The Pharmaceutical and Chemical Journal, 2019, 6(1):84-89

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Chemical Constituents and Antimicrobial Activity of Sudanese *Hyphaene thebaica* L. (Arecaceae) Fruit Oil

Abdel Karim, M.[1]*, Ezdehar, A.E.[2], Salah, H.[3]

[1]Sudan University of Science and Technology, Faculty of Science
[2]Jazan University, Samtah University College(Saudi Arabia)
[3]Western Kordofan University, Faculty of Education.

Abstract *Hyphaene thebaica* L. is a type of palm tree in the family Arecaceae. In Sudan, the plant grows along Nile river banks. *Hyphaene thebaica* fruit oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. The GC-MS analysis showed 50 components. Major constituents are: Z,Z-9,12-octadecadienoic acid methyl ester (30.62%), hexadecanoic acid methyl ester (23.19%), 9-octadecenoic acid methyl ester (16.12%), methyl stearate (5.84%). The studied oil showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It also exhibited significant anticandidal activity.

Keywords *Hyphaene thebaica*, Oil, GC-MS analysis, Antimicrobial Activity.

Introduction

Hyphaene thebaica L. (also known as Doum) is a type of palm tree in the family Arecaceae. In Sudan, the plant grows along Nile river banks [1,2]. Doum fruit pulp contains nutritional trace minerals,high quality protein beside the nutritionally essential linoleic acid [3]. The fruit also contains flavonoids, steroids, glycosides, terpenes and tannins [4,5]. Fruits are used traditionally against bilharzias and hypertension [6]. Also it could be of great merit for use as hypolipidemic agent [7]. It has been reported that the fruit possesses hypoglycemic properties [8]. Significant decrease in cholesterol, blood glucose, triglycerides and total lipids has been reported after administration of fruit decoction for a period of 1-2 months [9,10].

Analysis of fruit aqueous extract revealed the presence of some bioactive flavonoids including quercetin, hesperetin and naringin [5]. The free radical scavenging capacity of fruit extracts has been documented [11,12]. The accumulation of free radicals in the body is a risk factor in many harmful oxidative processes including degenerative diseases of ageing and cancer [13-15]. Previous studies demonstrated the antiinflammatory activity of Doum extracts [16]. Such activity has been associated with the presence of saponins, flavonoids and coumarins which are known for their anti-proliferative activity [17]. The antibacterial activity of Doum extracts has also been reported [12,18].

Plant material

Hyphaene thebaica fruits were collected from Khartoum, Sudan and authenticated by direct comparison with a herbarium sample. The fruits were shade – dried at room temperature and powdered.

GC-MS analysis

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

Test organisms

The oil from *Hyphane thebiaca* fruits was screened for antimicrobial activity using the standard microorganisms shown in Table 1.

Table 1: Test organisms

S. No.	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

Extraction of oil

Powdered shade-dried fruits of *Hyphane thebaica* (300 g) were exhaustively extracted with n-hexane at room temperature. The solvent was removed under reduced pressure to give the oil. The oil was esterified as follows: the oil (2 ml) was placed in a test tube and 7 ml of alcoholic sodium hydroxide were added followed by 7 ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. (2 ml) of supersaturated sodium chloride were added, then (2 ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated. (5 µl) of the hexane extract were mixed with 5 ml diethyl ether. The solution was filtered and the filtrate (1 µl) was injected in the GC-MS vial.

