

## Anti-melanogenesis properties of quercetin- and its derivative-rich extract from *Allium cepa*

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1 **Anti-melanogenesis properties of quercetin- and its derivative-rich extract from**  
2 *Allium cepa*

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20 **Abstract**

21 In an effort to find a new whitening agent, we have found that the methanol extract  
22 of the dried skin of *Allium cepa* showed inhibition of melanin formation.  
23 Bioassay-guided fractionation led to the isolation of quercetin (**1**) and quercetin  
24 4'-*O*- $\beta$ -glucoside (**3**) from *A. cepa* as the inhibitors of melanin formation in B16  
25 melanoma cells with an IC<sub>50</sub> of 26.5 and 130.6  $\mu$ M, respectively. In addition, we  
26 evaluated the effect of some quercetin derivatives, such as isoquercitrin (**2**); quercetin 3,  
27 4'-*O*-diglucoside (**4**); rutin (**5**) and hyperin (**6**) on B16 melanoma cells. These quercetin  
28 derivatives did not show any inhibition of melanin formation. Furthermore, the ORAC  
29 values of compounds **1-6** were 7.64, 8.65, 4.82, 4.32, 8.17 and 9.34  $\mu$ mol trolox  
30 equivalents/ $\mu$ mol, respectively. Dried skin of red onion showed inhibitory activity of  
31 melanin formation in B16 melanoma cells as well as antioxidant properties.

32

33 *Keywords* : *Allium cepa*, red onion, quercetin derivatives, anti melanogenesis,  
34 antioxidant

35

36 **1. Introduction**

37 Melanin pigments are formed in specialized pigment-producing cells known as

38 melanocytes, which originate in the neural crest during embryogenesis and are  
39 distributed throughout the embryo during its development (Sánchez-Ferrer,  
40 Rodríguez-López, & García-Carmona, 1995).

41 Melanin biosynthesis occurs in a cascade of enzymatic and spontaneous reactions  
42 that convert tyrosine to melanin pigments. The initial and rate-limiting step in melanin  
43 synthesis is the hydroxylation of tyrosine to dihydroxyphenylalanine or DOPA (Yoon et  
44 al., 2007). DOPA-oxidation produces a highly reactive intermediate that is further  
45 oxidized to form melanin by a free radical-coupling pathway. If free radicals are  
46 inappropriately processed in melanin synthesis, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is generated,  
47 leading to the production of hydroxyl radicals (HO•) and other reactive oxygen species  
48 (ROS) (Perluigi et al., 2003).

49 The onion is a versatile vegetable that is consumed fresh as well as in the form of  
50 processed products. The regular consumption of onions in food is associated with a  
51 reduced risk of neurodegenerative disorders, cancer, cataract, ulcer, osteoporosis,  
52 vascular disease and heart disease (Kaneko & Baba, 1999; Kawaii, Tomono, Katase,  
53 Ogawa, & Yano, 1999; Sanderson, Mclauchlin, & Williamson, 1999; Shutenko et al.,  
54 1999). Onion is one of the major sources of various biologically active phytochemicals  
55 e.g., phenolic acids, flavonoids, cepaenes, thiosulfinates and anthocyanins (Singh et al.,

56 2009). The major flavonoids found in the dry peel of the onion, which has usually been  
57 considered to be waste, contain large amounts of quercetin, quercetin glycoside and  
58 their oxidative product which are effective antioxidants against the lethal effect of  
59 oxidative stress (Gulsen, Makris, & Kefalas, 2007; Prakash, Upadhyay, Singh, & Singh,  
60 2007).

61 Onion (*Allium* species) plays an important role in traditional medicine in Indonesia;  
62 it is used as a diuretic, febrifuge, and poultice to cure wounds and to remove scars from  
63 the skin, and it suppresses the blood sugar level and platelet aggregation, (de Padua,  
64 Bunyaphatsara, & Lemmens, 1999).

65 Based on our preliminary screening data (not shown), the methanol extract of the  
66 dried skin of the red onion (*Allium cepa*) from Indonesia showed a potent melanin  
67 biosynthesis inhibitory activity on B16 melanoma cells. These findings led us to focus  
68 on the active compounds in the dried skin of the red onion.

