

GC-MS analysis and antimicrobial activity of sudanese *Senna italica* (Caesalpiniaceae) fixed oil

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Abstract

Senna italica is easily distinguishable through its many distinctive features. There are three subspecies in *Sennaitalica* based on the size of the inflorescence and the length of the petiole. The subspecies are italic, micrantha and arachoides. In this work *Senna italica* seed oil was studied by GC-MS. The oil was also assessed for antimicrobial activity. The GC-MS analysis showed 19 components dominated by: 9,12-octadecadienoic acid methyl ester (40.27%); hexadecanoic acid methyl ester (20.06%); 9-octadecenoic acid methyl ester (17.32%) and methyl stearate (13.47%). The antibacterial activity of the oil was evaluated via the diffusion bioassay against five standard pathogenic bacteria (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungus *Candida albicans*). The oil showed moderate antibacterial activity against *Bacillus subtilis*.

Keywords: *Senna italica*, fixed oil, GC-MS analysis, antimicrobial activity

Introduction

Cassia is large genus with about 550 species that belongs to the family Caesalpiniaceae. It was split into three genera (Cassia, Senna and Chamaecrista). Leaves and seeds of Cassia species are used as laxative and in diabetese and leprosy [1]. Cassia Leaf extracts were found to be effective against seed brone pathogenic fungi and also have been used as a ethnoverterinary medicine [2]. *Senna italica* is otherwise called as Cassia italic, Senegal senna, Italian senna, Cassia obovataor.). In many region these plants are cultivated commercially and medicinally. The decoction and maceration of leave and pods of *Senna italica* are used for skin problems such as burns and 53 ulcers. The roots are used for liver complains, gallbladder disorders, nausea and dysmenorrhoea [3]. The subspecies are italic, micrantha and arachoides. Plants which contain a variety of phytochemicals –like *Senna* species- have found. very important applications on the fields of agriculture, human and veterinary medicine [4].

Materials and Methods

Plant material

Seeds of *Senna italica* were purchased from the local market, Khartoum, Sudan. The plant was identified and authenticated by direct comparison with reference herbarium sample.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μ m, thickness) was used for GC-MS analysis.

Test organisms

Senna italica oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis*

(G+ve), *Staphylococcus aureus* (G+ve), *Pseudomonas aeruginosa* (G-ve), *Escherichia coli* (G-ve) and the fungal species *Candida albicans*.

Methods

Extraction of oil

Powdered seeds of *Senna italica* (300g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure to give the oil.

GC-MS analysis

The fixed oil from *Senna italica* fruits was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. chromatographic conditions are displayed below.

Table 2: Oven temperature program

Rate	Temperature (°C)	Hold Time (min. ⁻¹)
-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Prssure	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

The cup-plate agar diffusion bioassay was adopted, with some minor modifications, to assess the antimicrobial activity of the studied oil. (2ml) of the standardized bacterial

stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (6 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a test solution. The agar discs were removed, alternate cups were filled with 0.1 ml samples of test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours (for bacteria) and for 4 days at 30° for the fungus. After incubation, the diameters of the resultant growth inhibition zones were measured.

Results and Discussion

GC-MS analysis of *Senna italica* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 19 components (Table1). The typical total ion chromatograms (TIC) is depicted in Fig.1.

