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Research Article

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Constituents and Antimicrobial Activity of *Sudanese Sorghum* bicolor (Gramineae) Oil

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Abstract Sorghum is the fifth most grown crop worldwide. In Africa Sorghum bicolor has been cultivated long ago. *Sorghum bicolor* is rich in phenolics which are known for their diverse pharmacological properties. In this study *Sorghum bicolor* oil was studied by GC-MS. The oil was also evaluated for antimicrobial activity. The GC-MS analysis showed 33 components dominated by: 9,12-octadecadienoic acid (35.91%), pentadecanoic acid (11.46%), tetrapentacontane (11.45%), oleic acid (9.24%). The studied oil showed significant activity against *Bacillus subtilis*.

Keywords *Sorghum bicolor*, Oil, GC-MS analysis, antimicrobial activity

Introduction

Sorghum is a genus of plants in the family Gramineae which is widely cultivated as cereal crop [1]. Sorghum is the fifth most grown crop worldwide. In Africa Sorghum bicolor has been cultivated long ago [1]. This plant is a cone-like grass reaching 6m in height with branched clusters of grains. *Sorghum bicolor* is rich in phenolics which are known for their diverse pharmacological properties [2]. The flavonoids of this plant species contains , among others, apiginidin and 3-deoxyanthocyanidin [3,4]. In contrast to other grains, sorghum grains are endowed with higher flavonoid content ranking them as an important diet [4]. Many health promoting effects has been associated with sorghum intake. The *in vivo* antianemic properties of Sorghum bicolor has been reported [5,6]. It has been shown that the leaf extracts possess anti-inflammatory activity [7-9]. Leave extract also exhibited significant free radical scavenging capacity [9]. Moreover, the role of leave extract in neurocognitive disorder has been documented [10,11].

Materials and Methods

Plant Material

Sorghum bicolor seeds were purchased from the local market, Khartoum, Sudan and authenticated by comparison with a reference herbarium sample.

GC-MS analysis

GC-MS analysis of the oil 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 *Sorghum bicolor* was screened for antimicrobial activity using the standard microorganisms shown in Table 1.

Table 1: Test organisms

S. No.	Microorganism	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 seeds of *Sorghum bicolor* (350g) 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. 2 ml 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

Sorghum bicolor oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas.

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

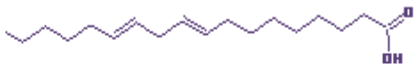
Antimicrobial assay

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. 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 ^{o}C for 24 h, while for fungi the plates were incubated at 25 ^{o}C for 3 days. The assay was carried out in duplicates and the diameters of inhibition zone were measured and averaged. Ampicilin, gentamicin and clotrimazole were used as positive control and DMSO as negative control.

Results and Discussion

The seed oil of *Sorghum bicolor* has been analyzed by GC-MS. Thirty three components were detected (Table 3). Major constituents of the oil are:

i)-9,12-Octadecadienoic acid (35.91%),



9,12-octadecadienoic acid

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Figure 1. The peak at m/z 294, which appeared at R.T. 19.003 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.

ii)-Pentadecanoic acid (11.46%),

Pentadecanoic acid

The EI mass spectrum of pentadecanoic acid methyl ester is displayed in Figure 2. The peak at m/z 256, which appeared at R.T. 17.249 in total ion chromatogram, corresponds $M^+[C_{16}H_{32}O_2]^+$. The peak at m/z225 corresponds to loss of a methoxyl function.

iii)- Tetrapentacontane (11.45%),

Tetrapentacontane

Figure 3 shows the mass spectrum of the tetrapentacontane. The peak at m/z 743 (RT., 22.163) is due to $[M - CH_3]^+$.

