspensions

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

2.2.3.3. Testing for antibacterial 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 a test solution. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics.

The agar discs were removed, alternate cup were filled with 0.1 ml samples of test solutions 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 different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured as average of two replicates.

3. Results and discussion

3.1. Constituents of *Jatropha curcas* oil

Jatropha curcas oil was analyzed by GC-MS. The analysis showed the presence of 22 components (Table 4) the typical total ion chromatograms (TIC) is displayed in Fig (1).

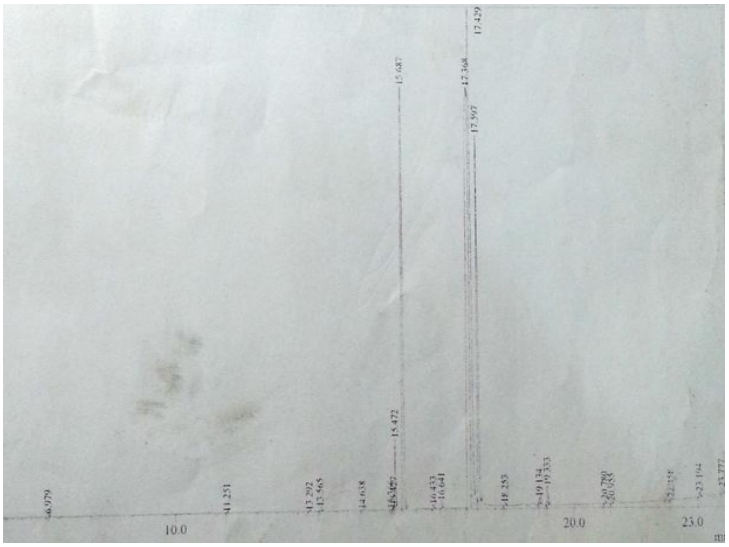

Figure 1: Total ions chromatogram of Jatropha Carcus oil

Table 4: Constituents of *Jatropha Carcus* oil

Peak#	R.Time	Area	Area%	Name
1	6.979	283745	0.08	.alpha.-Terpineol
2	11.251	20885	0.01	Dodecanoic acid, methyl ester
3	13.292	49577	0.01	5-Octadecenoic acid, methyl ester
4	13.565	436841	0.13	Methyl tetradecanoate
5	14.638	95238	0.03	Pentadecanoic acid, methyl ester
6	15.366	77696	0.02	7,10-Hexadecadienoic acid, methyl ester
7	15.427	429673	0.12	7-Hexadecenoic acid, methyl ester, (Z)-
8	15.472	5990822	1.73	9-Hexadecenoic acid, methyl ester, (Z)-
9	15.687	66165628	19.09	Hexadecanoic acid, methyl ester
10	16.433	415369	0.12	cis-10-Heptadecenoic acid, methyl ester
11	16.641	697036	0.20	Heptadecanoic acid, methyl ester
12	17.368	130178261	37.56	9,12-Octadecadienoic acid (Z,Z)-, methyl
13	17.429	90209769	26.03	9-Octadecenoic acid (Z)-, methyl ester
14	17.597	45754922	13.20	Methyl stearate
15	18.253	355506	0.10	9,12-Octadecadienoyl chloride, (Z,Z)-
16	19.134	669553	0.19	cis-11-Eicosenoic acid, methyl ester
17	19.333	2044008	0.59	Eicosanoic acid, methyl ester
18	20.780	429496	0.12	13-Docosenoic acid, methyl ester, (Z)-
19	20.955	231613	0.07	Docosanoic acid, methyl ester
20	22.458	256621	0.07	Tetracosanoic acid, methyl ester
21	23.194	666524	0.19	Squalene
22	23.777	1151668	0.33	Stigmasterol
		346610451	100.00	

Major constituent of *Jatropha Carcus* oil are:

Z,Z-9,12-octadecadienoic acid methyl ester (37.56 %)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.2.The peak at m/z294(R.T. 17.368 -in total ion chromatogram) corresponds M+$[C1_9H_{34}O_2]^+$. The signal at m/z263 corresponds to loss of a methoxyl function.

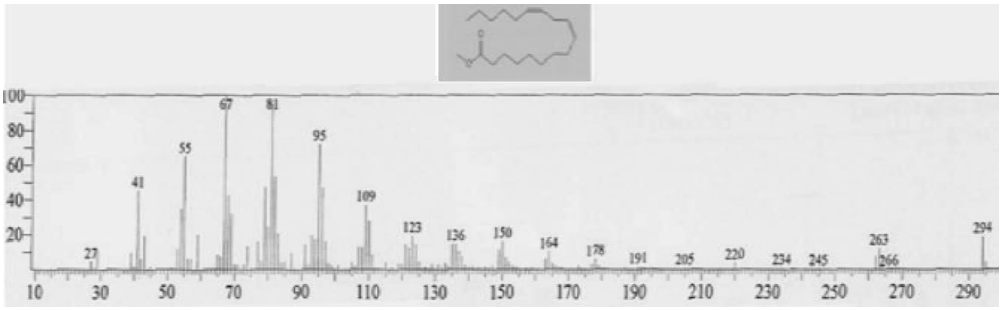

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

Z-9-Octadecenoic acid methyl ester (26.03 %)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.429 in total ion chromatogram, corresponds M+$[C_{19}H_{36}O_2]$+, while the peak at m/z266 accounts for loss of a methoxyl.

