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GC-MS analysis and antimicrobial activity of Sudanese *Khaya senegalensis* (Meliaceae) Fixed Oil

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Abstract
The medicinally important *Khaya senegalensis* is a large tree (up to 35m in height) in the family Meliaceae. The stem bark and leaves have been widely used in African system of medicine against many human disorders. The leaves are used against diarrhea and bacterial infections. *Khaya senegalensis* seed oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. Twenty three constituents were detected by GC-MS analysis. Major constituents are: 9-octadecenoic acid (Z) - methyl ester (47.43%); methyl stearate (18.86%); hexadecanoic acid, methyl ester (14.19%); 9, 12-octadecadienoic acid methyl ester (8.26%) and eicosanoic acid methyl ester (3.45%). In the cup plate agar diffusion bioassay, the oil showed good activity against G+ve *Staphylococcus aureus*.

Keywords: *Khaya senegalensis*, fixed oil, GC-MS analysis, antimicrobial activity

1. Introduction
Though it is mainly based on empirical operations and recipes, herbal medicine is still playing an important role [1-3] in primary health care in developing countries where modern medicine is often beyond affordability. Recently many research programs focused on the bioactivity of different phytochemicals that could serve as leads for drug discovery and drug development.
The medicinally important *Khaya senegalensis* is a large tree (up to 35m in height) in the family Meliaceae. The stem bark and leaves have been widely used in African system of medicine against many human disorders [4-10]. The leaves are used against diarrhea and bacterial infections. The antibacterial activity of the leaves aqueous extract has been documented [11]. Also the antioxidant activity of the leaves has been reported [12]. Leaves aqueous extract showed anti-hyperglycemic properties in model animals [13, 14] while the bark exhibited anti-proliferative and antiinflammatory effect [15] beside pro-apoptotic activity against some cells [16].

2. Materials and Methods
2.1 Materials
Plant material
Khaya senegalensis seeds were collected from Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, Institute of Medicinal and Aromatic Plants, Khartoum – Sudan.

Test organisms
The following standard microorganisms were used to assess the antimicrobial activity of *Khaya senegalensis* seed oil; G+ve: *Bacillus subtilis*, *Staphylococcus aureus*; G-ve: *Pseudomonas aeruginosa*, *Escherichia coli* and the fungal species *Candida albicans.*

2.2 Methods
Extraction of oil
Powdered seeds (300g) of *Khaya senegalensis* were macerated with n-hexan for 72h.The solvent was removed *in vacuo* and the oil was kept in the fridge at 4ºC for further studies. The oil was esterified by 7ml of alcoholic sodium hydroxide and 7ml of alcoholic sulphuric acid. After the usual workup (1µl) of the ester was injected in the GC-MS vial.

GC-MS analysis
The target oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument (RTX-5MS column: 30m, length; 0.25mm diameter; 0.25 µm, thickness). Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program and other chromatographic conditions are shown below.

Table 1: Oven temperature program

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

Column oven temperature: 150. 0ºC; Injection temperature: 300.0ºC; Injection mode: Split; 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 assay
Diffusion method was the method used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121ºC for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated

medium. (0.1ml) of the oil (concentration of 100mg/ml) was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37ºC for 24 hours for the bacteria and at 30ºC and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured with a transparent ruler and the results were recorded in millimeters.

3. Results and Discussion

GC-MS analysis of *Khaya senegalensis* oil

GC-MS analysis of *Khaya senegalensis* oil was carried out. Identification of the constituents was based on the retention times and the observed fragmentation pattern. 23 constituents were detected by GC-MS analysis. The typical total ion chromatogram (TIC) is displayed in Fig. (1). The constituents of the oil are shown in Table 2.

