

GC-MS Analysis, Antimicrobial and Antioxidant Activity of Sudanese *Adansonia digitata* L.; (*Malvaceae*) Fixed Oil

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Abstract

Medicinal plants are source of a great economic value. Plants herbs are naturally gifted at the synthesis of medicinal compounds. The extraction and characterization of bioactive compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Baobab *Adansonia digitata* L., (*Malvaceae*) seeds were collected from El-Obeid, North Kordofan state Sudan, and were extracted with hexane as solvent the extracted oil was analyzed by GC-MS. Twenty four constituents were detected the major constituents are: 9-octadecanoic acid methyl ester (25.08), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (23.08), Hexadecanoic acid, methyl ester (22.78), Methyl stearate (6.86) and Methyl 2-octylcyclopropene-1-heptanoate (6.08). In the well diffusion bioassay the oil showed significant activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and the yeast *Candida albicans* but it was partially active against *Escherichia coli*. The antioxidant activity of the extracted oil was evaluated using the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 0.5 ml. The antioxidant activity of the extracted oil was 19.00 ± 0.08 .

Keywords: Baobab, fixed oil, GC-MS, antimicrobial and antioxidant activity.

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INTRODUCTION

Medicine, in several developing countries, using local traditions and beliefs, is still the mainstay of health care. As defined by WHO, health is a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity. The practise of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand. In China about 40% of the total medicinal consumption is attributed to traditional tribal medicines [1].

Adansonia digitata L.; *Malvaceae* commonly known as Baobab indigenous to Africa where it is found in many countries such as Zimbabwe, Malawi, Mozambique, Mali, South Africa, Benin, Sudan, Senegal, the Ivory Coast, Kenya, Cameroon, Tanzania and Uganda [2]. The various parts of the plant (seeds, fruits, leaves, barks, stems and roots) have traditionally been used to treat various diseases include malaria, fever, tuberculosis, microbial infections, anaemia, diarrhoea, toothache and dysentery in many African countries [3, 4]. Baobab has numerous biological properties including antimicrobial, Analgesic, antipyretic, antiviral, anti-oxidant, Anti-insecticidal and

anti-inflammatory activities [5-8]. From different plant parts various nutritional and phytochemical ingredients were separated such as vitamin-C, minerals, amino acid, flavonoids, steroids, epicatechin, fatty acids, campesterol and tocopherol [2, 9]. This study was conducted to investigate the chemical constituents and biological activity of *Adansonia digitata* seeds oil.

MATERIAL AND METHODS

Plant material

Seeds of *Adansonia digitata* was collected in December (2018) from El-Obeid North Kordofan state Sudan. The plant was authenticated by a plant taxonomist at the Department of Botany Faculty of Science University of Kordofan Sudan. All chemicals used in this experiment were of analytical grade purchased from Sigma Chemical Co. (USA).

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MSQP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μ m, thickness). And anti-oxidant activity was measured on spectrophotometer.

Test organisms

The *Adansonia digitata* seeds oil was screened for antimicrobial activity using the standard microorganisms as shown in (Table No.1).

METHODS

Extraction of oil

Dry powdered seeds of *Adansonia digitata* (50.0g) were placed in thimble of soxhlet apparatus. A dry pre-weighed solvent flask containing hexane and condenser were attached and oil was extracted for 7 hours. The thimble was removed and hexane was retained. The excess of hexane was then evaporated on a hot water bath and the flask was dried in a desiccator and weighed. Oil percentage was calculated by subtracting the weight of empty flask from the weight of the flask containing the oil after solvent evaporation dividing it by the weight of the sample taken in grams.

GC-MS analysis

The qualitative and quantitative analysis of the sample was carried out by using GC-MS technique model (GCMS-QP2010-Ultra) from japons Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C/min to 300°C as final temperature degree with 3 minutes hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library of National Institute of Standards and Technology (NIST) [10].

In vitro antimicrobial assay

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed on to nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 108-109 colony forming unit per ml. The suspension was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry 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 drop (0.02 ml) 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. Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained [11].

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used [11].

Testing of antibacterial susceptibility

Disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton Agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines Bacterial suspension was diluted with sterile 5 minutes. Sterilized filter paper discs (Whitman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24h in the inverted position. The diameters (mm) of the inhibition zones were measured [11].

Antioxidant assay

The DPPH radical scavenging was determined according to the method of Shimada *et al.* with some modification. In 96-wells plate, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplayer reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate [12].

