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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SUDANESE DETARIUM MICROCARPUM GUILL. & PERR. (CAESALPINIOCEAE) OIL

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ABSTRACT

The present study was designed to provide data on the constituents and antimicrobial activity of of *Deterium microcarpum* oil. Thirty six components were detected by GC-MS analysis. *Detarium microcarpum* oil was dominated by fatty acids(65.11%). Other constituents include: alcohols (8.35%), aldehydes (8.14%), hydrocarbons (6.12%), heterocycles(3.05) and ketones(0.54%).Major components of the oil are: (i) oleic Acid (9-octadecenoic acid) - (21.44%) ii)9-octadecenoic acid methyl ester(12.79%) and iii) 9,12-octadecadienoic acid (Z,Z)-, methyl ester(11.38%).The oil has been screened for antimicrobial activity against : (Gram positive: *Staphylococcus aureus* and *Bacillus subtitis*; Gram negative: *Escherichs coli* and *Pseudomonas aeruginosa* and the fungal species *Candida albicans. Detarium microcarpum* oil showed significant activity against *Escherichs coli* and *Pseudomonas aeruginosa* and weak activity against *Staphylococcus aureus.* However, the oil was inactive against *Bacillus subtilis* and the fungal species *Candida albicans*.

KEYWORDS: *Detarium microcarpum*, Oil, GC-MS Analysis, Antimicrobial Activity.

INTRODUCTION

Detarium microcarpum is an important member of the family *Caesalpinioceae*, local name Abu Leila. It is a Savannah tree distributed in tropical Africa.[1,2] *Detarium microcarpum* is sometimes confused with *Detarium senegalense*, a species with smaller, thinner leaflets and larger fruit.[3] *Detarium microcarpum* seeds contain proteins, lipids, crude fibre, carbohydrates beside minerals: Na, K, Mg, Ca, S, P and Fe.[4,5] Seeds also contain saponins.[6]

Detarium microcarpum is used in African traditional system of medicine against leprosy, bronchitis, syphilis, dysentery, pneumonia, sore throat, diarrhea, malaria and meningitis.[5,7,8] The roots, stems, bark, leaves and fruits are all used to treat ailments including tuberculosis, meningitis and itching.

MATERIAL AND METHODS

Plant material

The seeds of *Detarium microcarpum* were collected from north Kordofan -Sudan. The plant was identified and authenticated by the National Tree Seed Center - Sudan.

Test organisms

*Detarium microcarpum*oil was investigated for antimicrobial activity using the standard microorganisms: *Bacillus subtilis* (G+ve); *Staphylococcus aureus* (G+ve); *Pseudomonas aeroginesa* (G-ve); *Escherichia coli* (G-ve) and the fungal species *Candida albicans.*

Methods

Extraction of oil

Maceration of seeds with hexane at room temperature was the method used for the extraction of *Detarium microcarpum* oil.

GC-MS analysis

Detarium microcarpum oil was analyzed by gas chromatography- mass spectrometry. A Shimadzo GC-MS-QP2010 ultra instrument with RTY- 5MS column (30m, length; 0.25ml diameter; 0.25µm, thickness) was used. Helium was the carrier gas. Oven temperature program is as follows

Rate: --; **Temperature**: 60^{o}C; **Hold time**: 0.00
Rate: 10; **Temperature**: 300^{o}C; **Hold time**: 3.00

Chromatographic conditions are depicted in Table 1.

Table 1: Chromatography conditions.

Column oven temperature	50.0c°
Injection temperature	300.00
Injection mode	Split
Flow control mode	Pressure
Pressure	100.00KPa
Total flow	50.0 ml/min
Column flow	1.61 ml/min
Linear velocity	46.3cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Antimicrobial assay

Bacterial growth was maintained on Muller-Hinton agar. One ml of a 24 hours broth culture of the test organisms were aseptically distributed onto agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml.

Serial dilutions of the stock suspension were made in sterile normal saline solution and one drop (0.02 ml) volumes of the appropriate dilution were transferred onto the surface of the dried agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drops (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 72h.. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspensions were stored in the refrigerator until used.

