SUPPLEMENTARY MATERIALS

Chemical constituents and antioxidant activity of essential oil and organic extract from the peel and kernel parts of *Citrus japonica* Thunb. (Kumquat) from Iran

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Abstract

The constituents of essential oils and organic extracts from peel and kernels of *Citrus japonica* were analysed by GC and GC/MS. The content of essential oil in peel and kernel were 1.1 and 0.8% based on dry weight, respectively. The essential oil of *C. japonica* peel and kernel were characterized by a higher amount of limonene (51.0% and 47.1%) and germacrene D (12.1% and 6.3%), and the hexane extracts of its peel and kernel were characterized by a higher amount of dodecanol-1 (12.9% and 20.8%), and linolenic acid (13.1% and 16.3%), respectively. The antioxidant activities of oils were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate that both oils from different parts of *C. japonica* possess considerable antioxidant activity. The fruit peel and kernel essential oil could thus be useful in the industries, chiefly in the food and pharmaceutical industry

Key word: *Citrus japonica*, Essential oil composition, Limonene, Germacrene D, hexane extract, antioxidant.

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Supplementary Results

Table S1 Composition (%) of the essential oil and hexane extract from *Citrus japonica* peel and kernel.

compound	KI*	Peel		Kernel	
		EO	HE	EO	HE
Hexane-1-chloro	865	-	1.2	-	-
α -Pinene	939	0.7	-	1.3	Tr
β -Myrcene	992	8.5	-	9.4	0.1
Decene-5-(E)	998	-	2.1	-	0.5
D-Limonene	1030	51.0	8.9	47.1	4.3
β -Phellandrene	1032	2.5	-	4.8	0.2
Nonene-4-methyl-5	1035	1.0	8.7	0.3	9.1
Decene-2-(Z)	1062	0.8	5.1	0.2	3.8
Decene-5-(Z)	1065	tr	2.5	0.6	-
1,6-Octadien-3-ol,3,7,dimethyl	1120	1.3	-	tr	-
Acetic acid, octyl ester	1142	0.5	-	0.3	-
Undecene-4-methyl-5	1162	-	6.7	-	6.1
Dodecene-1	1189	2.0	-	0.1	0.3
(+)-Carvone	1241	1.1	-	0.8	0.1
Cycloundecane, 1,1,2-trimethyl	1251	-	1.3	-	-
Tetradecene-7-(E)	1375	-	5.0	-	3.9
Geranyl acetate	1381	2.4	-	0.4	tr
Tetradecene-7-(Z)	1384	-	2.1	0.5	3.8
γ -Elemene	1432	0.9	-	4.0	-
Dodecanol-1	1468	-	12.9	-	20.8
Germacrene D	1483	12.1	-	6.3	0.2
γ -Muurolene	1485	tr	6.5	2.4	tr
Pentadecene-1	1508	-	14.3	-	11.6
γ -Cadinene	1510	1.1	tr	3.3	-
δ -Cadinene	1514	2.5	tr	1.1	tr
Myristicin	1520	1.6	-	4.1	2.8
Linolenic acid	1525	-	13.1	5.9	16.3
Linoleic acid	1532	3.2	6.9	1.4	9.9
Germacrene B	1562	1.8	-	3.0	tr
Hexadecene-3(Z)	1598	3.5	-	0.9	4.8
(E)-dihydrofarnesol	1715	-	2.2	-	-
Heptadecyl 2,2-dichloroacetate	1786	1.2	-	0.9	-
Total (%)	-	99.7	99.5	99.1	98.6

*KI: Kovats Indices, the composition was determined by comparison of the mass spectrum of each component with Wiley GC/MS library data and also from its retention indices (RI)., tr = trace (< 0.1%). **EO**: Essential Oil; **HE**: Hexane Extract.

Table S2 Class compositions, percentage and yield of the essential oils and hexane extracts of *Citrus japonica* peel and kernel.

Class composition	Peel (%)		Kernel (%)	
	*E.O	*H.EX	E.O.	H. EX.
Monoterpene	65.5	8.9	62.5	4.7
Hydrocarbons				
Oxygenated	3.5	-	1.2	2.9
Monoterpenes				
Sesquiterpenes	18.4	8.7	20.1	0.2
Hydrocarbons				
Oxygenated	1.6	2.2	-	-
Sesquiterpenes				
Unsaturated fatty acid (UFA)	3.2	20.0	7.3	26.2
Other compounds	10.3	61.9	3.8	64.7
Yield	1.1	3.6	0.8	4.1

* E.O. = Essential Oil; H.EX. = Hexane Extract.

Table S3. DPPH free radical scavenging activity of essential oil and hexane extract from peel and kernel of *C. japonica* and standard antioxidant, vitamin C.

Sample	IC ₅₀ (µg/mL)
Peel essential oil	112
Peel hexane extract	79
Kernel essential oil	165
Kernel hexane extract	63
Vitamin C(Ref.)	27

Supplementary Materials

Experimental

Plant materials

Kumquat was collected from Mazandaran province (Sari areal) at an altitude of 145m in March 2013 North in Iran. A Voucher specimen (410-pmp/a) is kept at the Herbarium of Botanical Research in Pharmaceutical Sciences Branch, I.A. University of Tehran, Iran.

Isolation of the Essential oil

Air dried peel and kernel parts of *C. japonica* (100g) of each sample were collected from the fruit garden, and subjected to 3h of hydrodistillation in a Clevenger-type apparatus (European Pharmacopoeia, 1996). The resulting oils from peel and kernel (yields: 1.1% and 0.8% V/W, respectively) were dried over anhydrous sodium sulfate (Na_2SO_4) and immediately placed into 2 glass tubes and sealed. The samples were stored in the dark at 2°C until chemical analysis and other experiments.

Preparation of hexane Extracts

Dried and powdered materials (peel and kernel) were extracted with hexane using a Soxhlet apparatus (70°C, 2h) to obtain the extracts, aliphatic hydrocarbons and the other apolar components. During extraction procedures, hexane (Merck, 99%) was used. The extracts were concentrated by rotary evaporator under vacuum at 40°C. The extraction yields were presented in Table S2.

GC analysis

GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (50m $\times$ 0.2mm, film thickness 0.32 μm). The column temperature was kept at 60°C for 5 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table S1.

GC/MS analysis

GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m $\times$ 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 5° C/min and kept constant at 220°C for 5 min. The flow rate of helium (He) as carrier gas was 1 mL/min. MS were taken at 70 eV. The extract constituents, essential oil compounds and terpenoids from peel and kernel were identified by comparing their retention times and mass peaks with those given in the literature and with those of authentic samples (Adams, 2001), and by NIST-Wiley library data search. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Antioxidant activity tests

The DPPH assay was carried out according to the modified method of Cheung et al. (2003). Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of samples in different concentrations (0.1-1.6 mg/mL) and kept in the dark for 10 min. The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of hexane. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as IC_{50} values (μg sample/mL) for comparison. IC_{50} value of each sample defined as the concentration of sample required for the 50 % decrease in absorbance of the blank, was calculated.