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CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *DETARIUM SENGALENSE* (FABACEAE) OIL

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ABSTRACT

Traditional medicine is still playing an important role in primary health care and numerous pharmacological reports on the impact of secondary metabolites on human physiology even potentiated the applications of medicinal plants. *Detarium sengalense* is a key species in ethnomedicine. The plant is consumed as food and is involved in pharmaceutical industries. *Detarium sengalense* is a natural remedy for a wide array of diseases including: malaria, leprosy, bronchitis, diarrahea, dysentery syphilis meningitis and sore throat. In this study, *Detarium sengalense* oil has been analyzed by GC-MS. The analysis showed 17 components. Major constituents are: i)9, 12-octadecadienoic acid (Z, Z)-, methyl ester (29.23%)ii)9-octadecenoic acid (Z)-, methyl ester (21.69%).iii) hexadecanoic acid, methyl ester (17.85%) and iv) methyl stearate (14.80%). The antmicrobial activity of the oil has been assessed. *Daterium senegalensis* oil showed significant anticandidal activity and significant antibacterial activity against *Escherichia coli*. The oil also exhibited very good activity against *Staphylococcus aureus*.

KEYWORDS: *Detarium sengalense*, Oil, Constituents, Antimirobial Activity.

INTRODUCTION

The genus Detarium (Fabaceae) is native to the African continent. It is widely distributed through west and central Africa.[1] Plants belonging to this genus are used in African traditional system of medicine.[2-4] Constituents reported from the genus Detarium include: coumarins, polysaccharides, diterpenes and proteins.[5-7]

Detarium sengalense is a key species in ethnomedicine The plant is consumed as food and is involved in pharmaceutical industries.[8] *Detarium sengalense* is a natural remedy for a wide array of diseases including : malaria, leprosy, bronchitis, diarrahea, dysentery ,syphilis meningitis and sore throat.[9-11] Decoction of leaves is used for convulsions.Fruit pulp is a traditional remedy for rheumatism, kidney disorders, spinal tuberculosis and fever.[10] Leaves are used for inflammations, anaemia, dysentery and skin infections.[11] Bark is used in ethnomedicine against burns, wounds, skin infections, bronchitis, pneumonia and digestive disorders.[11] Seeds are antidote for snake bite and root is said to be useful for snake bite.[10] It has been reported that seed non-starch polysaccharides reduced insulin in human subjects,[7] while seed extracts reduced levels of blood lipid.[12] Also it has been shown that seed extracts exhibited antimicrobial activity.[13] The antiviral potential of stem bark has been documented.[14]

MATERIALS AND METHODS

Plant Material

Detarium sengalense seeds were collected from a forest reserve around Damazin (Sudan) and authenticated by direct comparison with a herbarium sample.

Instruments

Detarium sengalense oil was studied by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness).

Microorganisms

The antimicrobial assay was performed by using the following standard microorganisms:
Bacillus subtilis (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa* (G-ve) *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil

Dry powdered *Detarium sengalense* seeds (300g) were exhaustively extracted with n-hexane at room temperature for 72hr. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

The constituents of *Detarium sengalense* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument .Chromatographic conditions are as follows: column oven temperature :150.0^{o}C ; injection temperature:300.0^{o}C ;injection mode : split; flow mode: linear velocity; pressure:139KPa; total flow: 50.0ml/min ; column flow:1.54ml/sec. ; linear velocity: 47.2cm/sec. ;purge flow:3.0 ml/min. ; split ratio: -1.0. Oven temperature program is presented Table 1:

Table 1: Oven temperature program.

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

A**ntimicrobial activity**

The antimicrobial screening was performed by using the cup plate agar diffusion assay . Bacterial culture was maintained in nutrient agar while fungal culture was accomplished on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer (No. 4).Test samples were added into wells of the seeded medium and then incubated for 24 hr (at 37^{0}C)-for bacteria- and for 72 hr(at25^{0}C) for fungal species. The diameters of inhibition zones were measured as average of two replicates.

