### Tyrosinase inhibitory effect of quercetin 4'-0 - $\beta$ - D-glucopyranoside from dried skin of red onion (Allium cepa)

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# 1 Tyrosinase inhibitory effect of quercetin 4'-O-β-D-glucopyranoside from dried skin 2 of Red Onion (Allium ascalonicum)

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#### 1 Abstract

In our effort to find a new whitening agent, we focused on *Allium ascalonicum*. Based on the biologically-guided fractionation by using mushroom tyrosinase, quercetin 4'-O- $\beta$ -D-glucopyranoside was isolated from dried skin of *A. ascalonicum*. Quercetin 4'-O- $\beta$ -D-glucopyranoside showed tyrosinase inhibitory activity using L-tyrosine or L-DOPA as a substrate with IC<sub>50</sub> of 4.3 and 52.7  $\mu$ M, respectively. Based on the results obtained, dried skin of red onion possessed the potential ingredients for skin-whitening cosmetics with anti-tyrosinase activity.

9 *Keywords* : Red onion, dried skin, quercetin  $4'-O-\beta$ -D-glucopyranoside, anti-tyrosinase.

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#### 11 **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 specialized pigment-producing cells known as melanocytes, which originate in the neural crest during embryogenesis and are distributed throughout the embryo during its development. At the cellular level, these compounds are biosynthesized 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 discoloration (Wang et al., 2006). Tyrosinase, a copper-containing monooxygenase, is a key enzyme that catalyzes melanin synthesis in melanocytes (Sturm, Teasdale, & Box, 2001). It catalyzes two major reactions, including hydroxylation of L-tyrosine and oxidation of the *o*-diphenol product, L-DOPA (3, 4-dihydrocyphenylalanine).

26 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 27 antioxidant content of onions because many epidemiological studies suggested that 28 29 regular consumption of onions in food is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer 30 31 development, reduction in symptoms associated with osteoporosis, prevention of vascular and heart diseases by inhibition of lipid peroxidation and lowering of low 32 density lipoprotein cholesterol levels (Kaneko & Baba, 1999; Kawaii, Tomono, Katase, 33 34 Ogawa, & Yano, 1999; Sanderson, Mclauchlin, & Williamson, 1999; Shutenko et al.,

1 1999). Onion is one of the major sources of various biologically active phytomolecules 2 e.g. phenolic acids, flavonoids, cepaenes, thiosulfinates and anthocyanins (Singh et al., 2009). The major flavonoids found in dry peel of onion that has been considered usually 3 as waste, contain large amounts of quercetin, quercetin glycoside and their oxidative 4 5 product which are effective antioxidants against the lethal effect of oxidative stress (Gulsen, Makris, & Kefalas, 2007; Prakash, Upadhyay, Singh, & Singh, 2007). Onions 6 are one of the richest sources of flavonoids in the human diet. Onions possess a high 7 8 level of antioxidant activity, which is attributed to the flavonoids quercetin, kaempferol, 9 myricetin, and catechin (Yang, Meyers, Heide, & Liu, 2004). They are also reported 10 to have liver protective effect, immune enhancement potential and anti-infection, 11 anti-stress, anti-cancer and other pharmacological properties (Balasenthil, Arivazhagan, Ramachandran, Ramachandran, & Nagini, 1999; Valko, Leibfritz, Moncola, Cronin, 12 Mazura, & Telser, 2007). 13

The use of Onion (*Allium* species) in Indonesia, plays important role in traditional medicine; it is used as diuretic, suppresses the blood sugar level, platelet aggregation, febrifuge, and as poultice to cure wounded and to remove scars wounded in the skin (de Padua et al. 1999, prosea). The outer layer of onion (dried skin) is used as food coloring especially in Javanesse tribe.

In Indonesia, where herbal medicine has been popular, more than 1300 species are known as medicinal plants, called Jamu (Roosita, Kusharto, Sekiyama, Fachrurozi & Ohtsuka, 2008). The uses of Jamu fall into four categories of medicine: health care, beauty care (cosmetics), tonics, and bodily protection (Soedarsono & Harini, 2002). To explore the use of onion as a source of potential drugs in human life, needs some experiments.

