NOTE

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2018**, *91*(*1*), 125–128
Published online: April 24, 2018
DOI: 10.5562/cca3294

HKD 1926

Chemical Composition and Antioxidant Activity of Essential Oil Obtained from Bitter Orange Peel (*Citrus aurantium* L.) Using Two Methods

Mila Radan,* Antonija Parčina, Franko Burčul

Department of Biochemistry, Faculty of Chemistry and Technology, University of Split, Ruđera Boškovića 35, HR-21000 Split, Croatia

* Corresponding author's e-mail address: mradan@ktf-split.hr

RECEIVED: January 30, 2018 ✯ REVISED: March 22, 2018 ✯ ACCEPTED: April 17, 2018

Abstract: In this study, it was shown that the chemical composition of the essential oil obtained from bitter orange peel (*Aurantii amari flavedo*, *Citrus aurantium* L., from Croatia) depends on the method of isolation. The peel essential oil was obtained by hydrodistillation and cold press method, and their chemical compositions were analyzed by gas chromatography-mass spectrometry (GC-MS). Twenty two components were characterized by mass spectra and linear retention indices. Limonene was found as dominant compound in both hydrodistillation and cold press essential oil with 91.1 % and 51.3 %, respectively. When comparing the chemical composition of two oils, a significant difference in percentage composition of three major compounds, limonene, linalool and hexadecanoic acid was observed. The antioxidant activity of the oils was tested using 2,2-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH). Both oils showed very poor antioxidant activity.

Keywords: *Citrus aurantium* L., bitter orange, peel, essential oil, limonene.

INTRODUCTION

BITTER orange (*Citrus aurantium* L.) belonging to the Rutaceae family has been traditionally cultivated in the Mediterranean region. The Adriatic coast has an especially rich tradition of using wild foods such as bitter orange. Some traditional uses of bitter orange peel (*auranti amari flavedo*) include its use as: aroma flavor in many food products including alcoholic and non-alcoholic beverages, for marmalades, gelatins and puddings, sweets, oils, cakes and condiments. Due to its pharmacological properties, Citrus have been used for treatment of various diseases since ancient times.[1] As a source of bioactive compounds *Citrus aurantium* L. essential oil has been recognized as antioxidative, antimicrobial, antiulcerogenic, neuroprotective, anti-anxiety and anti-larvicidal agent.[2–5]

In this study we analyzed chemical composition of essential oil obtained from the peel of bitter orange. In addition, we compared chemical composition as well as antioxidative potential of the oils obtained by two methods - hydrodistillation and cold pressing. Cold pressing is the standard method of essential oil isolation from citrus peels, while hydrodistillation represents comparatively more economical way to isolate the oil form orange byproducts.[6] The aim of this study was to compare chemical composition of the bitter orange peel oil obtained using two methods of isolation and to determine which method gives the oil with better antioxidative potential.

EXPERIMENTAL

Plant Material and Essential Oil Isolation

Fruits of bitter orange were collected in January 2016 in Split, Dalmatia region. The whole peel of bitter orange (200 g) was subjected to hydrodistillation (HYD) in Clevenger apparatus for 2 hours using solvent trap (pentane : diethyl ether = 2 : 1, *v* / *v*). Cold pressing isolation (CPI) was carried out using screwless cold press (IBG Monforts Oekotec GmbH, Monchengladbach, Germany). The essential oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4 °C until further analysis.

GC-MS Analysis

Gas chromatography and mass spectrometry (GC-MS) analyses were performed on gas chromatograph (model 3900; Varian Inc., Lake Forest, CA, USA) equipped with mass spectrometer (model 2100T) and non-polar capillary column VF-5MS (30 m × 0.25 mm i.d., and coating thickness 0.25 µm, Varian Inc.). Chromatographic conditions were as follows: helium was carrier gas at 1 mL min^{-1}, injector temperature was 250 °C. The column temperature was programmed at 60 °C isothermal for 3 min, after which it was increased to 246 °C at a rate of 3 °C min^{-1} and held isothermally for 25 min. The injected volume was 1 µL and the split ratio was 1 : 50.

Mass spectrometer conditions were: ionization voltage 70 eV; ion source temperature 200 °C; mass scan range: 40-350 mass units. The analyses were carried out in duplicate. The individual peaks were identified by comparison of their retention indices (relative to C_8-C_{30} *n*-alkanes) to those of authentic samples and literature, as well as by comparing their mass spectra with the Wiley 7 MS library (Wiley, New York, NY, USA) and NIST02 (Gaithersburg, MD, USA) mass spectral databases. The percentage composition of the samples was computed from the GC peak areas using the normalization method (without correction factors). The component percentages were calculated as mean values from duplicate GC-MS analyses.

2,2-Diphenyl-1-picrylhydrazyl Radical Scavenging Method (DPPH)

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was measured according to Burčul *et al.* method.[7] The radical scavenging activities of the samples were calculated according to the formula: % inhibition = $[(A_0 - A_{sample})/A_0] \times 100$, where A_0 was absorbance of the ethanolic DPPH solution measured at the beginning and A_{sample} was absorbance of the sample measured after 60 min. The results were expressed as percentage inhibition of DPPH radical.

