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CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF SAUDI *PRUNUS MAHALEB* L. (ROSACEAE) SEEDS

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ABSTRACT

Prunus mahaleb **L.**(Mahaleb) is a perennial tree reaching about 2-10m in height in the family Rosaceae. Different parts of the plant have been used traditionally against many human disorders. In this study the oil from *Prunus mahaleb* was studied by GC-MS and the antimicrobial activity was evaluated. The GC-MS analysis showed forty constituents. Major constituents are esters of: 9- octadecenoic acid (36.80%), 9,12-octadecadienoic acid (25.87%) and hexadecanoic acid (7.91%). In the cup plate agar diffusion assay, *Prunus mahaleb* oil showed partial activity against *Staphylococcus aureus*. It also exhibited partial anticandidal potency.

KEYWORDS: Prunus mahaleb, Oil, GC-MS analysis, Antimicrobial Activity.

INTRODUCTION

Prunus mahaleb **L.** (Mahaleb) is a perennial tree reaching about 2-10m in height in the family Rosaceae.[1,2] The famous Rosaceae family produced huge raw materials for the pharmaceutical and food industries.[3] For some countries, seed of mahaleb is a valuable export item.[4] Different parts of the plant have been used traditionally against many human disorders.[5] Seeds are heart tonic and are used against diabetes and gastrointestinal complaints. For centuries, the plant resin has been used in ethnomedicine against gastritis. Decoction of stem, fruit and leave have been used against asthma and cold. Seed kernel has been used as sedative, vasodilator and in treating diarrhea.[6,7]

The fatty aicd composition of mahaleb has been reported[8,9-11].Seed oil was found to be rich in oleic and linoleic acids.[8] GLC analysis of kernel fixed oil revealed the presence of twelve hydrocarbons beside some fatty acids.[12] Some coumarins have been isolated and identified from kernel extracts[13]. Also some phenolics have been reported from seeds and fruits.[11] The amino acid profile of the defatted seeds has been documented[9] and the mineral content of seeds has been studied.[9] Different extracts of mahaleb have been screened for antiinflammatory activity against a panel of human pathogens.[14,15] Several authors reported on the antioxidant potential of seed extracts.[14,16-18]

MATERIAL AND METHODS

Plant material

Seeds of mahaleb were purchased from the local market Riyadh(Saudi Arabia). The plant was authenticated by direct comparison with a reference herbarium sample.

Instruments

For 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.

Test organisms

The antimicrobial potential of mahaleb oil was estimated by the cup plate agar diffusion bioassay using the standard microorganisms: *Bacillus subtilis* (G+ve), *Staphylococcus aureus*(G+ve), *Pseudomonas aeroginosa*(G-ve), *Escherichia coli*(G-ve) and the yeast *Candida albicans.*

METHODS

Extraction of oil

Powdered seeds of mahaleb (250g) were macerated with n-hexane at room temperature for 48hr.The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

The oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument. Chromatographic conditions are outlined below.

Table 1: Oven temperature program.

Rate	Temperature(^{o}C)	Hold Time (min.$^{-1}$)
1.00	150.0	-
0.00	300.0	4.00

Table 2: Chromatographic conditions.

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	- 1.0

Antimicrobial activity

Mueller Hinton agar and Sabouraud dextrose agar were used as media for bacterial and fungal cultures respectively. The antimicrobial activity was performed according to the method described by Adeniyi et.al.[19] Agar Petri dishes maintained at 45^{o}C in a water bath were seeded with an overnight culture(1ml) of bacteria(10^{7}-10^{8} cfu/ml). Wells (8mm in diameter) were cut on the seeded agar via a sterile cork borer. The cups were filled with (0.1ml) of the test solution and the Petri dishes were left to settle and then incubated for 24 h. at 37^{o}C.. The assay was carried out in duplicates. After incubation the diameters of inhibition zones were measured and averaged as indicator of activity.The same procedure was adopted for antifungal activity, but Sabouarud dextrose agar was used instead of Mueller Hinton agar and incubation was continued for three days at 25^{o}C.

