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Research Article

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GC-MS Analysis and Antimicrobial Activity of Sudanese *Corchorus olitorius* L. (Tiliaceae) oil

Faiza, I.[1], Abdel Karim M[2]*, Weam, M.A. Khojali[3]

[1]University of Hail, Faculty of Science, Saudi Arabia
[2]Sudan University of Science and Technology, Faculty of Science, Sudan
[3]University of Hail, Faculty of Pharmacy, Saudi Arabia

Abstract *Corchorus olitorius* L. is a vegetable in the family Tiliaceae. It is widely grown in northern and central Sudan both under irrigation and rain-fed conditions. Seeds, leaves, roots and stems are used in traditional medicine. Leaves are used as demulcent, tonic and laxative. Leaves are also used against cystitis, dysuria and gonorrhea. Seeds are laxative and roots are used for toothache

In this study *Corchorus olitorius* oil has been analyzed by GC-MS. The analysis showed 22 components. Major constituents are: 9,12-octadecadienoic acid (Z,Z)-, methyl ester(46.95%); hexadecanoic acid, methyl ester (23.29%); methyl stearate (11.61%); 9,12 15- octadecatrienoic acid, 2,3-dihydroxypropyl ester (4.81%) and eicosanoic acid, methyl ester (4.34%).

The anitmicrobial activity of the essential oil has been assessed. The oil showed significant activity against *Bacillus subtilis*. It also exhibited significant anticandidal potency. It showed moderate activity against other test microorganisms.

Keywords *Corchorus olitorius*, Oil, GC-MS Analysis, Antimirobial Activity

Introduction

Recently their have been extensive research work in the field of phytochemistry and pharmacological effects of plant secondary metabolites. This is primarily due to their pivotal role in drug design and drug discovery. Medicinal plants constitute a vital source for pharmaceutical drugs [1-20].

Corchorus olitorius L. is a vegetable in the family Tiliaceae. It is widely grown in northern and central Sudan both under irrigation and rain-fed conditions. *Corchorus olitorius* is a tall herb, usually annual, reaching a height of 2.4 m. The plant could be unbranched, or with only a few side branches. The flowers are yellow, with five petals. The fruit is a multi-seeded capsule [21]. The plant is widely spread all over the tropics and it probably exists in all countries of tropical Africa. It is consumed as spontaneous vegetable by rural communities in most parts of Africa [22]. Seeds, leaves, roots and stems are used in traditional medicine [23-27]. Leaves are used as demulcent, tonic and laxative. Leaves are also used against cystitis, dysuria and gonorrhea [23-27]. Seeds are laxative and roots are used for toothache .Stems are used for treating cardiovascular disorder [21-22].

Materials and Methods

Plant material

Seeds of *Corchorus olitorius* were collected from Shambat-Khartoum (Sudan). The plant was authenticated by direct comparison with a 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

Corchorus olitorius oil was evaluated for antimicrobial potential 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

Extraction of oil

Powdered seeds of *Corchorus olitorius* (350g) were exhaustively extracted with n-hexane (maceration). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work. For GC-MS analysis the oil was esterified as follows: 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 shaken 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 shaken 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 oil of *Corchorus olitorius* was studied by gas chromatography– mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

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

Table 3: 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

Antimicrobial test

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antimicrobial activity of the oil.

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.

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.

(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 sample. Separate Petri dishes were designed for positive control.

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

After incubation, the diameters of the resultant growth inhibition zones were recorded as average of two replicates.

Results and Discussion

Corochorus olitorius fixed oil was successfully extracted from seeds by maceration using hexane as solvent. The oil was analyzed by GC-MS which revealed the different constituents of the oil. In cup plate agar diffusion bioassay, the oil was assessed for antimicrobial activity against 5 standard human pathogens.

GC- MS analysis of *corochorus olitorius* oil

The GC-MS analysis of *corochorus olitorius* oil showed 22 components dominated by:

-9,12-octadecadienoic acid (Z,Z)-, methyl ester (46.95%).

-Hexadecanoic acid, methyl ester (23.29%).

-Methyl stearate (11.61%).

-9,12,15- Octadecatrienoic acid , 2,3-dihydroxy propyl ester (4.81%).

-Eicosanoic acid, methyl ester (4.34%)

The total ions chromatograms is displayed in Fig. 1, while Table 4 shows different constituents of the oil.