RESULTS AND DISCUSSION

GC-MS analysis

Deterium senegalensis oil has been analyzed by GC-MS and the constituents were identified and quantized by their retention times and mass spectra . The GC-MS analysis revealed the presence of 17 components (Table 2). The typical total ion chromatograms (TIC) is presented in Fig. 1.

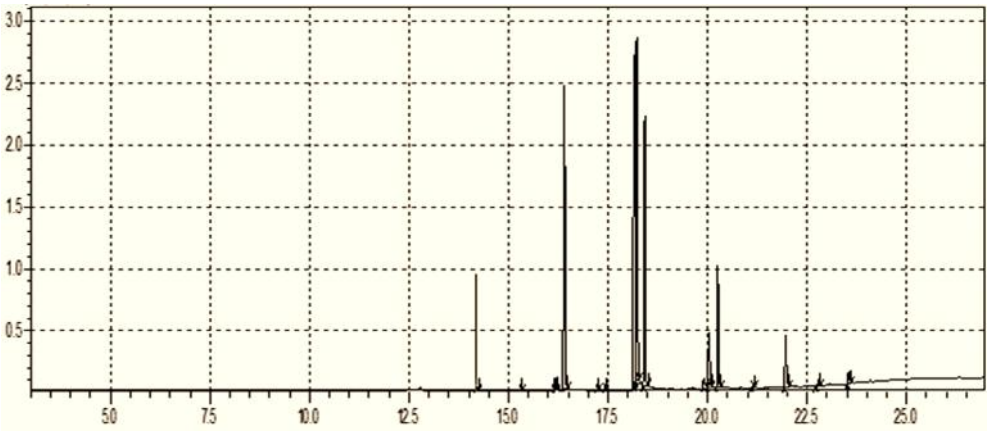


Fig. 1: Typical total ion chromatogram.

Table 2: Contituents of *Daterium senegalensis* oil.

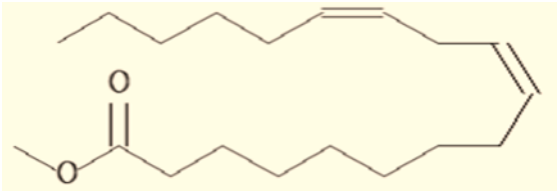
No.	Name	Ret. Time	Area%
1.	Methyl tetradecanoate	14.179	0.08
2.	Pentadecanoic acid, methyl ester	15.310	0.02
3.	7-Hexadecenoic acid, methyl ester, (Z)-	16.143	0.04
4.	9-Hexadecenoic acid, methyl ester, (Z)-	16.190	0.58
5.	Hexadecanoic acid, methyl ester	16.402	17.85
6.	cis-10-Heptadecenoic acid, methyl ester	17.208	0.06
7.	Heptadecanoic acid, methyl ester	17.420	0.11
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.168	29.23
9.	9-Octadecenoic acid (Z)-, methyl ester	18.215	21.69
10.	Methyl stearate	18.419	14.80
11.	9,12-Octadecadienoyl chloride, (Z,Z)-	19.901	0.76
12.	cis-11-Eicosenoic acid, methyl ester	20.029	3.71
13.	Eicosanoic acid, methyl ester	20.256	6.51

14.	Heneicosanoic acid, methyl ester	21.123	0.12
15.	Docosanoic acid, methyl ester	21.958	3.44
16.	Tricosanoic acid, methyl ester	22.762	0.18
17.	Tetracosanoic acid, methyl ester	23.538	0.82

Major constituents of the oil are

1. 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (29.23%)
2. 9-octadecenoic acid (Z)-, methyl ester (21.69%).
3. Hexadecanoic acid, methyl ester (17.85%).
4. Methyl stearate (14.80%)

