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Research Article

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Parkinsonia aculeate Oil: GC-MS Analysis and Antimicrobial Activity

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Abstract *Parkinsonia aculeata* is used traditionally as antipyretic, and leaves are said to be diaphoretic and abortifacient. Leaves are used against fever, malaria and rheumatic pain. The Leaves are also used in the treatment of bacterial infections, typhoid fever and diabetes. In this study *Parkinsonia aculeata* oil was analyzed by GC-MS. The analysis affirmed the presence of 33 components dominated by: (i) 9,12-octadecadienoic acid methyl ester (40.51%) (ii) hexadecanoic acid, methyl ester (16.80%) (iii) methyl stearate (10.97%) (iv) 9,12-octadecadienoyl chloride (5.05%). The oil was evaluated for antimicrobial activity against 5 standard human pathogens. It showed moderate activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Keywords *Parkinsonia aculeata*, GC-MS analysis, Antimicrobial Activity

Introduction

Parkinsonia aculeata is small spiny deciduous tree in the legume family (Leguminosae) [1]. The plant is native to tropical America. Now it is widely distributed in many African and Asian countries [2-3]. *Parkinsonia aculeata* grows up to 3-10 m in height and it is characterized by a green bark and smooth branches [4]. Alkaloids and steroids have been reported from the leaves, stems and flowers. The edible seeds contain some proteins. Seeds are mucilaginous and reported to contain sugars and fatty oil [5-6]. *Parkinsonia aculeata* is used traditionally as antipyretic, and leaves are said to be diaphoretic and abortifacient [7]. Leaves are used against fever, malaria and rheumatic pain [8-9]. The Leaves are also used in the treatment of bacterial infections, typhoid fever, diabetes, diabetes-related complications and trypanosomiasis [10].

Materials and Methods

Materials

Plant material

Parkinsonia aculeata seeds were collected from Hawata, eastern Sudan and authenticated by the Medicinal and Aromatic Plants Research Institute (Sudan). The seeds were shade – dried and powdered.

Instruments

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 studied oil was screened for antimicrobial activity using the standard microorganisms shown in Table(1).

Table 1: Test organisms

No	Microorganism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale, England

*ATCC. American type culture collection, Maryland, USA

Methods

Extraction of oil

Powdered shade-dried seeds of *Parkinsonia aculeata* (300g) 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 (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 shaken vigorously for five minutes and then left overnight. (2ml) of supersaturated sodium chloride were added, then (2ml) of normal hexane were added and the tube was vigorously shaken 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

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^{0}C ; Hold time(min.$^{-1}$) ,1.00

Rate : 4.00 ; Tempt. , 300.0^{0}C ; Hold time(min.$^{-1}$) ,0.00

Other chromatographic conditions are shown below:

Column oven temperature	150°C
Injection temperature	300°C
Rate	4/min.
Injection mode	split
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0ml/min.
Column flow	1.54ml/sec
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Split ratio	-1.0

Antimicrobial Assay

Mueller Hinton agar and Sabouraud dextrose agar were used as media for bacterial and fungal cultures respectively. The disc diffusion bioassay was used. The prepared microbial suspension was cast on the agar. On flooding the medium surface with the microbial suspension, the supernatant was discarded. The seeded agar was then incubated at 37^{o}C for 24hours.

Paper discs (6mm in diameter) impregnated with the test sample were placed onto the seeded agar. The plates were incubated for 24 hours at 37^{o} C. Tests were performed in duplicates and the inhibition zones were measured and averaged as indicator of activity.

Results and Discussion

***Parkinsonia aculeata* oil**

The total ion chromatograms of *Parkinsonia aculeata* oil is shown in Fig. 1 and the constituents of the oil are depicted in Table 2. The GC-MS analysis revealed the presence of 33 components dominated by (i) 9,12-octadecadienoic acid methyl ester (40.51%) (ii) of 33 components dominated by (i) 9,12-octadecadienoic acid methyl ester (40.51%) (ii) hexdecanoic acid methyl ester (16.89%) (iii) methyl stearate (10.97%) and (iv) 9,12-octadienoyl chloride (5.05%).