Testing of anti-bacterial susceptibility

Disc diffusion method

The paper disc diffusion was used to evaluate the antimicrobial activity of the targeted oil. The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines.[9] Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ml. One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6mm in diameter) were placed on the surface of the MHA and soaked with 20µl of a solution of test sample. The inoculated plates were incubated at 37°C for 24 hours. The diameters (mm) of the inhibition zones were measured as average of two replicates.

RESULT AND DISCUSSION

GC-MS analysis

Analysis of *Detarium microcarpum* oil by GC-MS revealed the presence of 36 components (Table 2). The typical total chromatograms (TIC) is shown in Fig. 1.

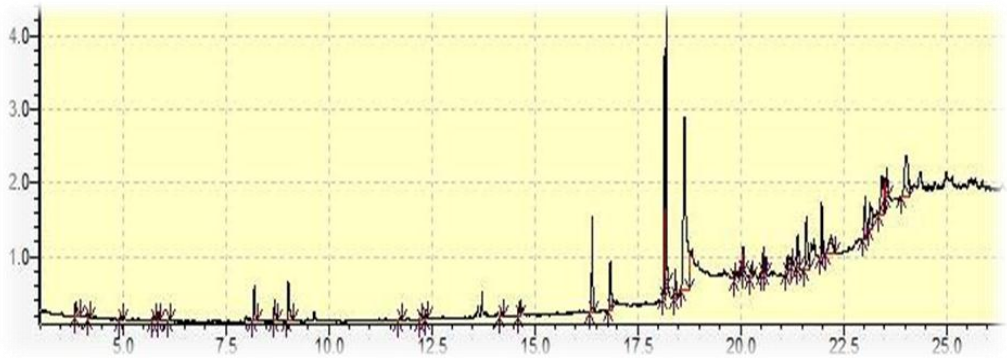

Fig. 1: Typical total ion chromatograms (TIC).

Detarium microcarpum oil was dominated by fatty acids (65.11%). Other constituents include: alcohols (8.35%), aldehydes (8.14%), hydrocarbons (6.12%), heterocycles (3.05) and ketones (0.54%).

Major components of the oil are

1. Oleic Acid (9-octadecenoic acid) - (21.44%).
2. 9-Octadecenoic acid methyl ester (12.79%)
3. 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (11.28%)

Table 2: Chemical constituents of *Detarium microcarpum* oil.

No.	Name	Ret. Time	Area%
1.	2-Heptenal, (Z)-	3.857	0.86
2.	2,3-Octanedione	4.156	0.13
3.	3-Octen-2-one	4.950	0.23
4.	Undecane	5.743	0.47
5.	Nonanal	5.859	0.36
6.	Octanoic acid, methyl ester	6.126	0.14
7.	2-Nonenal, (E)-	8.197	1.94
8.	2,4-Decadienal, (E,E)-	8.688	1.33
9.	2,4-Decadienal	9.022	2.79
10.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	11.715	0.18
11.	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	12.235	0.36
12.	2-Tridecenal, (E)-	12.371	0.20
13.	Methyl tetradecanoate	14.186	0.35
14.	Octanal, 2-(phenylmethylene)-	14.637	0.66
15.	Hexadecanoic acid, methyl ester	16.402	4.40
16.	n-Hexadecanoic acid	16.839	3.14
17.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.154	11.28
18.	9-Octadecenoic acid (Z)-, methyl ester	18.199	12.79
19.	Methyl stearate	18.421	1.24
20.	Oleic Acid	18.642	21.44
21.	Lauric acid, 2-methylbutyl ester	19.869	0.52
22.	11-Eicosenoic acid, methyl ester	20.066	1.04
23.	1H-Indene, 2,3,3a,4,7,7a-hexahydro-2,2,4,4,7,7-hexamethyl-	20.264	0.62
24.	4-Methyl-exo-tricyclo[6.2.1.0(2.7)]undecane	20.551	0.98
25.	2,3-Dihydroxypropyl elaidate	20.576	1.01
26.	Oleoyl chloride	21.133	0.89
27.	Cyclohexanol, 1-(2-nitropropyl)-	21.253	0.52
28.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	21.404	2.36
29.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	21.608	4.50
30.	Docosanoic acid, methyl ester	21.974	2.74
31.	Ergost-5-en-3-ol, (3.beta.)-	22.206	3.02
32.	3-(3-Methylbutyl)thiophene-1,1-dioxide	23.029	3.05
33.	Bicyclo[3.1.1]heptan-3-ol, 3-allyl-6,6-dimethyl-2-methylene-	23.145	2.45
34.	Oleic anhydride	23.433	3.81
35.	Tetracosanoic acid, methyl ester	23.550	1.43
36.	.gamma.-Sitosterol	24.030	6.77

