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Research Article

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GC-MS Studies and Antimicrobial Activity of *Hibiscus asper* Grown in Sudan

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Abstract *Hibiscus asper* is a perennial herb. The calyx and leaf of *H. asper* are commonly used traditionally in Africa as anti-inflammatory, anti-depressive, sedative, tonic, anti-anaemic. Leaves are used against jaundice. Calyx and leaves are also used in African system of medicine for the treatment of abscesses, urethritis, joint pain, male infertility and skin infections In this study *Hibiscus asper* oil was studied by GC-MS .Major constituents of the oil are: i) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (41.34%); 9-octadecenoic acid (Z)-, methyl ester (25.90%); hexadecanoic acid, methyl ester (20.84%) and methyl stearate (6.67%). The oil was assessed for antimicrobial activity. At a concentration of 100mg/ml, the oil showed partial activity against *Staphylococcus aureus* and *Escherichia coli.*

Keywords *Hibiscus asper*, Oil, GC-MS Analysis, Antimicrobial Activity

Introduction

The genus *Hibiscus* belongs to the family Malvaceae. It comprises about 250 species distributed in tropical and subtropical areas [1]. *Hibiscus asper* is a perennial herb. Stems with five prickles, simple or stellate hairs up to 2 m tall. Its leaves are alternate, simple and stipules up to 6 mm long [2].

The calyx and leaf of *H. asper* are commonly used traditionally in Africa as anti-inflammatory, anti-depressive, sedative, tonic, , anti-anaemic .Leaves are used against jaundice [3]. Calyx and leaves are also used in African system of medicine for the treatment of abscesses, urethritis, joint pain, male infertility and skin infections. It has been reported that [4] the plant is used in veterinary medicine as an anti-parasitic. Phytochemical screening of leaves revealed the presence of many secondary metabolites including saponins , alkaloids, tannins and flavonoids [5].

Materials and Methods

Plant material

Hibiscus asper Hook. was collected from western Kurdofan State –Sudan. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum – Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness) was used to identify and quantify the components of the studied oil.

Test organisms

The antimicrobial activity of *Hibscus asper* seed oil was evaluated using the following standard microorganisms: *Bacillus subtilis* (Gram +ve), *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve), *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans*.

Methods

Extraction of oil from *Hibiscus asper* seeds

Powdered shade-dried seeds of *Hibiscus asper* (300g) were exhaustively extracted with *n*-hexane (soxhlet). The solvent was removed under reduced pressure giving the oil.

GC-MS analysis [6]

Hibiscus Asper seed oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are as follows: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0 ^{o}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.

Antimicrobial Activity

A (24) hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated for 24h at 37º C. Bacterial growth was washed off with 100 ml sterile normal saline giving approximately 10^8- 10^9 C.F.U/ ml. The average number of viable organisms per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline solution

(0.02 ml) volumes of the appropriate dilution were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature and then incubated at 37°C for 24 hours.

The fungal cultures were maintained on Sabouraud dextrose agar. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) soaked with a solution of each test sample were placed on the surface of the seeded agar. The inoculated plates were incubated at 37 °C for 24 h. The diameters (mm) of the inhibition zones were measured as average of two replicates [7].

Results and Discussion

GC-MS analysis of *Hibiscus asper* oil was conducted and comparison of the mass spectra with the database on MS library revealed about 90-95% match. The GC-MS analysis revealed the presence of 16 components – Table 1. The total ion chromatograms is given in Fig. 1.

