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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE CASSIA FISTULA LINN (CAESALPINACEAE) FIXED OIL

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ABSTRACT

Cassia fistula fixed seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. Twenty five components were detected by GC-MS analysis. Main constituents are: 9, 12-Octadecadienoic acid methyl ester (24.46%); 9-Octadecenoic acid methyl ester (17.26%); Stigmast-7-en-3-ol (15.75%); Hexadecanoic acid methyl ester(13.55%) and Methyl stearate (7.75%). The antibacterial activity of the oil was evaluated via the diffusion assay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). The oil was active against all test organisms in the range: 100 - 50mg/ml. On the basis of its promising antimicrobial activity, it seems that this oil is a lead for further opimization.

KEYWORDS: Cassia fistula, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

Cassia fistula Linn (Caesalpinaceae) is a deciduous tree of middle size reaching up to 10m in height. Traditional claims include treatment of ulcers and other intestinal disorders.[1] The plant is also used as laxative and tonic.[2] *Cassia fistula* has also been used for haematemesis, diabetes, skin diseases and liver disorders.[3] It has been reported that the plant has analgesic,[4] antioxidant,[5] antifungal[6] and hepatoprotective properties.[7]

Phytochemical screening revealed the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, anthraquinones, steroids and reducing sugars.[8] Stem bark contains flavonols beside xanthones.[9] In a study, the ethyl acetate fraction of the bark showed significant hypoglycemic activity and the mechanism underlaying this action was addressed.[10,11] Different in vivo studies documented the hepatoprotective effect of the seeds and fruits.[12-15] Aqueous and methanol extracts showed significant radical scavenging capacity in the DPPH assay.[16,18] The methanol extract of buds exhibited potent antipyretic activity.[19] The antifungal properties of leave extracts was studied.[20] It has been reported that the methanol extract of seeds significantly decreased tumor volume and increased the life span in some in vivo studies.[21]

MATERIALS AND METHODS

Plant material

The seeds of *Cassia fistula* were collected from Nyala, western Sudan. The plant was authenticated by Institute of Aromatic and Medicinal Plants- 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 for GC-MS analysis.

Test organisms

The microorganisms shown in Table (1) were used for evaluating the antimicrobial acivity of *Cassia fistula* oil.

Table 1: Test microorganisms.

Ser. 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	fungus

Methods

Extraction of oil from *Casssia fistula* seeds

Powdered seeds of *Cassia fistula* (300g) were macerated with n-hexane at room temperature for 48h. The solvent

was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

GC-MS analysis

Cassia fistula oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument was used.Chromatographic conditions are displayed in Tables (2) and (3).

Table 2: Oven temperature program.

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 3: Chromatographic conditions.

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial screening

In cup plate agar diffusion bioassay, *Cassia fistula* oil was assessed for antimicrobial activity against five standard pathogenic microbes.

(1g) of the oil was weighed and dissolved in 10ml of DMSO to obtain a concentration of 100mg/ml. This was the initial concentration of the oil used to check the antimicrobial activities. Diffusion method was the method used for screening the oil. 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 the manufacturer's instructions, sterilized at 121^{o}C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer of 6mm in diameters, a well was cut at the centre of each inoculated medium. (0.1ml) of the test solution was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37^{o}C for 24 hours for the bacteria and at 30^{o}C and for 4 days for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured with a transparent ruler and the results were recorded in millimeters.

RESULTS AND DISCUSSION

GC-MS analysis of fixed oil

GC-MS analysis of *Cassia fistula* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST). The GC-MS analysis of the studied oil revealed the presence of 25 components (see Table 3 and Fig.4).

Table 4: Constiuents of *Cassia fistula* oil.

No.	R. Time	Area%	Name
1	5.349	0.05	1-Octanol
2	7.154	0.07	L-α-Terpineol
3	8.858	0.01	Decanoic acid, methyl ester
4	11.427	0.04	Dodecanoic acid methyl ester
5	13.751	0.92	Methyl tetracecanoate
6	14.563	0.11	5-Octadecenoic acid , methyl ester
7	14.829	0.31	Pentadecanoic acid, methyl ester
8	15.665	0.85	9-Hexadecanoic acid, methyl ester
9	15.874	13.55	Hexadecanoic acid, methyl ester
10	16.628	0.27	Cis-10-Heptadecenoic acid, methyl ester
11	16.671	0.09	6-Octadecenoic acid, methyl ester
12	16840	0.45	Heptadecanoic acid, methyl ester
13	17.543	24.46	9,12-Octadecadienoic acid, methyl ester
14	17.596	17.26	9-Octadecenoic acid , methyl ester
15	17.786	7.75	Methyl stearate
16	19.178	2.25	9,12-Octadecadienoyl chloride,z,z-
17	19.305	0.92	Oxiraneoctanoic acid,3-octyl-, methyl ester
18	19.339	1.02	11-Eicosenoic acid, methyl ester
19	19.539	3.72	Eicosanoic acid, methyl ester
20	19.710	0.54	Methyl 15-hydroxy-9,12-octadecadienoate
21	20.623	15.75	Stigma-7-en-3-ol,(3β-5-α-24S)
22	21.163	3.63	Docosanoic acid, methyl ester
23	21.929	0.92	Tricosanoic acid, methyl ester
24	22.667	3.63	Tetracosanoic acid, methyl ester
25	23.384	1.42	Methyl 22-methyltetracosanoate
		100.00	

