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Tyrosinase inhibitory effect of quercetin 4′-*O*-*β*-D-glucopyranoside from dried skin of red onion (*Allium cepa*)

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In an effort to find a new whitening agent, we focused our attention on *Allium cepa* (red onion). Based on biologically guided fractionation using mushroom tyrosinase, quercetin 4′-*O*-*β*-D-glucopyranoside was isolated from the dried skin of *A. cepa*. Quercetin 4′-*O*-*β*-D-glucopyranoside showed tyrosinase inhibitory activity using L-tyrosine or L-DOPA as a substrate, with IC_{50} values of 4.3 and 52.7 μM, respectively. Based on the results obtained, the dried skin of red onion possesses ingredients with potential for skin-whitening cosmetics with anti-tyrosinase activity.

Keywords: red onion; dried skin; quercetin 4′-*O*-*β*-D-glucopyranoside; anti-tyrosinase

1. Introduction

Visible pigmentation in mammals results from the synthesis and distribution of melanin in the skin and hair bulbs (Parves, Kang, Chung, & Bae, 2007). Melanin pigments are formed in specialised pigment-producing cells known as melanocytes that originate in the neural crest during embryogenesis and are distributed throughout the embryo during its development. At the cellular level, these compounds are biosynthesised in the membranous organelles known as melanosomes (Sánchez-Ferrer, Rodrígez-López, & García-Carmona, 1995).

Melanin may be overproduced due to chronic sun exposure, melasma, or other hyperpigmentation diseases. Therefore, a number of depigmenting agents have been developed for cases of undesirable skin discolouration (Wang et al., 2006). Tyrosinase, a copper-containing monooxygenase, is a key enzyme that catalyses melanin synthesis in melanocytes (Sturm, Teasdale, & Box, 2001). It catalyses two major reactions: the hydroxylation of L-tyrosine and the oxidation of the *o*-diphenol product, L-DOPA (3,4-dihydrocyphenylalanine).

Onion is a versatile vegetable which is consumed fresh as well as in the form of processed products. More recently, there has been renewed attention given to the

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antioxidant content of onions because many epidemiological studies have suggested that regular consumption of onions is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development, reduction in symptoms associated with osteoporosis, and prevention of vascular and heart diseases by inhibition of lipid peroxidation and lowering of low-density lipoprotein cholesterol levels (Kaneko & Baba, 1999; Kawaii, Tomono, Katase, Ogawa, & Yano, 1999; Sanderson, Mclauchlin, & Williamson, 1999; Shutenko et al., 1999). Onion is one of the major sources of various biologically active phytomolecules, e.g. phenolic acids, flavonoids, cepaenes, thiosulphinates and anthocyanins (Singh et al., 2009). The major flavonoids found in the dry peel of onion, which is often considered as waste, contain large amounts of quercetin, quercetin glycoside and their oxidative products, which are effective antioxidants against the lethal effects of oxidative stress (Gulsen, Makris, & Kefalas, 2007; Prakash, Upadhyay, Singh, & Singh, 2007). Onions are one of the richest sources of flavonoids in the human diet. Onions possess a high level of antioxidant activity, which is attributed to the flavonoids quercetin, kaempferol, myricetin and catechin (Yang, Meyers, Heide, & Liu, 2004). Onions are also reported to have liver protective effects, immune enhancement potential and anti-infection, anti-stress, anti-cancer and other pharmacological properties (Balasenthil, Arivazhagan, Ramachandran, Ramachandran, & Nagini, 1999; Valko et al., 2007).

In Indonesia, onion (*Allium* sp.) plays an important role in traditional medicine; it is used as a diuretic, suppresses the blood sugar level, platelet aggregation, febrifuge and as a poultice to cure wounds and to remove scars in the skin (de Padua, Bunyapraphatsara, & Lemmens, 1999). The outer layer of onion (the dried skin) is used as food colouring, especially in Javanese tribes.

In Indonesia, where herbal medicine is popular, more than 1300 species are known as medicinal plants, named 'Jamu' (Roosita, Kusharto, Sekiyama, Fachrurozi & Ohtsuka, 2008). The uses of Jamu fall into four categories of medicine: health care, beauty (cosmetics), tonics and bodily protection (Soedarsono & Harini, 2002). Exploration of the use of onion as a source of potential drugs for humans needs experimentation.

In this study, we evaluated the methanol extracts of red onion (*A. cepa*) from Indonesia in order to identify the tyrosinase inhibitor which could be developed as the potential depigmenting agent for use in skin-whitening cosmetics.