Constituents of the oil

The studied oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument.Helium was used as carrier gas. Chromatographic conditions are presented below:

- *Oven temperature program*

Rate: ---; Tempt., 150.0 °C; Hold time ($min.^{-1}$),1.00

Rate: 4.00; Tempt., 300.0 °C; Hold time ($min.^{-1}$), 0.00

- Column oven temperature: 150.0 °C
- Injection temperature: 300.0 °C
- Rate: 4/min
- Injection Mode: Split
- Flow control mode: Linear velocity
- Pressure: 139.3 KPa
- Total Flow: 50.0 ml/min
- Column Flow: 1.54 ml/sec
- Linear velocity: 47.2 cm/sec
- Purge flow: 3.0 ml/min
- Split ratio: -1.0

Broth cultures ($5.0x10^7$ cfu/ml) were streaked on the surface of the solid medium contained in Petri dishes. Filter paper discs (Oxid, 6 mm) were placed on the surface of the inoculated agar and then impregnated with 100mg/ml of test sample. For bacteria the plates were incubated at 37 ^oC for 24 h., while for fungi the plates were incubated at 25 ^oC for 3 days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamycin and clotrimazole were used as positive control and DMSO as negative control.

Antimicrobial assay

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 manufacture instructions.

Results and Discussion

GC-MS analysis of *Hyphane thebaica oil* was performed. Fifty constituents were detected. The constituents of the oil are presented in Table 3. Identification of the constituents was based on retention times and MS library data.

Major constituents are briefly discussed below:

The mass spectrum of 9,12-octadecadienoic acid-z,z- methyl ester(30.62%) is shown in Fig. 1. The peak at m/z294, which appeared at RT 17,303 in total ions chromatograms corresponds to $M^+[C_{19}H_{34}O_2]$. The signal at m/z263 is due to loss of a methoxyl.

Fig**.** 2 shows the mass spectrum of hexadecanoic acid methyl ester (23.19%). The molecular ion $M^+[C_{17}H_{34}O_2]$ appeared at m/z 270(RT,15.645). The signal at m/z 239 accounts for loss of a methoxyl.

The mass spectrum of 9-octadecanoic acid methyl ester (16.12%) is presented in Fig. 3. The signal at m/z296 (RT, 17.339) is due to the molecular ion $M^+[C_{19}H_{36}O_2]^+$. The peak at m/z266 is due to loss of a methoxyl.

Fig. 4 shows the mass spectrum of methyl stearate (5.84%).The peak at m/z 298 which appeared at RT 17.550 corresponds $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl.