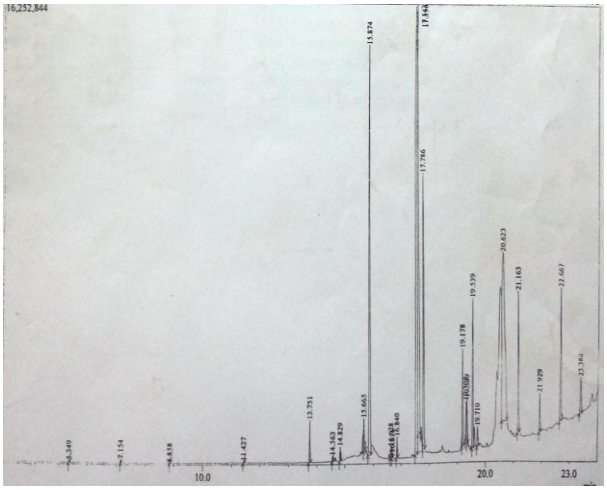

Fig. 1: Total ions chromatogramsof the oil.

Some important constituents are discussed below

9, 12-Octadecadienoic acid methyl ester (24.46%)

Fig. 2 shows the EI mass spectrum of 9, 12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T. 17.543 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.

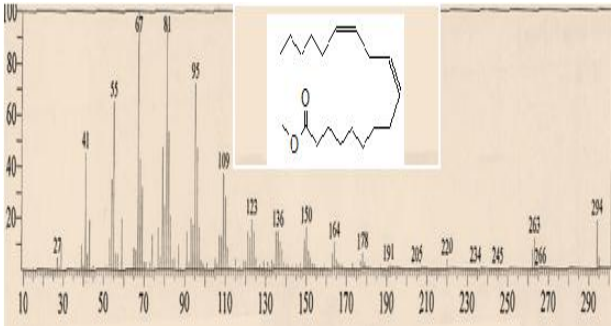

Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

Linoleic acid (9, 12-octadecadienoic acid) cannot be synthesized by humans and is available through diet.[22] It belongs to one of the two families of essential fatty acids. It exists in lipids of cell membrane and is used in the biosynthesis of arachidonic acid. It is converted enzymatically into mono-hydroxy products which are subsequently oxidized by some enzymes to keto metabolites. These metabolites are implicated in human physiology and pathology. Deficiency of linolate caused hair loss and poor wound healing in model animals.[23,24]

9-Octadecenoic acid methyl ester (17.26%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig.3.The peak at m/z 296, which appeared at R.T. 17.596 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl.

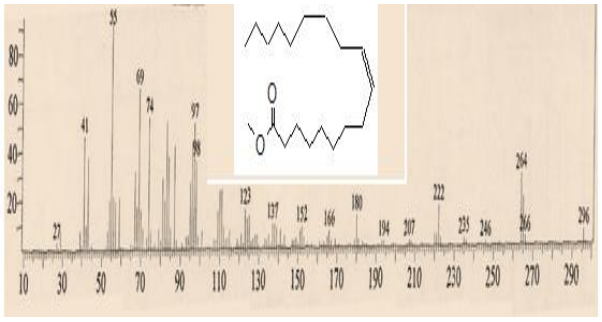

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester.

Oleic acid (9-octadecenoic acid) is a common monounsaturated fat in human diet. It may be responsible for the hypotensive potential of olive oil.[25] Oleic acid finds some applications in soap industry and it is used in small amounts as excipient in pharmaceutical industries. It is employed as emollient.[26] The consumption of oleate in olive oil has been associated with decreased risk of breast cancer.[27]

Stigmast-7-en-3-ol (15.75%)

The mass spectrum of stigmast-7-en-3-ol is depicted in Fig. 4.The peak at m/z 414 (R.T. 20.623) corresponds $M^+[C_{29}H_{50}O]^+$ while the signal at m/z399 is attributed to loss of a methyl group.