In the present study, we evaluated the methanol extracts of red onion (*Allium ascalonicum*) from Indonesia in order to identify the tyrosinase inhibitor which should develop as the potential depigmenting agents such as skin-whitening cosmetics.

- 28 **2. Materials and Methods**
- 29 **2.1.** Chemicals

30 DMSO, L-tyrosine and L-DOPA were purchased from Wako Pure Chemical Industries,

31 Ltd (Osaka, Japan). Mushroom tyrosinase (2870 units/mg) was purchased from Sigma

32 Chemical Co. (St. Louis, MO). Other chemicals are of the highest grade commercially

- 33 available.
- 34

#### 35 2.2. Plant materials

36 Red onion was purchased from traditional market in Jakarta, Indonesia, on September in

1 2008. Voucher specimen (ETA-CW-6) was deposited in Wood Chemistry Laboratory,

Department of Forest Product Technology, Faculty of Forestry, MulawarmanUniversity.

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#### 5 2.3. Preparation of plant extracts

6 Plant materials (dried skin of *A. ascalonicum*, were dried at room temperature and 7 powdered. The dried materials (17.38 g) were extracted with methanol at room 8 temperature with shaker at 150 rpm during 48 h. The extract solutions were filtered and 9 concentrated *in vacuum*, to obtain the crude methanol extracts. The crude extracts were 10 1.75 g.

11

#### 12 2.4. Isolation of quercetin 4'-O-β-D-glucopyranoside

The crude extract of dried skin of A. ascalonicum (1.4 g) which showed potent 13 14 inhibitory effect of melanin production in B16 melanoma cells, was applied to silica gel column (71 g of Wakogel C-200, 3.5 x 50 cm) and eluted with n-hexane/EtOAc 15 16 [10:0(100ml), 9:1(50 ml), 7:3(50 ml), 5:5(200ml), 3:7(200ml), 1:9(100ml)] and EtOAc/MeOH [9:1(100ml), 8:2(100 ml), 7:3(250 ml), 6:4(50 ml), 5:5(100ml), 4:6(50 17 ml), 3:7(50ml), 2:8(50 ml), 1:9(100ml), 0:10(100)] to give thirty three fractions (Fr 1 to 18 19 Fr 33). By using TLC and analytical HPLC, Fr 15 (84.7 mg) was isolated and identified 20 as quercetin  $4'-O-\beta$ -D-glucopyranoside by comparison with authentic sample 21 (Extrasynthese, France) and NMR analysis.

The NMR data were measured at 400 MHz on JNM-AL400 FT NMR spectrometer (Jeo1). The compound was dissolved in methanol- $d_4$  and chemical shift was referred to deuterated solvents. The compounds were assigned for <sup>1</sup>H, <sup>13</sup>C, HMQC, and HMBC.

25

#### 26 **2.5. Tyrosinase enzyme** *assay*

Although mushroom tyrosinase differs somewhat from other sources, this fungal source 27 28 was used for the present experiment due to its ready availability. It should be noted that 29 the commercial tyrosinase was reported to contain numerous proteins besides tyrosinase 30 (Flurkey et al., 2008), but was used without purification. The tyrosinase activity was determined with method as previously described (Arung, Shimizu, & Kondo, 2007). 31 Briefly, all the samples were first dissolved in DMSO and used for the actual 32 experiment at 30 times dilution. First, 333 µL of 330 µM L-tyrosine or 200 µM L-DOPA 33 solution was mixed with 600 µL of 0.1M phosphate buffer (pH 6.8), and incubated at 34  $25^{\circ}$ C. Then, 33 µL of the sample solution and 33 µL of the aqueous solution of 35 36 mushroom tyrosinase (1380 units/mL) was added to the mixture and measured the

increase in optical density at 475 nm, 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 addition of the enzyme. Since tyrosinase catalyzes 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.

