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GC-MS analysis and antimicrobial activity of Sudanese *Ruta graveolens* L. (Rutaceae) fixed oil

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Abstract
The present study was designed to investigate the chemical constituents of *Ruta graveolens* Linn. Seed oil and to assess its antimicrobial activity. Forty four components were detected by GC-MS analysis. Major components are: 9,12-octadecadienoic acid methyl ester(27.79%); 9,12,15-octadecatrienoic acid methyl ester(14.79%); hexadecanoic acid methyl ester(11.29%); methyl stearate(7.49%); 1,4-dihydroxy-3-(3-methyl-2-butenyl) - 2-naphthalenecarboxylic acid (7.04%); 9-octacecenoic acid methyl ester (5.43%).
The antimicrobial activity of the oil was evaluated using the diffusion assay against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseud*om*onasa aeruginos*a and the fungi *Candida albicans* and *Aspergillus niger*. The oil showed excellent activity against *Bacillus subtilis* at all test concentrations. It also gave significant activity against all test organisms in the range: 100-50mg/ml.

Keywords: *Ruta graveolens*, fixed oil, GC-MS, antimicrobial activity

Introduction
Rue-*Ruta graveolens* – is a perennial herb in the order Sapindales of the family Rutaceae. The plant, which is cultivated in many countries for its economic importance,, is native to the Mediterranean region. Rue, being an important medicinal plant is included in the European Pharmacopoeia [1]. The plant finds diverse application in herbal medicine. It has been used as emenagogue and in treatment of rheumatism, dermatitis, eye problems and inflammation [2]. The plant contains rutin [3] which increases visual sharpness [1]. The spasmolytic, antiinflammatory and antihistamine activities of Rue volatile oil have been documented [4]. Different extracts of Rue have been used as antidote against snake and scorpion venoms [5]. The oil is also used against eczema, psoriasis and intestinal disorders. It was reported that the oil has a depressing effect on the central nervous system and in high doses it is a narcotic poison [1].
Many phytochemicals including: alkaloids, terpenoids, flavonoids and coumarins have been reported from *Ruta graveolens* [6]. Some studies documented the antiinflammatory, antioxidant [3], antidiabetic [7], antimicrobial [8] and antiandrogenic [9] activities of Rue. It was also demonstrated that the plant displays ant nociceptive properties [10].
Ruta graveolens contains rutin (about 2%) as a major phenolic. According to some epidemiological studies, flavonoids - through their significant free radical scavenging capacity - can prevent cancer and cardiovascular diseases [11-13]. The antibacterial, antidiarrheal, antiulcer, antimutagenic, anti-inflammatory properties of rutin has been outlined [14].

Materials
Plant Material
Seeds of *Ruta graveolens* were purchased from the local market-Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

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
Ruta graveolens oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table (1).

Table 1: Test organisms

Ser. 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
Phytochemical screening
The plant was screened for major secondary metabolites according to the method described by Harborne [15].

Extraction of oil from seeds of *Ruta graveolens*

Powdered seeds of *Ruta graveolens* (400g) were macerated with n-hexane at room temperature. The solvent was removed under reduced pressure to afford the oil.

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

Ruta graveolens oil was studied by GC-MS. 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. Oven temperature program and other chromatographic conditions are depicted in Table 2.

Table 2: Chtomatographic conditions

Rate (min^{-1})	Temp.^oC	Hold Time
--	150	1.00
4.00	300	0.00
Column oven temperature		150.0^oC
Injection temperature		300.0^oC
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

Bacterial growth was maintained on Muller Hinton agar, while fungal growth was accomplished on a layer of Sabouraud dextrose agar. The media were prepared according to the manufacturer instructions. The media were inoculated with 100µl of each bacterial suspension to afford 106 UFC.

After drying, in sterile hood, disks(6mm in diameter) were soaked with test solutions, placed on the surface of the media and incubated at 35^oC for 24h(for bacteria) and at 25°C for four days (for fungi). After incubation, the diameters (in mm) of the resultant growth inhibition zones were measured

Results and Discussion

Phytochemical screening

The plant was screened for major secondary metabolites. The screening revealed the presence of alkaloids, steroids, terpenoids, flavonoids and tannins.