Table 4: constituent of *corochorus olitorius* oil

No.	Name	RT.	Area %
1	Dodecanoic acid methyl ester	11.219	0.02
2	Methyl tetradecanoate	13.530	0.17
3	6-Octadecenoic acid methyl ester	14.339	0.03
4	5-Octadecenoic acid methyl ester	14.444	0.02
5	7 10-Hexadecadienoic acid methyl ester	15.333	0.04
6	7-Hexadecenoic acid methyl ester	15.393	0.05
7	9-Hexadecenoic acid methyl ester	15.436	0.28
8	Hexadecanoic acid methyl ester	15.657	23.29
9	Hexadecanoic acid 14-methyl methyl ester	16.337	0.03
10	Heptadecanoic acid methyl ester	16.608	0.19
11	9 12-Octadecadienoic acid methyl ester	17.350	46.95
12	9-Octadecenoic acid methyl ester	17.370	2.99
13	9 12 15-Octadecatrienoic acid methyl ester	17.395	4.81
14	Methyl stearate	17.556	11.61

15	Cis-11-Eicosenoic acid methyl ester	19.009	0.66
16	Eicosanoic acid methyl ester	19.300	4.34
17	Heneicosanoic acid methyl ester	20.125	0.11
18	Docosanoic acid methyl ester	20.919	2.97
19	Tricosanoic acid methyl ester	21.685	0.19
20	Tetracosanoic acid methyl ester	22.422	0.19
21	Pentacosanoic acid methyl ester	23.138	0.10
22	Hexacosanoic acid methyl ester	21.824	0.24
			100%

Major components of *corochorus olitorius* oil are discussed below:

The mass spectrum of 9,12-octadcadienoic acid (z,z)-,methyl ester is shown in Fig. 2, the peak at m/z 294 corresponds $M^+[C_{19}H_{34}O_2]^+$, while the signal at M/Z 263 corresponds loss of methoxyl. Fig. 3 shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270 is due to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z 239 accounts for loss of methoxyl function. The mass spectrum of methyl stearate is displayed in Fig. 4. The signal which appeared at m/z 298 corresponds $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 is attributed to loss of methoxyl. Fig. 5 illustrates the mass spectrum of 9,12,15- octadecatrienoic acid , 2,3-dihydroxy propyl ester, the peak at m/z 352 corresponds $M^+[C_{21}H_{36}O_4]^+$. The signal at m/z 321 is due to loss of methoxyl. In Fig. 6 (mass spectrum of eicosanoic acid methyl ester), the peak at m/z 326 corresponds to $M^+[C_{21}H_{42}O_2]^+$ while the signal at M/Z 295 accounts for loss of methoxyl group.

