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GC-MS analysis and antimicrobial Activity of Sudanese *Terminalia laxiflora* engl. and diels fixed oil

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Abstract
The present study was carried out to investigate the constituents of *Terminalia laxiflora* oil and to evaluate its antimicrobial potency. GC-MS analysis revealed the presence of 54 components. Main constituents are: 9-Z-octadecenoic acid methyl ester (17.65%%), hexadecanoic acid methyl ester (16.16%), 9, 12-Z, Z-octadecadienoic acid methyl ester (15.32%), methyl stearate (8.10%).
The antimicrobial activity of the oil was assessed against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginos*a and the yeast *Candida albicans*. The oil showed good activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. However, It exhibited partial activity against *Pseudomonas aeruginosa* and *Candida albicans.* It seems that the oil is a lead for further optimization.

Keywords: *Terminalia laxiflora*, fixed oil, GC-MS, antimicrobial activity

Introduction
The genus *Terminalia*, which comprises about 200 species, is the second largest genus in the family Cumbretaceae. This genus is distributed in tropics and the greatest genetic diversity is found in south-east Asia [1]. Mostly, Terminalia species are large trees reaching up to 75m in height [2].
Terminalia is a genus of many attributes, due to diverse phytochemical constituents and various bioconstituents, it is widely used in ethnomedicine against a wide array of diseases including bacterial infections, skin diseases, sore throat, diarrhea, colds, abdominal disorders, dysentery, fever, jaundice, leprosy, hookworms, hypertension, cancer, ulcers, diabetes, and heart diseases [3, 4].
The bark of *Terminalia arjuna* is used traditionally as cardioprotective and antihyperlipidemic [6], while *Terminalia mollis* is aremedy for gonorrhea, diarrhea, HIV and malaria. *Terminalia chebula* fruits are used in Tibet region against a wide spectrum of ailments [7, 8]. *Terminalia brachystemma* is used traditionally against gastrointestinal disorders and schistosomiasis [9].
Different *Terminalia* species have been screened for major secondary metabolites [10] and several bioactive constituents such as flavonoids, tannins, alkaloids and terpenoids have been detected.
Terminalia laxiflora Engl. And Diels. is a large tree (up to 15m in height) in the family Combretaceae [11]. This species is very common in semi-humid and woodland savannah of Sudan.The plant is used by local healers to treat malaria and cough [12]. A wood "smoke bath" is practiced by Sudanese women for beautification purposes and traditional claims assume relief of rheumatic pains [13].
The plant is reported as: antimicrobial [14], anticancer [15], anti-inflammatory [16], antidiabetic [17], Hypocholesterolic [18], antiulcer [19], anticaries [20], anti-HIV-1, antimalarial [21] and antioxidant [22].
In a biologically-guided fractionation scheme of wood methanolic extract, the antiacne and free radical scavenging capacity of some bioconstituents have been demonstrated [32].

Materials and Methods
Materials
Plant Material
Seeds of *Terminalia laxiflora* were collected from Khartoum state (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
Terminalia laxiflora oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus*(G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli*(G-ve), *Candida albicans* (fungus).

Methods
Extraction of Oil from Seeds of *Terminalia laxiflora*
Powdered seeds of *Terminalia laxiflora* (300g) were exhaustively extracted with n-hexane (soxhlet). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

GC-MS Analysis

GC-MS analysis of *Terminalia laxiflora* oil was performed on a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are depicted in Tables 1 and oven temperature program is displayed below:

Rate	Temperature (^{0}C)	Hold Time (Min^{-1})
--	1	1.00
4.00	300.0	0.00

Table 1: Chromatographic conditions

Column oven temperature	**150.0ºC**
Injection temperature	300.0ºC
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

Testing for antimicrobial Activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil. Briefly, (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 one of a test solution. The agar discs were removed, alternate cup were filled with 0.1 ml of test samples 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 in two replicates and averaged as indicator of antimicrobial activity.

For antifungal activity Sabouraud dextrose agar was used as growth medium for fungi and incubation continued for 72h at 35ºC.

Results and Discussion

The oil of *Terminalia laxiflora* was analyzed by GC-MS and furthermore evaluated for its antimicrobial activity.

GC-MS Analysis of Oil

GC-MS analysis of *Terminalia laxiflora* oil was carried out. Identification of the constituents was based on the MS library (NIST) (a 90-95% match was observed). Also the observed fragmentation pattern was discussed. Fifty four constituents were detected by GC-MS analysis. The typical total ion chromatogram (TIC) is displayed in Fig. (1)- see also Table 2.

