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GC-MS analysis and antimicrobial activity of Sudanese *Eucalyptus camaldulensis* Dehn. (Myrtaceae) Fixed Oil

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Abstract
Eucalyptus camaldulensis fixed seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. Twenty nine components were detected by GC-MS analysis. Main constituents are: %), 9,12-octadecadienoic acid methyl ester(39.88%); 9-octadecenoic acid methyl ester(19.10%); Hexadecanoic acid methyl ester(14.99%) ; methyl stearate(9.06%) and tridecanedial (3.38%). antibacterial activity of the oil was evaluated via the diffusion assay against five standard human pathogens(Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseudomonasa aeruginos*a and the fungus *Candida albicans*). *Eucalyptus camaldulensis* oil showed excellent activity against all test microorganisms-except for *Bacillus subtilis* - in the concentration range: 100-25mg/ml. It also exhibited significant activity against the yeast *Candida albicans* at 12.5 and 6.25mg/ml. It seems that the oil is a lead for further optimization.

Keywords: *eucalyptus camaldulensis*, fixed oil, GC-MS, antimicrobial activity

Introduction
In developing countries where modern medicines are usually beyond affordability, traditional medicine is still playing an important role in primary health care. Recently, there has been a renewed interest in medicinal plants which are plausible candidates for leads necessary for drug discovery and drug design.
Scientific data on the constituents of plants used in Sudanese system of medicine is still scarce. Hence this study was designed to identify and quantify the constituents of the oil of the Sudanese material of *Eucalyptus camaldulensis* which is a key species in Sudanese traditional medicine. It was also aimed to evaluate the antimicrobial activity of the oil.
Eucalyptus camaldulensis is a tree up to 50m in height. The genus Eucalyptus is a large genus comprising about 900 species indigenous to Australia, New Guinea and Tasmania. Now the genus is grown worldwide specially in temperate regions due to its economic importance [1-4].
Eucalyptus oil has been used traditionally against: kidney disorders, gastritis, diabetes, cystitis, ringworms, malaria, leucorrhoea and laryngitis. It has also been used for asthma, bronchitis and inflammation of the respiratory tract [5-8].Externally the oil is used for lung tuberculosis, neuralgic pain and fever [9]. Though it occurs in many parts of the plants, the oil is plentiful in leaves [10]. The oil contains monoterpenes, α- and β-pinene, geraniol, camphene, sesquiterpenes and limonene, but the major constituent is 1,8-cineole [5, 11-15]. Some aromatic compounds have also been reported [16-18]. Many secondary metabolites (terpenoids, flavonoids, tannins and cyanogenic glycosides were reported from the genus Eucalyptus [19] (94).
The anticancer activity of the oil has been studies in model animals [20]. Also the ulcer healing properties has been documented [20]. Some constituents of *Eucapyptus camaldulensis* showed spasmolytic activity [21]. The inhibitory effect of cineole on some types of experimental inflammation has been demonstrated [22]. Cineole also showed a depressant effect on the central nervous system [22]. The cytotoxic effect of cineole has been studied on human colon cell lines HCT116 and specific induction of apoptosis has been observed. Cineole also significantly inhibited tumor progression in xenotransplanted models [23].

Materials and Methods
Materials
Plant materials
Seeds of *Eucalyptus camaldulensis* were collected from Nyala, western Sudan and identified by the Center of Seeds Research, Ministry of Agriculture, Sudan.

Instruments
A Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system was used.

Methods
Extraction of oil
Powdered seeds of *Eucalyptus camaldulensis* (300g) were exhaustively macerated with n-hexane at ambient temperature for 48h. The solvent was removed *in vacuo* to afford the oil.

GC-MS analysis
In GC-MS analysis, the temperature program was set up from 70°C to 280°C. Helium gas was used as carrier gas. The injection volume was 2 µL with injection temperature of 250ºC and a column flow of 1.80 ml/min for the GC. For the

mass spectroscopy ACQ mode scanner with scan range of 30-700 amu at the speed of 1478 was used. The mass spectra were then compared with the NIST05 mass spectral library (NIST, 2012)

Antimicrobial screening

In cup plate agar diffusion bioassay, *Eucalyptus camaldulensis* oil was assessed for antimicrobial activity against five standard pathogenic microbes.

(1g) of the oil was weighed and dissolved in 10ml of DMSO to obtain a concentration of 100mg/ml. This was the initial concentration of the oil used to check the antimicrobial activities. 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 test solution 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.

Results and Discussion

GC-MS analysis of fixed oil

GC-MS analysis of *Eucalyptus camaldulensis* fixed oil was carried out. The MS library (NIST) was checked for identification of the constituents (a 90-95% match was observed). Furthermore, the resulting fragmentation pattern was discussed. 29 components were detected by GC-MS anslysis (Table1).The typical total ion chromatogram (TIC) is depicted in Fig.1.