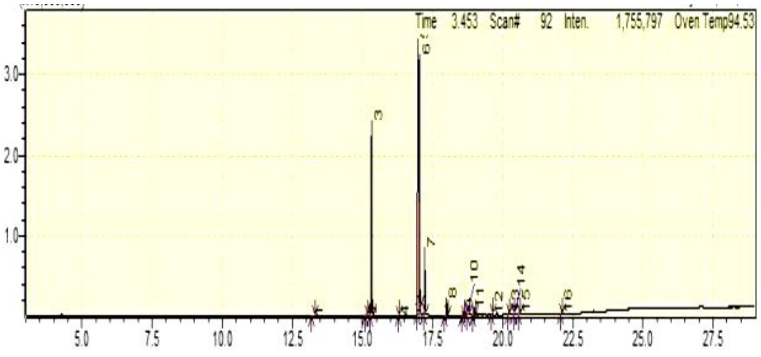

Figure 1: Total ions chromatograms of the oil

Table 1: Constituents of *Hibiscus asper* oil

ID#	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	13.227	261033	0.13
2.	9-Hexadecenoic acid, methyl ester, (Z)-	15.120	338367	0.17
3.	Hexadecanoic acid, methyl ester	15.322	42225774	20.84
4.	Heptadecanoic acid, methyl ester	16.289	137205	0.07
5.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.985	83755167	41.34
6.	9-Octadecenoic acid (Z)-, methyl ester	17.029	52480010	25.90
7.	Methyl stearate	17.222	13519511	6.67
8.	10-Octadecenoic acid, methyl ester	17.993	3552938	1.75
9.	13-Tetradece-11-yn-1-ol	18.580	1517499	0.75
10	Oleic Acid	18.748	463366	0.23
11	Eicosanoic acid, methyl ester	18.975	1496187	0.74
12	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	19.629	255119	0.13
13	(Z)6,(Z)9-Pentadecadien-1-ol	20.212	1251810	0.62
14	15-Tetracosenoic acid, methyl ester	20.417	327933	0.16
15	Docosanoic acid, methyl ester	20.595	601289	0.30
16	Triacontanoic acid, methyl ester	22.097	414901	0.20
			202598109	100.00

The GC-MS analysis showed the following major constituents: i) 9,12-octadecadienoic acid (Z,Z)-, methyl ester (41.34%); 9-octadecenoic acid (Z)-, methyl ester (25.90%); hexadecanoic acid, methyl ester (20.84%) and methyl stearate (6.67%).

Fig. 2 shows the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The peak which appeared at m/z 294 (R.T. 17.565) is due to $M^+[C_{19}H_{34}O_2]^+$. The loss of a methoxyl is evidenced by the peak at m/z 263.

9,12-octadecadienoic (linoleic acid) belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human bodies and are available through diet [6]. Linoleic acid is used in the biosynthesis of arachidonic acid. In vivo studies indicated that linoleate deficiency is associated with hair loss and poor wound healing [7, 8]. The EI mass spectrum of 9-octadecenoic acid methyl ester is shown in Fig. 3. The peak at m/z 296 (R.T. 17.605) accounts for the molecular ion: $M^+[C_{19}H_{36}O_2]^+$ .The signal at m/z 266 is due to loss of a methoxyl. Oleic (9-octadecenoic acid) acid is a common monounsaturated fat in human diet. The consumption of oleate in olive oil has been associated with decreased risk of breast cancer [9]. This acid may be responsible for the hypotensive potential of olive oil [10]. The mass spectrum of hexadecanoic acid methyl ester is illustrated in Fig. 4. The signal which appeared at m/z 270 (R.T. 15.848) is attributed to: $M^+[C_{17}H_{34}O_2]^+$. The loss of a methoxyl function is evidenced by the peak at m/z 239. Palmitic acid (hexadecanoic acid) is the most common fatty acid in plants and animals [11]. It is considered as the precursor of long-chain fatty acids .This acid is a major lipid component of human breast milk [12]. The EI mass spectrum of methyl stearate is shown in Fig. 5. The peak at m/z 298 (R.T. 17.763) accounts for: $M^+[C_{19}H_{38}O_2]^+$ while the signal at m/z 267 is attributed to loss of a methoxyl.