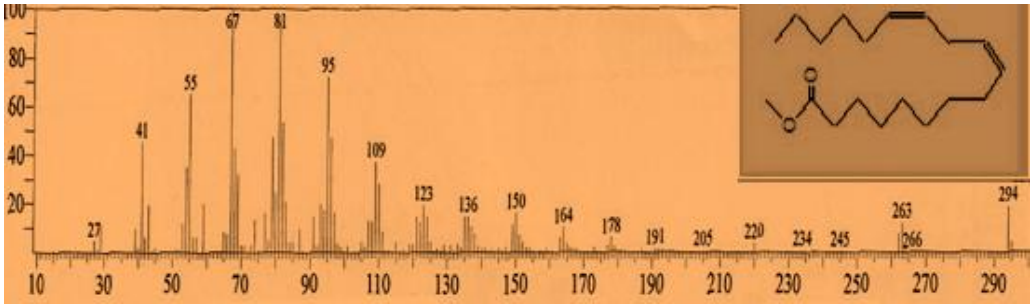

Figure 1: Mass spectrum of 9,12-octadecadienoic acid methyl ester

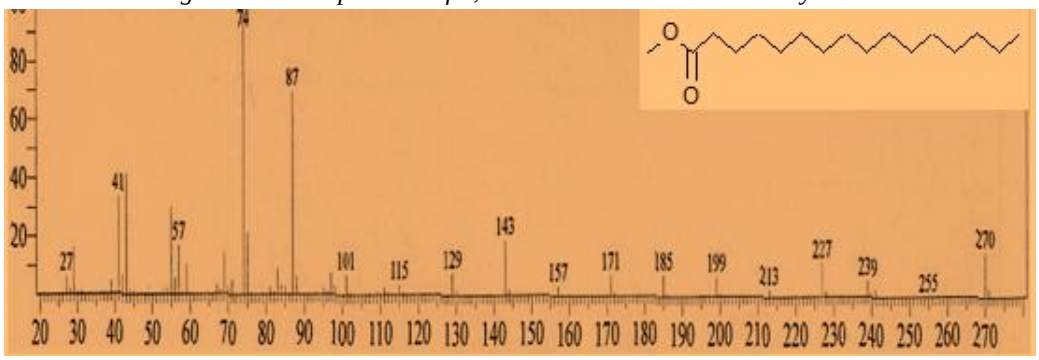

Figure 2: Mass spectrum of hexadecanoic acid methyl ester

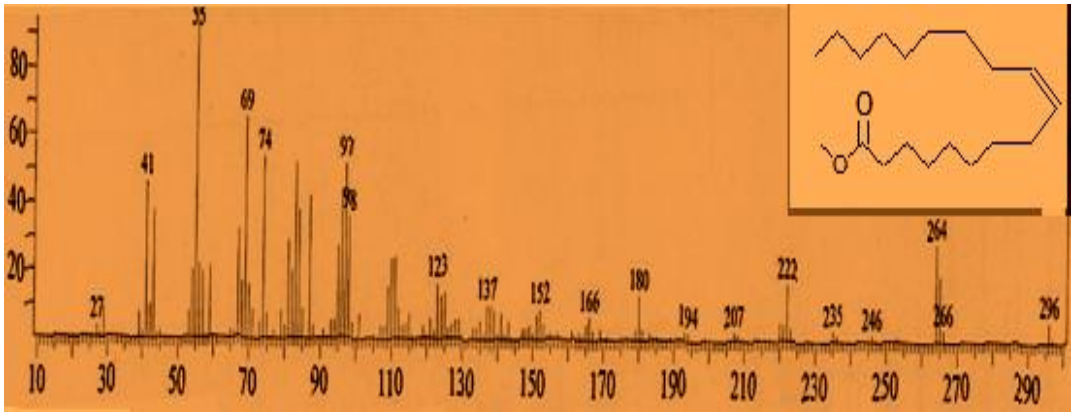

Figure 3: Mass spectrum of 9-octadecenoic acid methyl ester

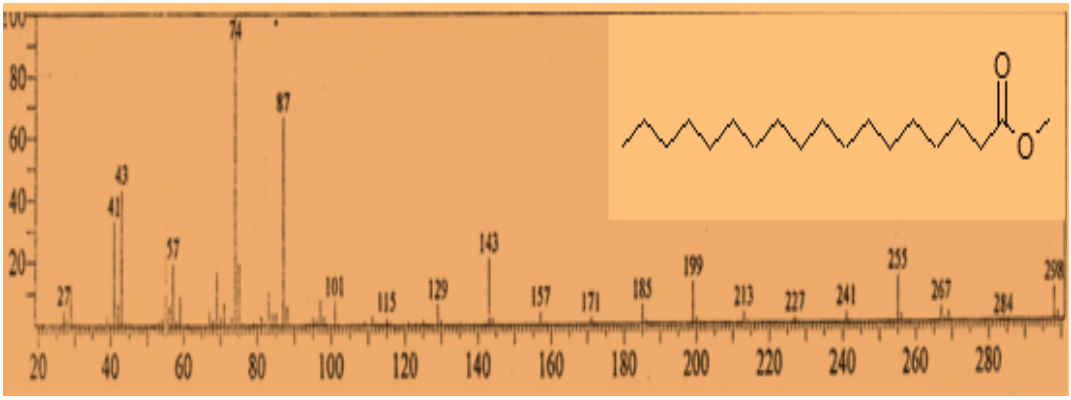

Figure 4: Mass spectrum of methyl stearate

Antimicrobial assay

The oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table 2. Results were interpreted as follows: (<9mm: inative; 9-12mm: partially active; 13-18mm: active ;>18mm: very active). Ampicilin, gentamycin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The oil also exhibited significant anticandidal activity.

