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RESEARCH ARTICLE

MS Analysis and Antimicrobial Activity of Fixed Oil From Saudi Peganum harmala L. (Zygophyllaceae)

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ABSTRACT

In this study the lipid composition of Saudi material of Peganum harmala (L.) seeds was identified and quantified. The antimicrobial activity of the extracted fixed oil was assessed . The following major components were detected : 9,12– octadecadienoic acid (56.08%),9-octadecenoic acid (22.35%) , hexadecanoic acid methyl ester(9.24%), , methyl stearate(4.56%). The fixed oil was extracted by two different methods (soxhlet and maceration). Each sample was screened for antimicrobial activity using cup plate agar diffusion assay against eight standard human pathogens. The influence of .the method of extraction on the antimicrobial activity was investigated..

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INTRODUCTION

Peganum harmala L. (Zygophyllaceae) is native to eastern Mediterranean region(1). Peganum harmala is a perennial plant which can grow to about 0.8 m tall. It blossoms between June and August in the northern hemisphere . The flowers are white and are about 2.5–3.8 cm in diameter (2,3). Peganum harmala is used traditionally in the treatment of a wide array of human disorders. β-Carboline alkaloids were identified in different parts(seeds,roots,barks) of Peganum harmala. Pharmacological surveys testified that harmala alkaloids namely; harmaline, harmine, harmalol and harmol are biologically active compounds (4,5).

The plant is employed in ethno-medicine to treat hypertension and cardiac disease (6,7) . Extracts of seeds are said to exert vasorelaxant effects (8) and alkaloids of Peganum harmala were shown to have anti-platelet aggregation effects(9). Furthermore,these alkaloids were shown to be psychoactive in mammalian body (10).Various studies demonstrated a wide range of effects produced by Peganum harmala extracts including ;analgesic (11,12) ,hallucination, excitation (13) and antidepressant (14) effects. Harmal alkaloids were shown to be involved in pathogensis of Parkinson`s disease.

Various studies indicated antiparasidal (15),antifungal (16,17),anti-bacterial (17,18) and insecticidal (19,20) effects for Harmal alkaloids. Significant antileishmanial activity was exhibited by Peganum harmala seeds extract (21) .Also Harmal methanolic extracts produced a dose-dependent decrease in litter size of model animals (22). Frequent abortion was observed in animals that feed the plant (23).

Recently there has been increasing interest in the use of medicinal plants in developing countries to treat a broad spectrum of human disorders. In parallel increased scientific interest in the bioconstituents(steroids,alkaloids,flavonoids….etc) of these plants was also observed . A knowledge of bio-active components would evidently site a rationale for traditional uses of medicinal plants and enrich the global database of phytochemicals. Plant constituents are usually influenced to some extent by geographical distribution .In this study the constituents and antimicrobial potential of Saudi Peganum harmala oil is addressed

MATERIALS AND METHODS

Plant material

Seeds of Peganum harmala were purchased from the local market – Riyadh - Saudi Arabi .The plant was identified and authenticated by Dr. Mohammad Abhary,Biotechnology Laboratory, Faculty of Science, Taiba University.

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

Peganum harmala oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 1: Test organisms

Ser. No	Micro-organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Klebsiella pneumoniae*	G-ve
6	*Acinetobacter baumannii*	G-ve
7	*Aspergillus flavus*	fungi
8	*Candida albicans*	fungi

Methods

Extraction of oil from Peganum harmala seeds

Powdered seeds of Peganum harmala (300g) were extracted with n-hexane (soxhlet) until exhaustion. The solvent was removed in vacuo to afford the oil. Another sample (300g) was exhaustively macerated with n-hexane.

Esterification of oil

(2ml) of oil was placed in a test tube and treated with (7ml) of alcoholic sodium hydroxide followed by(7ml) of alcoholic sulphuric acid. The tube was shaked for five minutes and then left overnight. Supersaturated sodium chloride(2ml) was added, followed by (2ml) of n-hexane .The contents of the tube were vigorously shaked for five minutes .The hexane layer was separated and (5µl) of the hexane layer were mixed with (5ml) diethyl ether . After filtration , (1µl) was directly injected in the GC-MS vial.

