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

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

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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*-β-D-glucopyranoside was isolated from dried skin of *A. ascalonicum*. Quercetin 4'-*O*-β-D-glucopyranoside showed tyrosinase inhibitory activity using L-tyrosine or L-DOPA as a substrate with IC_{50} of 4.3 and 52.7 μM, respectively. Based on the results obtained, dried skin of red onion possessed the potential ingredients 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 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).

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 antioxidant content of onions because many epidemiological studies suggested that regular consumption of onions in food is associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development, reduction in symptoms associated with osteoporosis, 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, thiosulfinates and anthocyanins (Singh et al., 2009). The major flavonoids found in dry peel of onion that has been considered usually as waste, contain large amounts of quercetin, quercetin glycoside and their oxidative product which are effective antioxidants against the lethal effect 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). They are also reported to have liver protective effect, immune enhancement potential and anti-infection, anti-stress, anti-cancer and other pharmacological properties (Balasenthil, Arivazhagan, Ramachandran, Ramachandran, & Nagini, 1999; Valko, Leibfritz, Moncola, Cronin, Mazura, & Telser, 2007).

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.

2. Materials and Methods

2.1. Chemicals

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

2.2. Plant materials

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

2008. Voucher specimen (ETA-CW-6) was deposited in Wood Chemistry Laboratory, Department of Forest Product Technology, Faculty of Forestry, Mulawarman University.

2.3. Preparation of plant extracts

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

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 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 [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 ml), 3:7(50ml), 2:8(50 ml), 1:9(100ml), 0:10(100)] to give thirty three fractions (Fr 1 to Fr 33). By using TLC and analytical HPLC, Fr 15 (84.7 mg) was isolated and identified as quercetin 4'-*O*-β-D-glucopyranoside by comparison with authentic sample (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 ^{1}H, ^{13}C, HMQC, and HMBC.

2.5. Tyrosinase enzyme *assay*

Although mushroom tyrosinase differs somewhat from other sources, this fungal source was used for the present 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 method as previously described (Arung, Shimizu, & Kondo, 2007). Briefly, all the samples were first dissolved in DMSO and used for the actual experiment at 30 times dilution. First, 333 μL of 330 μM L-tyrosine or 200 μM L-DOPA solution was mixed with 600 μL of 0.1M phosphate buffer (pH 6.8), and incubated at 25^{o}C. Then, 33 μL of the sample solution and 33 μL of the aqueous solution of 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.

3. Results and discussion

3.1. Isolated compounds

The methanol extract of *A. ascalonicum* showed tyrosinase inhibitory activity with 90% 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 compound that showed tyrosinase inhibitory activity. NMR assignment was performed to elucidate the structure of isolated compound. NMR data revealed that F15 was quercetin 4'-*O*-β-D-glucopyranoside (84.7 mg, 6.1%, Figure 1) which compared with the previous report by Tanabe, Ogawa, Tesaki & Watanabe (1997). Naturally occurring of components in dried skin of onion were found about five quercetin 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%) (Wiczkowski et al., 2008).

3.2. Anti tyrosinase activity

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

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;

Hubbard, Wolffram, Lovegrove, & Gibbins, 2004; Cermak, Landgraf, Wolffram; 2004; 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*-β-D-glucopyranoside showed tyrosinase inhibitory activities.

4. Conclusion

In this study, we have found a new facet of the biological activity of quercetin 4'-*O*-β-D-glucopyranoside which showed tyrosinase inhibitory activity. Quercetin 4'-*O*-β-D-glucopyranoside is a promising compound that could be useful for treating hyperpigmentation, as a skin-whitening agent. However, it needs further experiments to clarify its function. It should be noted that safety is a 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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References

Alfonso, D., & Kapetanidis, I. (1994). Flavonoids from *Iohcroma gesneriodes*. *Pharmaceutica Acta Helvetiae*, 68, 211-214.

Arung, E.T., Shimizu, K., & Kondo, R. (2006). Inhibitory effect of artocarpanone from *Artocarpus heterophyllus* on melanin biosynthesis. *Biological and Pharmaceutical Bulletin*, 29, 1966-1969.