Table 2: Contituents of *Terminalia laxiflora* Oil

Peak#	R.Time	Area	Area%	Name
1	3.562	159085	0.07	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-meth
2	4.136	236717	0.10	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-me
3	4.684	41773	0.02	2-Carene
4	4.845	45335	0.02	Cyclohexene, 1-methyl-4-(1-methylethenyl)
5	5.246	35396	0.02	.gamma.-Terpinene
6	5.780	43048	0.02	1,6-Octadien-3-ol, 3,7-dimethyl-
7	6.080	39361	0.02	Octanoic acid, methyl ester
8	6.255	34167	0.02	Cyclohexane, (1-methylethylidene)-
9	6.455	32391	0.01	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-n
10	6.747	49541	0.02	Benzenepropanal
11	6.970	45117	0.02	Terpinen-4-ol
12	7.154	247881	0.11	.alpha.-Terpineol
13	7.248	92824	0.04	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-di
14	7.885	125174	0.05	2-Butanone, 4-phenyl-
15	7.953	79108	0.03	Geraniol
16	8.857	47129	0.02	Decanoic acid, methyl ester
17	9.154	47099	0.02	Cyclohexene, 4-ethenyl-4-methyl-3-(1-meth
18	9.286	2038237	0.89	3-Cyclohexene-1-methanol, .alpha.,.alpha.,
19	9.889	130952	0.06	.gamma.-Muurolene
20	10.427	138317	0.06	Guaia-1(10),11-diene
21	11.013	330595	0.15	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-met
22	11.100	581174	0.26	1,6-Cyclodecadiene, 1-methyl-5-methylene-
23	11.172	155154	0.07	.beta.-curcumene
24	11.267	151481	0.07	.alpha.-Farnesene
25	11.331	127211	0.06	Isocaryophillene
26	11.491	151366	0.07	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7
27	11.537	154528	0.07	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-
28	11.572	113459	0.05	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-d
29	11.909	339011	0.15	Cyclohexanemethanol, 4-ethenyl-.alpha.,.a
30	11.971	455207	0.20	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-,
31	12.887	397902	0.17	.gamma.-HIMACHALENE
32	13.026	135646	0.06	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-
33	13.200	438742	0.19	2-Naphthalenemethanol, decahydro-.alpha
34	13.574	212193	0.09	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8
35	13.747	565638	0.25	Methyl tetradecanoate
36	14.471	95304	0.04	cis,cis-7,10,-Hexadecadienal
37	14.559	182422	0.08	6-Octadecenoic acid, methyl ester, (Z)-
38	14.665	137458	0.06	5-Octadecenoic acid, methyl ester
39	14.825	197474	0.09	Pentadecanoic acid, methyl ester
40	15.663	1200525	0.53	9-Hexadecenoic acid, methyl ester, (Z)-
41	15.874	36807104	16.16	Hexadecanoic acid, methyl ester
42	16.835	275864	0.12	Hexadecanoic acid, 15-methyl-, methyl este
43	17.528	34887333	15.32	9,12-Octadecadienoic acid (Z,Z)-, methyl e
44	17.583	40194800	17.65	9-Octadecenoic acid (Z)-, methyl ester
45	17.782	18454847	8.10	Methyl stearate
46	17.959	8464868	3.72	Z,Z-8,10-Hexadecadien-1-ol
47	19.137	1213509	0.53	9-Octadecynoic acid, methyl ester
48	19.182	2549996	1.12	1-Naphthalenepropanol, .alpha.-ethenylde
49	19.304	2076909	0.91	1,5,9-Cyclododecanetriol
50	19.420	1211262	0.53	Andrographolide
51	19.536	4355102	1.91	Eicosanoic acid, methyl ester
52	19.595	830592	0.36	PGH1, methyl ester
53	20.361	660777	0.29	Henicosanoic acid, methyl ester
54	20.791	2391471	1.05	E,E,Z-1,3,12-Nonadecatriene-5,14-diol

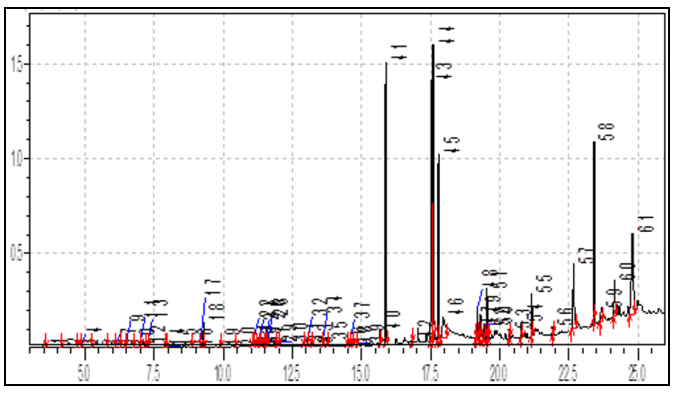

Fig 1: Total ion chromatograms

Main constituents of the oil are discussed below:

9-Z-Octadecenoic Acid Methyl Ester (17.65 %)

Fig. 2 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.583 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl.