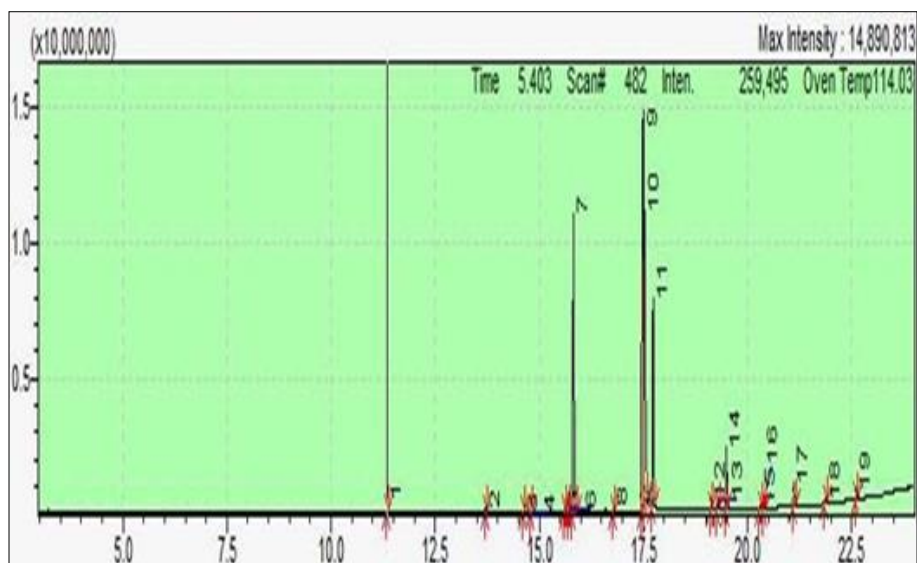


Fig 1: Chromatograms of *Senna italica* oil

Table 1: Constituents of *Senna italica* oil

No.	RT	Area %	Name
1	11.362	0.60	Butylated Hydroxytoluene
2	13.715	0.18	Methyl tetradecanoate
3	14.631	0.03	5-Octadecenoic acid, methyl ester
4	14.791	0.07	Pentadecanoic acid, methyl ester
5	15.627	0.13	9-Hexadecenoic acid, methyl ester, (Z)-
6	15.720	0.17	7-Hexadecenoic acid, methyl ester, (Z)-
7	15.826	20.06	Hexadecanoic acid, methyl ester
8	16.797	0.27	Heptadecanoic acid, methyl ester
9	17.498	40.26	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
10	17.532	17.32	9-Octadecenoic acid (Z)-, methyl ester
11	17.737	13.47	Methyl stearate
12	19.130	0.47	cis-11,14-Eicosadienoic acid, methyl ester
13	19.295	0.56	11-Eicosenoic acid, methyl ester
14	19.491	3.43	Eicosanoic acid, methyl ester
15	20.320	0.25	Heneicosanoic acid, methyl ester
16	20.406	0.24	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
17	21.114	1.48	Docosanoic acid, methyl ester
18	21.881	0.40	Tricosanoic acid, methyl ester
19	22.620	0.61	Tetracosanoic acid, methyl ester
		100	

Major constituents are briefly discussed below:

9,12-Octadecadienoic acid methyl ester (40.27%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2. The peak at m/z 294 (R.T. 17.288) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z 263 is due to loss of a methoxyl.

9,12-Octadecadienoic exists in lipids and cell membrane. It belongs to one of the two families of essential fatty acids. Such acids can not be synthesized by human body and are available through diet [10].

Hexadecanoic acid methyl ester (20.06%)

Figure 3 shows the mass spectrum of hexadecanoic acid methyl ester. The molecular ion: $M^+[C_{17}H_{34}O_2]^+$ appeared at m/z 270 at R.T. 15.630 in total ion chromatogram. The fragment at m/z 239 is due to loss of a methoxyl function.

hexadecanoic acid (palmitic acid) is a saturated fatty acid and it is considered as the most common fatty acid in animals and humans. Palmitic acid is the precursor of long-chain fatty acids^[11]. This acid is a major lipid component of human breast milk^[12, 13]. Palmitic acid, beside being used in soap industry, is widely used in food industry.

9-Octadecenoic acid methyl ester (17.32%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Fig. 4. The peak at m/z 296 (R.T. 17.328)

corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 266 is attributed to loss of a methoxyl. 9-Octadecenoic acid (oleic acid) is included in animal fats and vegetables, hence it is included in the normal human diet. Oleic acid is used as emollient^[14]. It is used in small amount as excipient in pharmaceutical industries. 9-Octadecenoic acid could be responsible for the hypotensive effect of olive oil^[15]. It has been claimed that the presence of oleate in olive oil is associated with decreased risk of breast cancer^[16].

Methyl stearate (13.47%)

Figure 5 displays the EI mass spectrum of methyl stearate. The molecular ion: $M^+[C_{19}H_{38}O_2]^+$ appeared as expected at m/z 298 (R.T. 17.543). The peak at m/z 267 corresponds to loss of a methoxyl function.