RESULTS AND DISCUSSION

The oil from *Prunus mahaleb* which is a key species in ethnomedicine has been investigated. The oil of this species was extracted and studied by GC-MS. Furthermore, the oil has been assessed for antimicrobial activity via the agar diffusion bioassay against five standard pathogenic microbes. Identification of the constituents was accomplished by consulting the MS library (NIST) and also by matching retentions times with the database of the library.

Constituents of oil

Forty components have been detected by GC-MS analysis (Table 3).The typical total ion chromatograms(TIC) is presented in Fig 1.

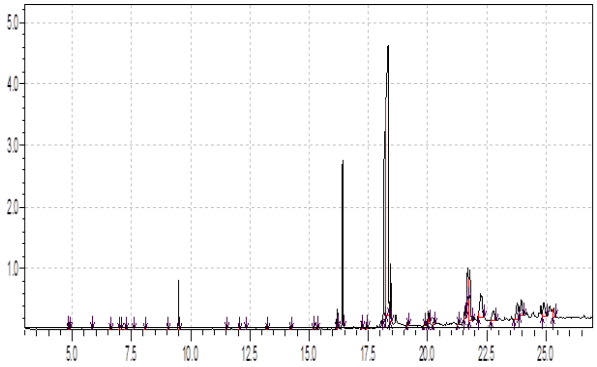

Fig.1: Typical total ion chromatograms.

Table 3: Contituents of *Prunus mahaleb* oil.

No.	Name	RT	Area%
1	D-Limonine	4.827	0.08
2	Eucalyptol	4.883	0.23
3	1,6-Octadien-3-ol,3,7-dimethyl	5.813	0.17
4	(+)-2-Bornanone	6.590	0.03
5	Cyclohexanol,5-methyl-2-(1-methylethyl)-	6.970	0.02
6	3-Cyclhexen-1-ol,4-methyl-1-(methylethyl)-(R)--	7.052	0.01
7	alpha Terpineol	7.250	0.15
8	6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-(E)-	7.588	0.03
9	1,6-Octadien-3-ol,3,7-dimethyl, 2-aminobenzoate	8.057	0.07
10	3-Cyclohexene-1-methanol,alpha,alpha,4-trimethylacetate	9.029	0.01
11	trans-beta-Terpenylbutanoate	9.487	1.96
12	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-,[4aR-(4a alpha,7-alpha,Ba beta)]-	11.486	0.09
13	Ledol	12.042	0.02
14	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,(E)-	12.315	0.02
15	Apiol	13.183	0.20
16	Methyltetradecanoate	14.182	0.17
17	cis—5-Dodecenoic acid methyl ester	15.150	0.02
18	Pentadecanoic acid methyl ester	15.315	0.09
19	7-Hexadecenoic acid mrthyl ester	16.180	0.43
20	9-Hexadecenoic acid methyl ester	16.198	0.77
21	Hexadecanoic acid methyl	16.410	7.91
22	Cis-10-Heptadecenoic acid methyl ester	17.214	0.31
23	Heptadecanoic acid methyl ester	17.430	0.17
24	9,12-Octadecadienoic acid methyl ester	18.245	25.87
25	9-Octadecenoic acid methyl ester	18.335	36.80
26	Methyl stearate	18.440	2.25
27	cis-10-Nonadecenoic acid methyl ester	19.156	0.24
28	8,11-Eicosadienoic acid methyl ester	19.861	0.15
29	cis-11-Eicosenoic acid methyl ester	20.037	0.73
30	Eicosanoic acid methyl ester	20.261	0.38
31	Phenol,2,2`-methylene-bis-[6-(1,1-dimethylethyl)-4-methyl-	21.286	0.11
32	gamma-Sitosterol	21.599	1.36
33	beta-Sitosterol	21.686	2.54
34	Stigmast-7-en-3-ol,(3-beta,5-alpha,24S)-	21.785	4.11
35	6a,14a-Methanopicene,perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-	22.233	4.21
36	Beta-Amyrin	22.766	1.54
37	9,19-Cyclolanost-24-en-3-ol,(3-beta)-	23.776	2.37
38	alpha-Amyrin	23.955	1.79
39	Vitamin E	24.905	1.80
40	Pregn-4-ene-3,20-dione,14,17-dihydroxy	25.319	0.79