Table 2: Constituents of *Parkinsonia aculeata* oil

No.	RT.	Area%	Name
1	7.110	0.07	Alpha -Terpineol
2	13.704	0.28	Methyl tetradecanoate
3	14.514	0.05	4-Octadecenoic acid methyl ester
4	14.620	0.05	5-Octadecenoic acid methyl ester
5	14.780	0.16	Pentadecanoic acid methyl ester
6	15.511	0.04	7,10-hexadecadienoic acid methyl ester
7	15.574	0.06	Z-7-Hexadecenoic acid methyl ester
8	15.615	0.73	Cis-10-Nonadecenoic acid methyl ester
9	15.708	0.08	9-Hexadecenoic acid methyl ester
10	15.817	16.80	Hexadecanoic acid methyl ester
11	16.577	0.21	Cis-10-Heptadecenoic acid methyl ester
12	16.786	0.23	Heptadecanoic acid methyl ester
13	17.499	40.51	9,12-Octadecadienoic acid methyl ester
14	17.534	9.98	9-Octadecenoic –(Z)- acid methyl ester
15	17.567	3.47	9-Octadecenoic acid methyl ester
16	17.640	0.68	Phytol
17	17.729	10.97	Methyl stearate
18	18.290	0.14	Methyl 9-cis-11-trans-octadecadienoate
19	18.620	0.05	Nonadecanoic acid methyl ester
20	18.762	0.13	Gamma-Linolenic acid methyl ester
21	18.874	0.17	Methyl 9-cis-11-trans-13-trans-octadecadienoate
22	19.123	5.05	9,12-Octadecanoyl chloride
23	19.245	1.04	Oxiraneoctanoic acid , 3-octyl methyl ester
24	19.282	0.38	Cis-11-Eicosenoic acid methyl ester
25	19.481	1.89	Eicosanoic acid methyl ester
26	19.535	1.51	PGH, methyl ester
27	19.646	2.08	1-Naphthalenol, decahydro-4a-methyl-
28	20.048	0.47	Stigmast-7-en-3-ol , (3.beta, 5.alpha.,24S)
29	20.306	0.23	Heneicosanoic acid methyl ester
30	20.406	0.06	Phenol,2,2`-methylene-bis(6-(1,1-dimethylene)
31	21.102	1.15	Docosanoic acid methyl ester
32	21.868	0.42	Tricosanoic acid methyl ester
33	22.605	0.87	Tetracosanoic acid methyl ester
		100.00	

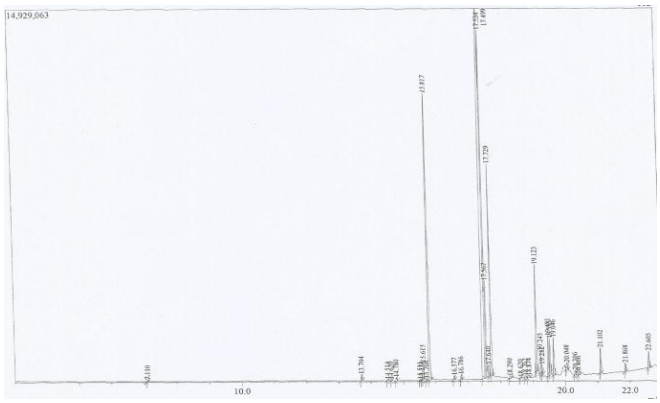

Figure 1: Total ions chromatograms

Major components are briefly discussed below:

(i) 9,12-octadecadienoic acid methyl ester (40.51%)

Fig. 2 shows the mass spectrum of the 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak at m/z 294 (RT. 17.499) corresponds to the molecular ion: $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 accounts for loss of a methoxyl.

(ii) Hexadecanoic acid, methyl ester (16.80%)

The mass spectrum of the hexadecanoic acid methyl ester is presented in Fig. 3. The peak at m/z 270 (RT. 15.817) is due to the molecular ion: $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z: 239 is attributed to loss of a methoxyl function.

(iii) Methyl stearate (10.97%)

In Fig. 4 (mass spectrum of methyl stearate), the signal at m/z 298 (RT. 17.729) accounts for $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 is due to loss of a methoxyl.