GC-MS analysis

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used to analyze the oil. Analytical grade Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program and other chromatographic conditions are shown below:

Rate: - ; Temperature :1500 ; Hold Time (min-1) : 1.00

Rate : 4.00 ; Temperature :3000 ; Hold Time (min-1) : 0.00

Other chromatographic conditions include:

Column oven temperature	150.0oC
Injection temperature	300.0oC
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	- 1.0

Antimicrobial assay

Preparation of bacterial and fungal suspensions

Aliquotes (1ml) of 24h broth culture of the microrganisms were incubated for 24h at 37°C after being aseptically distributed onto nutrient agar slopes. The harvested bacterial growth was washed off with sterile normal saline and suspended in normal saline(100ml) to afford about 108-109colony forming units per ml. This suspension was stored at 40C for further manuplation.Using the surface viable counting technique, the average number of viable organism per ml of the stock suspension was determined. Serial dilutions were made and (0.02ml) of the appropriate dilutions were transferred onto the surface of dry nutrient agar plates. After 2h at room temperature, the plates were incubated at 37°C for 24 hours.

Cultures of fungi were maintained on potato dextrose agar and were incubated for four days at 25°C. The harvested fungal growth was washed with sterile normal saline, and stored in the refrigerator(40C) until used.

Testing for antibacterial activity

The antibacterial activity of the test oil was assessed via cup-plate agar diffusion bioassay , with some minor modifications. The standardized bacterial stock suspension(2ml) was mixed with 200 ml of sterile nutrient agar which was maintained at 45°C in a water bath. (20ml) of incubated nutrient agar were distributed into sterile Petri dishes and was left to settle .Plates were divided into two halves. Two cups in each half (diameter, 10mm) were cut using cork borer (No 4), each of these cups was used for a test solution. Similar Petri dishes were used for standard antimicrobial chemotherapeutics.

Finally, agar discs were removed and alternate cups were filled with samples (0.1 ml) and left to diffuse at room temperature for 2h. They were incubated in the upright position for 24 h. at 37°C. Following incubation, the diameters of the growth inhibition zones were measured in triplicates and averaged.

RESULTS AND DISCUSSION

GC-MS analysis of *Peganum harmala* fixed oil:-

Using GC-MS analysis the constituents of *Peganum harmala* oil were identified and quantified . The observed fragmentation pattern was also interpreted . Excellent match (90-95%) with the database on MS library was observed. Ten components were revealed by GC-MS analysis (Table 2). Typical total ion chromatogram is displayed Fig.1 .