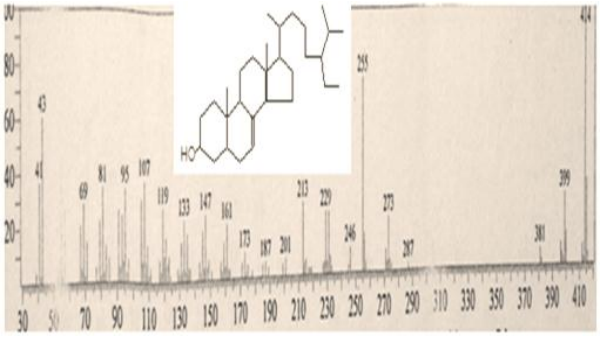

Fig. 4: Mass spectrum stigmast-7-en-3-ol.

Hexadecanoic acid methyl ester (13.55%)

Fig. 5 shows the mass spectrum of hexadecanoic acid methyl ester .The peak at m/z 270 (R.T. 15.874) corresponds $M^+[C_{17}H_{34}O_2]^+$ while the signal at m/z239 is due to loss of a methoxyl function.

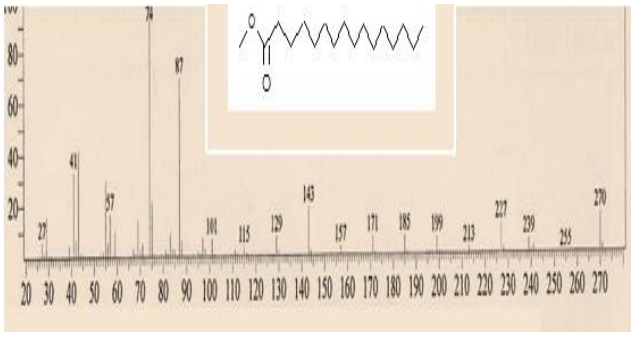

Fig. 5: Mass spectrum of hexadecanoic acid methyl ester.

Hexadecanoic acid (palmitic acid) is a saturated fatty acid .It is widely spread in plants and humans. This acid is produced first during the synthesis of fatty acids[28] and is considered as precursor of long-chain fatty acids.

Palmitic acid is a major lipid component of human breast milk.[29,30] The acid finds applications in soaps and cosmetics industries. It is also used in food industry.

Methyl stearate (7.75%)

Mass spectrum of methyl stearate is shown in Fig. 6.The The signal at m/z 298 with R.T. 17.786 corresponds $M^{+}[C_{19}H_{38}O_2]^{+}$ .The peak at m/z267 is due to loss of a methoxyl.

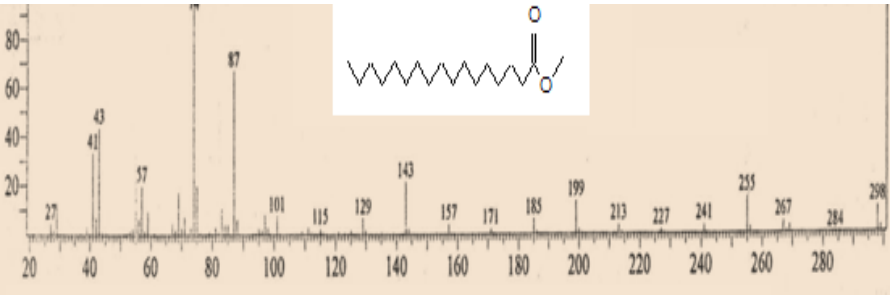

Fig. 6: Mass spectrum of methyl stearate.

Antimicrobial activity

The mean diameters of inhibition zone (MDIZ) produced by oil on standard microorganisms are presented in Table (5). The results were interpreted in commonly used terms : < 9 mm considered inactive; 9-12 mm partially active; 13-18 mm active and more than 18 mm very active.

Results displayed in Table 5 demonstrate activity of oil against all test organisms in the range: 100 - 50mg/ml. On the basis of its promising antimicrobial activity, it seems that this oil is a lead for further opimization. Tables (6) and (7) show the antimicrobial potency of standard drugs.

Table 5: Antimicrobial activity of *Cassia fistula* oil.

Sample	Inhibition zone diameter (mm / mg oil)				
	Bs **(G^+)**	***Sa*** **(G^+)**	***Ec.*** **(G^-)**	***Pa*** **(G^-)**	***Ca.***
Control Methanol	00	00	00	00	00
Cassia fistula oil (100mg/ml)	15	15	16	15	14
50mg/ml	13	14	13	14	13
25 mg/ml	13	13	9	13	12
12.500 mg/ml	12	12	--	10	--
6.25 mg/ml	10	7	--	8	--

Table 6: Antibacterial activity of standard drugs.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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 7: Antifungal activity of standard drug.

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

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

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