GC-MS analysis

The GC-MS analysis of the oil showed the presence of 44 constituents (Table 3). The total ion chromatogram is displayed in Fig.1.

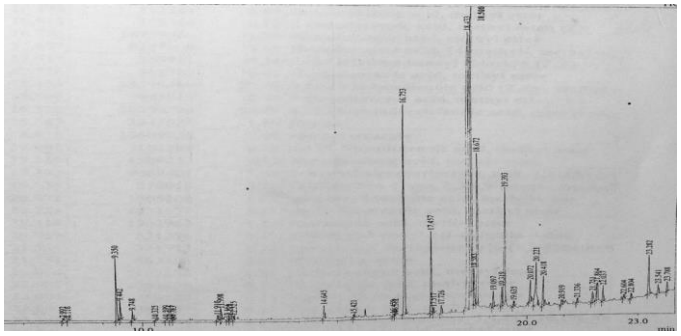

Fig 1: Total ions chromatogram of Rue oil

Table 3: Constituents of Rue oil

k#	R.Time	Area	Area%	Name
1	7.992	143652	0.10	L-.alpha.-Terpineol
2	8.116	135016	0.10	Decanal
3	9.350	3796565	2.68	2-Undecanone
4	9.442	1151117	0.81	2-Tridecanol
5	9.748	613222	0.43	Decanoic acid, methyl ester
6	10.325	67199	0.05	2-Dodecanone
7	10.599	58872	0.04	.alpha.-Cubebene
8	10.715	127808	0.09	2-Dodecanone
9	10.783	38326	0.03	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1
10	11.910	330184	0.23	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met
11	11.998	628490	0.44	2-Tridecanone
12	12.166	131828	0.09	.alpha.-Farnesene
13	12.225	145592	0.10	.beta.-Bisabolene
14	12.325	271524	0.19	Dodecanoic acid, methyl ester
15	14.645	814906	0.58	Methyl tetradecanoate
16	15.421	201912	0.14	Tridecanoic acid, 12-methyl-, methyl ester
17	16.458	122304	0.09	Heneicosanoic acid, methyl ester
18	16.512	176254	0.12	6-Octadecenoic acid, methyl ester, (Z)-
19	16.753	15999666	11.29	Hexadecanoic acid, methyl ester
20	17.457	5227009	3.69	Hexadecanoic acid, 14-methyl-, methyl est
21	17.517	339921	0.24	9,12-Octadecadienoyl chloride, (Z,Z)-
22	17.726	591701	0.42	Heptadecanoic acid, methyl ester
23	18.433	39379489	27.79	9,12-Octadecadienoic acid (Z,Z)-, methyl e
24	18.500	7693955	5.43	9-Octadecenoic acid, methyl ester, (E)-
25	18.526	20959590	14.79	9,12,15-Octadecatrienoic acid, methyl este
26	18.583	2247522	1.59	Phytol
27	18.672	10609030	7.49	Methyl stearate
28	19.097	1104246	0.78	cis-10-Nonadecenoic acid, methyl ester
29	19.319	1309233	0.92	Nonadecanoic acid, methyl ester
30	19.393	9969401	7.04	2-Naphthalenecarboxylic acid, 1,4-dihydr
31	19.635	370018	0.26	1H-Xanthen-1-one, 2,3,4,9-tetrahydro-9-(2
32	20.072	1505208	1.06	.gamma.-Linolenic acid, methyl ester
33	20.221	4432677	3.13	cis-11-Eicosenoic acid, methyl ester
34	20.418	1962962	1.39	Eicosanoic acid, methyl ester
35	20.919	531296	0.37	2-Methyl-3-nonyl-1H-quinolin-4-one
36	21.336	331722	0.23	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
37	21.751	863203	0.61	Menthyl acetate
38	21.864	1182296	0.83	13-Docosenoic acid, methyl ester
39	22.037	1082590	0.76	Docosanoic acid, methyl ester
40	22.604	288129	0.20	Methyl 20-methyl-docosanoate
41	22.804	381907	0.27	Tricosanoic acid, methyl ester
42	23.282	3081344	2.17	2(1H)-Phenanthrenone, 3,4,4a,9,10,10a-he
43	23.541	640983	0.45	Tetracosanoic acid, methyl ester
44	23.788	660198	0.47	Fumaric acid, decyl 2-decyl ester
		141700067	100.00	

Major constituents are:

9, 12-octadecadienoic acid methyl ester (27.79%)

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T.18.433 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^{+\cdot}$The signal at mz263 corresponds to loss of a methoxyl.