RESULTS AND DISCUSSION

GC-MS analysis of *Adansonia digitata* seeds oil

The GC-MS analysis of the studied oil revealed the presence of 24 constituents (Table No.2). The typical total ion of the following constituents was detected in the chromatogram (Figure No.1).

As major constituents

The mass spectrum of 9-octadecanoic acid methyl ester is displayed in (Figure No.2).The peak at m/z 296, which appeared at R.T. 18.358 corresponds

$M^+[C_{19}H_{36}O_2]^+$. The signal at m/z264 accounts for loss of a methoxyl function.

The mass spectrum of 9,12-octadecanoic acid methyl ester is shown in (Figure No.3).The peak at m/z 294, which appeared at R.T. 18.292 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$. The signal at m/z263 corresponds to loss of a methoxyl function.

The mass spectrum of hexadecanoic acid methyl ester is depicted in (Figure No.4).The signal at m/z 270 (R.T. 15.688) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z239 is due to loss of a methoxyl.

The mass spectrum of methyl stearate as shown in (Figure No.5).The signal at m/z 298(R.T. 18.527) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z267 corresponds to loss of a methoxyl.

The mass spectrum of Methyl 2-octylcyclopropene is depicted in (Figure No.6).The signal at m/z 294 (R.T. 18.015) corresponds

$M^+[C_{17}H_{34}O_2]^+$. The signal at m/z239 is due to loss of a methoxyl.

Antimicrobial activity

The oil was screened for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are shown in (Table No.3). The results were interpreted in terms of the commonly used terms (>9mm: inactive;9-12mm:partially active;13-18mm:active;<18mm: very active). (Table No.3). also represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Antioxidant activity

The percentage of DPPH radical scavenging activity (RSA) of essential oil was 19% lower than Standard (Table No.4). The results indicated that of *Adansonia digitata* seeds contain anti-oxidant compounds this is in agreement with previously reported studies [11].

Table-1: Test organisms

S.No	Microorganisms	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Escherichia coli</i>	G-ve
4	<i>Pseudomonas aeruginosa</i>	G-ve
5	<i>Candida albicans</i>	Fungus

Table-2: Constituents of *Adansonia digitata* seeds oil

S.No	Name	R.Time	Area	Area%
1.	Methyl tetradecanoate	14.276	1791813	0.50
2.	cis-5-Dodecenoic acid, methyl ester	15.137	82173	0.02
3.	5-Octadecenoic acid, methyl ester	15.248	70642	0.02
4.	Pentadecanoic acid, methyl ester	15.413	349131	0.10
5.	7,10-Hexadecadienoic acid, methyl ester	16.192	109135	0.03
6.	7-Hexadecenoic acid, methyl ester, (Z)-	16.256	302005	0.08
7.	9-Hexadecenoic acid, methyl ester, (Z)-	16.297	1089533	0.30
8.	Hexadecanoic acid, methyl ester	16.534	82424943	22.78
9.	Methyl 9,12-heptadecadienoate	17.247	1692192	0.47
10.	cis-10-Heptadecenoic acid, methyl ester	17.300	2182275	0.60
11.	Heptadecanoic acid, methyl ester	17.529	1642547	0.45
12.	Methyl 2-octylcyclopropene-1-heptanoate	18.015	21986105	6.08
13.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.292	83529035	23.08
14.	9-Octadecenoic acid (Z)-, methyl ester	18.358	90728104	25.08
15.	Methyl stearate	18.527	24833196	6.86
16.	cis-11,14-Eicosadienoic acid, methyl ester	18.973	11806173	3.26
17.	cis-10-Nonadecenoic acid, methyl ester	19.347	18843516	5.21
18.	cis-11-Eicosenoic acid, methyl ester	20.165	3080587	0.85
19.	Eicosanoic acid, methyl ester	20.366	6735316	1.86
20.	Docosanoic acid, methyl ester	22.072	2216227	0.61
21.	Tricosanoic acid, methyl ester	22.875	571977	0.16
22.	Tetracosanoic acid, methyl ester	23.651	1823150	0.50
23.	Gamma-Sitosterol	24.288	3810842	1.05
24.	Gamma-Tocopherol	26.714	175818	0.05

Table-3: Antioxidant activity of *Adansonia digitata* seeds oil

Drug	Conc. (mg/ml)	E.c	P.s	S,a	B.s	C.a
Seeds Oil	100	11	15	17	16	18
Gentamicin	40	22	21	19	25	—
Clotrimazole	40	—	—	—	—	28

Note: E.c = *Escherichia coli*, P.s = *Pseudomonas aeruginosa*, S.a = *Staphylococcus aureus*, B.s = *Bacillus subtilis*, C.a = *Candida albicans*.