Table 2: Antimicrobial activity of *Hyphaene thebaica* oil

Sample	Sa	Bs	Ec	Ps	Ps
Oil (100mg/ml)	17	--	--	21	19
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamycin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

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

Table 3: Constituents of *Hyphane theibica* oil

No.	R. Time	Area%	Name
1	6.981	0.14	Ethyl dioxovalerate
2	7.324	0.15	1-Pentanol, 2,2-dimethyl
3	7.432	0.08	Butanoic acid, 2-ethyl-2-methyl
4	7.930	0.12	2-Decanol, Methyl ether
5	7.997	0.22	Oxalic acid, cyclohexyl dodecyl ester
6	8.307	0.12	Dodecane
7	8.441	0.07	Bicycl[4.4.1]undeca-1,3,5,7,9-pentaene
8	8.659	0.08	Nonanoic acid, methyl ester
9	8.898	0.25	2-H-Pyranmethanol, tetrahydro-2,5-dimethyl
10	9.251	0.08	(1R,2R,3S,5R)-(-)-2,3-Pinanediol
11	9.560	0.07	Biphenyl
12	9.631	0.17	Tetradecane
13	9.806	0.02	Benzoic acid,3-methyl-4-nitro, methyl ester
14	9.905	0.05	Naphthalene,2,6-dimethyl

15	10.103	0.06	Naphthalene,2,3-dimethyl
16	10.145	0.08	Octadecanoic acid,9,10-dihydro, methyl ester
17	10.372	0.03	Naphthalene-1,3-dimethyl
18	10.419	0.06	Dodecane,2,6,10-trimethyl
19	10.896	0.27	Heptadecane,7-methyl
20	11.219	0.34	Dodecanoic acid , methyl ester
21	11.530	0.21	Nonanedioc acid , methyl ester
22	12.877	0.56	4-Fluoro , alpha, -methylbenzyl alcohol, methyl ester
23	12.986	0.07	Carbamic acid, N-[1-(4-methylphenyl)ethyl-]
24	13.230	0.19	Heptadecane,2,6,10,15-tetramethyl-
25	13.290	0.12	Hexadecane,2,6,10,14-tetramethyl-
26	13.360	0.33	2-Fluoro-,alpha,-methylbenzyl alcohol, methyl ester
27	13.534	1.42	Methyl tetradecanoate
28	14.053	0.08	2-(1H)-Quinoline, 4-hydroxy-6-(1-methylethyl)-3-phenyl
29	14.313	0.20	Heneicosane
30	14.608	0.36	Pentadecanoic acid, methyl ester
31	15.342	0.22	2-methyltetracosane
32	15.441	0.85	9-Hexadecenoic acid , methyl ester
33	15.645	23.19	Hexadecenoic acid , methyl ester
34	16.295	1.00	Hexadecenoic acid , ethyl ester
35	16.399	0.34	9,12-Octadecadienoic acid , methyl ester
36	16.613	1.10	Heptadecanoic acid , methyl ester
37	17.303	30.62	9,12-Octadecadienoic acid(Z,Z)- , methyl ester
38	17.339	16.21	9-Octadecenoic acid(Z)- , methyl ester
39	17.550	5.84	Methyl stearate
40	17.890	1.54	9,12-Octadecadienoic acid , ethyl ester
41	19.107	0.85	Cis-11-Eicosenoic ,methyl ester
42	19.305	1.49	Eicosenoic ,methyl ester
43	19.356	0.60	7,10,13-Eicosatrienoic acid , Eicosenoic ,methyl ester
44	20.924	2.18	Docosanoic acid, Eicosenoic ,methyl ester
45	21.688	0.48	Tricosanoic acid ,methyl ester
46	22.426	3.08	Tetracosanoic acid ,methyl ester
47	23.137	0.88	Pentacosanoic acid ,methyl ester
48	23.195	0.61	Oxirane,hexadecyl
49	23.572	0.55	Tricontane,-1-bromo
50	23.824	2.38	Hexacosanoic acid ,methyl ester

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