RESULTS AND DISCUSSION

Auranti amari flavedo essential oil chemical composition was determined by GC-MS and results are shown in Table 1, while chromatograms of the analyses are shown in Figure 1.

Twenty two components were identified by mass spectra and linear retention indices (Table 1). Eighteen components were found in the oil obtained by cold pressing and fourteen components in the oil obtained by hydrodistillation method. Comparison of the chemical composition of the oils obtained by two methods shows significant difference in the content of three prevailing compounds: limonene, linalool and hexadecanoic acid. According to previous compositional studies of the bitter orange peel oil, monoterpene hydrocarbon limonene was a major compound.[8,9] These findings agree with our results where limonene was also found to be a dominant component in both samples, *i.e.* the oil obtained by hydrodistillation (91.1 %) and in the oil obtained by cold pressing method (51.3 %). Reports from other studies show that mycrene commonly appears as the second most prominent compound in the orange peel oil.[3,10] However, in our study it was identified in small percentage in the cold pressing oil sample (0.8 %) only. The lower content of

Table 1. Chemical composition of the orange peel oils obtained by two methods.

No.	Compound	Identification[a]	RI[b]	HYD[d] / %	CPI[c] / %
1.	*α*-Pinene	MS, RI, St	937	0,8	0,8
2.	Sabinene	MS, RI	978	0,8	0,9
3.	*β*-Pinene	MS, RI, St	989	0,8	0,6
4.	Myrcene	MS, RI	996	-	0,8
5.	Limonene	MS, RI, St	1034	91,1	51,3
6.	*cis*-Linalool oxide	MS, RI	1076	0,1	1,1
7.	Terpinolene	MS, RI	1086	0,1	-
8.	*trans*-Linalool oxide	MS, RI	1089	0,2	1,4
9.	Linalool	MS, RI, St	1107	1,5	17,2
10.	Terpinen-4-ol	MS, RI, St	1188	0,3	1,5
11.	*α*-Terpineol	MS, RI, St	1208	1,1	1,5
12.	Linalyl acetate	MS, RI, St	1246	0,1	-
13.	Neryl acetate	MS, RI, St	1354	-	0,5
14.	Neryl acetone	MS, RI	1447	-	0,6
15.	*α*-himachalene	MS, RI	1473	-	0,9
16.	Hexacosane	MS, RI, St	1600	-	0,5
17.	Heptacosane	MS, RI, St	1700	-	0,7
18.	Octacosane	MS, RI, St	1800	-	0,5
19.	Hexahydrofarnesyl acetone	MS, RI	1837	-	1,5
20.	1-Eicosene	MS, RI	1979	0,5	-
21.	Hexadecanoic acid	MS, RI	2002	1,3	15,6
22.	Octadecanoic acid	MS, RI	2180	0,2	-
			Total (%):	98,9	97,9

[a] Identification type: MS - mass spectra, RI - retention index, St - authentic standard.

[b] RI - retention indices determined relative to *n*-alkanes (C8-C30) for VF-5MS column.

[c] HYD - hydrodistillation isolation method.

[d] CPI - cold pressing isolation method.

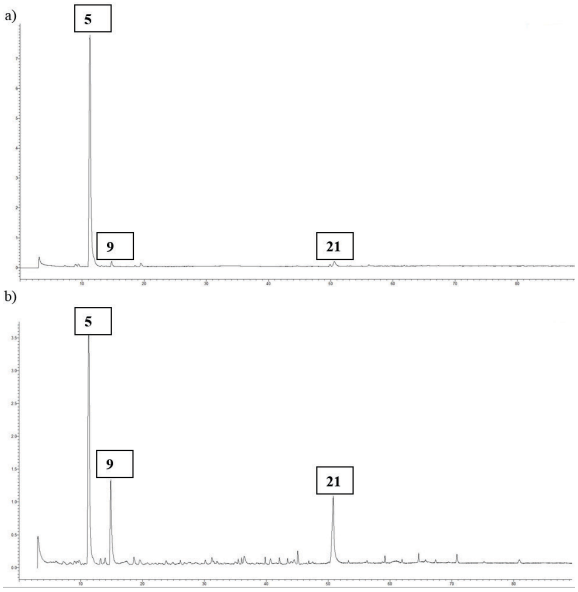

Figure 1. GC-MS chromatograms of the: a) essential oil obtained by hydrodistillation and b) by cold pressing method. Main components of both oils: limonene, linalool and hexadecanoic acid, 5, 9, 21, respectively; are marked with numbers corresponding to numbers in Table 1.

limonene in the oil obtained by cold pressing was replaced with linalool (17.2 %) and hexadecanoic acid (15.6 %).

Generally, the increased temperatures and extended isolation time during hydrodistillation can cause modifications of the oil components as well as loss of some volatile constituents.[11] This may be reason for comparatively less identified number of compounds in the oil obtained by hydrodistillation.