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.2. The peak at m/z294 with retention time 18.168 corresponds to the molecular ion M^+ $[C_{19}H_{34}O_2]^+$ while the signal at m/z263 is due to loss of a methoxyl group. The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.3. The peak at m/z 296 with retention time 18.215 accounts for the molecular ion M^+ $[C_{19}H_{36}O_2]^+$. The mass spectrum of hexadecanoic acid, methyl ester, is depicted in Fig.4. The peak at m/z270 with retention time 16.402 is due to the molecular ion M^+ $[C_{17}H_{34}O_2]^+$. Fig.5 shows the mass spectrum of methyl stearate. The signal at m/z298 (retention time:18.419) is due to the molecular ion M^+ $[C_{19}H_{38}O_2]^+$.The peak at m/z267 is due to loss of a methoxyl.



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

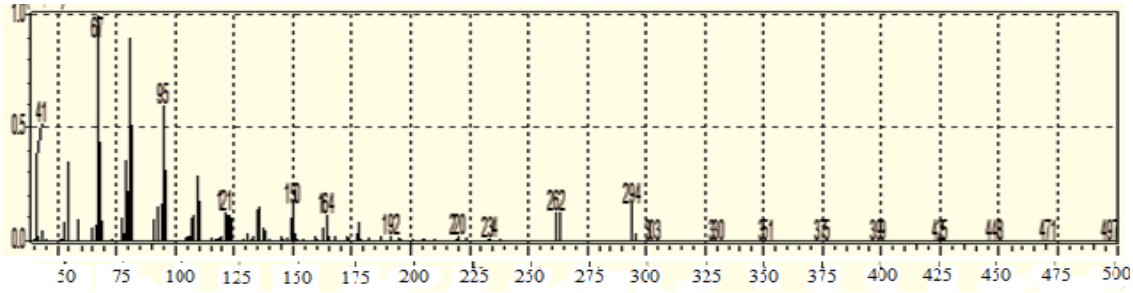
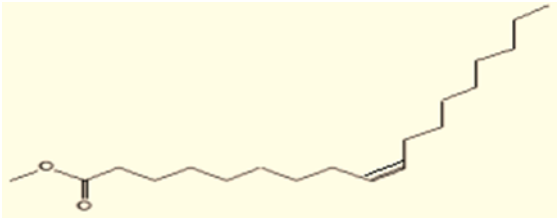


Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester.



9-octadecenoic acid (Z)-, methyl ester.

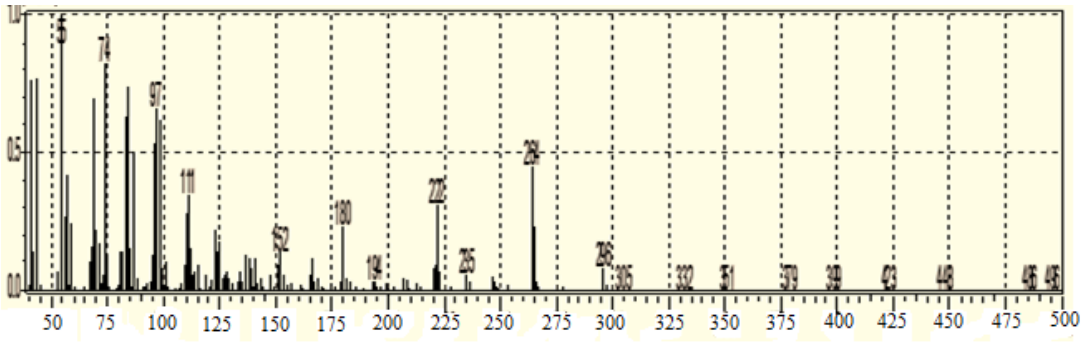
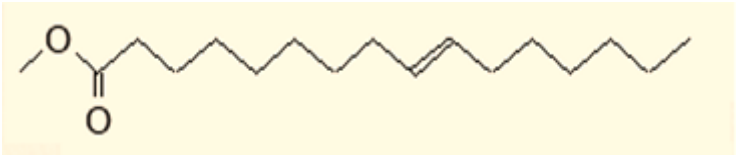


Fig. 3: Mass spectrum of 9-octadecenoic acid (Z)-, methyl ester.