## 69 **2. Materials and Methods**

### 70 *2.1. Chemicals*

71 NaOH, DMSO, L-tyrosine, L-DOPA, and rutin (**5**) purchased from Wako (Osaka,  
72 Japan). Mushroom tyrosinase, Fluorescein sodium salt (FL), 2,2'-azobis  
73 (2-methylpropionamide) dihydrochloride (AAPH), and

74 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and  
75 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide or MTT were obtained  
76 from Sigma (St. Louis, MO, USA). Quercetin-3, 4'-*O*-diglucoside (**4**) and hyperin (**6**)  
77 were from Tokiwa Phytochemical (Tokiwa, Japan). EMEM was from Nissui Chemical  
78 Co (Osaka, Japan). The ethylenediaminetetraacetic acid or EDTA was from Dojindo  
79 (Kumamoto, Japan). The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was from TCI (Tokyo,  
80 Japan) and isoquercitrin (**2**) was from Fluka (Steinheim, Germany). Other chemicals are  
81 of the highest grade commercially available.

82

## 83 2.2. *Plant materials*

84 Red onion (*A. cepa*) was purchased from traditional market in Jakarta, Indonesia,  
85 on September in 2008. Voucher specimen (ETA-CW-6) was deposited in Wood  
86 Chemistry Laboratory, Department of Forest Product Technology, Faculty of Forestry,  
87 Mulawarman University, Indonesia.

88

## 89 2.3. *Preparation of plant extracts*

90 Plant materials (dried skin or flesh part of *A. cepa*) were dried at room temperature  
91 and powdered. The dried materials (17.38 g) were extracted with methanol (650 mL) at

92 room temperature with shaker at 150 rpm during 48 h. The extract solutions were  
93 filtered and concentrated *in vacuum*, to obtain the crude methanol extracts. The crude  
94 extracts were 1.75 g.

95

#### 96 2.4. Isolation of Quercetin (**1**) and Quercetin 4'-O- $\beta$ -glucoside (**3**)

97 The dried skin of methanol extract of *A. cepa* (1.4 g) was applied to silica gel  
98 column chromatography (71 g of Wakogel C-200, 3.5 x 50 cm). The chromatography  
99 was eluted with *n*-hexane/EtOAc [10:0 (100 mL), 9:1 (50 mL), 7:3 (50 mL), 5:5 (200  
100 mL), 3:7 (200 mL), 1:9 (100 mL)] and EtOAc/MeOH [9:1 (100 mL), 8:2 (100 mL), 7:3  
101 (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  
102 mL), 0:10 (100)] to give thirty three fractions (Fr 1 to Fr 33). The fractions Fr. 3 (66.9  
103 mg) and Fr. 15 (84.7 mg) was analyzed by TLC (Thin Layer Chromatography) and  
104 HPLC (High Performance Liquid Chromatography). Based on NMR (Nuclear Magnetic  
105 Resonance) analysis, Fr. 3 was identified as quercetin (**1**) and Fr. 15 was  
106 quercetin-4'-O-glucoside (**3**) by comparison of previous NMR data (Alfonso &  
107 Kapetanidis, 1994; Tanabe, Ogawa, Tesaki & Watanabe, 1997), respectively (Figure 1).  
108 The NMR spectra of compounds were recorded at 400 MHz on JNM-AL400 FT NMR  
109 spectrometer (Jeol). All compounds were dissolved in DMSO-*d*<sub>6</sub>, or methanol-*d*<sub>4</sub>, and

110 chemical shifts were referred to deuterated solvents. The compounds were assigned for  
111  $^1\text{H}$ ,  $^{13}\text{C}$ , HMQC, and HMBC.

112

### 113 *2.5. HPLC analysis*

114 All the crude extracts of dried skin and flesh of onion were dissolved in HPLC  
115 grade methanol, filtered through sterile 0.22  $\mu\text{m}$  millipore filter and subjected to  
116 qualitative analysis by using Waters 600 HPLC instrument. The instrument is equipped  
117 with a photodiode array (detector Waters 996), controller (Waters 600s), pump (Water  
118 626), and auto sampler injector Model 231 (Gilson). A ODS Inertsil C18 (4.6 mm i.d.  
119 x 250 mm) was used as a column. Data were integrated by Empower Build 1154-J  
120 series software (Waters). Separation was achieved by flow rate of 1 mL/min with  
121 methanol (60%)/water (40%) containing 1% trifluoroacetic acid in isocratic program by  
122 monitoring the absorbance at 250 nm.

123

### 124 *2.6. Oxygen radical absorbance capacity (ORAC) assay*

125 Samples were directly dissolved in acetone/water/acetic acid (70:29.5:0.5, v/v/v),  
126 and diluted with 75 mM potassium phosphate buffer (pH 7.4) for analysis. Trolox, FL  
127 and AAPH solutions were prepared with 75 mM phosphate buffer (pH 7.4). The ORAC



