JOURNAL OF FOOD AND DRUG ANALYSIS 25 (2017) 162–175

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Review Article

Phenolic compounds and biological activities of small-size citrus: Kumquat and calamondin

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
29 October 2016
Accepted 31 October 2016
Available online 7 December 2016

Keywords:
biological activity
calamondin
kumquat
phenolic compositions

ABSTRACT

Kumquat and calamondin are two small-size citrus fruits. Owing to their health benefits, they are traditionally used as folk medicine in Asian countries. However, the research on flavonoids and biological activities of kumquat and calamondin have received less attention. This review summarizes the reported quantitative and qualitative data of phenolic compositions in these two fruits. Effects of maturity, harvest time, various solvent extractions and heat treatment of phenolic compositions, and bioactivities were discussed; distributions of the forms of phenolic compounds existing in kumquat and calamondin were also summarized. Furthermore, biological activities, including antioxidant, anti-tyrosinase, antimicrobial, antitumor, and antimetabolic disorder effects, have also been discussed. Effective phenolic components were proposed for a certain bioactivity. It was found that C-glycoside flavonoids are dominant phenolic compounds in kumquat and calamondin, unlike in other citrus fruits. Up to now, biological activities and chemical characteristics of C-glycoside flavonoids in kumquat and calamondin are largely unknown.

1. Introduction

Citrus species are rich sources of flavanone glycosides, in particular, hesperidin and naringin, but the presence of dihydrochalcones and C-glycoside flavonoids is not common in fruits of the genus *Citrus*. Kumquat and calamondin bear small-size fruits and contain high amounts of C-glycoside flavonoids and dihydrochalcone compounds [1–5]. Kumquat is an elliptical shaped fruit, closely related to *Citrus*. It has four major cultivated types, including *Fortunella japonica*, *Fortunella margarita*, *Fortunella crassifolia*, and *Fortunella hindsii* [3]. The whole fruit including peel is used and may be candied or preserved in sugar syrup. Kumquat is also used as traditional folk medicine to manage inflammation of the respiratory tract [6–8]. Health benefits of citrus are well documented; however, few studies report the bioactivity and phenolic composition of kumquat. Moreover, the biological activity of flavonoid C-glycoside in kumquat has not been investigated. Aglycones of flavonoid C-glycosides in kumquat, such as phloretin and acacetin, exhibit a broad spectrum of biological activities such

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http://dx.doi.org/10.1016/j.jfda.2016.10.024

as antioxidant activity, antiinflammatory effect, and anticancer effect [9–13].

Calamondin (*Citrus mitis*, *Citrus microcarpa*, or *Citrus manurensis*), a cultivar related to *Citrus*, is a hybrid between *Citrus reticulate* Blanco and *Fortunella* spp. [14]. Hot water extract of immature fruits with green peel has been a popular beverage in Taiwan for many years due in part to its potential health beneficial properties. It may be attributed to the presence of bioactive compounds such as flavonoids in the peel. Calamondin contains a large quantity of 3′,5′-di-C-β-glucopyranosylphloretin (DGPP) in its peel, juice sac, and leaves [3]. The flavonoid composition is quite different from that of *Citrus*. However, there are only a few reports on the quantitative data and biological activity of calamondin peel, particularly that of the immature fruits. Therefore, it is desirable to study the flavonoid compositions, especially C-glycoside flavonoids and dihydrochalcone, as well as biological activity of kumquat and calamondin for their potential applications.