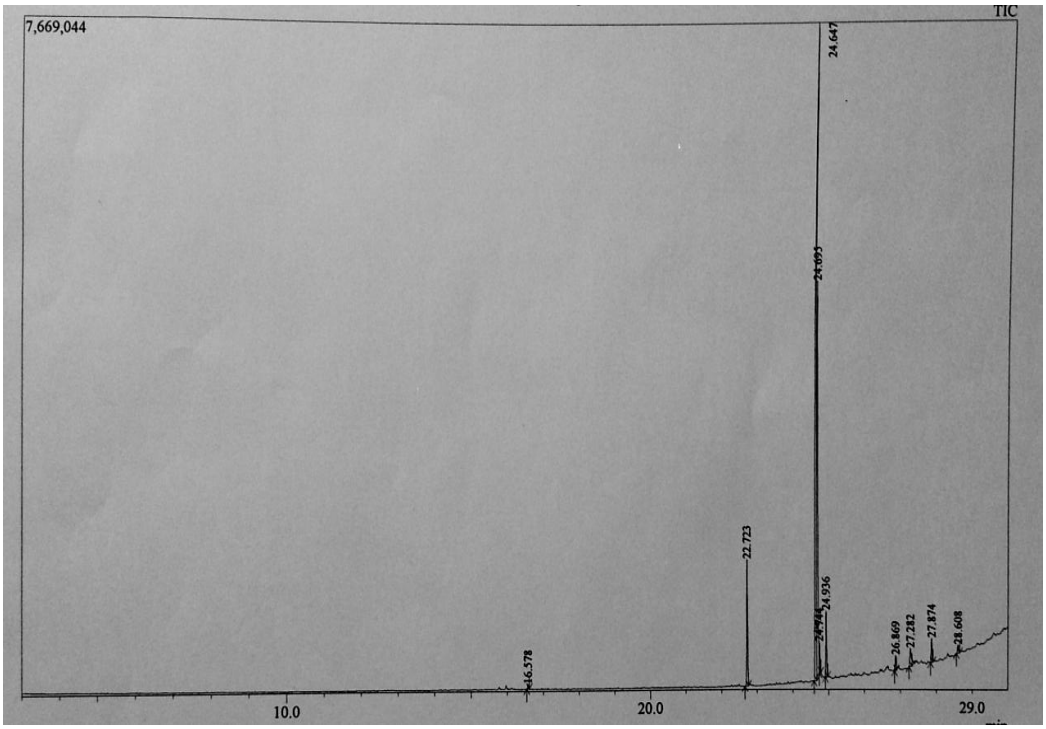

Fig. 1:Cromatograms of *Peganum harmala* oil

Table 2: Constituents of *Peganum harmala* oil

Peak#	*R.Time	Area	Area%	Name
1	16.578	103817	0.35	Butylated Hydroxytoluene
2	22.723	2739580	9.24	Hexadecanoic acid, methyl ester
3	24.647	16630037	56.08	9,12-Octadecadienoic acid (Z,Z)-, methyl e
4	24.695	6626894	22.35	9-Octadecenoic acid (Z)-, methyl ester
5	24.744	546567	1.84	6-Octadecenoic acid, methyl ester, (Z)-
6	24.936	1350888	4.56	Methyl stearate
7	26.869	329228	1.11	Eicosanoic acid, methyl ester
8	27.282	591458	1.99	9-Octadecenamide, (Z)-
9	27.874	503199	1.70	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
10	28.608	232138	0.78	Docosanoic acid, methyl ester
		29653806	100.00	

The following important constituents were detected:

9,12-Octadecadienoic acid methyl ester (56.08%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2 .The peak at m/z294, which appeared at R.T. 24.647 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.

9,12-Octadecadienoic(linoleic acid) belongs to essential fatty acids. Such acids are not synthesized by humans and are available through diet (24).Linoleic acid is involved in the biosynthesis of arachidonic acid .It exists in lipids of cell membrane. Nuts and fatty seeds are rich sources for this acid.Some enzymes like lipoxygenase convert linoleic acid into mono-hydroxy products which are enzymatically oxidized to keto metabolites. Such

metabolites are implicated in human physiology and pathology.Linoleate deficiency caused mild skin scaling and hair loss in model animals (25,26) .

9-Octadecenoic acid methyl ester(22.35%)

The peak at m/z 296(Fig.3) , which appeared at R.T. 24.695 , corresponds to $M^+[C_{19}H_{36}O_2]^+$ while the signal at m/z266 is due to loss of a methoxyl group.

As part of animal fats and vegatables , 9-octadecenoic acid(oleic acid) is included in the normal human diet. It is used as emollient (27), a major component of soap and as excipient in pharmaceutical industries. Oleic acid is considered as a common monounsaturated fat in human diet. The hypotensive potential of olive oil is probably due to the presence of this acid in olive oil (28).

Hexadecanoic acid methyl ester(10.86%)

In total ion chromatogram, the peak at m/z 270 (R.T. 22.723) corresponds to $M^+[C_{17}H_{34}O_2]^+$ while the signal at m/z239 accounts for loss of a methoxyl function .

Methyl stearate(6.37%):-

The EI mass spectrum of methyl stearate is shown in Fig. 5.The peak at m/z 298(R.T. 24.936) corresponds to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 is attributed to loss of a methoxyl group.