Arung, E.T., Shimizu, K., & Kondo, R. (2007). Structure-activity relationship of prenyl-substituted polyphenols from *Artocarpus heterophyllus* as inhibitors of melanin biosynthesis in cultured melanoma cells. *Chemistry and Biodiversity*, 4, 2166-2171.

Balasenthil, S., Arivazhagan, S., Ramachandran, C.R., Ramachandran, V., & Nagini, S. (1999). Chemopreventive potential of neem (*Azadirachta indica*) on 7,12-dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis. *Journal of Ethnopharmacology*, 67, 189–195.

Cabanes, J., Chazarra, S., & García-Carmona, F. (1994). Kojic acid, a cosmetic skin whitening agent, is a slow-binding inhibitor of catecholase activity of tyrosinase.

The Journal of Pharmacy and Pharmacology, 46, 982-985.
Cao, G., Sofic, E., & Prior, R.L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine*, 22, 749–760.
Cotelle, N., Berbier, J.L., Catteau, J.P., Pommery, J., Wallet, J.C., & Gaydou, E.M. (1996). Antioxidant properties of hydroxyl-flavones. *Free Radical Biology and Medicine*, 20, 35-43.
Curto E.V., Kwong, C., Hermersdörfer, H., Glatt, H., Santis, C., & Virador, V. (1999). Inhibitors of mammalian melanocyte tyrosinase: *in vitro* comparisons of alkyl esters of gentisic acid with other putative inhibitors. *Biochemical Pharmacology*, 57, 663-672.
de Padua L,S., Bunyapraphatsara, N., & Lemmens, R.H.M.J. (1999). Plant resources of South-East Asia: Medicinal and poisonous plants, vol. 12 (1), pp. 93-97. Bogor, Prosea Press.
Donsing, P., Limpeanchob, N., & Viyoch, J., Evaluation of the effect of Thai breadfruit's heartwood extract on melanogenesis-inhibitory and antioxidation activities. *J. Cosmet. Sci.*, 59, 41-58 (2008).
Flurkey, A., Cooksey, J., Reddy, A., Spoonmore, K., Rescigno, A., Inlow, J., & Flurkey, W. H. (2008). Enzyme, protein, carbohydrate, and phenolic contaminants in commercial tyrosinase preparations: potential problems affecting tyrosinase activity and inhibition studies. *Journal of Agricultural Food and Chemistry*, 56, 4760-4768.
Gulsen, A., Makris, D.P., & Kefalas, P. (2007). Biomimetic oxidation of quercetin: isolation of a naturally occurring quercetin heterodimer and evaluation of its in vitro antioxidant properties. *Food Research International*, 40, 7–14.
Kaneko, T., & Baba, N. (1999). Protective effect of flavonoids on endothelial cells against linoleic acid hydroperoxide-induced toxicity. *Bioscience Biotechnology Biochemistry*, 63,323–328.
Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., & Yano, M. (1999). Antiproliferative activity of flavonoids on several cancer cell lines. *Bioscience Biotechnology Biochemistry*, 63, 896–899.
Kim, Y.J. (2007). Antimelanogenic and antioxidant properties of gallic acid. *Biological and Pharmaceutical Bulletin*, 30, 1052-1055.
Nihei, K.I., & Kubo, I. (2003). Identification of oxidation product of arbutin in mushroom tyrosinase assay system *Bioorganic & Medicinal Chemistry Letter*, 13, 2409-2412.

Parvez, S., Kang, M., Chung, H.S., & Bae, H. (2007). Naturally occurring tyrosinase inhibitors: mechanism and applications in skin health, cosmetic and agriculture industries. *Phytotherapy Research*, 21, 805–816.

Prakash, D., Upadhyay, G., Singh, B.N., & Singh, H.B. (2007). Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (Glycine max). *Food Chemistry*, 104, 783–790.

Rice-Evans, C.A., Miller, N.J., Bolwell, P.G., Branley, P.M., & Pridham, J.B. (1995). The relative antioxidant activities of plant-derivatived polyphenolic flavonoids. *Free Radical Research*, 22, 375-383.

