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GC-MS analysis and antimicrobial activity of Sudanese *Diospyros mespiliformis* Hochst. Ex. A. DC. fixed oil

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Abstract
The present study was aimed to investigate the constituents of *Diospyros mespiliformis* oil and to evaluate its antimicrobial potency. *Diospyros mespiliformis* is widely used in Sudanese system of medicine. GC-MS analysis showed the presence of 29 components dominated by: isoproyl linoleate (9, 12-octadecadienoic acid -1-methylethyl ester) - 29.03%; 9, 12-octadecadienoic acid methyl ester (12.10%); 1, 6, 11-dodecatriene (12.02%) and pentadecanoic acid methyl ester (6.68%).
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 aeruginosa* and the yeast *Candida albicans*. The oil showed significant activity against *Bacillus subtilis*. It also exhibited significant anticandidal activity and good activity against other test microorganisms. Hence it is a lead for antimicrobial agents.

Keywords: *Diospyros mespiliformis*, fixed oil, GC-MS, antimicrobial activity

Introduction

Recently a certain interest in medicinal plants has been renewed specially in developing countries where modern synthetic drugs are beyond affordability, beside the side effects of these drugs [1, 2]. Medicinal plants are considered as potential source of drug leads and many modern drugs are of plant origin (morphin, atropine. etc). The WHO has reported over 20,000 medicinal plants used in ethnomedicine of different communities [3].

Diospyros mespiliformis Hochst. Ex. A. DC. (also known as Jackal –berry) is an evergreen tree in the family Ebenaceae. It grows up to 12-15m and may reach 20m in height in rainy forests [4]. The plant has been used medicinally for a long time in traditional practice of several communities including African, Chinese, and Indian systems of medicine [5]. Phytochemical screening revealed the presence of many secondary metabolites including saponins, alkaloids, tannins, alkaloids, flavonoids and glycosides [6].

In Africa *Diospyros mespiliformis* is a traditional food [7, 8]. Leave extract is used by traditional healers against wounds, fever, syphilis, ringworms, headache, and sleeping sickness [9-11]. Barks and roots are claimed to treat syphilis, leprosy, pneumonia, malaria and diarrhea [12]. The cytotoxicity and free radical scavenging capacity of different extracts of *Diospyros mespiliformis* have been documented [13]. It has been reported that various parts of the plant contain bioactive constituents with antimicrobial potency [9].

Materials and Methods

Plant Material

The seeds of *Diospyros mespiliformis* were collected from a forest reserve around Damazin – Sudan. The plant was authenticated by Institute of Aromatic and Medicinal Plants-Khartoum, Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length; 0.25mm diameter; 0.25 μm, thickness) was used for GC-MS analysis .

Test organisms

Diospyros mespiliformis oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Escherichia coli* (G-ve), *Candida albicans* (fungus).

Methods

Extraction of oil from *Diospyros mespiliformis*

Dry-powdered seeds of *Diospyros mespiliformis* (300g) were macerated with n-hexane at room temperature for 72h. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol. A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with (99ml) methanol.

The oil (2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by (7ml) of alcoholic

sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added, then (2ml) of n-hexane were added and the tube was vigorously shaked for five minutes .The hexane layer was then separated. (5μl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate (1μl) was injected in the GC-MS vial.

GC-MS analysis

Diospyros mespiliformis fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1: Oven temperature program

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial Assay

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in (100 ml) of normal saline to produce a suspension containing about 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on Sabaraud dextrose agar incubated at 35°C for 72h. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

The cup-plate agar diffusion method was adopted, with some minor modifications, to assess the antibacterial activity. (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 the test solutions. Separate Petri dishes were designed for standard antimicrobial chemotherapeutic agents.

The agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution using adjustable volume microtiter pipette 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 (for bacteria) and at 35ºC for 72h (for fungi). After incubation, the diameters of the resultant growth inhibition zones were measured in two replicates and averaged as indicator of antimicrobial activity.

Results and Discussion

GC-MS analysis of *Diospyros mespiliformis* fixed oil

GC-MS analysis of *Diospyros mespiliformis* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

The GC-MS analysis revealed the presence of 29 components (Table 3). The typical total ion chromatograms is depicted in Fig.1.

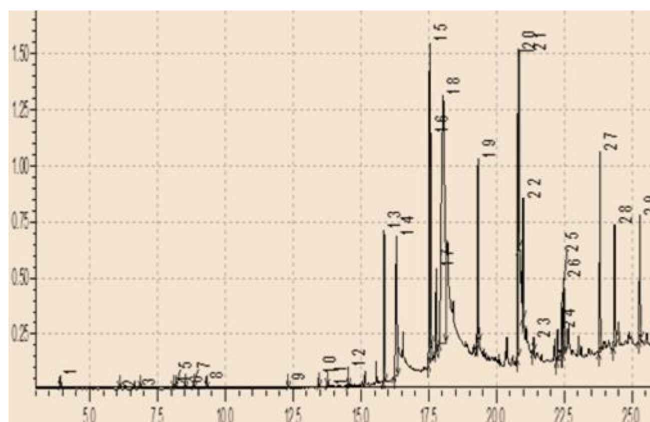


Fig 1: Total ions chromatogram

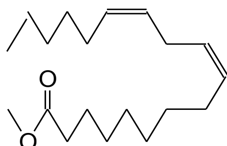
Table 3: Constituents of *Diospyros mespiliformis* oil