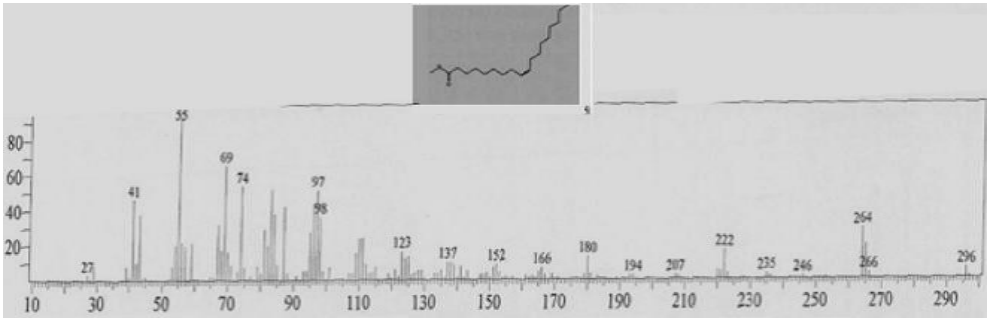

Figure 3: Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester (19.09 %)

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270 (R.T.15.687) corresponds M+$[C_{17}H_{34}O_2]^+$ .The signal at m/z239 corresponds to loss of a methoxyl**.**

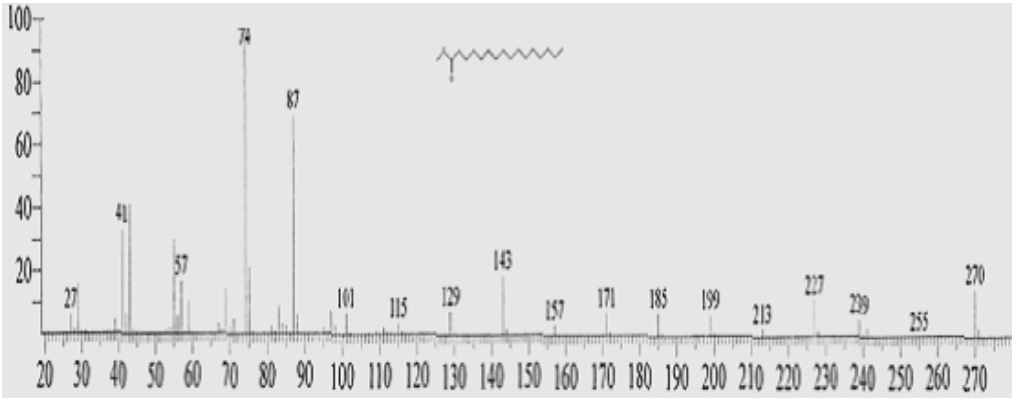

Figure 4: Mass spectrum of hexadecanoic acid methyl ester

Methyl stearate (13.20 %)

Fig. 5 shows the mass spectrum of methyl stearate .The signal at m/z 298(R.T.17.597) corresponds M+$[C_{19}H_{38}O_2]^+$, while the peak at m/z267 corresponds to loss of a methoxyl group.

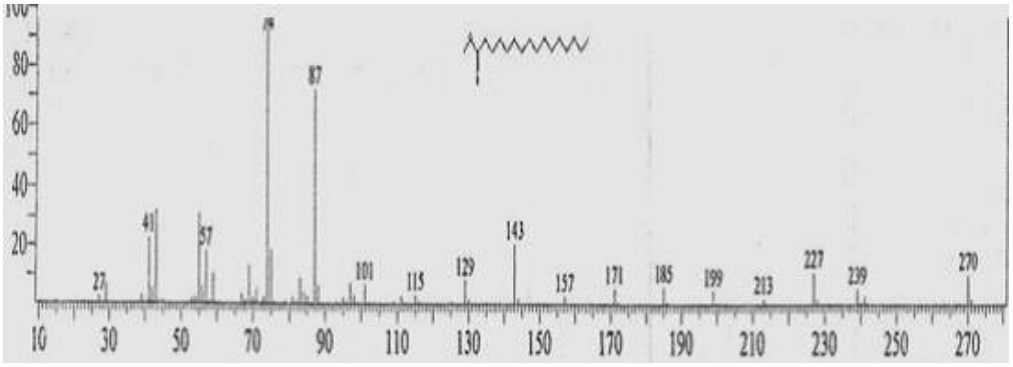

Figure 5: Mass spectrum of methyl stearate

3.2. Antimicrobial activity

The oil was screened for antimicrobial activity against standard microorganisms using the cup plate diffusion bioassay. The average of the diameters of the growth inhibition zones are shown in Table (5). The results were interpreted as follows: (<9mm: inative; 9-12mm: partially active; 13-18mm: active;>18mm:very active). Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

The oil showed excellent activity against *Escherichia coli* and excellent anticandidal activity (Table 5)..

Table 5: Antibacterial activity of *Jatropha curcas* oil

Type		Sa	Bs	Ec	Ps	Ca
Oil	100	14	--	20	--	21

Table 6: Antibacterial activity of standard chemotherapeutic

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

Table 7: Antifungal activity of standard chemotherapeutic agent

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

Sa.*: Staphylococcus aureus*
Ec.*: Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: Bacillus subtilis
M.D.I.Z: Mean diameter or growth inhibition zone (mm).

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