(iv) 9,12-Octadecadienoyl chloride (5.05%)

Fig. 5 shows the mass spectrum of the 9,12-octadecadienoyl chloride - (Z,Z). The peak at m/z 298 (RT. 19.123) corresponds $M^+[C_{18}H_{31}ClO]^+$. The signal at m/z 264 accounts for loss of chlorine**.**

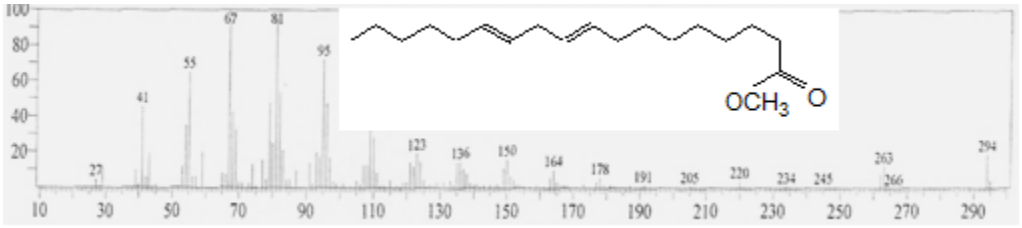

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

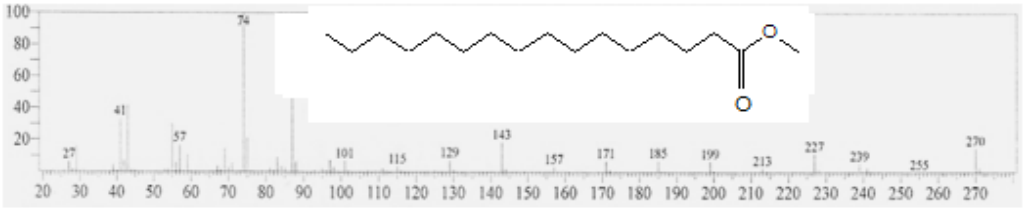

Figure 3: Mass spectrum of hexadecanoic acid, methyl ester

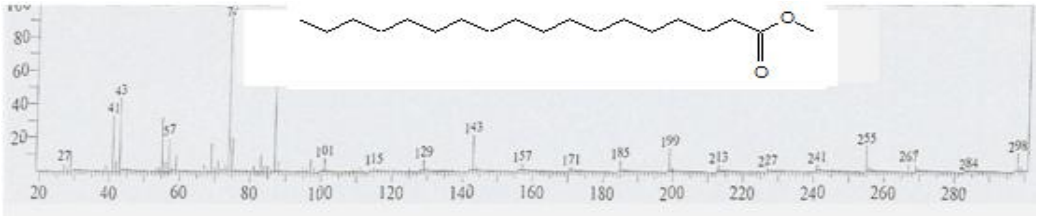

Figure 4: Mass spectrum of methyl stearate

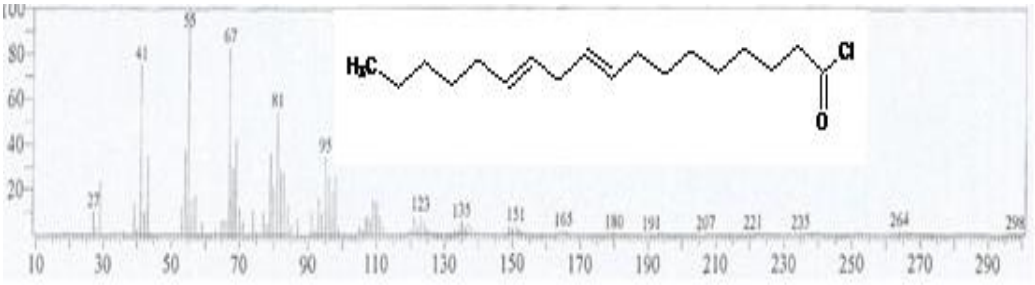

Figure 5: Mass spectrum of 9,12-octadecadienoyl chloride

Antimicrobial Activity

Parkinsonia aculeata oil was evaluated for antimicrobial activity against five standard microorganisms. The results are depicted in Table 3. Results were interpreted in the following conventional terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active).

Table 3: Inhibition zones of oil and standard drugs

Sample	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	15	--	15	14	10
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (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*

The studied oil showed moderate activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Table 3). Ampicilin, gentamicin and cltrimazole were used as positive control (Table 4).

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