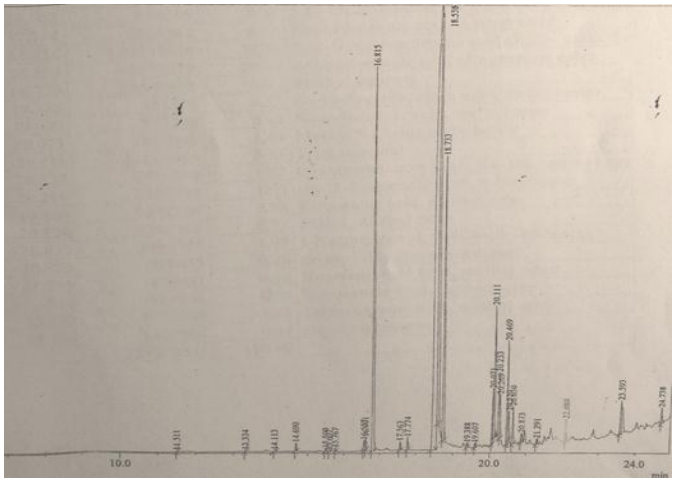

Fig 1: Total ion chromatograms

Table 1: Contituents of *Eucalyptus camaldulensis*

S. No	R. Time	Area%	Name
1	11.511	0.04	Alloaroanadendrene
2	.33413	0.05	Cyclheptane,4-methylene-1-methyl
3	14.113	0.07	2-Naphthalenemethanol,decahydro-alpha-
4	14.690	0.20	Methyl tetrdecanoate
5	15.500	0.09	Cis-5-Dodecenoic acid methyl ester
6	15.607	0.04	5-Octadeanoic acid,methyl ester
7	15.767	0.09	Pentadecanoic acid, methyl ester
8	16.557	0.32	7-Hexadecanoic acid,methyl ester
9	16.601	0.30	9-Hexadcanoic acid, methyl ester
10	16.815	14.99	Hexadecanoic acid,methyl ester
11	17.563	0.25	9,12-Octadecadienoyl chloride
12	17.774	0.35	Heptadecanoic acid,methyl ester
13	18.536	39.88	9,12-Octadecadienoic acid methy ester(Z,Z-)
14	18.553	19.10	9-Octadecenoic acid methyl ester(z)
15	18.733	9.06	Methyl stearate
16	19.388	0.13	Cis-10-Nonadecesoic acid,methyl ester
17	19.607	0.12	Nonadecanoic acid, methyl ester
18	20.071	1.48	Methyl-5,13-docosadienoate
19	20.111	3.38	Tridecanedial
20	20.233	1.82	Oxiraacoctanoic acid,3-octyl-methyl ester
21	20.269	1.03	Cis-11-Eicosenoic acid,methyl ester
22	20.469	2.85	Eicosanoic acid,methyl este
23	20.520	0.73	PGHI,methyl ester
24	20.630	1.05	1-Naphthalenol dechydro-4a-,methyl ester
25	20.873	0.28	Cis,cis,7,10-Hexedecdienal
26	21.291	0.14	Heneicosanoic acid, methyl ester
27	22.088	0.59	Docosanoic acid,methyl ester
28	23.593	0.15	Tetracosanoic acid, methyl ester
29	24.738	0.44	Hexatriacontane
		100%	

Main constituents of the oil are discussed below

9,12-Z,Z-Octadecadienoic acid methyl ester (39.88%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.2.The peak at m/z294(R.T. 18.536 -in total ion chromatogram) corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z263 corresponds to loss of a methoxyl function.

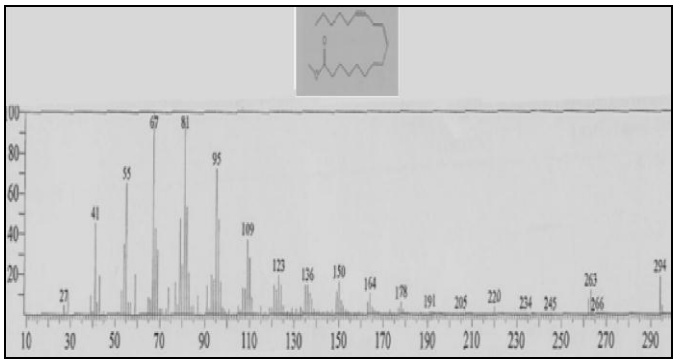

Fig 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

9-Z-Octadecenoic acid methyl ester (19.10%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 18.553 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl.