Comparison of the essential oil content obtained in this study with previous compositional studies of the orange peel oils shows presence of of hexadecanoic acid, especially in the oil obtained by cold pressing.[8–10] The absence of hexadecanoic acid from oils in other reports may be due to insufficient running time during GC analysis since hexadecanoic acid, due to hydrophobic interactions, has retention time shifted to higher values. The high percentage of hexadecanoic acid in the cold pressing oil sample may also correlate to concomitant use of a non-polar solvent (diethyl ether) for recovery of essential oil from the mixture. Isolation of non-aroma active fats is a common disadvantage of cold pressing method.[11] Citrus essential oils have a wide range of uses. Because of its pleasant citric fragrance and safety,[9] limonene is commonly used as a flavouring agent in foods, pharmaceuticals and drinks. Due to its high limonene content *auranti amari flavedo* oil represents a valuable source of limonene. However, the content of limonene may vary significantly with respect to the isolation method employed.

Table 2. Antioxidant activity of *auranti amari flavedo* essential oil determined by DPPH method.

Conc. / g L^{-1}	10	20	50	100
HYD[(a)] / %	1.88±0.8	3.55±0.5	11.2±0.7	12.4±0.2
CPI[(b)] / %	1.85±0.3	3.47±0.8	4.5±0.4	n.m.
Vitamin C / mg L^{-1}	75	100	125	150
DPPH[(c)] / %	38.18±0.5	46.73±0.2	66.1±0.8	81.82±0.6

[(a)] HYD - hydrodistillation isolation method.

[(b)] CPI - cold pressing isolation method, n.m. - not measured due to turbidity.

[(c)] Comparison to standard Vitamin C; Values are represented as mean's ± SD (n = 3).

Free radical scavenging properties of bitter orange peel oils obtained by two different isolation methods are shown in Table 2.

In comparison to vitamin C, antioxidant activity was very low for the oils obtained by both methods. Slightly higher DPPH scavenging activity (11.2 %) was measured for the oil obtained by hydrodistillation. Even at higher concentration (100 g L^{-1}), scavenging activity of the oils remained weak and did not reach the 50 % inhibition level. It is known from the literature that major components of *auranti amari flavedo* oil, *i.e.* limonene, linalool, and hexadecanoic acid, have no significant antioxidative importnace when measured *in vitro*.[13,14] These results are comparable to results obtained from the other studies on DPPH scavenging ability of bitter orange peel oil.[15]

CONCLUSION

To the best of our knowledge this is the first report on chemical composition of bitter orange peel essential oil grown in Croatia. Interesting difference, in composition of bitter orange peel oil from Croatia and the oils obtained from other geographical sites, lies in relatively high amount of linalool and hexadecanoic acid present in the oil obtained by cold pressing isolation. In general, each study shows fair variations in the results due to geographical location and growth stage. Though not significantly, the scavenging effect of the *auranti amari flavedo* oil may contribute to the prevention of oxidation in different matrices.

Acknowledgment. This work was fully supported by the Croatian Science Foundation, grant number HRZZ: IP-2014-09-6897.

REFERENCES

[1] B. Á. Arias, L. Ramón-Laca, *J. Ethnopharmacol.* **2005**, *97*, 89.

[2] A. B. Hsouna, N. Hamdi, N. B. Halima, S. Abdelkafi, *J.*

Oleo Sci. **2013**, *62*, 763.
[3] T. M. Moraes, H. Kushima, F. C. Moleiro, R. C. Santos, L. R. Rocha, M. O. Marques, W. Vilegas, C. A. Hiruma-Lima, *Chem.-Biol. Interact.* **2009**, *180*, 499.
[4] M. Pultrini Ade, L. A. Galindo, M. Costa, *Life Sci.* **2006**, *78*, 1720.
[5] A. Sanei-Dehkordi, M. M. Sedaghat, H. Vatandoost, M. R. Abai, *J. Arthropod.-Borne Dis.* **2016**, *10*, 577.
[6] A. Gök, Ş. İsmail Kirbaşlar, F. Gülay Kirbaşlar, *J. Essent. Oil Res.* **2015**, *27*, 17.
[7] F. Burčul, I. G. Mekinić, M. Radan, P. Rollin, I. Blažević, *J. Enzyme Inhib. Med. Chem.* **2018**, *33*, 577.
[8] S. Bourgou, F. Z. Rahali, I. Ourghemmi, M. Saďdani Tounsi, *Sci. World J.* **2012**, *2012*, 528593.
[9] I. Jabri karoui, B. Marzouk, *BioMed Res. Int.* **2013**, *2013*, 12.
[10] V. Rowshan, S. Najafian, *J. Essent. Oil-Bear. Pants.* **2015**, *18*, 1006.
[11] J. Richter, I. Schellenberg, *Anal. Bioanal. Chem.* **2007**, *387*, 2207.
[12] M. H. Boelens, R. Jimenez, *Flavour Fragrance J.* **1989**, *4*, 139.
[13] S. Aazza, B. Lyoussi, M. G. Miguel, *Molecules* **2011**, *16*, 7672.
[14] H. Zengin, A. Baysal, *Molecules* **2014**, *19*, 17773.
[15] E. Sarrou, P. Chatzopoulou, K. Dimassi-Theriou, I. Therios, *Molecules* **2013**, *18*, 10639.