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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *PRUNUS DULCIS* (ROSACEAE) OIL

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ABSTRACT

Prunus dulcis is a small deciduous tree in the family Rosaceae. The plant is widely distributed along the Mediterranean region, northern Africa and southern Europe. *Prunus dulcis* is rich in unsaturated fatty acids. It also contains fiber, sterols, minerals (Cu and Mg) beside high quality protein. *Prunus dulcis* oil is anti-inflammatory and hepatoprotective. The plant has anti-stress and antioxidant properties. It is immune stimulant, antihyperlipidemic and laxative. GC- MS analysis of *Prunus dulcis* oil revealed the presence of the following major components: i) 9-octadecenoic acid (Z)-, methyl ester. (60.84%). ii) 9,12-octadecadienoic acid (Z,Z)-, methyl ester .(25.11%). iii) hexadecnoic acid, methyl ester. (10.20%).The oil was evaluated for its antimicrobial activity against five standard human pathogens. The oil showed moderate anticandidal activity. However, it was inactive against other test organisms.

KEYWORD: *Prunus dulcis,* Oil, Constituents, Antimicrobail activity.

INTRODUCTION

Since the dawn of civilization, herbal medicine played a vital role in primary health care. Nowadays, many communities are totally relying on herbal medicine which is more affordable and endowed with less side effects compared to modern medicines. Recently, phytochemical and pharmacological studies focused on bioactive molecules isolated from medicinal plants as leads in drug design and drug development.

This study was designed to investigate the constituents and antimicrobial potential of *Prunus dulcis* oil. This potential medicinal plant is extensively used in African system of traditional medicine.

Prunus dulcis is a small deciduous tree (4-10cm high) in the family Rosaceae.[1] The plant is widely distributed along the Mediterranean region, northern Africa and southern Europe.[2,3] *Prunus dulcis* is rich in unsaturated fatty acids. It also contains fiber, sterols, minerals (Cu and Mg) beside high quality protein.[4] *Prunus dulcis* oil is anti-inflammatory and hepatoprotective.[5] The plant has antistress and antioxidant properties.[6,7] It is immune stimulant, antihyperlipidemic,[8] and laxative.[9]

It has been reported that *Prunus dulcis* may reduce cancer risk.[10] The plant is said to reduce cholesterol level and consequently reduces cardiovascular disorders.[11] *Prunus dulcis* is antidiabetic,[12] and antihypertensive.[13]

MATERIALS AND METHODS

Plant material

Seeds of *Prunus dulcis* were purchased from the local market- Riyadh – Saudi Arabia. The plant was authenticated by direct comparison with a reference herbarium sample.

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with RTX-5MS column (30m,length; 0.25mm diameter; 0.25 µm, thickness).

Test organisms

Prunus dulcis oil was screened for antimicrobial activity using the standard microorganisms: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeroginosa, Escherichia coli* and *Candida albicans.*

Methods

Extraction of oil

Powdered seeds of *Prunus dulcis* (400g) were macerated with n-hexane at room temperature.The solvent was removed under reduced pressure to give the oil. The oil was esterified by alcoholic sodium hydroxide and alcoholic sulphuric acid.

GC-MS analysis

The extracted oil was analyzed by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument. Helium was used as carrier gas. Chromatographic conditions are as follows: **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.

Antimicrobial assay

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9}colony forming units per ml. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and (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 for the drop to dry, and then incubated at 37°C for 24 hours. Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for 72h. (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 then left to settle and in each of these plates cups (10 mm in diameter) were cut using sterile cork borer (No 4). The agar discs were removed. The cups were filled with sample (0.1 ml) 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. After incubation, the diameters of the resultant growth inhibition zones were measured and recorded as an average of two replicates.

RESULTS AND DISCUSSION

Prunus dulcis oil was studied by GC-MS technique. The analysis showed 12 components – see Table 1. Total ions chromatograms is depicted in Fig.1.

Major components of the oil are:

i) 9-Octadecenoic acid (Z)-, methyl ester. (60.84%).
ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (25.11%).
iii) Hexadecnoic acid, methyl ester. (10.20%).

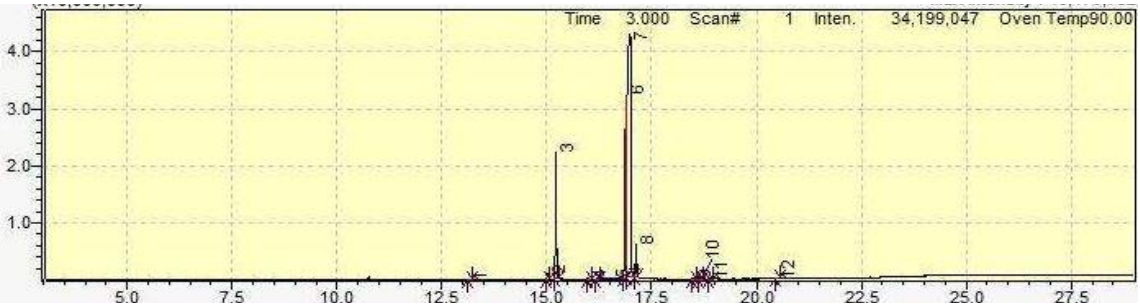

Fig. 1: Total ions chromatograms.

Table 1: Cconstiuents of *Prunus dulcis* oil.

