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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SAUDI TRIGONELLA FOENUM L. (FABACEAE) FIXED OIL

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ABSTRACT

In this study, Trigonella foenum seed oil was analyzed by GC-MS and antimicrobial potency of the oil was evaluated. 26 components were detected by GC-MS analysis being dominated by: z,z-9,12-octadecadienoic acid(35.85%), 9,12,15-octadecatrienoic acid methyl ester(19.66%),hexadecanoic acid methyl ester(13.29%), z-9-octadecenoic acid methyl ester (10.13%). The antimicrobial activity of the oil was evaluated via the diffusion bioassay against five standard human pathogens(Gram positive: Staphylococcus aureus and Bacillus subtilis; Gram negative : Escherichia coli and Pseudomonasa aeruginosa and the fungal species Candida albicans) . The oil showed different antimicrobial responses against test organisms. The oil showed excellent activity against the bacterial strain Staphylococcus aureus in the concentration range: 100-25mg/ml. It also exhibited excellent activity against the yeast Candida albicans at 100mg/ml. The oil showed activity against all test organism at 100 mg/ml.

KEYWORDS: Trigonella foenum, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

Medicinal plants have been used by humans since time immemorial to fight diseases.They are source for a large number of conventional medicines (quinine, from cinchona; morphine, from Opium poppy …etc). According to WHO reports, the use of herbal medicine is 2-3 fold greater than conventional medicines.

Trigonella foenum L. is an annual herb in the family Fabaceae. It is a key species in the Sudanese system of medicine as well as the traditional medicine of many other communities. Seed is the most applicable part in phytotherapy.[1-7] The plant contains protein (20-30%) with high content of lysine and tryptophan.The species is said to be a rich source of iron, calcium, β-carotene.[8] Fat and amino acid content is 6.53% and 25.8% respectively. The plant also contains vitamins A and D beside B complex.[9] Phytochemical screening revealed the presence of steroids, saponins and coumarins. Trigonelline, scopoletin, phytic and nicotinic acids were also reported.[10]

Trigonella foenum is characterized by a high quality fibre and its protein is comparable to that of soybean.[11]

Seed is an excellent nutrient and has a wide spectrum of therapeutic effects. Seeds are reported as: antidiabetic, anti-inflammatory, antitumor, anticholesterolemic, carminative, laxative, demulcent, galactogogue, emollient, febrifuge, parasiticidal and uterine tonic.[12-15]

MATERIALS AND METHODS

Plant material

The seeds of Trigonella foenum were collected from around Najran,Saudi Arabia .The plant was authenticated by direct comparison with a herbarium sample.

Test organisms

The following standard bacterial pathogens were used to assess the antimicrobial potency of Trigonella foenum oil: 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 Trigonella foenum seeds

Dry-powdered seeds of Trigonella foenum (400g) were extracted with n-hexane at room temperature for 48h. The solvent was removed under reduced pressure leaving

the oil. For GC-MS analysis, a methanolic solution of sodium hydroxide and a methanolic sulphuric acid were used to esterify the oil.

GC-MS analysis

Trigonella foenum fixed oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo Ultra instrument was used with RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). Analytical grade helium (purity; 99.99 %) was a carrier gas. Oven temperature program and other chromatographic conditions are displayed below.

Table 1: Oven temperature program.

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

Table 2: 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 assay

Preparation of bacterial suspensions

Diffusion method was used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were used as media for the growth of bacteria and fungi respectively. The media were prepared according to the manufacturer's instructions.

One ml aliquots of 24 hours broth culture of the test microorganisms were aseptically distributed onto nutrient agar slopes and then incubated at 37°C for 24 hours. The harvested bacterial growth was washed off using sterile normal saline, then it was suspended in (100 ml) of normal saline to give about 108-109 colony forming units per ml. Using the surface viable counting technique , the average number of viable organism per ml of the stock suspension was determined. Serial dilutions of the stock suspension were made in sterile normal saline. (0.02 ml) of the appropriate dilutions 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. Fungal cultures were accomplished on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity

(2ml) of the standardized bacterial stock suspension were mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with (0.1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37^{o}C for 24 hours.

