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Research Article

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GC-MS Analysis and Antimicrobial Activity of Sudanese *Guiera senegalensis* Oil

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Abstract *Guiera senegalensis* is a shrub (3-5m in height) found mainly in west Africa.Literature reports revealed several traditional uses for this plant.It is used against fever, respiratory congestion , cough, lung disorders and malaria. GC- MS analysis of *Guiera senegalensis* oil revealed the presence of the following major components: i)- 9,12-octadecadienoic acid (Z,Z)-, methyl ester (23.77%). ii)- hexadecanoic acid, methyl ester (22.92%) , iii- 9-octadecenoic acid methyl ester (21.14%) and iv)- methyl stearate (12.27%). *Guiera senegalensis* oil was assessed for antimicrobial activity against five standard microorganisms. The oil showed moderate activity against *Staphylococcus aureus* beside weak activity against other test organisms.

Keywords *Guiera senegalensis*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

Guiera senegalensis is a shrub (3-5m in height) found mainly in West Africa [1]. Literature reports revealed several traditional uses for this plant [2]. It is used against fever, respiratory congestion, cough, lung disorders and malaria [3-9].

Leaves, bark and root of this plant are recommended for stomach pain, beriberi, leprosy, impotency, syphilis and diarrhea [10,11]. *Guiera senegalensis* is also used in veterinary medicine to improve body weight and milk secretion [3]. Gall nuts are mixed with charcoal to form a very strong diuretic agent. The mixture is also used for oliguria and cerebral malaria [11,12]. Leaf tea is used in the treatment of eczema, chest conditions, fever and colds [13]. Fresh mashed leaves are used as a natural remedy for wounds [14]. Leaves are applied as poultice for Guinea worms and tumors [14]. It has been reported that *Guiera senegalensis* possesses antimicrobial, antioxidant, anticancer and trypanocidal properties [15-23].

Materials and Methods

Plant Material

Guiera senegalensis seeds were collected from a forest around Damazin-Sudan. The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Instruments

The GC-MS analysis was carried out on A Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness).

Test organisms

Test organisms used in this study are: *Bacillus subtilis* (G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil

Powdered seeds of *Guiera senegalensis* (300g) were macerated with n-hexane for 48h. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

(2ml) of the oil was mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving sodium (2 g) in 100 ml methanol. (7 ml) Alcoholic sulfuric acid (1ml H_2SO_4 in 100 ml methanol) was then added. The mixture was shaked for 5 minutes. The content of the test tube was left to stand overnight. Then (1ml) of supersaturated sodium chloride was added and the tube was shaked for 5 min. (2ml) of normal hexane were added and the contents were shaked thoroughly for 5 minutes. (5 µl) of the n-hexane were diluted with (5ml) of diethyl ether and dried over anhydrous sodium sulphite. (1µl) of the diluted sample was injected in the GC MS vial.

The qualitative and quantitative analysis of the sample was carried out by using a Shimadzu machine-model (GC/MS-QP2010-Ultra) The sample was injected under the following chromatographic conditions: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0^{o}C; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0ml/min; column flow: 1.54ml/sec. ; linear velocity: 47.2cm/sec.; purge flow: 3.0 ml/min.; split ratio: -1.0. Oven temperature program is presented Table 1.

Table 1: Oven temperature program

Rate	Temperature (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

Antimicrobial assay

The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 10^{8}cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 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 test sample. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and recorded as average of two replicates.

Results and Discussion

Guiera senegalensis oil was studied by GC-MS. The analysis showed the presence of 10 constituents (Table 2). The total ions chromatogram is depicted in Fig. 1.