Fig. 2 shows the mass spectrum of oleic acid. The signal at m/z282 (RT.18.642) accounts for the molecular ion $[C_{18}H_{34}O]^+$.The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 3. The signal at m/z296 (RT.18.199) corresponds M^+ $[C_{19}H_{36}O_2]^+$. Fig. 4 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT. 18.154)corresponds M^+ $[C_{19}H_{34}O_2]^+$.

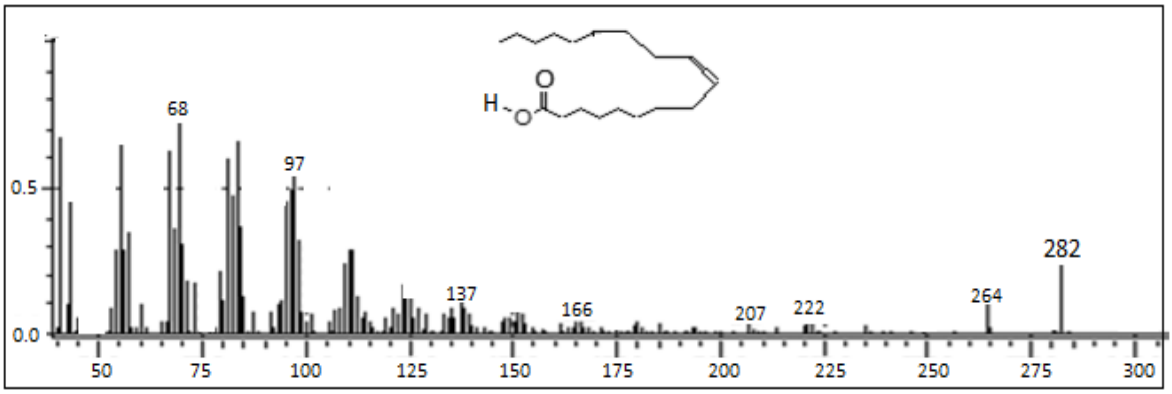

Fig. 2: Mass spectrum of oleic acid.

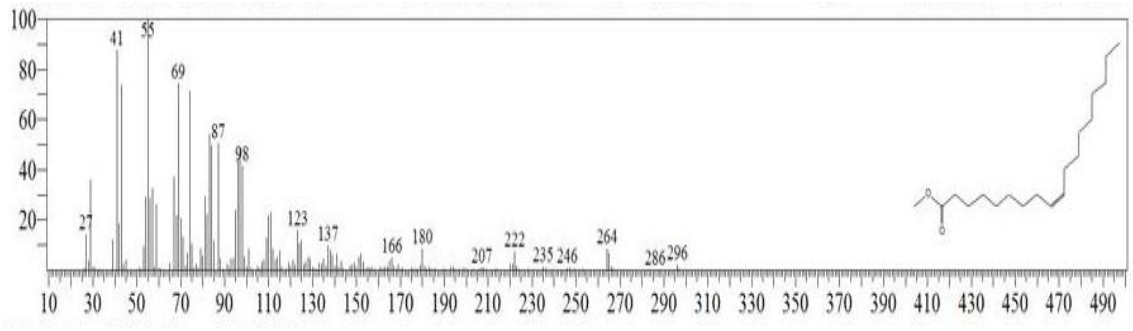

Fig. 3: Mass spectrum for 9-octadecenoic acid[z]-, methyl ester.