Table-4: Antioxidant activity of *Adansonia digitata* seeds oil

S. No	Sample Code	%RSA ±SD (DPPH)
1	Seeds oil	19.00 ± 0.08
Standard	Propyl gallate	95.00 ± 0.02

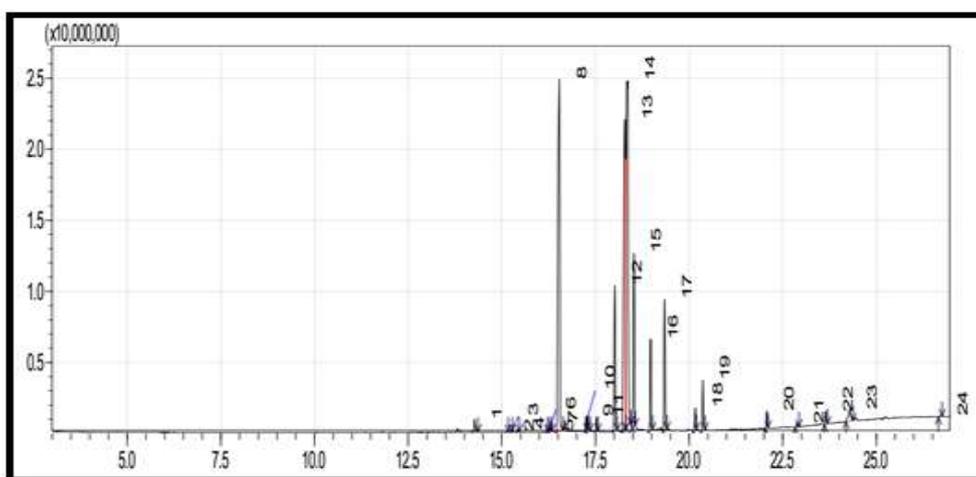


Fig-1: Chromatograms of *Adansonia digitata* seeds oil

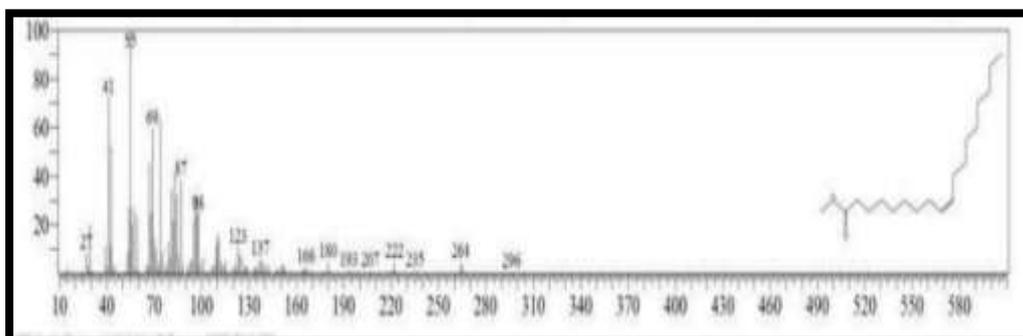


Fig-2: Mass spectrum of 9-octadecanoic acid methyl ester

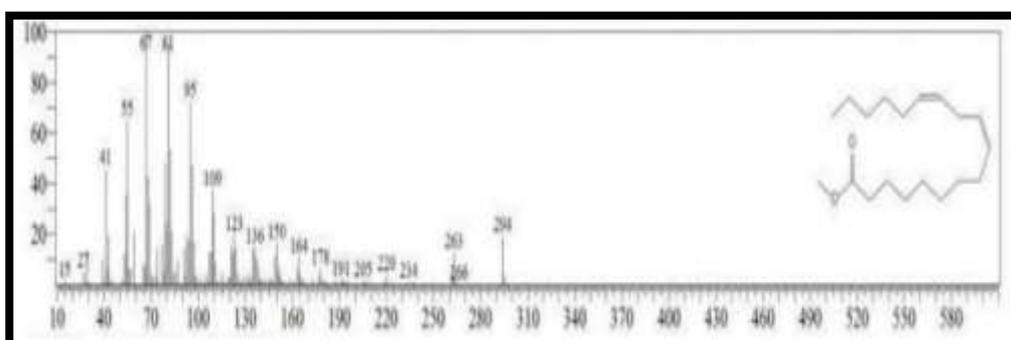


Fig-3: Mass spectrum 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

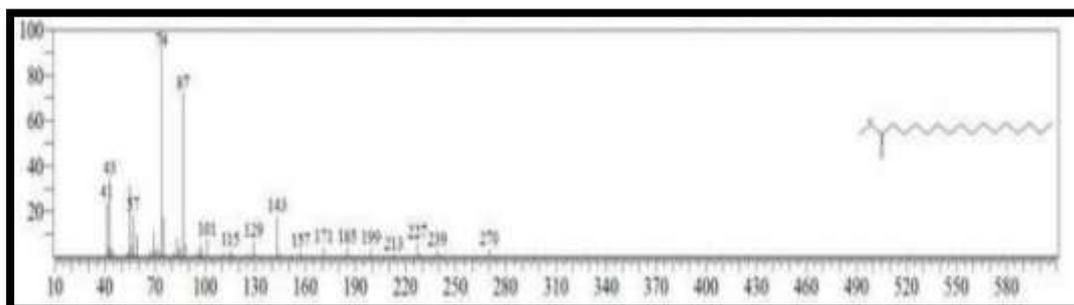


Fig-4: Mass spectrum Hexadecanoic acid, methyl ester

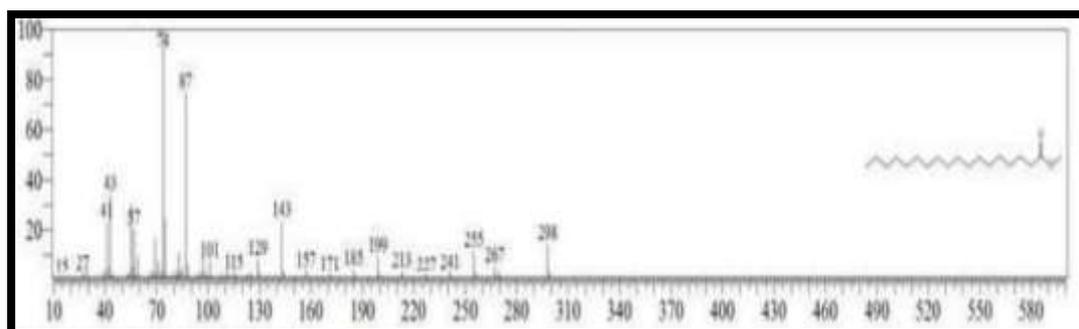


Fig-5: Mass spectrum of methyl stearate