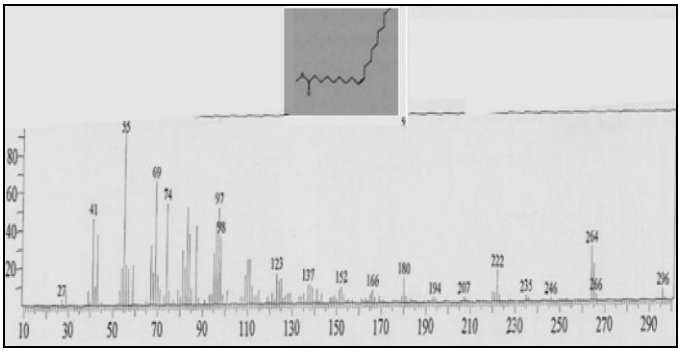

Fig 3: Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester (14.99%)

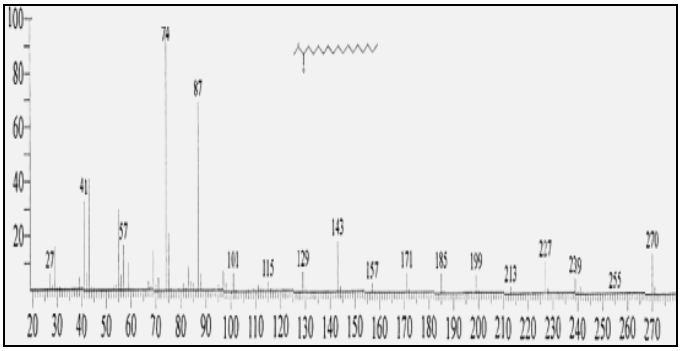

Fig 4: Mass spectrum of hexadecanoic acid methyl ester

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270 (R.T.16.815) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z239 corresponds to loss of a methoxyl.

Methyl stearate (9.06%)

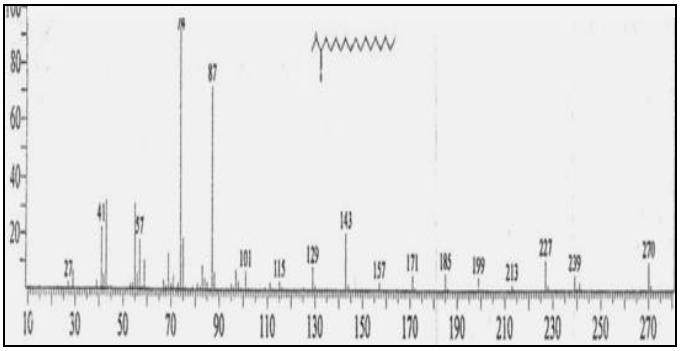

Fig 5: Mass spectrum of methyl stearate

Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298(R.T.18.733) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 corresponds to loss of a methoxyl group.

Tridecanedial (3.38%)

The mass spectrum of tridecanedial is depicted in Fig.6.The peak at m/z 212 (R.T. 20.111) corresponds $M^+[C_{13}H_{24}O_2]^+$.

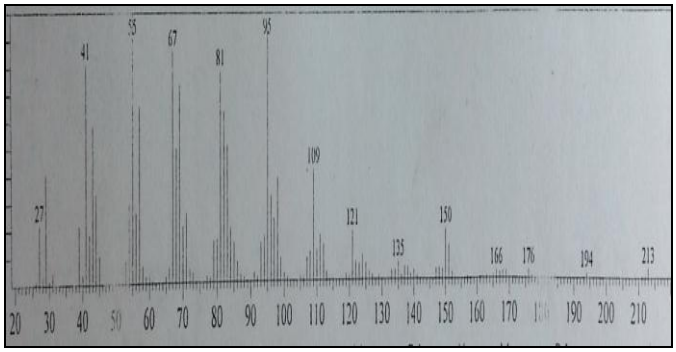

Fig 6: Mass spectrum of tridecanedial

Antibacterial activity

Eucalyptus camaldulensis oil was screened for antimicrobial activity against five standard bacterial strains. The diameters of the growth of inhibition zones are shown in Table (2). Conventional terms were used for interpretation of the results : (<9mm: inative; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (3) and (4) represent the antimicrobial activity of standard drugs.

Table 2: Antibacterial activity of *Eucalyptus camaldulensis* oil

Type	Conc. (mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	20	-	19	19	20
	50	19	-	18	18	19
	25	18	-	17	17	18
	12.5	18	-	13	16	18
	6.25	15	-	12	13	17

Table 3: 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 4: 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 excellent activity against all test microorganisms-except for *Bacillus subtilis* - in the concentration range: 100-25mg/ml.It also exhibited significant activity against the yeast *Candida albicans* at 12.5 and 6.25mg/ml.

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