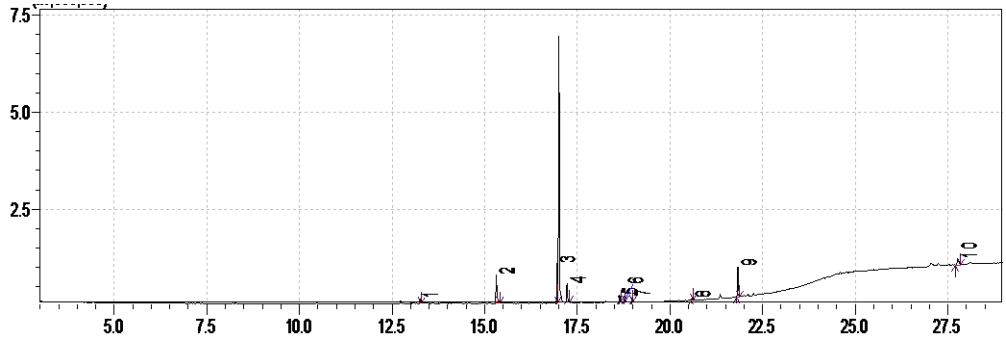

Figure 1: Total ions chromatograms

Table 2: Constituents of the oil

No.	Name	Ret. Time	Area%
1.	1-Octanamine, N-methyl-N-octyl-	13.247	3.38
2.	Hexadecanoic acid, methyl ester	15.311	22.92
3.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.955	23.77
4.	9-Octadecenoic acid methyl ester	17.218	21.14
5.	Methyl stearate	17.220	12.27
6.	Triisooctylamine	18.636	3.97
7.	11-Octadecenoic acid, methyl ester	18.772	1.31
8.	Eicosanoic acid, methyl ester	18.975	2.92
9.	Docosanoic acid, methyl ester	20.595	1.61
10.	alpha.-Amyrin	27.776	6.71

The GC-MS analysis of the oil showed the following major components:

i- 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (23.77%).

ii- Hexadecanoic acid, methyl ester (22.92%).

iii-9-Octadecenoic acid methyl ester (21.14%)

iii- Methyl stearate (12.27%)..

Fig. 2 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294 (RT. 16.955) corresponds $M^+ [C_{19}H_{34}O_2]^+$. The mass spectrum of hexadecanoic acid methyl ester is presented in Fig. 3.The peak at m/z 270 which appeared at (RT.15.311) is due to $M^+ [C_{17}H_{32}O_2]^+$. Fig. 4 shows the mass spectrum 9-octadecenoic acid methyl ester. The signal at m/z296 (RT.17.218) corresponds $M^+ [C_{19}H_{36}O_2]^+$ .The mass spectrum of methyl stereate is illustrated in Fig. 5. The signal at m/z 298 (R.T.17.220) corresponds M+$[C_{19}H_{38}O_2]$+ .