The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

RESULTS AND DISCUSSION

GC-MS analysis of Trigonella foenum fixed oil

Trigonella foenum oil was analyzed by GC-MS .MS library (NIST) was checked for identification of the constituents. Furthermore, the observed fragmentation pattern was interpreted (MS library revealed about 90-95% match).

The GC-MS analysis showed the presence of 26 components (Table 3).The typical total ion chromatograms (TIC) is depicted in Fig.1.

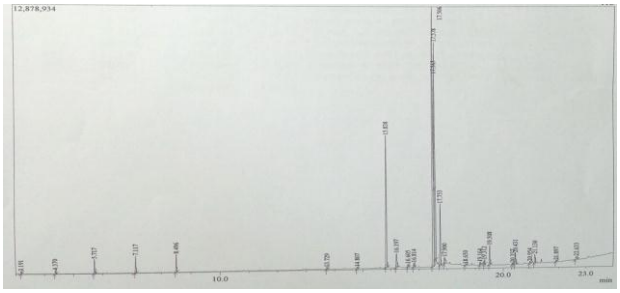

Fig. 1: Total ion chromatograms.

Table 3: Contituents of Trigonella foenum oil.

Pk#	R.Time	Area	Area%	Name
1	3.191	159990	0.20	Styrene
2	4.370	278667	0.34	Octane, 3,5-dimethyl-
3	5.717	961604	1.18	Undecane
4	7.117	1206003	1.47	Dodecane
5	8.496	1265360	1.55	Tridecane
6	13.729	162751	0.20	Methyl tetradecanoate
7	14.807	154412	0.19	Pentadecanoic acid, methyl ester
8	15.838	10872233	13.29	Hexadecanoic acid, methyl ester
9	16.197	1457523	1.78	Pentadecanoic acid
10	16.605	284594	0.35	7-Hexadecenoic acid, methyl ester, (Z)-
11	16.814	375596	0.46	Heptadecanoic acid, methyl ester
12	17.506	29327852	35.85	9,12-Octadecadienoic acid (Z,Z)-, methyl e
13	17.565	8285287	10.13	9-Octadecenoic acid (Z)-, methyl ester
14	17.578	16086319	19.66	9,12,15-Octadecatrienoic acid, methyl este
15	17.753	5064721	6.19	Methyl stearate
16	17.900	712880	0.87	Oleic Acid
17	18.650	121001	0.15	Octadecanoic acid, 17-methyl-, methyl est
18	19.164	244733	0.30	Methyl 8,11,14-heptadecatrienoate
19	19.312	356302	0.44	cis-11-Eicosenoic acid, methyl ester
20	19.508	1585209	1.94	Eicosanoic acid, methyl ester
21	20.337	191320	0.23	Heneicosanoic acid, methyl ester
22	20.431	1005461	1.23	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
23	20.954	196923	0.24	13-Docosenoic acid, methyl ester, (Z)-
24	21.130	783109	0.96	Docosanoic acid, methyl ester
25	21.897	156486	0.19	Tricosanoic acid, methyl ester
26	22.633	511571	0.63	Tetracosanoic acid, methyl ester
		81807907	100.00	

Major constituents of the oil are discussed below.

Z, Z-9, 12-Octadecadienoic acid methyl ester (35.85%)

Fig. 2 shows the EI mass spectrum of 9, 12-octadecadienoic acid methyl ester. The peak at m/z294, which appeared at R.T. 17.506 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.

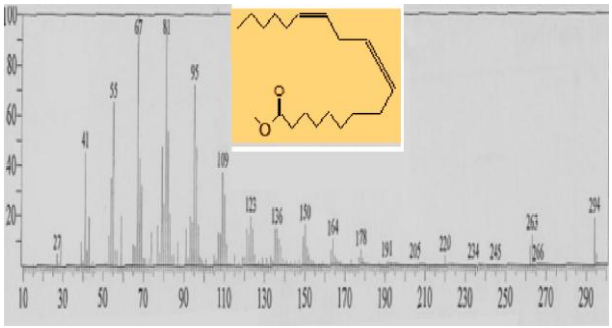

Fig. 2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester.