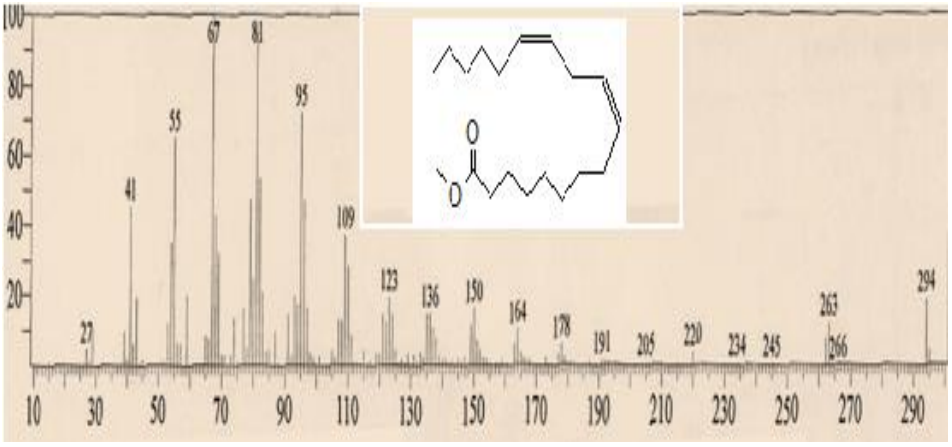

Fig 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

9, 12, 15-Octadecatrienoic acid methyl ester (14.79%)

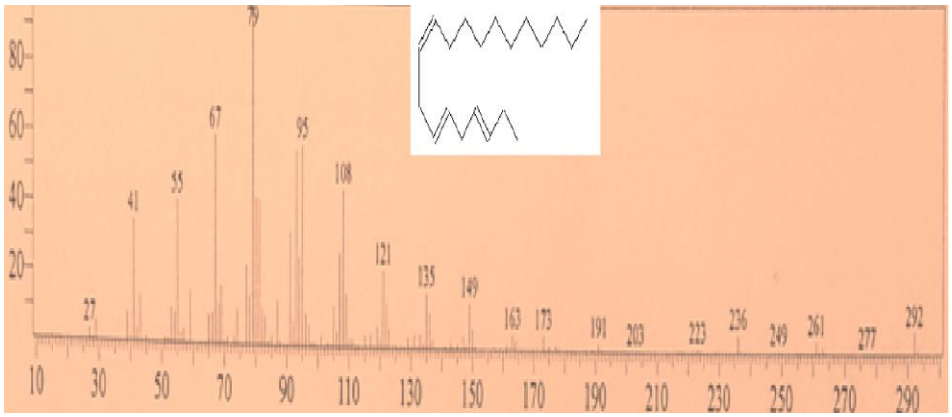

Fig 3: Mass spectrum of 9, 12, 15-octadecatrienoic acid methyl ester

The mass spectrum of 9, 12, 15-Octadecatrienoic acid methyl ester is depicted in Fig.3. The signal at m/z 292 (RT. 18.526) corresponds M^+ $[C_{19}H_{32}O_2]^+$. The peak at m/z277 is attributed to loss of a methyl group.

Hexadecanoic acid methyl ester(11.29%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4.The signal at m/z 270 (R.T. 16.753) corresponds $M^+[C_{17}H_{34}O_2]^{+\cdot}$The signal at m/z239 is due to loss of a methoxyl.