No.	Name	Ret.Time	Area%
1.	Methyl tetradecanoate	13.142	0.03
2.	9-Hexadecenoic acid, methyl ester, (Z)-	15.022	0.57
3.	Hexadecanoic acid, methyl ester	15.221	10.20
4.	cis-10-Heptadecenoic acid, methyl ester	15.990	0.14
5.	Heptadecanoic acid, methyl ester	16.197	0.07
6.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.910	25.11
7.	9-Octadecenoic acid (Z)-, methyl ester	16.988	60.84
8.	Methyl stearate	17.131	2.53
9.	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	18.490	0.20
10.	cis-11-Eicosenoic acid, methyl ester	18.674	0.16
11.	Eicosanoic acid, methyl ester	18.872	0.12
12.	Docosanoic acid, methyl ester	20.487	0.03

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig.2.The signal at m/z296 (RT.16.988) corresponds M^{+} $[C_{19}H_{36}O_{2}]^{+}$. Fig. 3 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester. The peak at m/z 294(RT.16.910)corresponds M^{+} $[C_{19}H_{34}O_{2}]^{+}$ The mass spectrum of hexadecanoic acid methyl ester is presented in Fig.4.The peak at m/z 270 (RT.15.221) is due to M^{+} $[C_{17}H_{32}O_{2}]^{+}$.

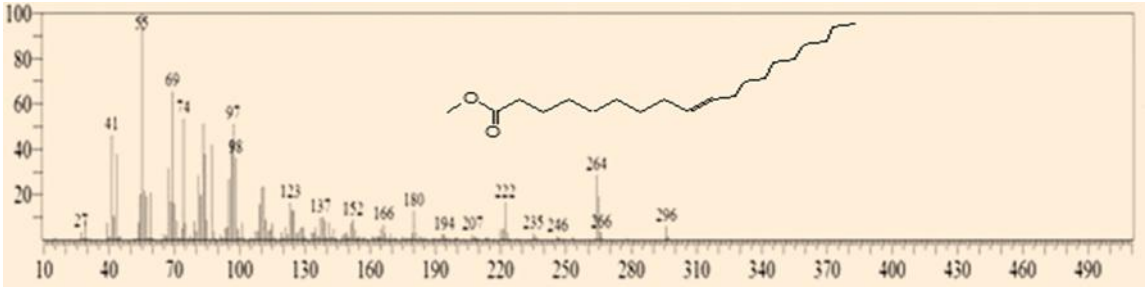

Fig. 2: Mass spectrum for 9-octadecenoic acid[z]-,methyl ester.

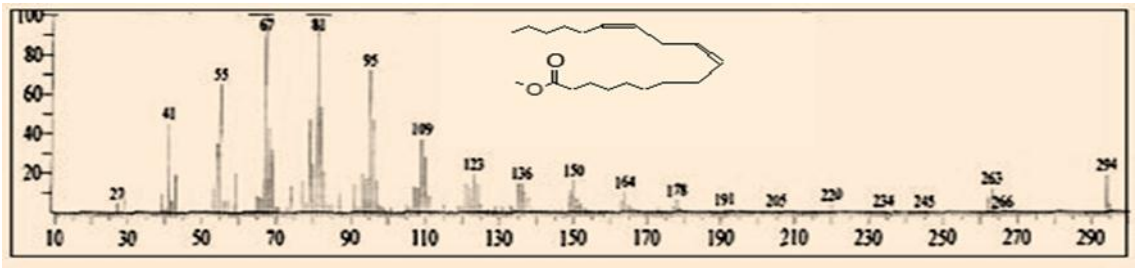

Fig. 3: Mass spectrum of 9,12-octadecadienoic acid.

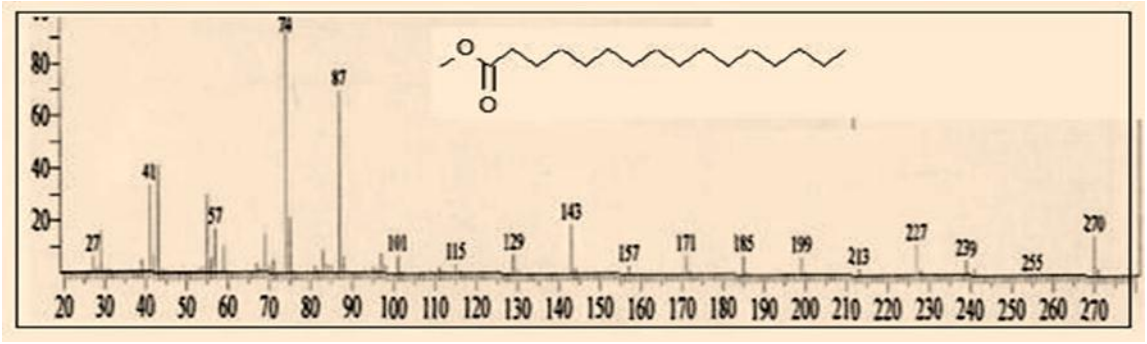

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

Antimicrobial activity

Prunus dulcis oil was evaluated for antimicrobial activity against five standard pathogenic microbes. The average of the diameters of the growth inhibition zones are presented in Table (2).Results were interpreted in the following terms: (<9mm: inative;9-12mm:partially active; 13-18mm: active;>18mm:very active). Ampicilin, gentamicin and clotrimazole were used as positive controls. The oil showed moderate anticandidal activity. However, it was inactive against other test organisms.

Table 2: Inhibition zones (mm/mg sample).

Type	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	--	--	--	--	16
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

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

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