Table 2: Constituents of *Khaya senegalensis* oil

No.	Area %	Name
1	0.03	1,3-Cyclohexadiene
2	0.01	Alpha Farnesene
3	0.01	Beta Bisabolene
4	0.01	Dodecanoic acid methyl ester
5	0.03	3-(1,5-dimethyl-4-hexyl-) -Cyclohexene
6	0.14	Methyl tetradecanoate
7	0.07	9-Hexadecenoic acid methyl ester
8	0.35	7-Hexadecenoic acid methyl ester
9	14.19	Hexadecanoic acid methyl ester
10	0.50	n-Hexadecanoic acid
11	0.06	14-methyl- Hexadecanoic acid methyl ester
12	0.12	Cis-10-Heptadecenoic acid methyl ester
13	0.35	Heptadecenoic acid methyl ester
14	8.26	9,12-Octadecenoic acid methyl ester
15	47.43	9-Octadecenoic acid methyl ester
16	18.86	Methyl stearate
17	1.20	Oleic acid
18	0.47	11-Eicosenoic acid methyl ester
19	3.45	Eicosenoic acid methyl ester
20	2.07	1,2,3-Propanetriyl-9-Octadecenoic acid ester
21	1.13	Docosanoic acid methyl ester
22	0.48	Cholest-5-en-3-ol
23	0.77	Tetracosanoic acid methyl ester
	100.00	

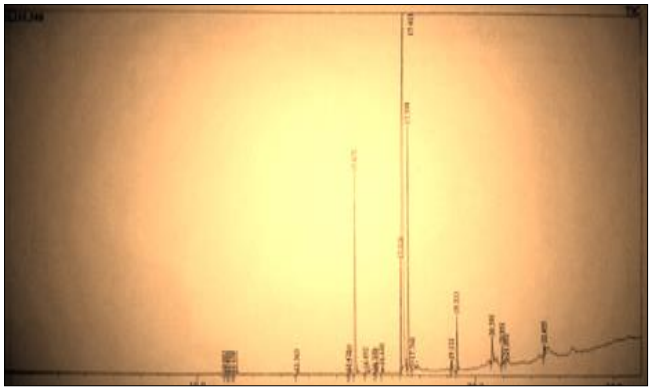

Fig 1: Total ion chromatograms

Main constituents of the oil are discussed below:

9-Octadecenoic acid methyl ester (47.43%)

The mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig.2. The peak at m/z296, which appeared at R.T. 17.415 in total ion chromatogram, corresponds M^+ [$C_{19}H_{36}O_2]^+$. The peak at m/z266 corresponds to loss of methoxyl function.

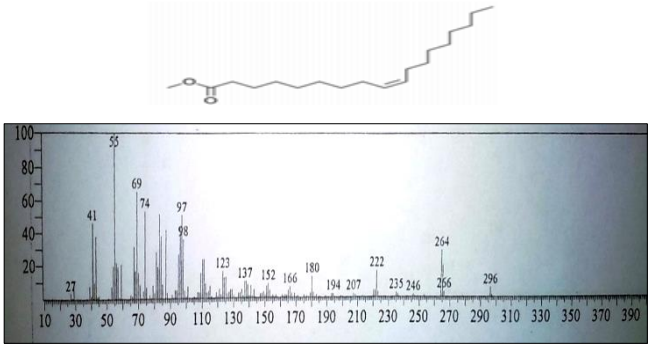

Fig 2: mass spectrum of 9-octadecenoic acid methyl ester

Methyl stearate (18.86%)

The mass spectrum of methyl stearate is shown in Fig.3. The peak at m/z 298 (R.T.17.595) is due to M^+ [$C_{19}H_{38}O_2$]$^+$, while the signal at m/z 267 correspond to loss of a methoxyl.

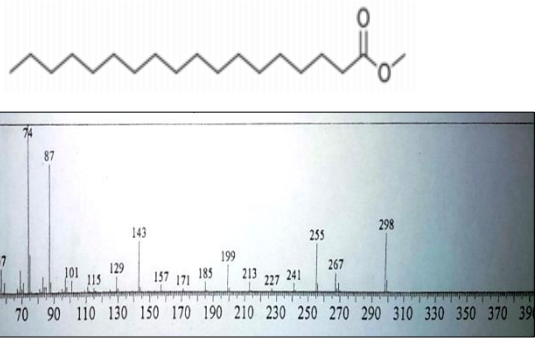

Fig 3: mass spectrum of methyl stearate

Hexadecanoic acid methyl ester (14.19%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.4. The peak at m/z 270, which appeared at R.T. 15.670 accounts for M^+ [$C_{17}H_{34}O_2]^{+\cdot}$ The signal at m/z 239 is due to loss of methoxyl.