Peak#	R.Time	Area	Area%	Name
1	3.889	755121	0.23	2-Heptenal, (E)-
2	6.085	119408	0.04	Octanoic acid, methyl ester
3	6.797	372634	0.11	Octanoic acid
4	8.053	370473	0.11	2-Decenal, (E)-
5	8.149	100574	0.03	Nonanoic acid
6	8.511	445775	0.13	2,4-Decadienal, (E,E)-
7	8.827	614773	0.19	2,4-Decadienal
8	9.287	744955	0.22	3-Cyclohexene-1-methanol, .alpha.,.alpha.,
9	12.301	530749	0.16	Hexadecane
10	13.443	860829	0.26	Heptadecane
11	13.746	117125	0.04	Methyl tetradecanoate
12	14.528	1230654	0.37	Heneicosane
13	15.855	11313250	3.41	Hexadecanoic acid, methyl ester
14	16.311	22165613	6.68	Pentadecanoic acid
15	17.533	40158432	12.10	9,12-Octadecadienoic acid (Z,Z)-, methyl e
16	17.568	10688332	3.22	9-Octadecenoic acid, methyl ester, (E)-
17	17.774	5283299	1.59	Methyl stearate
18	18.029	96306614	29.03	9,12-Octadecadienoic acid (Z,Z)-
19	19.316	15991474	4.82	l-(+)-Ascorbic acid 2,6-dihexadecanoate
20	20.802	39898401	12.02	1,6,11-Dodecatriene, (Z)-
21	20.828	13022930	3.92	9-Octadecenoic acid, 1,2,3-propanetriyl est
22	20.981	9115142	2.75	L-Ascorbic acid, 6-octadecanoate
23	21.358	1192452	0.36	n-Propyl 9,12-octadecadienoate
24	22.255	3532115	1.06	Ethyl 9,12,15-octadecatrienoate
25	22.405	3693455	1.11	Dotriacontane
26	22.482	9510322	2.87	Butyl 9,12-octadecadienoate
27	23.817	18770366	5.66	Hexatriacontane
28	24.355	12155035	3.66	Isopropyl linoleate
29	25.285	12737868	3.84	Tetratriacontane
		331798170	100.00	

Some important constituents are discussed below:

9, 12-Octadecadienoic acid (29.03%)

Fig. 2 shows the EI mass spectrum of 9,12-octadecadienoic acid .The peak at m/z294, which appeared at R.T. 18.029 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_{2}]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.



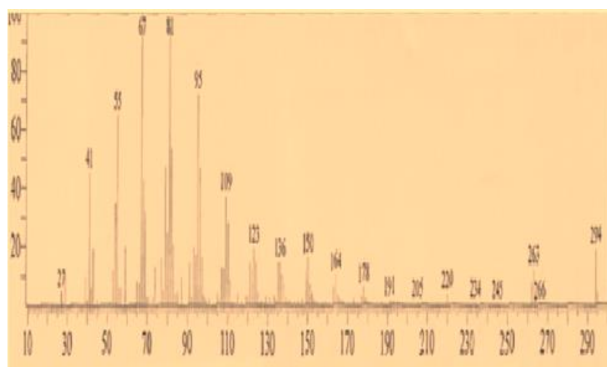
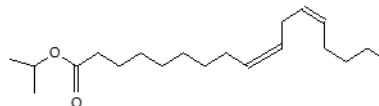


Fig 2: Mass spectrum of 9,12-octadecadienoic acid

9, 12-octadecadienoic acid methylethyl ester (12.10%)

The signal m/z322 with RT17.533 corresponds $M^{+}(C_{21}H_{38}O_{2})^{+}$.The peak at m/z263 is due to loss of an isopropyl group(Fig.3).



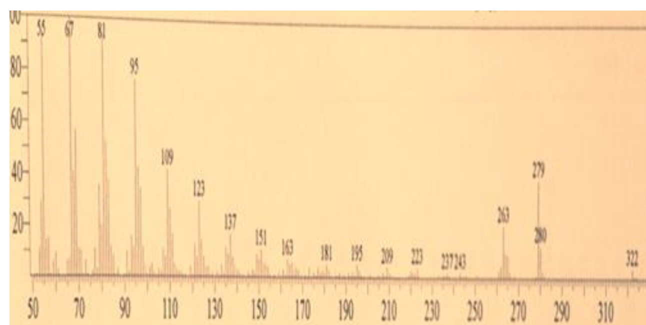
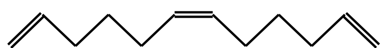


Fig 3: Mass spectrum of 9,12-octadecadienoic acid methylethyl ester

1, 6, 11-Dodecatriene (12.02%)

Mass spectrum of 1, 6, 11-dodecatriene is shown in Fig. 4. m/z 164, which appeared at R.T. 20.802 corresponds $M^{+}[C_{12}H_{20}]^{+}$.