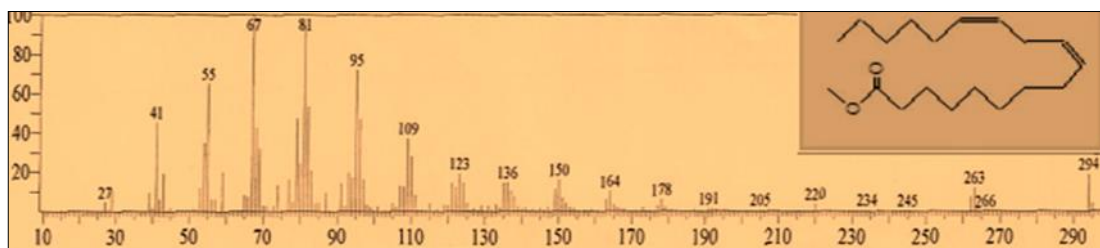


Fig 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

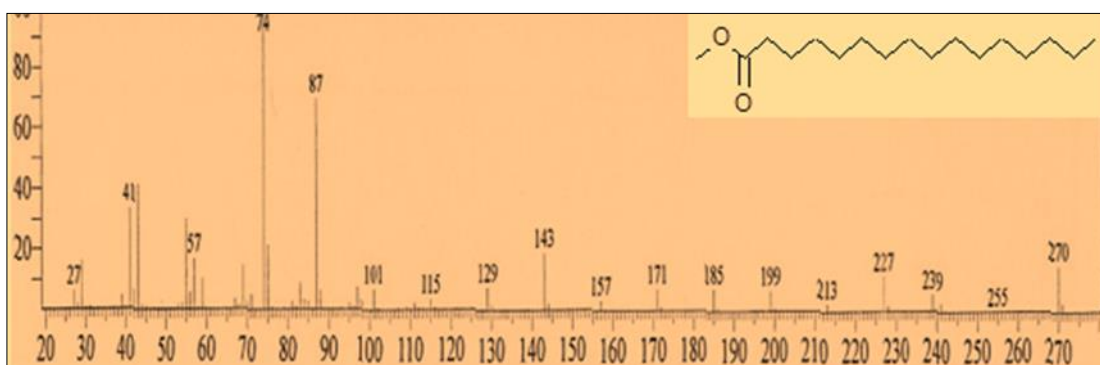


Fig 3: Mass spectrum of hexadecanoic acid methyl ester

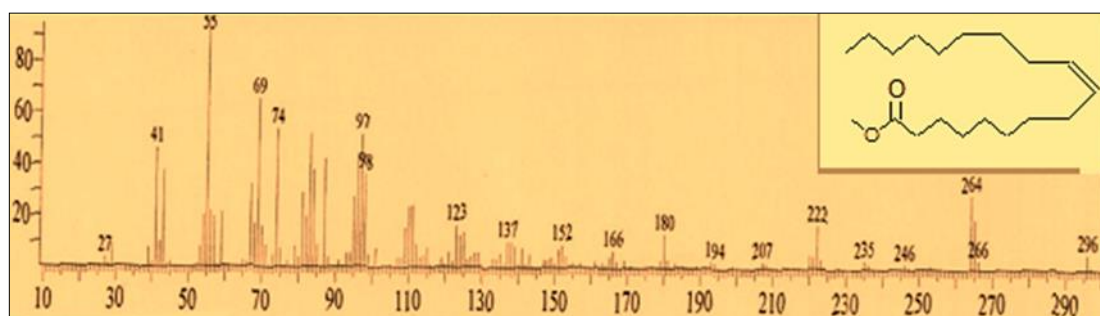


Fig 4: Mass spectrum of 9-octadecenoic acid methyl ester

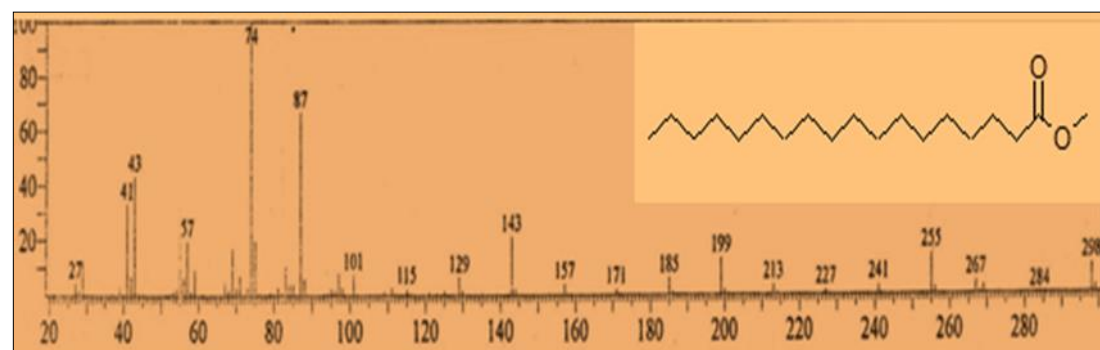


Fig 5: Mass spectrum of methyl stearate

Antimicrobial activity

In cup plate agar diffusion assay, the 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 chemotherapeutics respectively.

The oil showed moderate antibacterial activity against *Bacillus subtilis*

Table 3: Antimicrobial activity of the oil

Sample	Ec	Pa	Sa	Bs	Ca
<i>Senna italica</i> oil (100mg/ml)	10	12	12	14	-

Table 4: 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 5: 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*

Ca.: *Candida albicans*

Bs.: *Bacillus subtilis*

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