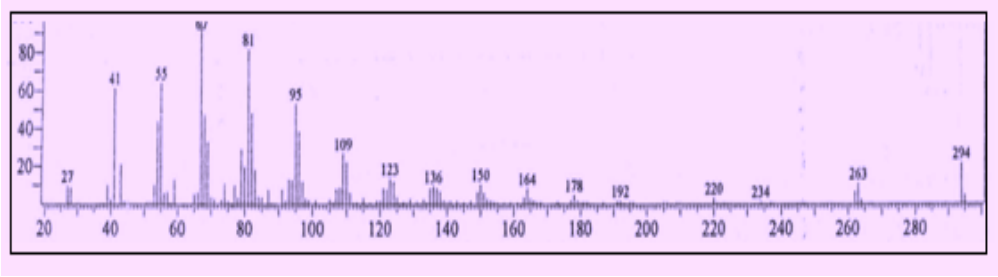


Figure 1: Mass spectrum of 9,12-octadecanoic acid methyl ester

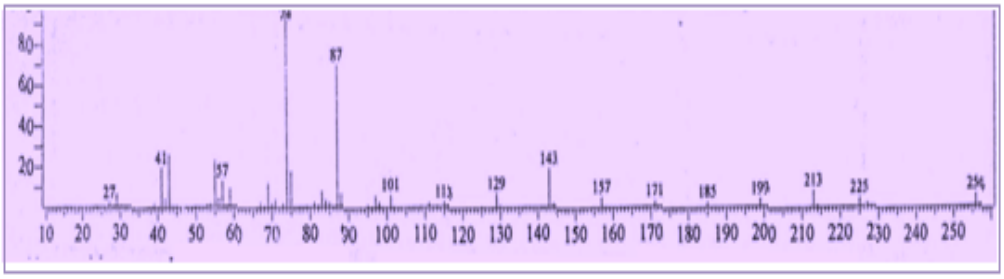


Figure 2: Mass spectrum of pentadecanoic acid methyl ester

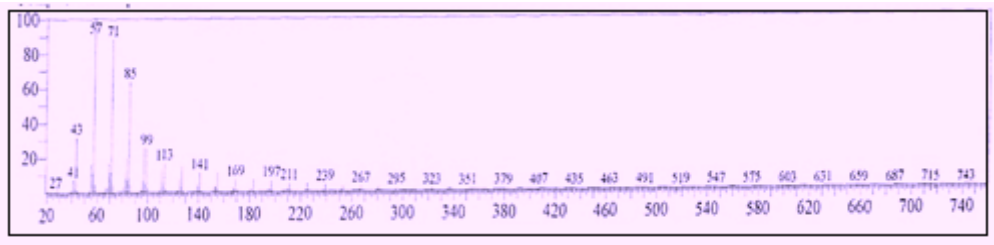


Figure 3: Mass spectrum of tetrapentacontane

Antimicrobial activity

Sorghum bicolor 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 in conventional terms: (<9mm: inative; 9-12mm: partially active; 13-18mm: active;>18mm: very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The studied oil showed significant activity against *Bacillus subtilis* and moderate activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans.*

Table 2: Inhibition zones(mm/mg sample)

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

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

Table 3: Constituents of the oil

No.	Name	R. Time	Area %
1	2-Heptanal	4.583	0.07
2	Butyric acid, 4-pentadecyl ester	6.560	0.07
3	Nonanal	6.700	0.10
4	Octanoic acid methyl ester	6.975	0.16
5	Octanoic acid	7.698	0.22
6	Alpha – Terpineol	8.063	0.06
7	3,4-Nonadienal	8.344	0.03
8	2-Decenal	8.995	0.27
9	2,4-Dodecadienal	9.459	0.64
10	2,4-Dodecadienal , E,E-	9.776	1.31
11	2-Undecenal	10.392	0.19
12	Nonanoic acid , 9-oxo, methyl ester	11.317	0.17
13	2-Pentadecanone, 6,10,14- trimethyl	16.014	0.07
14	9-Hexadecenoic acid , methyl ester -Z-	16.630	0.16
15	Hexadecenoic acid , methyl ester	16.824	6.22
16	Palmitoleic acid	17.026	0.19
17	Pentadecanoic acid	17.249	11.46
18	9,12-Octadecadienoic acid (Z,Z) , methyl ester	18.485	8.43
19	9-Octadecenoic acid (Z) , methyl ester	18.533	6.85
20	9-Octadecenoic acid , methyl ester	18.571	0.61
21	Methyl stearate	18.739	1.02

22	9,12-Octadecadienoic acid (Z,Z)	19.003	35.91
23	Oleic acid	19.038	9.24
24	Oxiraneoctanoic acid, 30ctyl- , methyl ester	20.142	0.16
25	Eicosanoic acid, methyl ester	20.500	0.33
26	PGHI, methyl ester	20.557	0.26
27	Methyl 13,16-docosadienoate	20.666	0.28
28	9,12,15-Octadecatrienoic acid methyl ester	21.005	0.68
29	Phenol , 2,2` - methylenebis[6-(1,1-dimethylene)]	21.422	0.19
30	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	21.753	0.61
31	Dotriacontane	21.861	0.34
32	Tetrapentacontane	22.163	11.45
33	Octacosanoic acid , methyl ester	22.913	2.24
			100.00

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