Hexadecanoic acid, methyl ester.

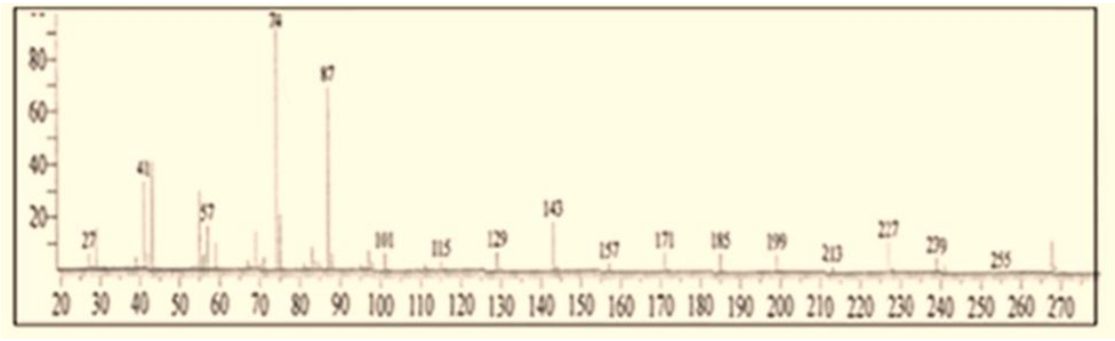
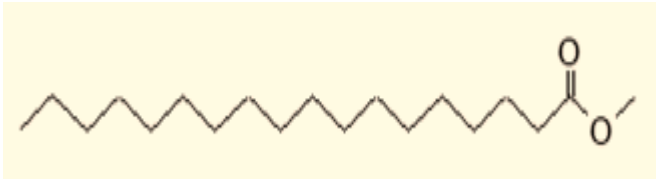


Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester.



Methyl stearate.

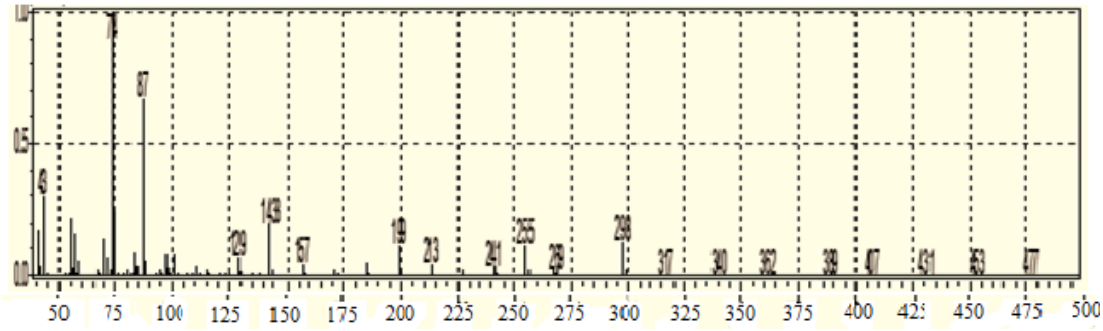


Fig. 5: Mass spectrum of methyl stearate.

Antimicrobial activity

In cup plate agar diffusion assay the oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 3.

Table 2: Antimicrobial activity of the oil.

Type	Ec.	Ps.	Sa.	Bs.	Ca.
Oil 100mg/ml	18	--	15	--	18

Sa: Staphylococcus aureus.
Ec: Escherichia coli.
Ps: Pseudomonas aeruginosa.
Bs: Bacillus subtilis.
Ca: Candida albicans.

Daterium senegalensis oil showed significant anticandidal activity and significant antibacterial activity against *Escherichia coli*. The oil also exhibited very good activity against *Staphylococcus aureus*.

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