2. Kumquat

2.1. *Flavonoids and phenolic acids*

Fortunellin, a flavone (acacetin-7-O-neohesperidoside), was isolated from kumquat by Matsuno in 1958 [1]. It was known as a major representative flavonoid in kumquat for a long time. In 1985, eight flavonoid glycosides, including 6,8-di-C-glucosylapigenin, 3,6-di-C-glucosylacacetin, 2″-O-α-L-rhamnosyl-4′-O-methylvitexin, 2″-O-α-L-rhamnosyl-4′-O-methylisovitexin, 2″-O-α-L-rhamnosylvitexin, 2″-O-α-L-rhamnosylorientin, 2″-O-α-L-rhamnosyl-4′-O-methylorientin, and poncirin, were isolated and identified in hot water extract of the peel of kumquat (*F. japonica*) [2]. Kawail et al [15] quantified flavonoids in various citrus, including kumquat (*F. japonica*, *F. margarita*, and *F. crassifolia*), extracted by a mixture of methanol and dimethyl sulfoxide. The major quantified flavonoids in the edible part of kumquat were narirutin, rhoifolin, kaempferol, luteolin, poncirin, hesperidin, neoponcirin, eriocitrin, and quercetin. The highest content of narirutin was found in the range of 289–460 μg/100 mg dried sample. However, as a result of reinvestigation by the same laboratory, "narirutin" was shown to be a dihydrochalcone glucoside, DGPP, which has previously been isolated from kumquat (*F. margarita*) as a major flavonoid [3]. They assumed that the accumulation of DGPP is a generic trait of the genus *Fortunella*, while the genera *Citrus* and *Poncitrus* probably lack it. The other three major compounds were identified as 2′-O-α-L-rhamnopyranosylcytisoside, 2′-O-α-L-rhamnopyranosylisocytisoside, and fortunellin. Fortunellin and DGPP in the peel of *F. japonica* had also been identified by Cho et al [16], while acacetin-8-C-neohesperidoside and kaempferide 3-O-rhamnopyranoside were found at the same time. In a study of kumquat peel extracted by ethyl acetate, DGPP and poncirin were also reported [17]. However, they had also found acacetin-6-C-rutinoside, acacetin-8-C-rutinoside, and acacetin-7-O-rutinoside, instead of neohesperidoside, which most former researchers identified.

However, other studies identified naringin, hesperidin, neohesperidin, diosmin, sinensetin, rutin, quercetin, and kaempferol in the peel of kumquat [18]. Schirra et al [19] demonstrated that only narirutin and rhoifolin were present in kumquat. The found that flavonoids in kumquat are quite different from those reported in other studies. Only five flavonoids, including poncirin, didymin, isorhoifolin, hesperidin, and narirutin, had been quantified in the pulp of kumquat (*F. margarita*) [20]. These indicated a lack of consistent knowledge on the flavonoid compositions of kumquat. It is probably because a few studies in this field focus on kumquat and also because inadequate techniques are used for flavonoid identification.

In the last decade, liquid chromatography tandem mass spectrometry (LC/MS/MS) was widely used to investigate the natural constituents of plants because of its good reliability in quantitative and qualitative analyses. Barreca et al [21] quantified 13 flavonoids in kumquat juice (*F. japonica*) by LC/MS/MS, the main components of which were DGPP, fortunellin, acacetin-6-C-neohesperidoside, acacetin-8-C-neohesperidoside, apigenin-8-C-neohesperidoside, hesperidin, poncirin, didymin, and rhoifolin. In another study, DGPP, poncirin, narirutin, rutin, and apigenin-8-C-rutinoside were observed and quantified in five kumquat extracts using different solvents by LC/MS/MS [22]. In our previous study, 15 flavonoids could be separated from hot water extract of kumquat (*F. margarita*) and characterized by high-performance liquid chromatography (HPLC) (Figure 1) [4]. Seven major flavonoids have been identified and quantified by LC/MS/MS. Their chemical structures and quantitative data are shown in Figure 2 and Table 1 [5]. A similar result about the seven major flavonoids in kumquat has also been reported [23]. However, the authors did not quantify the phenolic compounds. Another study of the leaves of *F. japonica* has identified three flavonoids, including apigenin-7-O-β-D-glucopyranoside, apigenin-7-O-β-D-rhamnoglucoside, and the methoxylated flavonoid cirsimaritin, which are quite different from the flavonoids found in the peel and pulp of kumquat [24].

Seven quantified flavonoids of immature and mature kumquats are listed in Table 1. They were mainly soluble conjugated flavonoids. In immature kumquats, over 90% of total identified flavonoids were C-glycosyl compounds, including DGPP, acacetin-8-C-neohesperidoside, acacetin-6-C-neohesperidoside, and apigenin-8-C-neohesperidoside. The most abundant compound was DGPP in 70% of total flavonoids. The level of O-glycosyl compounds are about 10%, including fortunellin, poncirin, and rhoifolin. The major O-glycosyl flavonoid was fortunellin (7%) [5].

C-glycosides are usually resistant against acidic and enzymatic hydrolysis in contrast to corresponding O-glycosides [25]. DGPP, a phloretin with 6- and 8-C-glycosylation, is a dihydrochalcone glucoside. It is a hydrophilic compound mainly because of its two glucose moieties. Although 98% remained at pH 4.8 after 24 hours of storage, it degraded to 68% at pH 6.8 [26]. Phloretin has been reported to have many biological activities, including antioxidant activity in apple [27], which might partially be because of the stabilization of radical by 2,6-dihydroxyacetophenone via tautomerization [10]. Acacetin-8-C-neohesperidoside and acacetin-6-C-neohesperidoside, also named as margaritene and isomargaritene, respectively, are C-glycosyl flavones