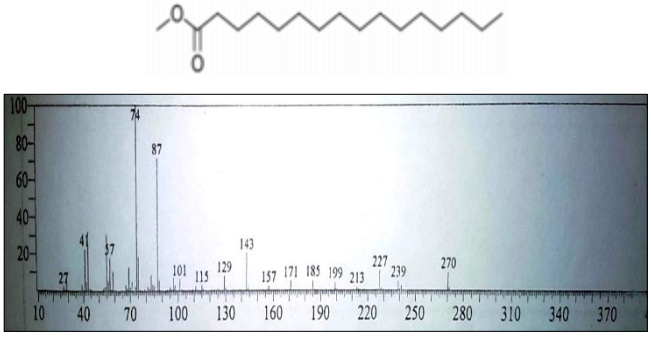

Fig 4: mass spectrum of Hexadecanoic acid, methyl ester

9, 12 Octadecadienoic acid methyl ester (8.26%)

Fig. 5 shows the mass spectrum of 9, 12-octadecadienoic acid methyl ester. The signal at m/z294, which appeared at R.T. 17.325 in total ion chromatogram, corresponds M^+ $[C_{19}H_{34}O_2]^+$. The peak at m/z 263 is attribted to loss of methoxyl function.

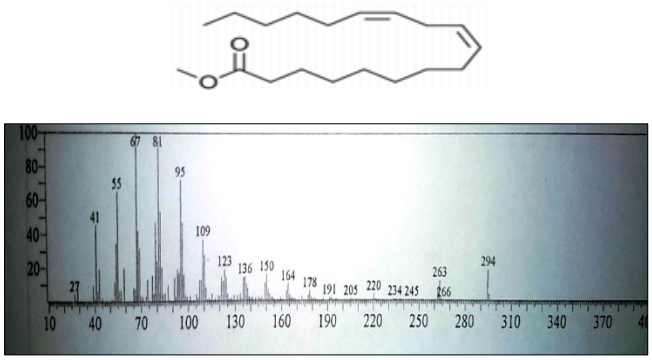

Fig 5: mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-methyl ester

Eicosanoic acid, methyl ester (3.45%)

The mass spectrum of eicosanoic acid, methyl ester is depicted in Fig.6. The peak at m/z 326 (R.T. 19.335) corresponds M^+ [$C_{21}H_{42}O_2$]$^+$, while the peak at m/z 295 accounts for loss of methoxyl.

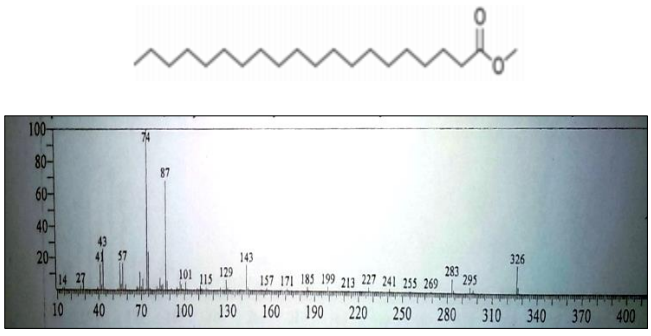

Fig 6: mass spectrum of eicosanoid acid, methyl ester

Antimicrobial activity of oil

Khaya senegalensis fixed 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 according to the following data:(< 9mm: inactive; 9-12 mm: partially active; 13- 18 mm: active; >18mm: very active). Evidently the oil showed significant activity against G+ve *Staphylococcus aureus*.

Table 3: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
Khaya senegalensis oil (100mg/ml)	-	-	15	-	-
	-	-		-	

Table 4: Antibacterial activity of standard drugs

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 drug

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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