SHORT COMMUNICATION

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 was 1.1 and 0.8% based on dry weight. The essential oil of *C. japonica* peel and kernel was characterised 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 characterised 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 industries.

1. Introduction

The essential oils and extracts are naturally found in vegetables and fruits and may be formed during processes like fermentation or may be added into drug and food during the manufacturing process. During recent years, plant compounds have come more into the focus of phytomedicine (Sylvestre et al. 2006). Their widespread use has raised the interest of scientists in basic research of organic fatty acids. Especially, the biological activities of essential oil and extracts have been investigated in recent years (Karlova et al. 2010; Shafaghat 2011).
The genus Citrus (family: Rutaceae) is represented in Iran by numerous species and one of the important genera which frequently is used as fruits such as oranges, lemons, lime, citrus and grapefruits (Mozaffarian 2007). Citrus japonica (kumquat) is native to South Asia and Asia-Pacific regions. Popular mainly for their raw fruits, they have also been used for their medicinal and therapeutic effects. It has been recognised for its antiphlogistic, antivirus, carminative, deodorant and expectorant properties (Quijano & Pino 2009). The peel of kumquat is sweet and usually edible with a typical aroma due to the presence of flavonoids and terpenoids (Koyasako & Bernhard 1983). C. japonica has a typical citrus character and thus with other citrus fruits (known as Morakkabat in Persian) can be prepared as marmalade, added to fruit salad and conserved as a whole in sugar syrup in the food industry. The organic extracts from plants with a suitable composition of essential fatty acids are currently being sought (de Melo et al. 2000; Melgarejo & Artés 2000). The oily composition has a particular importance because of the potentially healthy unsaturated fatty acids (UFAs). UFAs are important since they play a preventive role in cardiovascular diseases, contributing to the reduction of blood cholesterol (De Hoya & Mata 1989). These compounds from various plants may also have industrial, pharmaceutical and food applications, such as in cosmetics and in health care products. Thus, the characteristics and chemical constituents of kumquat peel and kernel oils were investigated. The phytochemistry of Citrus species is diverse, in that these species contain limonene (76.7%) in fruit peel oil (Quijano & Pino 2009) and (62%) in leaves of Citrus aurantium L. (Gholivand & Piryaei 2013), linalool (35.1%) in leaf oil (Satyal et al. 2012), flavonoids and polyphenols in methanolic extract (Schirra et al. 2008). Earlier studies have identified the chemical compositions (Choi 2005; Satyal et al. 2012), antifungal activities (Van Hung et al. 2013), antiproliferative activity of limonene (Manassero et al. 2013), antimicrobial activity and chemical composition (Settanni et al. 2014) and three tocopherol analogues (Seo et al. 2015) in the different species of Citrus genus. To the best of our knowledge, however, there are no reports on the constituents of hexane extract from peel and kernel of C. japonica from Iran and no reliable references have so far been found reporting the content and constituent of kumquat kernel essential oil and those antioxidant activities.

2. Results and discussion

Hydrodistillation of C. japonica peel and kernel produced a yellow coloured oil with a yield of 1.1 and 0.8% (V/W), based on the dry weight of samples. According to the results, the hexane extract yields of the studied C. japonica were found to be 3.6 and 4.1% on the basis of dry weight of the peels and kernels, respectively (Table S2). The results obtained in the analyses of the essential oil and hexane extract of C. japonica peels and kernels are listed in Table S1, in which the percentage and retention times of components are given. These values are listed in order of their elution from HP-5MS capillary column. As table S1 shows, about 99.7% (20 components) of the essential oil of C. japonica in the peel and 99.1% (23 components) of the oil were identified in the kernel sample. The major constituents identified in peel oil were limonene (51.0%), germacrene D (12.1%) and β-myrcene (8.5%). The kernel essential oil, however, was predominated by limonene (47.1%), β-myrcone (9.4%), germacrene D (6.3%) and β-phellandrene (4.8%). The essential oil from kumquat peel consisted mainly of six monoterpenoids (65.5%) which were characterised by limonene (51.0%), β-myrcene (8.5%), β-phellandrene (2.5%), (+)-carvone (1.1%), α-pinene (0.7%) and five sesquiterpenoids (18.4%) characterised by germacrene D (12.1%), δ-cadinene (2.5%), germacrene B (1.8%),
γ-cadinene (1.1%), γ-elemene (0.9%) and two oxygenated monoterpenes (3.5%) which were characterised by geranyl acetate (2.4%), (+)-carvone (1.1%) and only one oxygenated aromatic compound (myristicin, 1.6%) as the oil constituents.