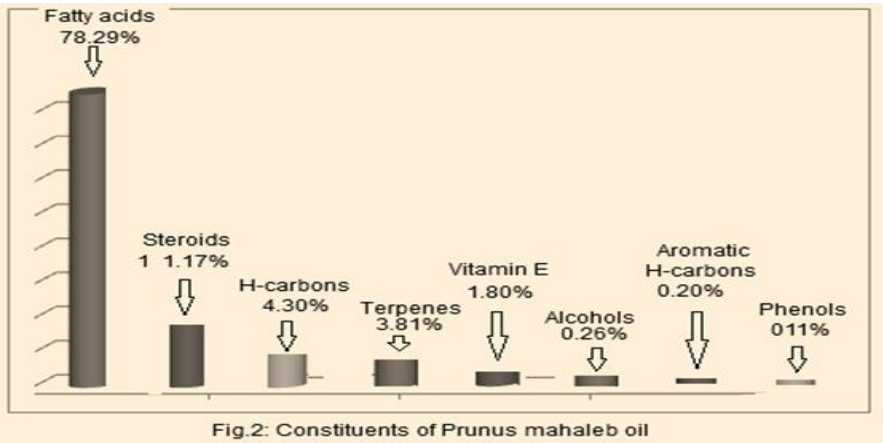

Fig.2: Constituents of Prunus mahaleb oil

Major constituents are discussed below

9- Octadecenoic acid methyl ester (36.80%)

The mass spectrum of 9-octadecenoic acid methyl ester is depicted in Fig. 3. The signal at m/z 296 which appeared at R. T 18.335 is due to $M^+[C_{19}H_{36}O_2]^+$, while the signal which appeared at m/z 265 is due to loss of methoxyl.

9,12-octadecadienoic acid methyl ester(25.87%)

The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.4. The peak at m/z294 (R.T. 18.245) coincides with $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 is due to loss of a methoxyl.

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.[20] Linoleic acid is used in the biosynthesis of arachidonic acid. It exists in lipids of cell membrane.

Hexadecanoic acid methyl ester (7.91%)

Fig. 5 presents the EI mass spectrum of hexadecanoic acid methyl ester. The peak at m\z 270, with retention time 16.410, is attributed to $M^+[C_{17}H_{34}O_2]^+$ while the peak at m\z 239 is due to loss of methoxyl function.

Hexadecanoic acid (palmitic acid) is a saturated fatty acid. It is widely spread in plants and humans. The acid is produced first during the synthesis of fatty acid and is considered as precursor of long chain fatty acid. Palmitic acid is a major lipid components of human breast milk. The acid finds applications in soap and cosmetics industries, it is also used in food industry.

Methyl stearate (2.25%)

The EI mass spectrum of methyl stearate is shown in Fig. 6. The peak at m/z 298which appeared at R.T. 18.440 corresponds to $M^+[C_{19}H_{38}O_2]$. The peak at m/z 267 accounts for loss of methoxyl.

The following steroids were detected as minor components:

i-Stigmast-7-en-3-ol(4.11%)

ii-Beta- sitosterol(2.54%)

iii-Gamma sitosterol(1.36%)

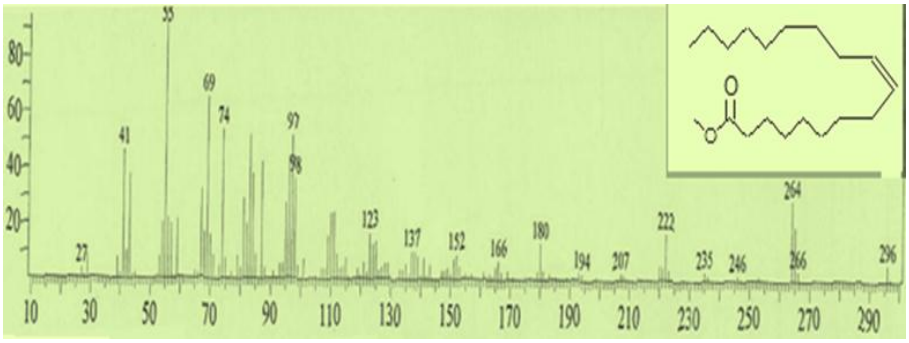

Fig.3: Mass spectrum of 9-octadecenoic acid methyl ester.