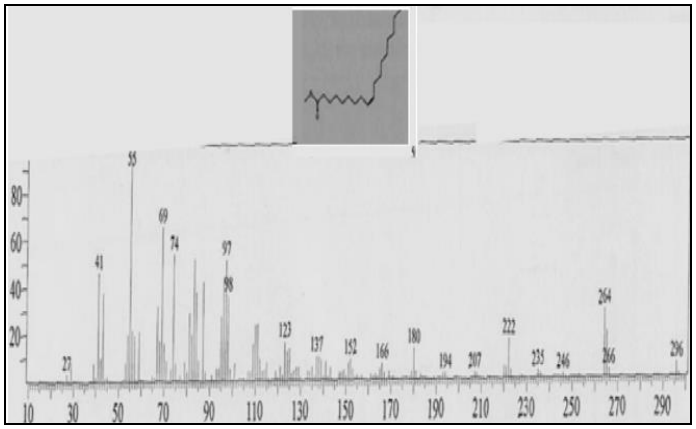

Fig 2: Mass spectrum of 9-Z-octadecenoic acid methyl ester acid methyl ester

Hexadecanoic Acid Methyl Ester (16.16%)

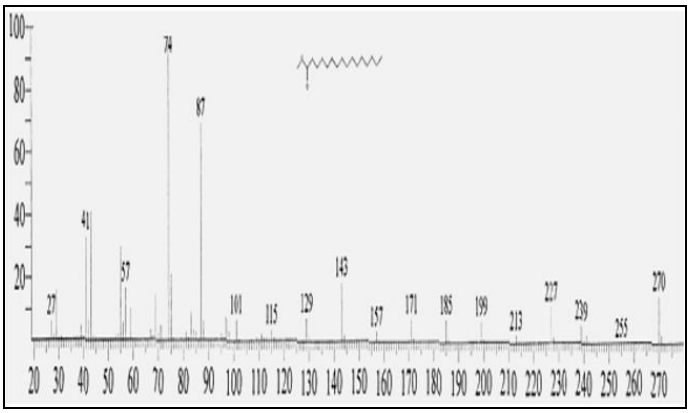

Fig 3: Mass spectrum of hexadecanoic acid methyl ester

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.3.The peak at m/z 270 (R.T.15.874) corresponds M^+ $[C_{17}H_{34}O_2]^+$. The signal at m/z239 corresponds to loss of a methoxyl.

9,12-Z, Z-Octadecadienoic Acid Methyl Ester (15.32%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is displayed in Fig. 4.The peak at m/z294 (R.T. 17.528-in total ion chromatogram) corresponds M^+ $[C_{19}H_{34}O_2]^+$. The signal at m/z263 corresponds methoxyl function.

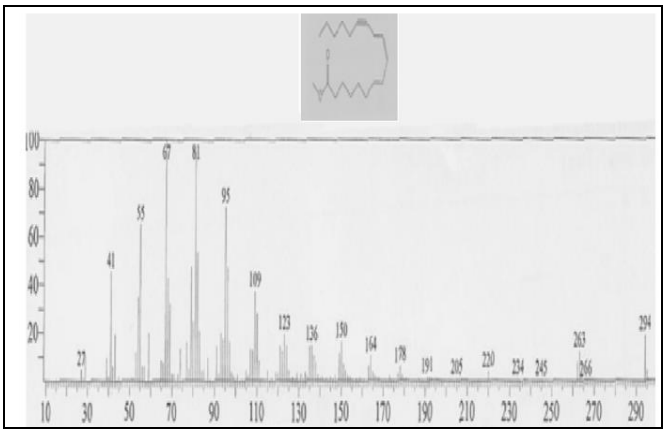

Fig 4: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

Methyl Stearate (8.10%)

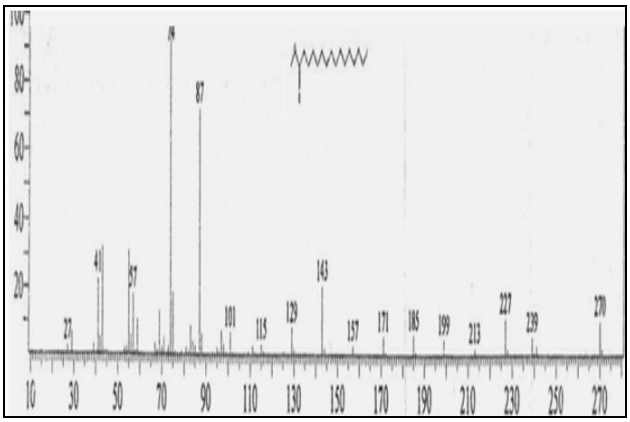

Fig 5: Mass spectrum of methyl stearate

Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T.17.782) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 corresponds to loss of a methoxyl group.

Antimicrobial Activity

Terminalia laxiflora oil was evaluated for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (3). Results were interpreted as follows: (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (4) and (5) show the antibacterial and antifungal activities of standard drugs respectively.

Table 3: Antibacterial activity of *Terminalia laxiflora* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	15	14	15	12	12

Table 4: Antibacterial activity of standard chemotherapeutic agents

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

Table 5: 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 Escherichia coli*
Ca: *Candida albicans*
Bs: *Bacillus subtilis*

The oil showed good activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. However, it exhibited partial activity against *Pseudomonas aeruginosa* and *Candida albicans.* It seems that the oil is a lead for further optimization.

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