Roosita, K., Kusharto, C.M., Sekiyama, M., Fachrurozi, Y., Ohtsuka, R (2008). Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia. *Journal of Ethnopharmacology*, 115: 72–81.

Sánchez-Ferrer, A., Rodrígez-López, J.N., & García-Carmona, F. (1995). Tyrosinase: a comprehensive review of its mechanism. *Biochimia Biophysica Acta*, 1247, 1-11.

Sanderson, J., Mclauchlin, W., & Williamson, G. (1999). Quercetin inhibits hydrogen peroxide-induced oxidization of the rat lens. *Free Radical Biology and Medicine*, 26, 639–645.

Shutenko, Z., Henry, Y., Pinard, E., Seylaz, J., Potier, P., Berthet, F., Girard, P., & Sercombe, R. (1999). Influence of the antioxidant quercetin in vivo on the level of nitric oxide determined by electron paramagnetic resonance in rat brain during global ischemia and reperfusion. *Biochemical Pharmacology*, 57,199–208.

Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Singh, D.P., Sarma, B.K., Upadhyay, G., & Singh, H.B. (2009). Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. *Food and Chemical Toxicology*, 47, 450–452.

Soedarsono, R.S., & Harini, S.R. (2002). Jamu as traditional medicine in Java, Indonesia. *South Pacific Study*, 23, 1-9.

Sturm, R.A., Teasdale, R.D., & Box, N.F. (2001). Human pigmentation genes: identification, structure and consequences of polymorphic variation. *Gene*, 277, 49–62.

Tanabe, S., Ogawa, N., Tesaki, S., & Watanabe, M. (1997). Isolation and identification of an onion blacking-active component by chelate formation with ferric ions. *Nippon Kasei Gakkaishi*, 48, 339–342.

Valko, M., Leibfritz, D., Moncola, J., Cronin, M.T.D., Mazura, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemical and Cell Biolology*, 39, 44–84.

Vinson, J.A., Dabbagh, Y.A., Serry, M.M., & Jang, J.H. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*, 43, 2800-2802.

Virador, V.M., Kobayashi, N., Matsunaga, J., Hearing, V.J. (1999). A standardized protocol for assessing regulators of pigmentation. *Analytical Biochemistry*; 270, 207-219.

Wang, K.H., Lin, R.D., Hsu, F.L., Huang, Y.H., Chang, H.C., Huang, C.Y., & Lee, M.H. (2006). Cosmetic applications of selected traditional Chinese herbal medicines. *Journal of Ethnopharmacology*, 106, 353-359.

Wiczkowski, W., Romaszko, J., Bucinski, A., Szawara-Nowak, D., Honke, J., Zielinski, H., & Piskula, M.K. (2008). Quercetin from shallots (*Allium cepa* L. var.aggregatum) is more bioavailable than its glucosides. *The Journal of Nutrition*, 138, 885-888.

William, G., Plum, G.W., Uda, Y., Price, K.R., & Rhodes, M.J.C. (1996). Dietary quercetin glycosides: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinine reductase in Hepalclc7 cells. *Carcinogenesis*, 17, 2385-2387.

Yang, J., Meyers, K.J., Heide, J.V., & Liu, R.H. (2004). Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *Journal of Agricultural and Food Chemistry*, 52, 6787-6793.

Browning, A.M., Walle, U.K., & Walle, T. (2005). Flavonoid glycosides inhibit oral cancer cell proliferation--role of cellular uptake and hydrolysis to the aglycones. *Journal of Pharmacy and Pharmacology*, 57, 1037-1042.

Hubbard, G.P., Wolffram, S., Lovegrove, J.A., & Gibbins, J.M. (2004). Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *Journal of Thrombosis and Haemostasis*, 2, 2138-2145.

Cermak, R., Landgraf, S., & Wolffram, S. (2004). Quercetin glucosides inhibit glucose uptake into brush-border-membrane vesicles of porcine jejunum. *The British Jounal of Nutrition*, 91, 849-855.

Yesilada, E., Tsuchiya, K., Takaishi, Y., & Kawazoe, K. (2000). Isolation and characterization of free radical scavenging flavonoid glycosides from the flowers of Spartium junceum by activity-guided fractionation. *Journal of Ethnopharmacology*, 73, 471-478.