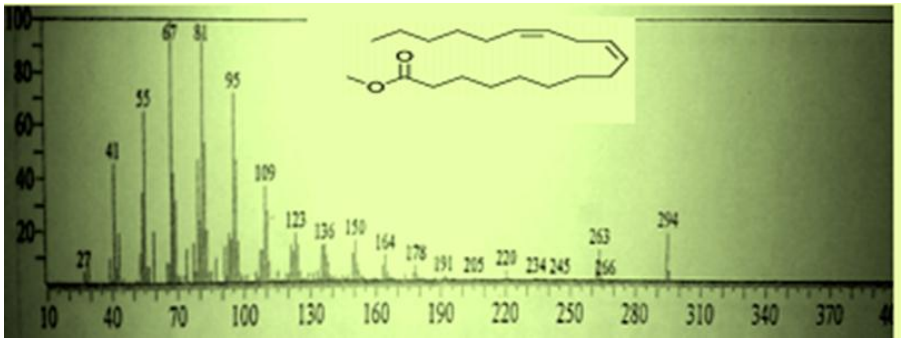

Fig. 4: Mass spectrum of 9,12-octadecenoic acid methyl ester.

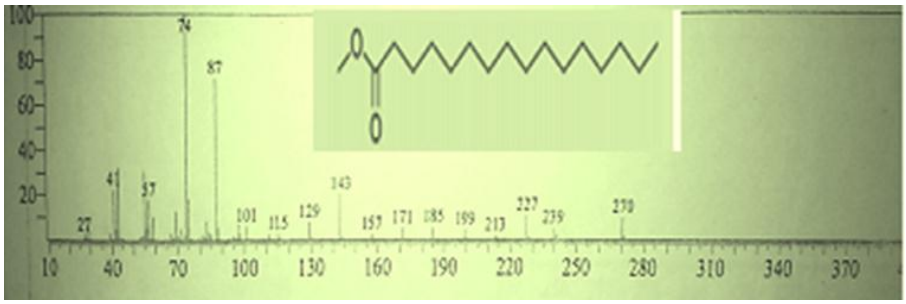

Fig. 5: Mass spectrum of hexadecanoic acid methyl ester.

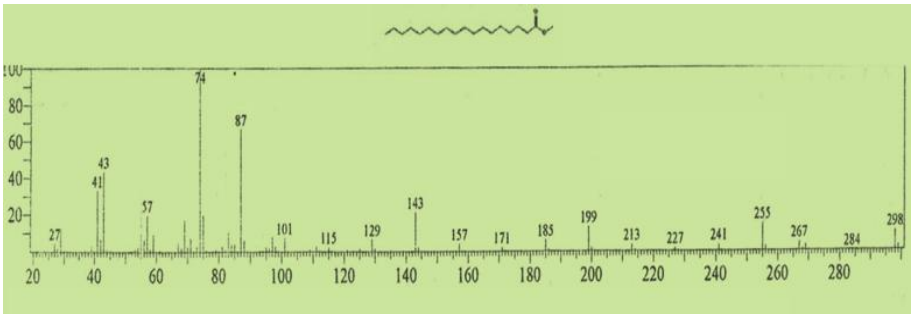

Fig. 6: Mass spectrum of methyl stearate.

Antimicrobial activity

In the cup plates agar diffusion bioassay, the target oil was assessed for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 4. The results were interpreted in term of commonly used terms <9mm: inactive; 9-12mm :partially active; 13-18 :active;>18mm: very active. Tables 5 and.6 represent the antimicrobial activity of standard positive controls.

Table 4: Oil : diameters of inhibition zones(mm).

Type	Ec.	Ps.	Sa.	Bs.	Ca.
Oil 100mg/ml	--	--	10	--	10

Table 5: 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 6: 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.
Ps: Pseudomonas aeruginosa.
Bs: Bacillus subtilis.
Ca: Candida albicans.

Prunus mahaleb oil showed partial activity against *Staphylococcus aureus* as well as partial anticandidal potency.

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