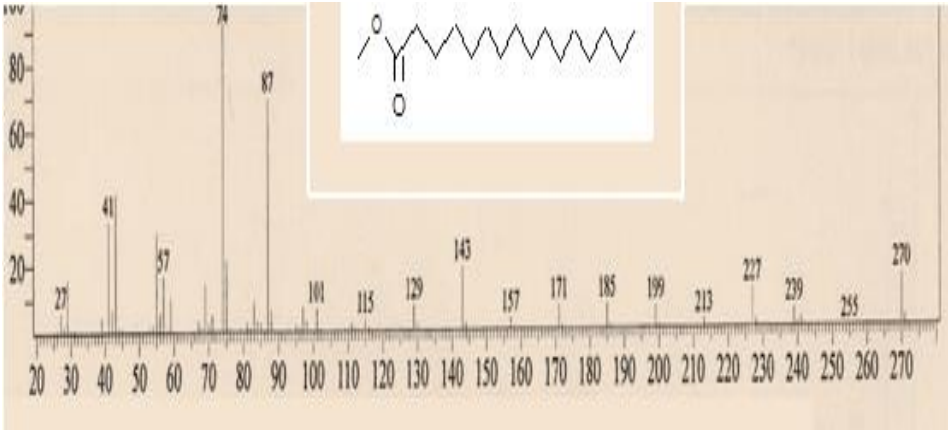

Fig 4: Mass spectrum of hexadecanoic acid methyl ester

Methyl stearate (7.49%)

Fig. 5 displays the mass spectrum of methyl stearate. The peak at m/z 298, which appeared at R.T.18.672 corresponds $M^+[C_{19}H_{38}O_2]^{+\cdot}$The signal at m/z267 corresponds to loss of a methoxyl function.

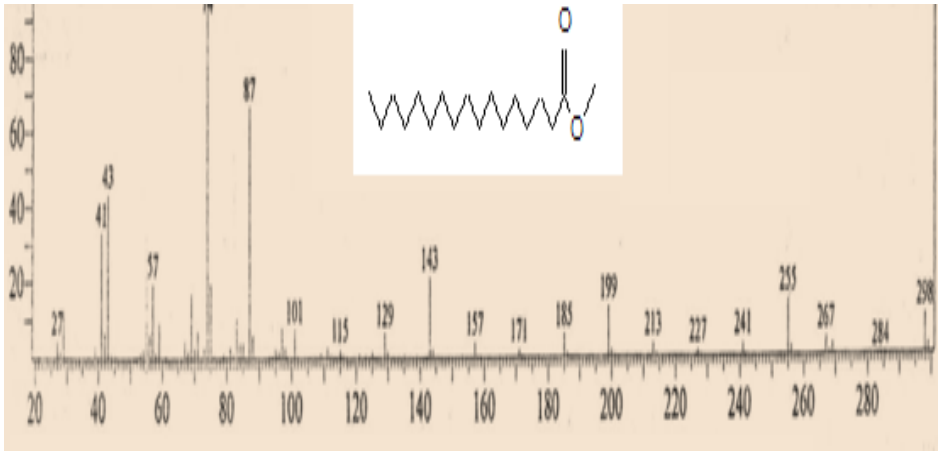

Fig 5: Mass spectrum of methyl stearate

1,4-Dihydroxy-3-(3-methyl-2-butenyl) - 2-naphthalenecarboxylic acid (7.04%)

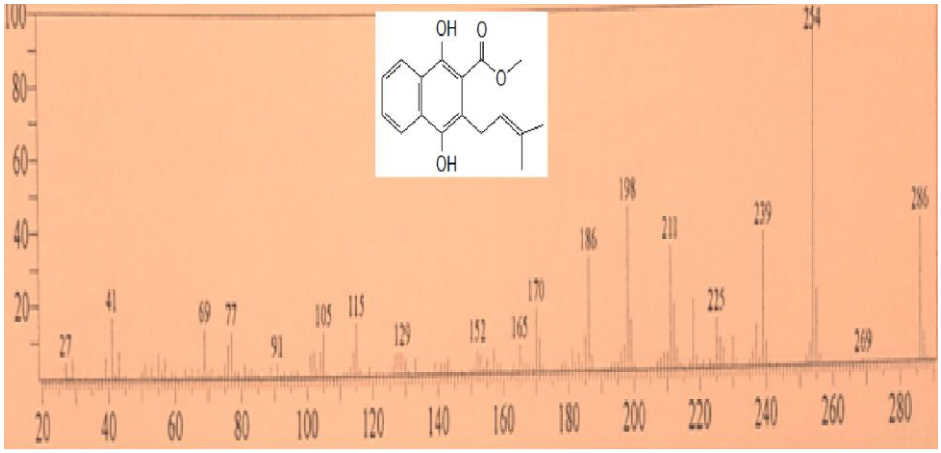

Fig 6: Mass spectrum of 1,4-Dihydroxy-3-(3-methyl-2-butenyl) - 2-naphthalenecarboxylic acid