2. Results and discussion

2.1. *Isolated compounds*

The methanol extract of *A. cepa* showed tyrosinase inhibitory activity with 90% or 39% at 100 μg mL^{-1} using L-tyrosine or L-DOPA, respectively. Based on the biologically guided fractionation using mushroom tyrosinase, we isolated the active compound that showed tyrosinase inhibitory activity. NMR assignment was performed to elucidate the structure of the isolated compound. NMR data revealed that Fr 15 was quercetin 4′-*O*-*β*-D-glucopyranoside (84.7 mg, 6.1%, Figure 1), which was compared with the previous report by Tanabe, Ogawa, Tesaki and Watanabe (1997). Naturally-occurring of components in the dried skin of onion were found to

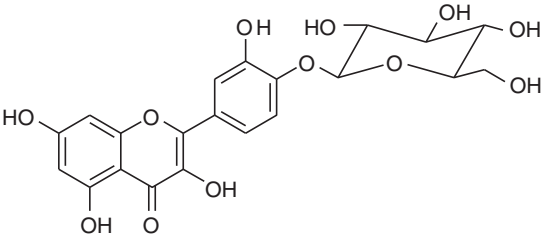

Figure 1. Chemical structure of quercetin 4′-*O*-*β*-D-glucopyranoside.

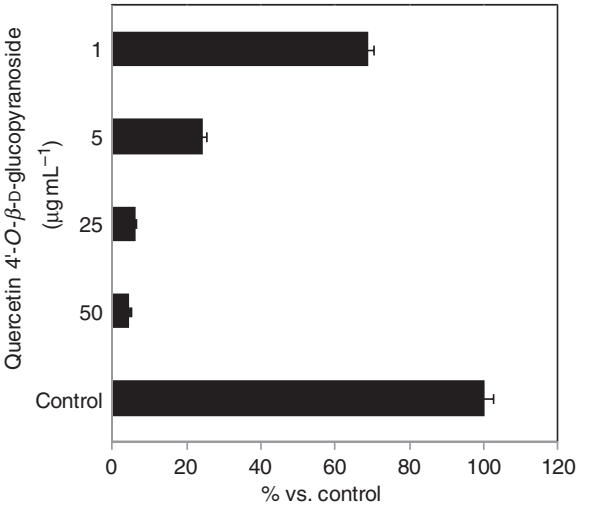

Figure 2. The effect of quercetin 4′-*O*-*β*-D-glucopyranoside from *A. cepa* dried skin extract (substrate: L-tyrosine) on tyrosinase.

be five quercetin derivatives: quercetin 3,4′-*O*-diglucoside (3.2%), quercetin 3-*O*-glucoside (0.2%), quercetin 4′-*O*-glucoside (13.2%) and isorhamnetin 4′-*O*-glucoside (0.1%) (Wiczkowski et al., 2008).

2.2. *Anti-tyrosinase activity*

In this study, the anti-melanogenesis effects of the isolated compound of the extract prepared from the dried skin of *A. cepa* was determined by tyrosinase enzyme assay. Figures 2 and 3 present the inhibition of quercetin 4′-*O*-*β*-D-glucopyranoside in mushroom tyrosinase in both L-tyrosine and L-DOPA as substrates, respectively. Both Figures 2 and 3 show that quercetin 4′-*O*-*β*-D-glucopyranoside inhibited tyrosinase activity dose dependently from 1 to 50 μg mL^{-1}. Furthermore, Table 1 summarises the tyrosinase inhibitory activity, as shown in IC_{50} values of 4.3 and 52.7 μM. In this study, we used kojic acid as a positive control for tyrosinase inhibition (Cabanes, Chazarra, & García-Carmona, 1994; Curto et al., 1999; Virador, Kobayashi, Matsunaga, & Hearing, 1999).

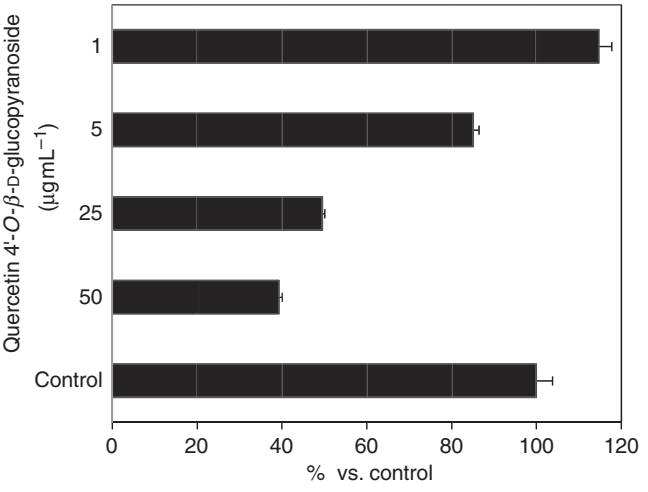

Figure 3. The effect of quercetin 4′-*O*-*β*-D-glucopyranoside from *A. cepa* dried skin extract (substrate: L-DOPA) on tyrosinase.

Table 1. Effect of an isolated compound from dried skin of *A. cepa* on mushroom tyrosinase.

Compounds	L-Tyrosine[a] IC_{50} (μmol)[b]	L-DOPA[a] IC_{50} (μmol)[b]
Quercetin-4′-*O*-glucoside	4.3	52.7
Kojic acid	5.3	14.1

Notes: [a]Substrate; [b]IC_{50} was interpolated from graphed concentrations and determined graphically with statistical software.