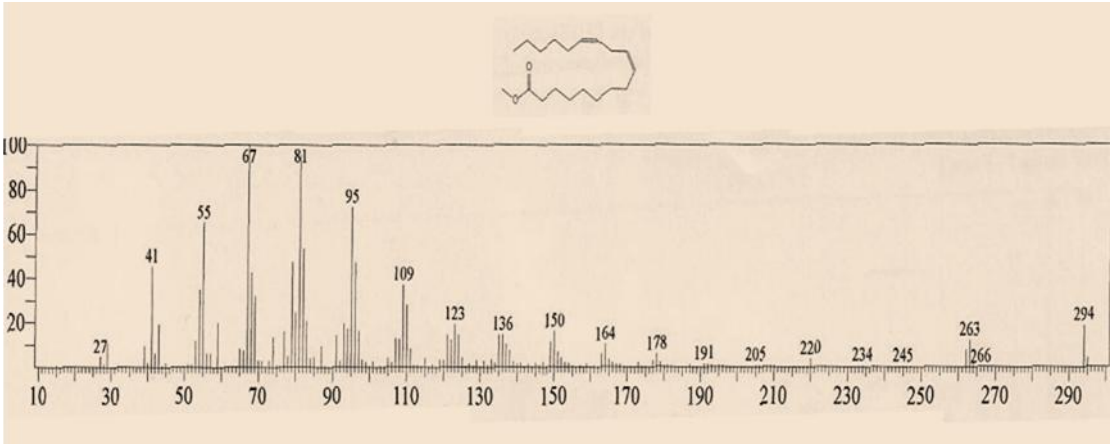

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

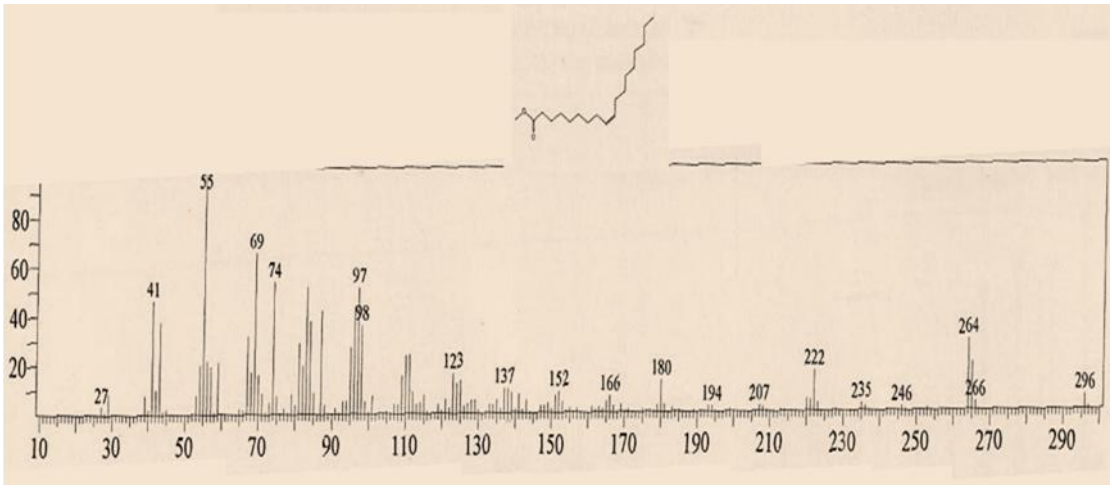

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester

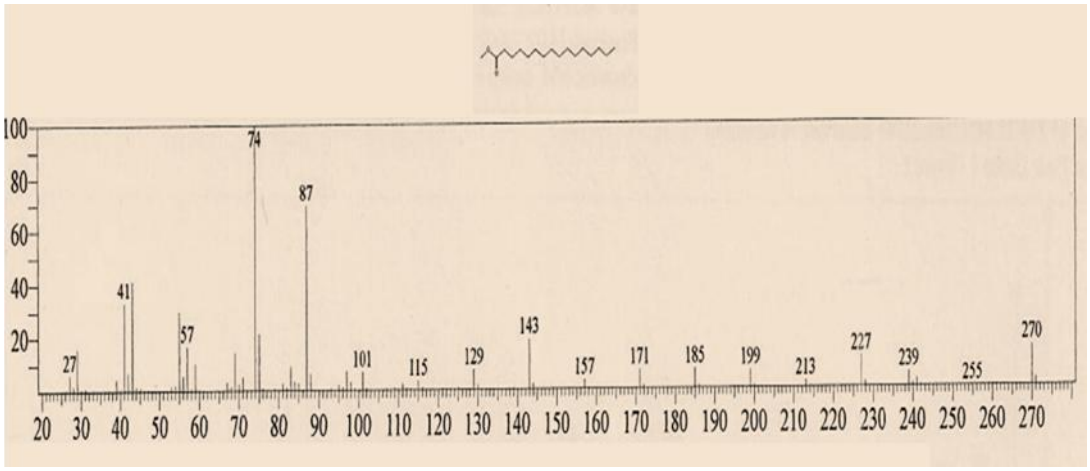

Fig. 4: Mass spectrum of hexadecanoic acid

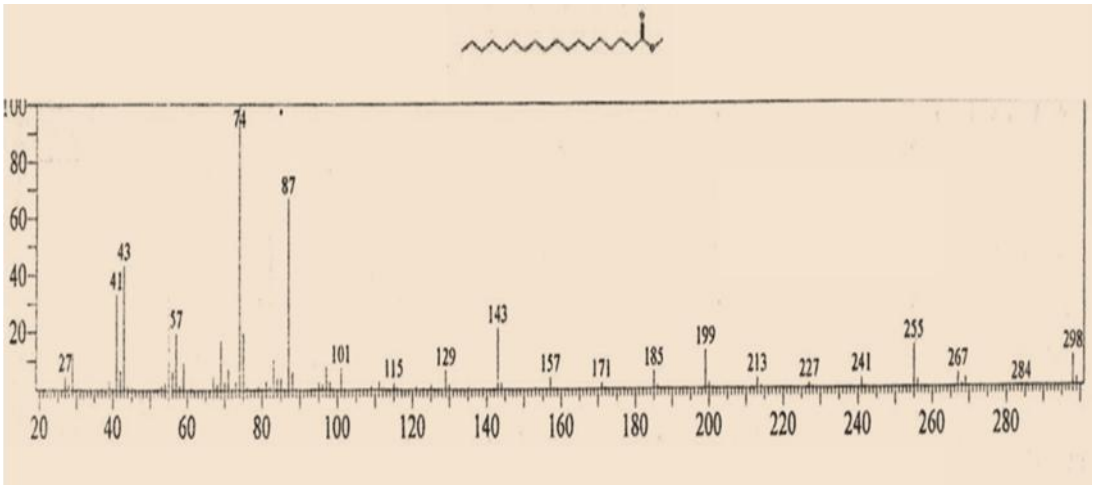

Fig. 5: Mass spectrum of methyl stearate

Antibacterial activity:-

Peganum harmala oil was assessed for antimicrobial activity. Diameters of the growth inhibition zones are displayed in Table (3) .Results were interpreted as follows:

<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) . Table 3 shows the minimum inhibition concentration.

The test oil was extracted by two different methods (B ; soxhlet ; B_1 ; maceration) to evaluate the effect of heating(soxhlet) on the antimicrobial potency of the extracted oil. The oil extracted by maceration showed good activity against *Escherichia coli* at 50 µg/ml while the soxhlet sample was partially active at this concentration(Table 3)..For *Klebsiella pneumonia* , the macerated sample was more potent than the soxhlet sample at concentrateons of 5 , 10 and 50 µg/ml .The macerated sample was partially active against the fungus *Candida albicans* at concentrations of 10 and 50 µg/ml , while the soxhlet sample was devoid of activity at these concentrations. Noteworthy that the soxhlet sample showed excellent activity at 50 µg/ml against *Bacillus subtilis* ,while the macerated sample showed only a weak activity at this concentration.