As can be seen in Table S1, the dominant compounds in both the oils of peel and kernel are monoterpenic hydrocarbons, but these components were in minor amounts in the samples from hexane extract. The essential oil from kumquat kernels contained five monoterpenic hydrocarbons (62.5%), which were characterised by limonene (47.1%), β-myrcene (9.4%), β-phellandrene (4.8%), (+)-carvone (0.8%), α-pinene (1.3%) and six sesquiterpenoids (20.1%) characterised by germacrene D (6.3%), δ-cadinene (1.1%), germacrene B (3.0%), γ-cadinene (3.3%), γ-elemene (4.0%), γ-muurolene (2.4%) and two oxygenated monoterpenes (1.2%) which were characterised by geranyl acetate (0.4%) and (+)-carvone (0.8%) as the oil constituents. The hexane extract constituents from *C. japonica* peels and kernels were studied using GC/FID and GC/MS techniques for the first time. Analysis of the chemical composition of hexane extracts from *C. japonica* in both samples (peels and kernels) showed the presence of fatty acids, aliphatic hydrocarbons and terpenoid constituents (Table S1). As can be seen in Table S1, about 16 compounds of the extract from peels and 18 components from kernel extract were identified. There were some differences in the essential oil constituents, aliphatic hydrocarbons and fatty acid profiles of the different parts of kumquat. Polysaturated fatty acids (PUFAs), essential oil components (EOCs) and some of the other contents were observed in both samples of this fruit. In fact, both parts mainly include UFAs, with a clear predominance of ω-3. One of the essential fatty acids (EFAs), linolenic acid (ω-3), was a predominant component in kernel sample. Linoleic acid is an ω-6 fatty acid, ranging from 6.9% (in peels oil) to 9.9% (in kernel oil) that was found in little amounts in this work. The hexanic extract of kernel sample had a higher proportion of UFAs compared to peels extract sample (Tables S1 and S2). Two of the fatty acids were major unsaturated fatty acids, namely linolenic acid (ω-3) and linoleic acid (ω-6). The total content of hexane extracts varied from 98.6% (in kernel oil) to 99.5% (in peel oil). The major PUFAs in peel and kernel including linolenic (ω-3) (13.1 and 16.3%) and linoleic acids (ω-6) (6.9 and 9.9%), respectively, are shown in the Table. The major terpenoid compounds in peel extract were limonene (8.9%), γ-muurolene (6.5%) and (E)-dihydrofarnesol (2.2%). Six major aliphatic hydrocarbons pentadecene-1 (14.3%), dodecanol-1 (12.9%), nonene-4-methyl-5 (8.7%), undecene-4-methyl-5 (6.7%), decene-2(Z) (5.1%) and tetradecene-7(E) (5.0%) were detected in the hexane extract of peels sample.

D-limonene is the main compound in essential oils of citrus fruits, where it occurs in concentrations of more than 90% and in an enantiomerically pure form (Adams et al. 2003). A previous report showed that the major chemical component of citrus oils is limonene, ranging from 32 to 98%, with sweet orange containing 68–98%, lemon 45–76% and bergamot 32–45% (Fisher & Phillips 2008; Moufida & Marzouk 2003). In the investigation on the essential oil of the leaf of *C. japonica* collected from a mature fruiting tree from Nepal, monoterpenes predominated sesquiterpenes and linalool (35.1%), eugenol (14.8%), geraniol (12.7%) and its aldehyde counterpart geranial (7.9%) were the major constituents among them 42 characterised, comprising (99.6%) of the total components detected in the oil (Satyal et al. 2012). The Hydrodistillation essential oil obtained from the peels of *Fortunella crassifolia* (a kumquat variety) has been studied. The major components were identified to be limonene (74.8%), myrcene (7.1%) and camphene (1.4%). The oil was richer in monoterpenes than sesquiterpenes and oxygenated hydrocarbons (Wang et al. 2012). In another study on the characteristic odour components in the essential oil from *Fortunella japonica* peel,
limonene was the most dominant component, amounting to 93.7%. The limonene content of *C. japonica* peel and kernel oils were comparatively less than that reported for *F. japonica* peel oil. The α-pinene content of the essential oil was similar to that reported for *F. japonica* peel oil (Choi 2005). According to our results, the main constituents of essential oil of *C. japonica* (peel and kernel) were monoterpenes and oxygenated hydrocarbon compounds and, in hexane extracts from both parts of this fruit were UFAs and some of the aliphatic hydrocarbons and terpenoids compounds. It is clear that there is a significant correlation between the chemical compositions and biological activity and human health. Thus, it seems that *C. japonica* essential oil and extract from kernel and peel may be a good dietary source for food and pharmaceutical industries. These results also showed that the essential oil and extract constituents from the same fruit peel of the same chemotype vary significantly. This variation is also observed in other species and other localities.

The antioxidant activities of essential oil and hexane extract were reported for the first time. Results obtained in the antioxidant study of the samples are shown in Table S3. Antioxidant activity was tested according to the DPPH (1,1-diphenyl-2-pycrylhydrazile) radical scavenging method. All the four samples from peel (essential oil and hexane extract) and kernel (essential oil and hexane extract) obtained from *C. japonica* scavenged the DPPH radical in a dose-dependent manner, and the DPPH radical scavenging activity (IC$_{50}$) was decreased in the following order: kernel extract > peel extract > peel essential oil > kernel essential oil (Table S3).

According to this data, kernel extract oil was the most efficient free radical scavenger with the lowest IC$_{50}$ value of 63 μg/ml among all the essential oils and hexane extracts. The activity of the reference antioxidant (vitamin C) was much higher than that of sample oils. Although kernel oil did not differ considerably in fatty acid composition, it exhibited the best DPPH scavenging activity.

3. Conclusion

In the essential oils from peel and kernel of *C. japonica*, limonene is identified as the major oil constituent. These oils were characterised by high levels of monoterpenes hydrocarbons (limonene and β-myrcene) and sesquiterpenes (germacrene D). These terpenoid compounds as well as unsaturated fatty acid are widespread components of the essential oils and extract and used as flavours and fragrances in the drug, cosmetic, perfume and food industries. In this paper, we also described the fatty acid compositions of *C. japonica* peel and kernel. The major UFAs were alpha-linolenic (ω-3) and linoleic (ω-6) acids. The hexane extract of peel and kernel has also beneficial health effects as well as its rich unsaturated fatty acid content and therefore, it could be considered a good alternative for ω-3 and 6 for drug, cosmetics and nutraceutical industries. These results remain significant as the first step in screening antioxidant activity of *C. japonica* fruits. It can be concluded that, *C. japonica* fruit (peel and kernel), which are consumed as a fruit in Iran, can be used as an accessible source of natural antioxidants with consequent health benefits.

Disclosure statement

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