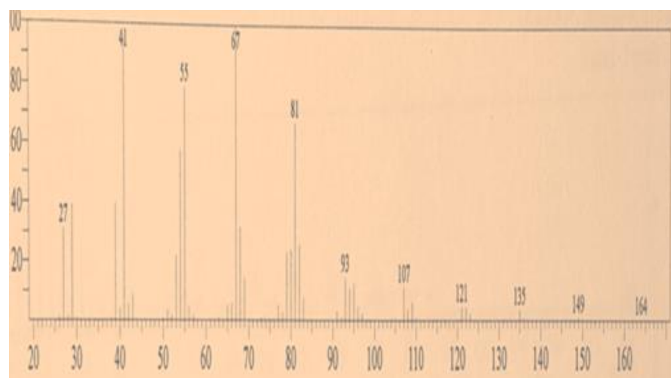
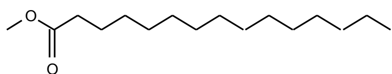


Fig 4: Mass spectrum of 1, 6, 11-dodecatriene

Pentadecanoic acid methyl ester (6.68%)



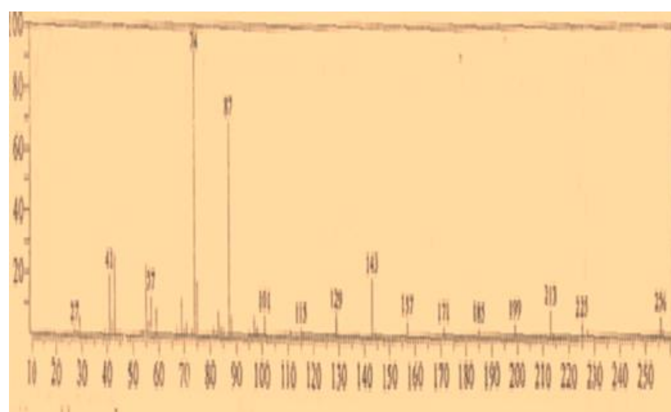


Fig 5: Mass spectrum of pentadecanoic acid methyl ester

The EI mass spectrum of pentadecanoic acid methyl ester is shown in Fig. 5.The peak at m/z 256, which appeared at R.T. 16.311 in total ion chromatogram, corresponds to $M^+[C_{16}H_{32}O_2]^+$. The peak at m/z225 corresponds to loss of a methoxyl group.

Antibacterial 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 (4). The results were interpreted in commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active;>18mm:very active). Tables (5) and (6) display the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents respectively.

Table 4: Antibacterial activity of *Cyperus esculentus* oil

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	16	20	15	15	18

Table 5: 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 6: 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 significant activity against *Bacillus subtilis* . It also exhibited significant anticandidal activity beside a good activity against other test microorganisms. Hence it is a lead for antimicrobial agents.

References

1. Akinpelu DA, Onakoya TM. African Journal of Biotechnology. 2006; 5(11):1078-1081.
2. Balbaa SI. Investigating the efficacy of three plants species (sclerocarya birrea, Neo carya Mocrophylla and Grewia Mollis) as anti snake venom. M.sc Dissertation (Unpublished), Usmanu Danfodiyo University Sokoto, 1976.
3. Cos P, Vlietinck AJ, Vanden Berghe D, Maes L. Journal of Ethnopharmacology. 2006; 106:290-302.
4. Bala Sidi-Aliyu. Common Ethnomedicinal Plants of the Semiarid Regions of West Africa: Their Description and Phytochemicals, Triumph Publishing Company, 2006.
5. Mallavadhni UV, Panda AK, Rao YR. Phytochemistry. 1998; 49:901-51.
6. Ebbo AA, Mammam M, Suleiman MM, Ahmed A, Bello A. Anatomy and Physiology. 2004; 4(4):1000156.
7. National Research Council (NRC). Lost crops of Africa. Volume III: Fruits. National Academic press. 2008, 1-5.
8. National Research Council. Ebony, Lost Crops of Africa, Volume III, Fruits, National Academies Press, 2008. ISBN 978-0-309-10596-5.
9. Dangoggo SM, Hassan LG, Sadiq IS, Manga SB. Journal of Chemical Engineering. 2012; 1(1):31.
10. Etkin NL. Tropical Doctor. 1997; 27(1):12-16.
11. Kerharo Adam JG. Pharmacopée sénégalaise traditionnelle, plantes médicinales et toxiques. Vigot et Frères; Paris. 1974, 1011.
12. Mohamed IE, El Nur EE, Choudhary MI, Khan SN., Rec. Nat. Prod. 2009; 3:198-203.
13. Mohammad A, Ahmed K, Suliman I, Amna A, Omer S, Mohammed G. Inter. Journal of Botany Studies. 2016; 1(1):23-28.