Quercetin 4′-*O*-*β*-D-glucopyranoside has been found to have some biological functions, such as inhibiting oral cancer cell proliferation, inhibiting platelet aggregation, inhibiting glucose uptake into the brush-border-membrane and antioxidant activity (Browning, Walle, & Walle, 2005; Cermak, Landgraf, & Wolffram, 2004; Hubbard, Wolffram, Lovegrove, & Gibbins, 2004; Williamson, Plumb, Uda, Price, & Rhodes, 1996; Yesilada, Tsuchiya, Takaishi, & Kawazoe, 2000). To our knowledge, this is the first report that the dried skin of red onion and its isolated compound, quercetin 4′-*O*-*β*-D-glucopyranoside, have shown tyrosinase inhibitory activities.

3. Experimental

3.1. *Chemicals*

DMSO, L-tyrosine and L-DOPA were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Mushroom tyrosinase (2870 U mg^{-1}) was purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals used were of the highest grade commercially available.

3.2. *Plant materials*

Red onion was purchased from a traditional market in Jakarta, Indonesia, in September 2008. A voucher specimen (ETA-CW-6) was deposited in the Wood Chemistry Laboratory, Department of Forest Product Technology, Faculty of Forestry, Mulawarman University.

3.3. *Preparation of plant extracts*

Plant materials (dried skin of *A. cepa*) were dried at room temperature and powdered. The dried materials (17.38 g) were extracted with methanol at room temperature with a shaker at 150 rpm for 48 h. The extract solutions were filtered and concentrated *in vacuo*, to obtain the crude methanol extracts. The crude extracts weighed 1.75 g.

3.4. *Isolation of quercetin 4′-O-β-D-glucopyranoside*

The crude extract of the dried skin of *A. cepa* (1.4 g), which showed potent inhibitory effect of melanin production in B16 melanoma cells, was applied to silica gel column (71 g of Wakogel C-200, 3.5×50 cm) and eluted with *n*-hexane/EtOAc [10 : 0 (100 mL), 9 : 1 (50 mL), 7 : 3 (50 mL), 5 : 5 (200 mL), 3 : 7 (200 mL), 1 : 9 (100 mL)] and EtOAc/MeOH [9 : 1 (100 mL), 8 : 2 (100 mL), 7 : 3 (250 mL), 6 : 4 (50 mL), 5 : 5 (100 mL), 4 : 6 (50 mL), 3 : 7 (50 mL), 2 : 8 (50 mL), 1 : 9 (100 mL), 0 : 10 (100 mL)] to give 33 fractions (Fr 1–Fr 33). Using TLC and analytical HPLC, Fr 15 (84.7 mg) was isolated and identified as quercetin 4′-*O*-β-D-glucopyranoside by comparison with an authentic sample (Extrasynthese, France) and NMR analysis.

The NMR data were measured at 400 MHz on a JNM-AL400 FT NMR spectrometer (Jeol). The compound was dissolved in methanol-d_4 and the chemical shift was referred to deuterated solvents. The compounds were assigned for ^{1}H, ^{13}C, HMQC and HMBC.

3.5. *Tyrosinase enzyme assay*

Although mushroom tyrosinase differs somewhat to that from other sources, this fungal source was used for this experiment due to its ready availability. It should be noted that the commercial tyrosinase was reported to contain numerous proteins besides tyrosinase (Flurkey et al., 2008), but was used without purification. The tyrosinase activity was determined with the method as previously described (Arung, Shimizu, & Kondo, 2006). Briefly, all samples were first dissolved in DMSO and used for the actual experiment at 30 times dilution. First, 333 μL of 330 μmol L-tyrosine or 200 μmol L-DOPA solution was mixed with 600 μL of 0.1 M phosphate buffer (pH 6.8), and incubated at 25°C. Then, 33 μL of the sample solution and 33 μL of the aqueous solution of mushroom tyrosinase (1380 U mL^{-1}) were added to the mixture, and the increase in optical density at 475 nm was measured on the basis of the formation of DOPAchrome. The reaction solution was incubated at 25°C for 10 min and the absorbance at 475 nm was measured before and after incubation. The reaction was started by the addition of the enzyme. Since tyrosinase catalyses a reaction between two substrates, a phenolic compound and oxygen, the assay was

carried out in air-saturated solution. Controls, without inhibitor, were routinely carried out. Each experiment was carried out in duplicate or triplicate. Kojic acid was used as a positive control.

4. Conclusion

In this study, we have found a new facet of the biological activity of quercetin 4′-*O*-*β*-D-glucopyranoside: that it showed tyrosinase inhibitory activity. Quercetin 4′-*O*-*β*-D-glucopyranoside is a promising compound that could be useful for treating hyperpigmentation in the form of a skin-whitening agent. However, it needs further experiments to clarify its function. It should be noted that safety is the primary consideration for its practical use in humans. Still, our findings are in line with the traditional uses of medicinal plants in Indonesia for their skin care functions.

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