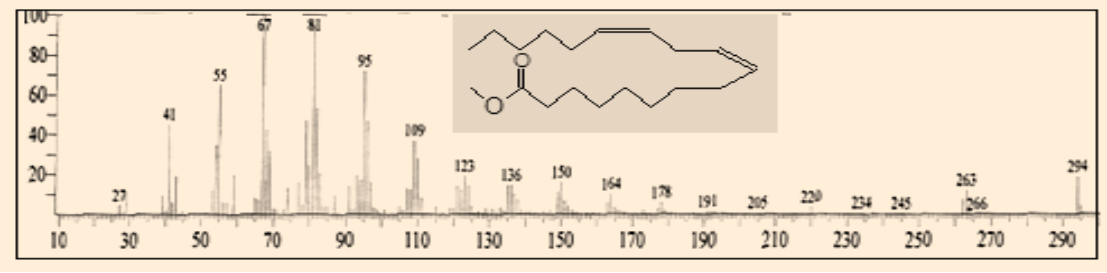

Figure 2: Mass spectrum of 9,12-octadecanoic acid methyl ester

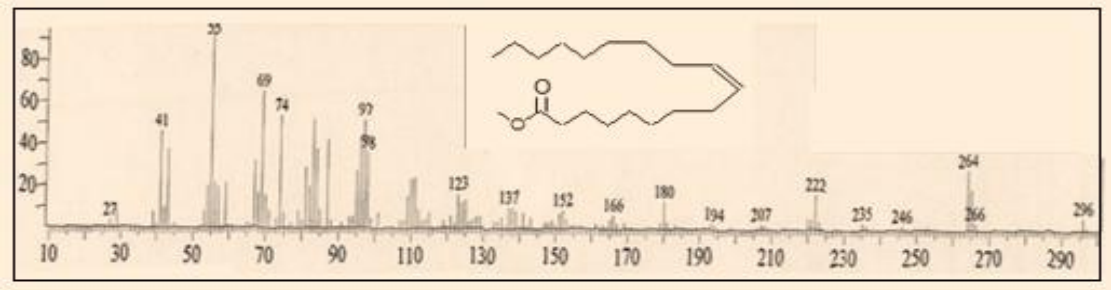

Figure 3: Mass spectrum of 9-octadecenoic acid methyl ester

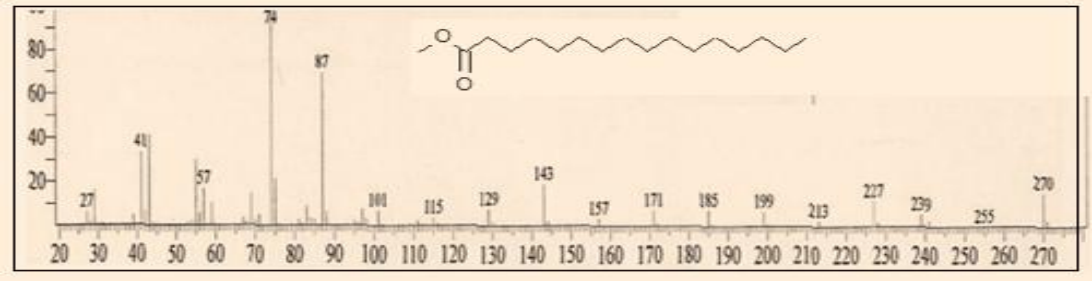

Figure 4: Mass spectrum of hexadecanoic acid methyl ester

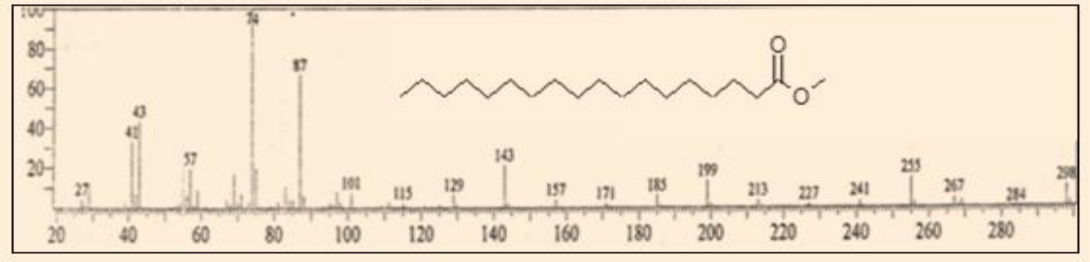

Figure 5: Mass spectrum of methyl stereate

Antibacterial Activity

The studied oil was evaluated for antimicrobial activity. The averages of the diameters of the growth inhibition zones are shown in Table 2. The results were interpreted in terms of the commonly used terms; >9mm: inactive; 9-12mm: partially active; 13-18mm: active;<18mm: very active. Tables 3 and 4 represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively. At a concentration of 100mg/ml the oils showed partial activity against *Staphylococcus aureus* and *Escherichia coli.*

Table 2: Inhibition zones (mm) of the oil

Bs	Sa	Ps	Ec	Ca	Conc (mg/ml)	Drug
-	13	-	10	8	100	Oil

Table 3: Inhibition zones (mm) of standard antibacterial agent

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Inhibition zones (mm) of standard antifungal agent

Drug	Conc. mg/ml	Ca.
Clotrimazole	30	38
	15	31
	7.5	29

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

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