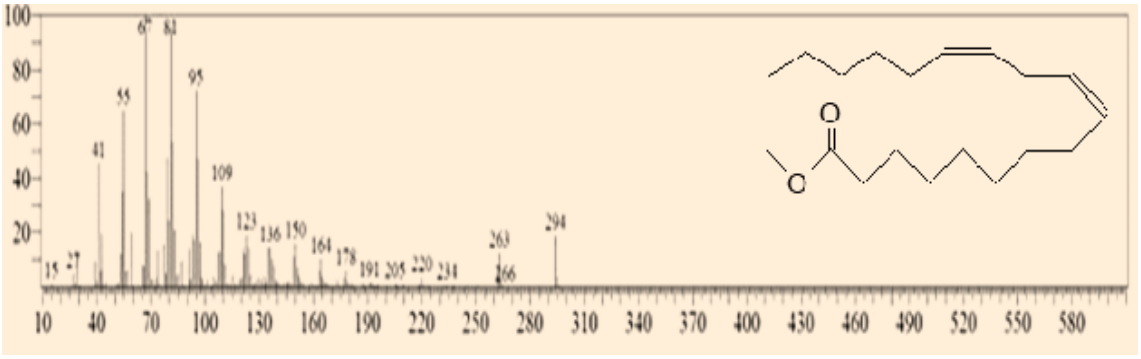

Figure 2: Mass spectrum of 9,12-octadcadienoic acid (z,z)-,methyl ester

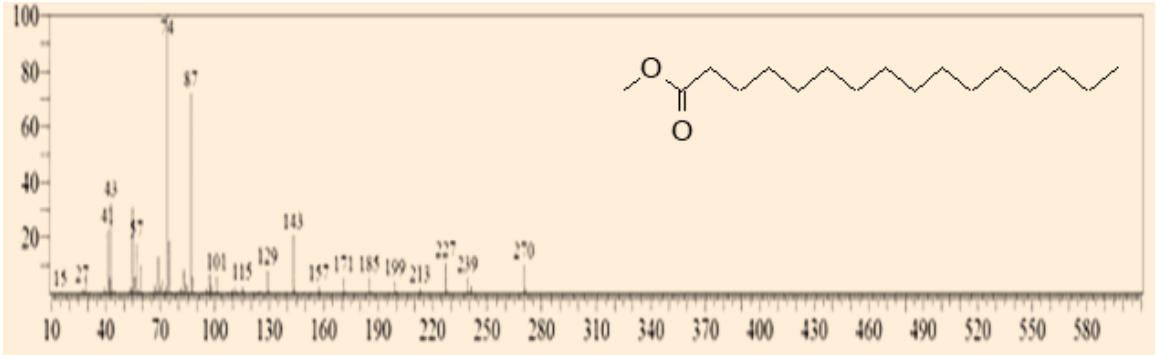

Figure 3: Mass spectrum of hexadecanoic acid ,methyl ester

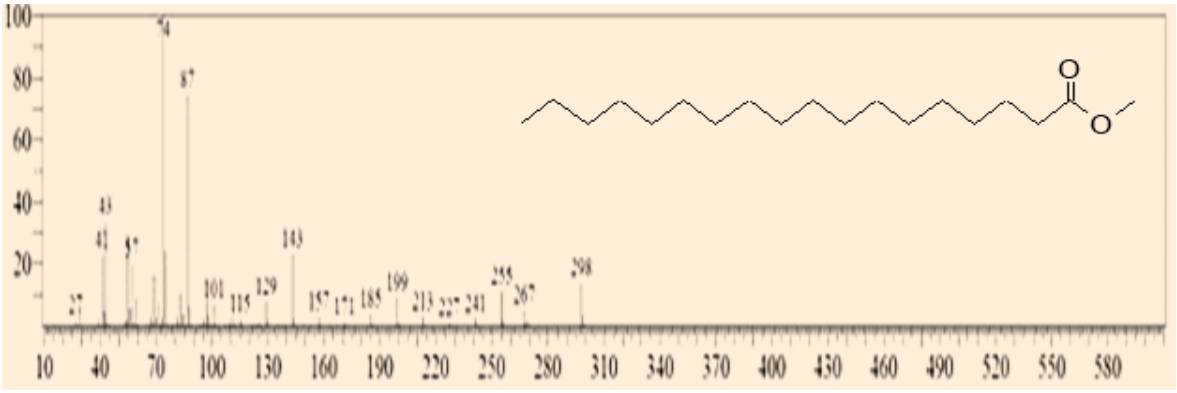

Figure 4: Mass spectrum of methyl stearate

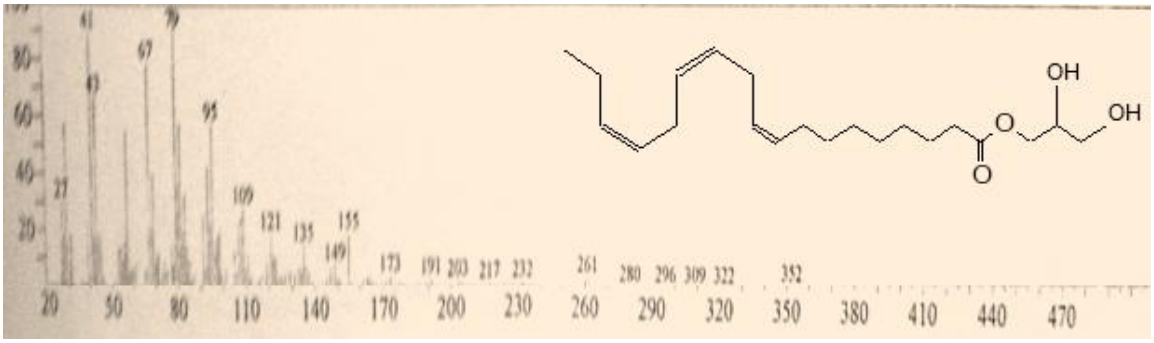

Figure 5: Mass spectrum 9,12,15,-octadecatrieonic acid-2,3-dihydroxy ester

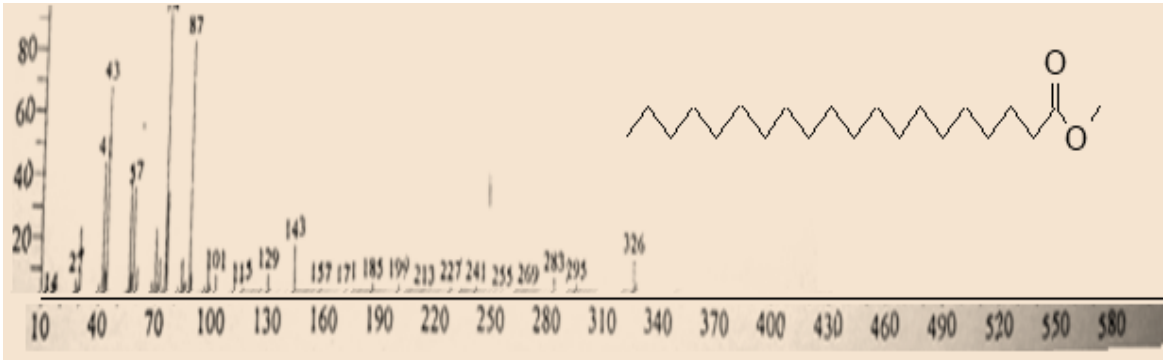

Figure 6: Mass spectrum of eicosanoic acid, methyl ester

Antimicrobial test

corochorus olitorius oil was assessed for antimicrobial activity via the cup plate agar diffusion bioassay using five standard human pathogens. The average of the diameters of the growth of inhibition zones are shown in Table (5). The results were interpreted in terms of the commonly used terms (<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.

Table 5: Antibacterial activity of *corochorus olitorius* seed oil: M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca
corochorus olitorius oil	100	14	14	13	17	20

Table 6: Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)

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 7: Antifungal activity of standard chemotherapeutic agent

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

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

The oil showed significant activity against *Bacillus subtilis*. It also exhibited significant anticandidal potency. It showed moderate activity against other test microorganisms.

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