8

#### 9 3. Results and discussion

#### 10 3.1. Isolated compounds

11 The methanol extract of A. ascalonicum showed tyrosinase inhibitory activity with 90% 12 or 39% at 100 µg/ml using L-tyrosine or L-DOPA, respectively. Based on the biologically-guided fractionation by using mushroom tyrosinase led us to isolate active 13 14 compound that showed tyrosinase inhibitory activity. NMR assignment was performed 15 to elucidate the structure of isolated compound. NMR data revealed that F15 was 16 quercetin 4'-O-β-D-glucopyranoside (84.7 mg, 6.1%, Figure 1) which compared with 17 the previous report by Tanabe, Ogawa, Tesaki & Watanabe (1997). Naturally 18 occurring of components in dried skin of onion were found about five quercetin 19 derivatives, such as quercetin 3,4'-O-diglucoside (3.2%), quercetin 3-O-glucoside (0.2%), quercetin 4'-O-glucoside (13.2%), isorhamnetin 4'-O-glucoside (0.1%) 20 21 (Wiczkowski et al., 2008).

22

#### 23 **3.2.** Anti tyrosinase activity

In this present study, the anti melanogenesis effects of isolated compound from the 24 25 extract prepared of the dried skin of A. ascalonicum was determined by tyrosinase 26 enzyme assay. Figure 2 and 3 presented the inhibition of quercetin 4'-O-β-D-glucopyranoside in mushroom tyrosinase in both L-tyrosine and L-DOPA as 27 substrates. 28 respectively. Both Figure 2 and 3 showed that quercetin 4'-O-β-D-glucopyranoside inhibited tyrosinase activity at dose dependently from 1-50 29 µg/ml. Furthermore, Table 1 summarized the tyrosinase inhibitory activity as shown in 30  $IC_{50}$  of 4.3 and 52.7µM. In this study, we used kojic acid as a positive control for 31 tyrosinase inhibition (Cabanes, Chazarra, & Garcia-Carmona, 1994; Virador, Kobayashi, 32 Matsunaga, & Hearing, 1999; Curto et al., 1999). 33

Quercetin 4'-*O*-β-D-glucopyranoside has to have some biological functions such as
inhibited oral cancer cell proliferation, inhibited platelet aggregation, inhibited glucose
uptake into brush-border-membrane, and antioxidant (Browning, Walle, & Walle, 2005;

- 1 Hubbard, Wolffram, Lovegrove, & Gibbins, 2004; Cermak, Landgraf, Wolffram; 2004;
- 2 Yesilada, Tsuchiya, Takaishi, Kawazoe, 2000; Williamson, Plumb, Uda, Price, &
- Rhodes, 1996). To our knowledge, this is the first report that the dried skin of red onion and its isolated compound, quercetin  $4'-O-\beta$ -D-glucopyranoside showed tyrosinase
- 5 inhibitory activities.
- 6

#### 7 **4. Conclusion**

8 In this study, we have found a new facet of the biological activity of quercetin 9 4'-O- $\beta$ -D-glucopyranoside which showed tyrosinase inhibitory activity. Quercetin 10 4'-O- $\beta$ -D-glucopyranoside is a promising compound that could be useful for treating 11 hyperpigmentation, as a skin-whitening agent. However, it needs further experiments to 12 clarify its function. It should be noted that safety is a primary consideration for its 13 practical use in humans. Still, our findings are in line with the traditional uses of 14 medicinal plants in Indonesia for their skin care functions.

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5	Legend of Tables
6	
7	Table 1. Effect of an isolated compound form dried skin of A. ascalonicum on
8	mushroom tyrosinase
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15	Legend of Figures
16	
17	<b>Figure 1</b> . Chemical structure of quercetin 4'- <i>O</i> -β-D-glucopyranoside
18	<b>Figure 2</b> . The effect of quercetin 4'- $O$ - $\beta$ -D-glucopyranoside from A. ascalonicum dried
19	skin extract (substrate : L-tyrosine) on tyrosinase.
20	<b>Figure 3</b> . The effect of quercetin 4'- $O$ - $\beta$ -D-glucopyranoside from A. ascalonicum dried
21	skin extract (substrate : L-DOPA) on tyrosinase.
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- Figure 2.



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- Figure 3.