The mass spectrum of 1,4-dihydroxy-3-(3-methyl-2-butenyl) - 2-naphthalenecarboxylic acid is displayed in Fig.6.The peak at m/z 286(R.T. 19.393) corresponds $M^{+}[C_{17}H_{18}O_4]^{+\cdot}$

9-Octacecenoic acid methyl ester (5.43%)

Fig.7 shows the mass spectrum of 9-octadecenoic acid methyl ester. The signal at m/z 296 (R.T. 18.500 in total ion chromatogram) corresponds $M^{+}[C_{19}H_{36}O_2]^{+\cdot}$ while the peak at m/z266 accounts for loss of a methoxyl.

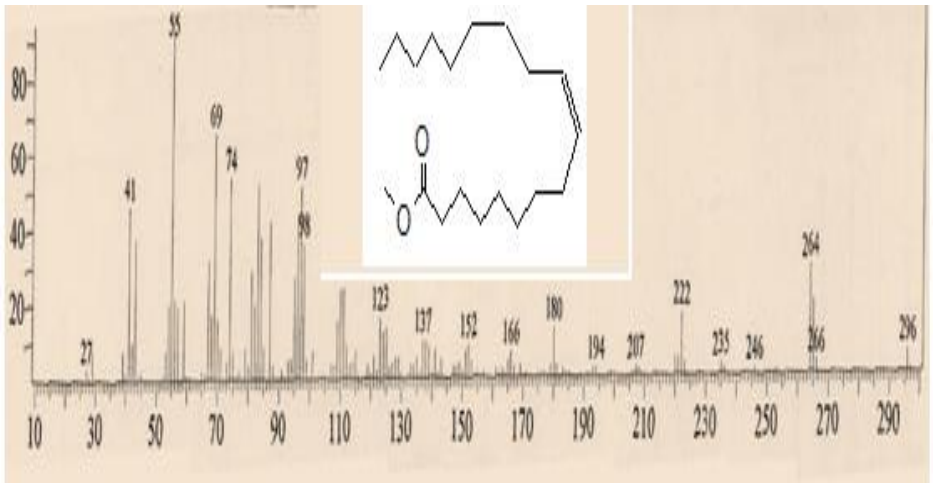

Fig 7: Mass spectrum of 9-octadecenoic acid methyl ester

Antimicrobial activity

The mean diameters of inhibition zone (MDIZ) produced by oil on standard microorganisms are presented in Table (4). The results were interpreted in commonly used terms : < 9 mm considered inactive; 9-12 mm partially active; 13-18 mm active and more than 18 mm very active. Results displayed in Table 4 demonstrate activity of oil against all test organisms in the range: 100 - 25mg/ml. The oil showed excellent activity against *Bacillus subtilis* in the range 100 – 12.50mg/ml. A significant anticandidial activity was also observed.

On the basis of its promising antimicrobial activity, it seems that this oil is a lead for further opimization. Tables 5 and 6 represent the antimicrobial activity of standard chemotherapeutic agents.

Table 4: Antimicrobial activity of *Ruta graveolens* oil

Sample	Inhibition zone diameter (mm / mg oil)				
	Bs **(G^+)**	***Sa*** **(G^+)**	***Ec.*** **(G^-)**	***Pa*** **(G^-)**	***Ca.***
Control Methanol	00	00	00	00	00
Oil *Ruta graveolens* (100mg/ml)	28	17	20	20	19
50mg/ml	27	16	18	16	18
25 mg/ml	26	15	15	15	18
12.500 mg/ml	25	11	12	14	17-
6.25 mg/ml	24	--	--	--	11

Table 5: Antibacterial activity of standard drugs

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 6: 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

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