It seems that the potency of such extracts depends largely on the type of test organism and the concentration of the sample.The same trend was observed for the minimum inhibition concentration(Table 4).

Table 3 : Antibacterial activity of *Peganum harmala* oil : M.D.I.Z(,mm)

		B			B_1			Amp	Kan	Strip	Nys
Gram	Strain	5	10	50	5	10	50	10	10	10	10
-ve	*Escherichia coli*	-	10±0.3	12±0.3	10±0.4	12±0.5	14±0.6	15±0.4	19±0.3	25±0.4	0
-ve	*Klebsiella pneumoniae*	7±0.3	11±0.4	13±0.5	9±0.5	13±0.3	14±0.5	10±0.4	19±0.0	26±0.4	0
-ve	*Acinetobacter baumannii*	9±0.3	12±0.3	15±0.3	-	9±0.0	11±0.3	12±0.3	19±0.3	12±0.3	0
-ve	*Pseudomonas aureginosa*	-	10±0.5	12±0.7	-	9±0.5	11±0.6	12±0.3	14±0.3	20±0.5	0
+ve	*Staphylococcus aureus*	-	10±0.0	13±0.5	-	9±0.3	11±0.4	15±0.5	17±0.5	15±0.5	0
+ve	*Bacillus subtilis*	10±0.5	14±0.5	19±0.5	9±0.0	9±0.3	13±0.4	13±0.4	19±0.4	30±0.4	0

C	*Candida albicans*	-	-	-	8±0.4	9±0.5	9±0.0	N	16±0.5	N	12±0.5
F	*Aspergillus flavus*	-	-	-	-	-	-	N	15±0.5	N	15±0.5

-ve : gram negative, +ve: gram positive, C: colony forming, F: filamentous, -: no activit

Table :4 Minimum Inhibition concentration

Gram Strain	B			B_1			Amp.	Kan.	Strip.	Nya.
	100	250	500	100	250	500	10	10	10	10
-ve *Escherichia coli*	R	R	I	R	R	I	S	S	S	N
-ve *Klebsiella pneumoniae*	R	R	I	R	I	S	S	S	S	N
-ve *Acinetobacter baumannii*	R	I	S	R	R	I	S	S	S	N
-ve *Pseudomonas aureginosa*	R	I	I	R	R	I	S	S	S	N
+ve *Staphylococcus aureus*	R	R	S	R	R	I	S	S	S	N
+ve *Bacillus subtilis*	R	S	S	R	R	S	S	S	S	N
C *Candida albicans*	R	R	R	R	R	R	N	S	N	S
F *Aspergillus flavus*	R	R	R	R	R	R	N	S	N	S

R = resistant ; I = intermediate ; N = not measured

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