Williamson, G., Plumb, G.W., Uda, Y., Price, K.R., & Rhodes, M.J. (1996). Dietary

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Legend of Tables

Table 1. Effect of an isolated compound form dried skin of *A. ascalonicum* on mushroom tyrosinase

Legend of Figures

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

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

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

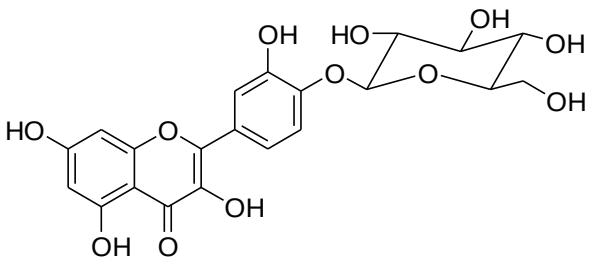

Figure 1.

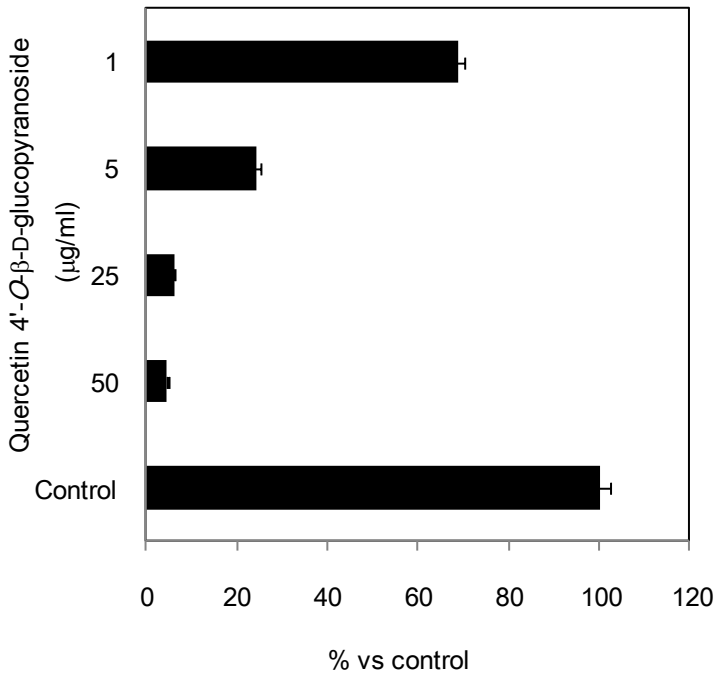
Quercetin 4'-O-β-D-glucopyranoside (μg/ml)
1
5
25
50
Control
0
20
40
60
80
100
120
% vs control

Figure 2.

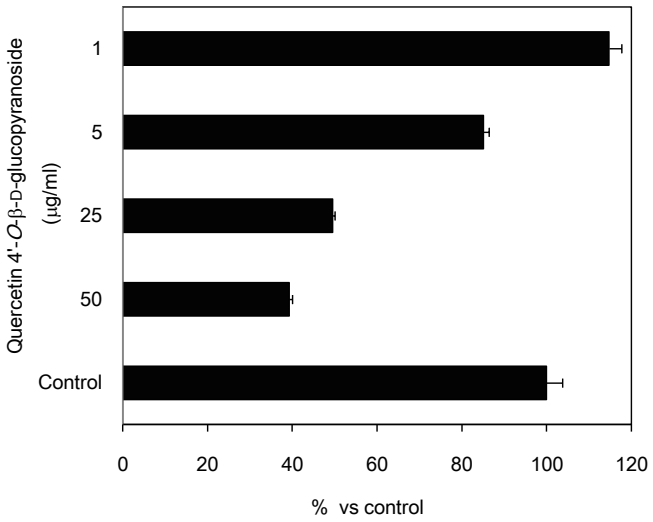
Quercetin 4'-O-β-D-glucopyranoside (μg/ml)
1
5
25
50
Control
0
20
40
60
80
100
120
% vs control

Figure 3.