9, 12, 15-Octadecatrienoic acid methyl ester (19.66%)

Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester is depicted in Fig.3.The peak at m/z 292, which appeared at R.T. 17.578 corresponds $M^+[C_{19}H_{32}O_2]^+$ while the peak at m/z277 is attributed to loss of a methyl function.

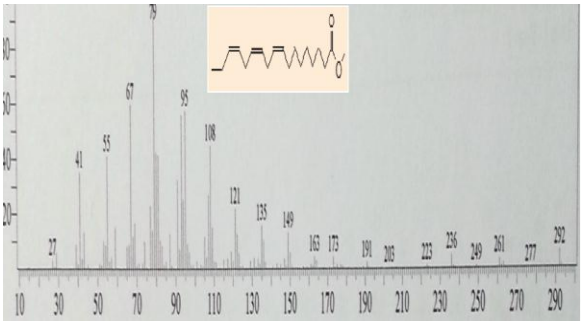

Fig. 3: Mass spectrum of 9, 12, 15-octadecatrienoic acid methyl ester.

Hexadecanoic acid methyl ester (13.29%)

Mass spectrum of hexadecanoic acid methyl ester is depicted in Fig. 4.The peak at m/z 270, which appeared at R.T. 15.838 corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m/z239 is attributed to loss of a methoxyl group.

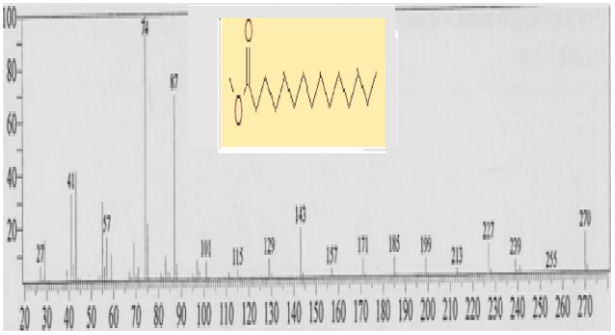

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

Z-9-Octadecenoic acid methyl ester (10.13%)

Fig. 5 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at R.T. 17.613 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z266 accounts for loss of a methoxyl function

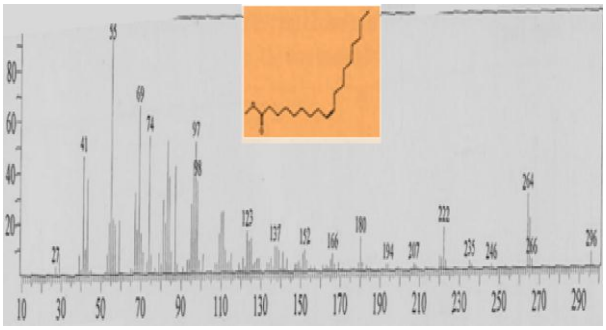

Fig. 5: Mass spectrum of 9-octadecenoic acid methyl ester.

Antibacterial activity

Trigonella foenum fixed oil was screened for antimicrobial activity against five standard human pathogens. The results are depicted in Table (4). The results were interpreted as follows: (<9mm: inative; 9-12mm: partially active;13-18mm: active; >18mm:very active). Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and anifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4: Antibacterial activity of Trigonella foenum oil.

Type	Conc.(mg/m)	Sa	Bs	Ec	Ps	Ca
Oil	100	20	14	15	15	17
	50	18	-	14	14	15
	25	17	-	13	13	10
	12.5	15	-	12	12	9
	6.25	11	-	10	7	-

Table 5: Antibacterial activity of standard chemotherapeutic agents.

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 6: Antifungal activity of standard chemotherapeutic agent.

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

The oil showed excellent activity against the bacterial strain Staphylococcus aureus in the concentration range: 100-25mg/ml. It also exhibited excellent activity against the yeast Candida albicans at 100mg/ml. The oil showed activity against all test organism at 100 mg/ml.

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