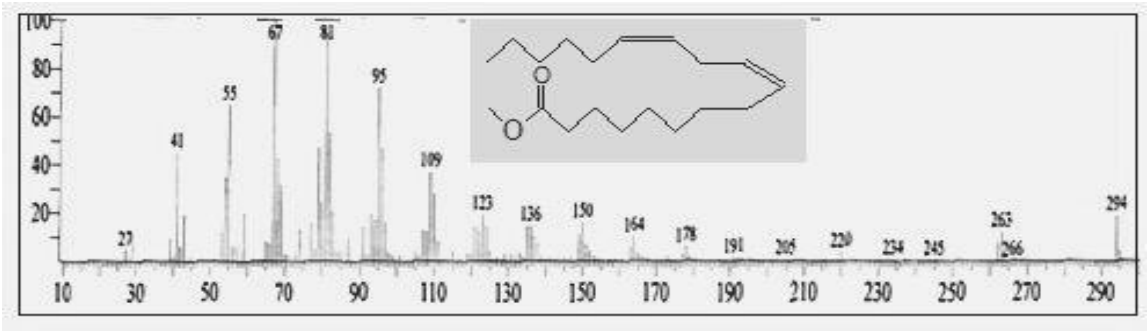

Figure 2: Mass spectrum of 9,12-octadecadienoic acid

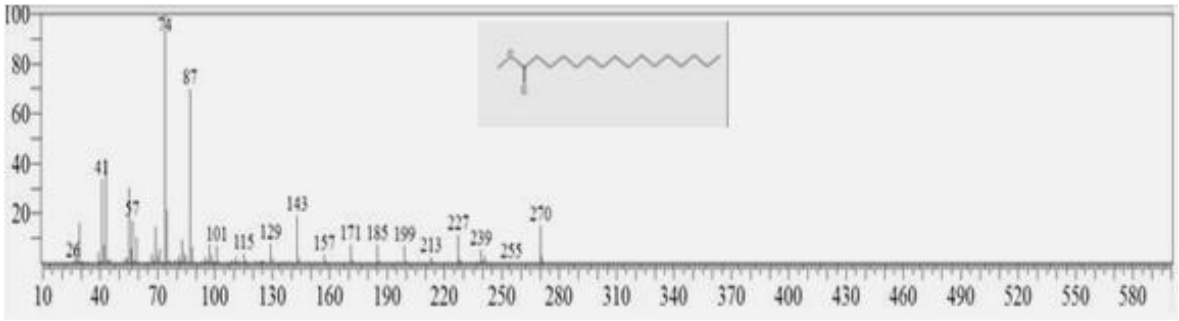

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

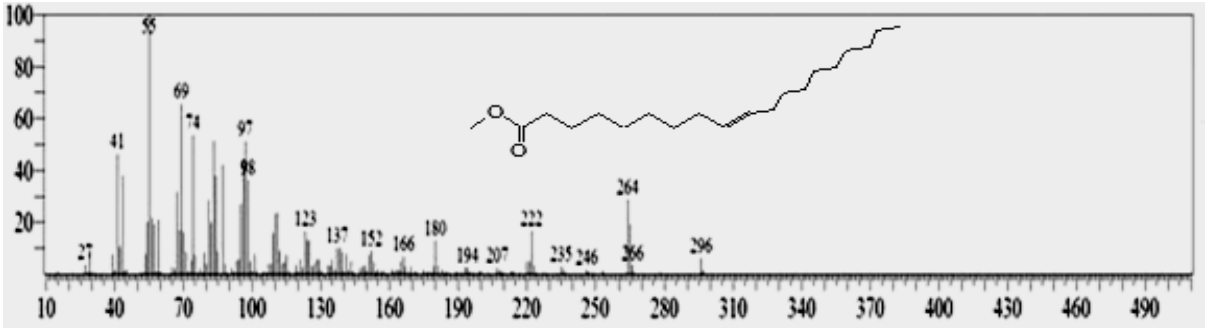

Figure 4: Mass spectrum for 9-octadecenoic acid[z]-, methyl ester

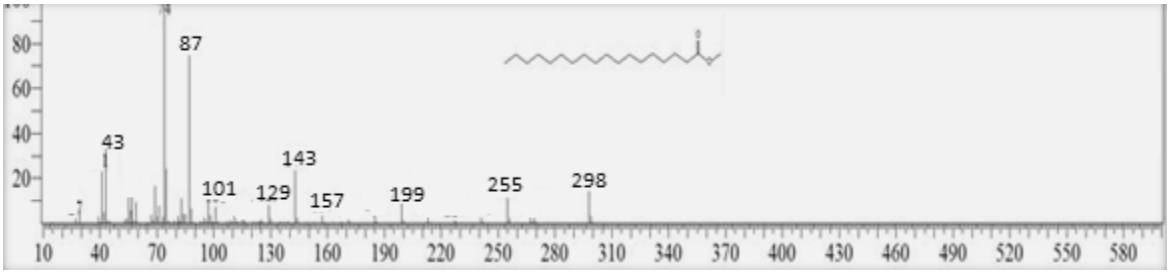

Figure 5: Mass spectrum of methyl stearate

Antimicrobial activity

Guiera senegalensis oil was assessed for antimicrobial activity against five standard microorganisms. The average of the diameters of the growth inhibition zones are presented in Table 3. 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 oil showed moderate activity against *Staphylococcus aureus* beside weak activity against other test organisms.

Table 3: Inhibition zones (mm/mg sample)

Type	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	15	11	13	11	13
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*

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