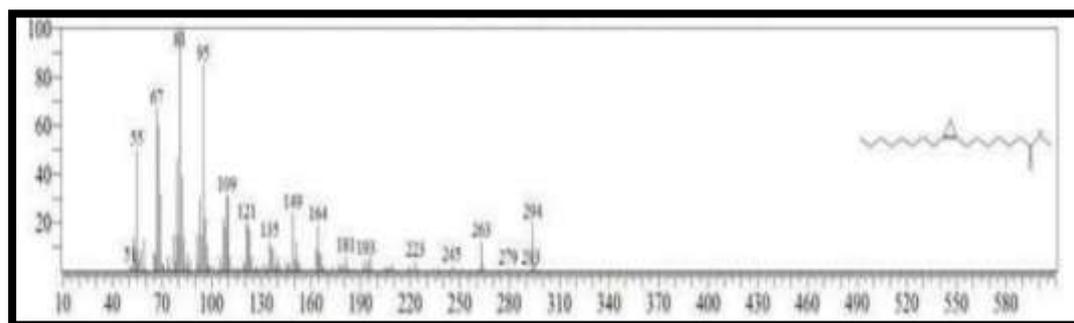


Fig-6: Mass spectrum Methyl 2-octylcyclopropene-1-heptanoate

CONCLUSION

The results indicated that the hexane extract of the Baobab seeds oil content twenty four compounds and has potential antimicrobial and anti-oxidant activity. The oil showed activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and the yeast *Candida albicans*. But it was partially active against against *Escherichia coli*.

ACKNOWLEDGMENT

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Conflict of Interest

There is no conflict of interest.

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