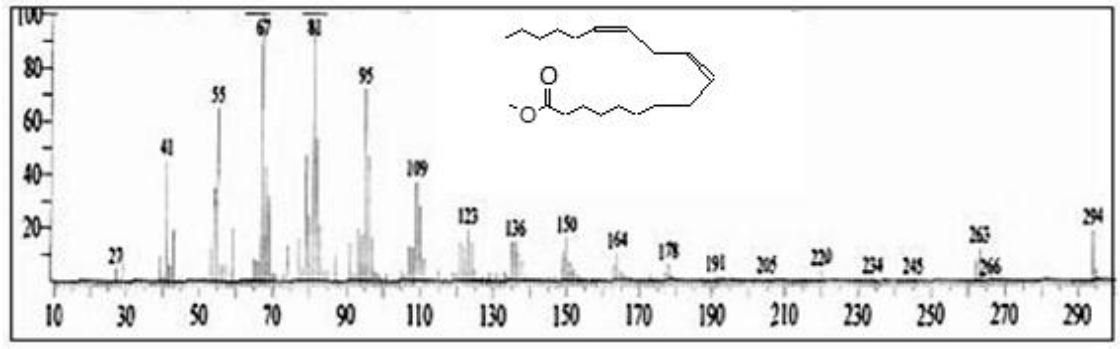

Fig. 4: Mass spectrum of 9, 12-octadecadienoic acid.

Antimicrobial activity

The paper disc diffusion method was used to screen *Detarium microcarpum* oil for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are illustrated in Table (3). The result were interpreted in terms of the commonly used terns (<9mm: inactive, 9-12mm: partially active, 13-18mm: active: > 18 mm very active}. Ampicillin, gentamicin and clotrimazole were used as positive contol.

Detarium microcarpum oil showed significant activity against *Escherichs coli* and *Pseudomonas aeruginosa* and weak activity against *Staphylococcus aureus.* However, the oil was inactive against *Bacillus subtilis* and the fungal species *Candida albicans*.

Table 3: Inhibition zones (mm/mg sample).

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	13	--	17	18	--
Ampicilin(40mg/ml)	30	15	--	--	--
Gentacycin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

REFERENCES

1. Keay RWJ, Onochie CFA, Stanfield DP Nigeria trees, National press Ltd. Federal Department of Forest Research, Ibadan, 1964; 2: 42-50,227-228.
2. Hopkins B, Stanfield DP Savannah Trees of Nigeria. University Press, Ibadan, 1966; 6-7: 12.
3. Vautier, H., Sanon, M., & Sacande, M. *Detarium microcarpum* Guill. & Perr., Amer.J. of Plant Sciences, 2015; 6: 1069-79.
4. Abreu P, Relva A. Carbohydrates from *Detarium microcarpum* bark extract. Carbohydrate Research, 2002; 337: 1663-1666.
5. Abreu PM, Rosa VS, Araujo EM, Canda AB, Kayser O, Bindseii KV,Siems K, Seeman A, Phytochemical analysis and Antimicrobial evaluation of *Detarium microcarpum* bark. Pharm.Pharmacol. Lett., 1998; 8: 107-111.
6. Anhwange BA, Ajibola VO, Oniye S.: Chemical studies of the seeds of *Moringa oleifera* (*Lam*) and *Detarium microcarpum* (*Guill*and *Sperr*). J. Biol. Sci., 2004; 4(6): 711-715.
7. Daziel JM The useful plants of west tropical Africa. Crown Agents, London, 1937.
8. Abreu PM, Martins ES, Kayser O, Bindseil KU, Siems K, Seemann A, Frevert J: Anti-microbial, anti-tumor and Anti-leishmania screening of medicinal plants from Guinea-Bissau. Phytomedicine, 1999; 6(3): 187-195.
9. National Committee for Clinical Laboratory Standards (NCCLS) Performance standards for antimicrobial susceptibility testing; ninth informational supplement, Wayne, Pensilvania document M100-S9, 1999; 19.