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SHORT COMMUNICATION

Total flavonoid content and essential oil composition of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach from North of Iran

Pardis Hafez-Taghva, Mina Zamzad* and Lida Khalafi

Department of Chemistry, Shahr-e-Qods branch,
Islamic Azad University, Iran

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Essential oil of the fruits of *Chaenomeles japonica* (Thunb.) Lindl. ex Spach was obtained by hydro distillation and analyzed by GC and GC-MS. Thirty five components representing 94.6 % of total were identified. The oil was rich in monoterpenes (88.3 %) and the main components were: carvacrol (62.6 %), limonene (8.0 %), p-cymene (4.9 %) and γ-terpinene (3.2 %). Quantitative assessment of flavonoids was conducted by aluminum chloride colorimetric method in terms of quercetin equivalent in various extracts of *C. japonica* fruits. After one week duration of extraction, the highest amount of flavonoid content (1.316 mg/g) was observed in chloroform extract.

Keywords: *Chaenomeles japonica* (Thunb.) Lindl. ex Spach, Colorimetric method, Essential oil, Flavonoid.

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Introduction

Secondary metabolites from plants have important biological and pharmacological activities. Flavonoids are the most common group of polyphenols which are important in plants for normal growth, development and defense against infection and injury[1]. These secondary metabolites also show antioxidant[2], anti-allergic, anti-inflamatory, antimicrobial and anticancer activities[3]. Researchers have become interested in flavonoids and other phenolic compounds for their medicinal properties, especially their potential role in the prevention of cancer and heart diseases[1].

The genus *Chaenomeles* (Family Rosaceae) comprising of 4 East Asian species namely *C. speciosa*, *C. cathayensis*, *C. thibetica* and *C. japonica*[4] is appreciated because of their characteristic fragrance and flavor, which makes them well suited for industrial processing. Of these, *C. japonica* (Thunb.) Lindl. ex Spach has aromatic yellow fruits and is rich in juice and fiber[5-8].

The present research was undertaken to determine the essential oil composition and total flavonoid content of different solvent extracts of *C. japonica* fruits, which is cultivated in Iran as an ornamental plant. Essential oil composition of the *C. japonica* fruits from Iran has not been reported earlier and extensive literature review revealed lack of report on total flavonoid concentration of the *C. japonica*.

Materials and Methods

All the chemicals used were Merck (Germany) products. Unico UV-2100 UV/Vis spectrophotometer (China) was used for absorbance measurements. The extracts were filtered through Whatman (No. 41) filter paper.

Plant material

The fruits of *C. japonica* were collected from the seaside area of Roodsar, Gilan Province, North of Iran during July 2012 and was identified by using the identification key in the Manual of Cultivated Trees and Shrubs[9]. A voucher specimen (No. 102886) was deposited in the herbarium of Research Institute of Forests & Rangelands, Tehran, Iran. The collected fruits were air dried in darkness at room temperature. Fifty grams of the powdered dried fruits of *C. japonica* was hydrodistilled by a Clevenger-type apparatus for 4 h. The essential oil was separated, dried by anhydrous sodium sulphate and then subjected to GC and GC-MS.

Gas Chromatography (GC)

GC analysis was performed on a Shimadzu GC-15A equipped with a split/split-less injector (250 ºC). N_2 was used as carrier gas (1 mL/min). The DB-5 (50 m × 0.2 mm, film thickness 0.32 µm) capillary column was used. The column temperature was kept at 60 ºC for 3 min and then heated to 220 ºC with a rate of 5 ºC/min.

Gas Chromatography-Mass Spectroscopy (GC-MS)

GC-MS analysis was performed using a mass selective detector (Agilent 5973) coupled with a gas chromatograph (Agilent 6890), equipped with a capillary column (HP-5MS) (30 × 0.25 mm, film

*Correspondent author
Email: minajamzad@yahoo.com, m.jamzad@qodsiau.ac.ir

thickness 0.25 µm). The column temperature was kept at 60 ºC for 6 min and programmed to 220 ºC at a rate of 5 ºC/min and then was involved to 300 ºC at the rate of 15 ºC /min and kept constant at 300 ºC for 3 min. The injector temperature was 290 ºC and the flow rate of Helium as carrier gas was 0.8 mL/min. The MS operating parameters were: ionization energy 70 ev, ion source temperature 220 ºC.

Identification of constituents

The constituents were identified by comparing their retention indices (RRI) relative to n-alkanes, computer matching with the Wiley library and confirmed by comparing their Mass spectra with those of authentic samples or with data already available in the literature[10]. The percentage composition of the identified compounds was computed from the GC peak area without any correction factor.

Extracts preparation

Plant materials (4 g dried and powdered fruit) were mixed with 25 mL of the solvents (distilled water, 80 % methanol and chloroform) separately and stored at room temperature for 48 h and once more for one week. All the infusions were filtrated and the extracts were stored at 4 °C for further analysis.

Determination of flavonoids concentration

Total flavonoids contents were determined by aluminum chloride colorimetric method[11]. Briefly, 0.5 mL of each extract was dissolved in methanol (1.5 mL) and then 10 % aluminum chloride (0.1 mL) and 1.0 M sodium acetate (0.1 mL) were added to the solutions. Finally distilled water (2.8 mL) was added and the solutions were incubated at room temperature. After half an hour the absorbance of the reaction mixtures were measured at 415 nm by a UV-Visible spectrophotometer. Each reported absorbance was average of 3 independent measurements. The calibration curve was plotted for quercetin (0-100 ppm) and the content of flavonoids in the extracts was expressed in terms of quercetin equivalent (mg quercetin/g dried fruit).

Results and Discussion

Essential oil composition of *C. japonica* fruits was identified by GC and GC-MS. Thirty five components representing 94.6 % of total oil were identified. The oil was rich in monoterpenes (88.3 %). The main components were Carvacrol (62 %), limonene (8.0 %), p-cymene (4.9 %) and γ-terpinene (3.2 %). Sesquiterpenes (3.9 %) and nonterpenoids including aldehydes (1.4 %), acids (0.8 %) and esters (0.2 %) were also found in the oil. In a previous investigation, Lesinska *et al*. reported 20 volatile compounds in *C. japonica* from Canada, of which alcohols and esters were the major identified compounds[2]. In another study, volatile compounds of the fruit juices of different taxa in the genus *Chaenomeles* from Sweden and Finland were extracted by solid-phase microextraction. The study revealed that, *C. japonica*

Table 1—Essential oil composition of *Chaenomeles japonica* fruits

Compounds	RRI	%
β-Myrcene	991	1.1
α-Terpinene	1018	0.6
p-Cymene	1026	4.9
Limonene	1031	8.0
γ-Terpinene	1062	3.2
cis-Sabinene hydrate	1068	0.8
Terpinolene	1088	0.1
Linalool	1098	0.2
Nonanal	1098	0.8
Menthone	1154	0.6
Borneol	1165	1.8
Terpin-4-ol	1177	0.9
α-Terpineol	1189	0.3
Methyl chavicol	1195	0.6
Thymol methyl ether	1235	2.1
Pulegone	1237	0.4
n-Decanal	1272	0.6
Neo- iso-3-thujyl acetate	1278	0.1
Carvacrol	1298	62.6
Decanoic acid	1313	0.4
Decanoic acid ethyl ester	1338	0.1
β-Caryophyllene	1418	1.2
Aromadendrene	1439	0.2
Geranyl acetone	1453	0.2
Viridiflorene	1493	0.3
β-Bisabolene	1509	0.1
γ-Cadinene	1513	0.1
δ-Cadinene	1524	0.2
Dodecanoic acid	1568	0.4
Spathulenol	1576	0.3
Caryophyllene oxide	1581	0.5
Ethyl dodecanoate	1595	0.1
γ-Eudesmol	1630	0.2
epi-α-Cadinol	1640	0.5
β-Acoradienol	1757	0.1
Total		94.6

RRI: Relative Retention Index, determined with reference to a homologous series of normal alkanes on HP-5MS column.

was the richest in terpenic hydrocarbons like α-pinene, β-pinene, β-caryophyllene and α-humullene[12]. The present study revealed some differences in the composition of the present sample from those reported earlier. Carvacrol, as the main component in the present study was not reported earlier while limonene, p-cymene, γ-terpinene and β-myrcene were found quantitatively different. As the qualitative differences can be a common feature within a taxon, the difference in essential oil composition of *C. japonica* can be ascribed to a different chemo type of this plant species. Regarding morphological variation, it is probable that the studied specimens belong to different varieties and consequently their essential oil composition has been different. On the other hand, we used a different method for isolation of volatile compounds and it is also known that environmental conditions such as geographical distribution, habitat conditions, collecting time, drying conditions, mode of distillation and climate factors have significant influence on the essential oil composition of the plants.

Concentration of flavonoids in various extracts of *C. japonica* fruits was determined. A standard calibration curve ($Y=0.0125 X + 0.0145$, $R^2= 0.9996$) was derived for quercetin. The content of flavonoids is expressed in terms of quercetin equivalent (mg quercetin/g dried plant). The flavonoids concentration in plant extracts by Chloroform, methanol 80 % and distilled water were found as 0.618, 0.176 and 0.091 mg/g after 48 h and as 1.316, 0.302 and 0.229 mg/g after one week extraction, respectively.

Conclusion

The present investigation revealed that the fruits of *C. japonica* contain significant amount of flavonoids and chloroform was the best solvent for extraction. Extraction during one week was also more effective. The essential oil of fruits was rich in carvacrol and the fruits can be considered for nutritional and pharmaceutical uses.

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