128 assay was performed as described by Ou, Hampsch-Woodill, & Prior (2001) with some  
129 modification as follows: 300  $\mu$ L of the standard (Trolox) or sample solution is mixed  
130 with 1.8 mL of 48 nM FL solution, and then they were incubated independently at 37°C  
131 for 15 min. AAPH solution (900  $\mu$ L; 12.9 mM, final concentration) was added to the  
132 mixture and vortexed for 10s. Then, it immediately placed in a fluorescence  
133 spectrophotometer (Model FP-6500, JASCO Co., Ltd., Tokyo, Japan), and measured  
134 every 5s for 60 min at 37°C (Ex: 485 nm, Em: 520 nm). A blank (FL + AAPH) using  
135 phosphate buffer, standard solutions (6.25-50  $\mu$ M Trolox), and sample solutions were  
136 measured at same conditions. Three independent assays were performed for each  
137 sample. The area under the fluorescence decay curve (AUC) was calculated as

138 
$$\text{AUC} = 1 + \sum \frac{f_i}{f_0}$$

139 where  $f_0$  is the initial fluorescence reading at 0 min and  $f_i$  is the fluorescence reading at  
140 time  $i$ .

141 The ORAC values were calculated as Prior et al. (2003) by using a equation (Y) a +  
142 b(X) between Trolox concentration (Y)( $\mu$ M) and the net area under the FL decay curve  
143 (X). Linear regression was used in the range of 6.25-50  $\mu$ M Trolox. Data are expressed  
144 as micromoles of Trolox equivalents (TE) per micromole of sample ( $\mu$ mol TE/ $\mu$ mol).

145

146 *2.7. Cell culture*

147 A mouse melanoma cell line, B16, was obtained from RIKEN Cell Bank. The cells  
148 were maintained in EMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and  
149 0.09 mg/mL theophylline. The cells were incubated at 37°C in a humidified atmosphere  
150 of 5% CO<sub>2</sub>.

151

152 *2.8. Inhibitory effect of melanin biosynthesis using cultured B16 melanoma cells*

153 This assay was determined as described by Arung, Shimizu, & Kondo (2007).  
154 Briefly, confluent cultures of B16 melanoma cells were rinsed in phosphate-buffered  
155 saline (PBS) and removed from the plastic using 0.25 % trypsin/EDTA. The cells were  
156 placed in two plates of 24-well plastic culture plates (1 plate is for determining of  
157 melanin and other is for cell viability) at a density of  $1 \times 10^5$  cells/well and incubated for  
158 24 h in media prior to being treated with the samples. After 24 h, the media were  
159 replaced with 998  $\mu$ L of fresh media and 2  $\mu$ L of DMSO was added with or without  
160 (control) the test sample at various concentrations (n=3). Arbutin was used as a positive  
161 control. The cells were incubated for an additional 48 h, and then the medium was  
162 replaced with fresh medium containing each sample. After 24 h, the remaining adherent  
163 cells were assayed (see below).

164 *2.9. Determination of melanin content in B16 melanoma cells*

165 The melanin content of the cells after treatment was determined as follows. After  
166 removing the medium and washing the cells with PBS, the cell pellet was dissolved in  
167 1.0 mL of 1N NaOH. The crude cell extracts were assayed using a micro plate reader  
168 (Bio-Tek, USA) at 405 nm to determine the melanin content. The results from the cells  
169 treated with the test samples were analyzed as a percentage of the results from the  
170 control culture.

171 *2.10. Cell viability*

172 Cell viability was determined by use of the micro culture tetrazolium technique  
173 (MTT). The MTT assay provides a quantitative measure of the number of viable cells  
174 by determining the amount of formazan crystals produced by metabolic activity in  
175 treated versus control cells. Culture was initiated in 24-well plates at  $1 \times 10^5$  cells per  
176 well. After incubation, 50  $\mu$ L of MTT reagent [3-(4, 5-dimethyl-2-thiazolyl)-2,  
177 5-diphenyl-2H-tetrazolium bromide in PBS (5 mg/mL)] was added to each well. The  
178 plates were incubated in a humidified atmosphere of 5% of CO<sub>2</sub> at 37°C for 4 h. After  
179 the medium was removed, 1.0 mL isopropyl alcohol (containing 0.04 N HCl) was added  
180 into the plate, and the absorbance was measured at 570 nm relative to 630 nm.

181

182 **3. Results and discussion**

183 *3.1. The effect of the methanol extract of the dried skin and flesh of A. cepa on B16*  
184 *melanoma.*

185 The diameter of Indonesian red onion (*A. cepa*) is approximately 1.5 – 2.0 cm. The  
186 thickness of the dried skin is approximately 0.5 - 1.0 mm. Figures 2 and 3 show the  
187 effect of the methanol extract of the dried skin and flesh of *A. cepa* on B16 melanoma  
188 cells, respectively. The dried skin extract dose dependently inhibited melanin formation  
189 in B16 melanoma cells. The inhibition of 40-50% of melanin formation was evident at  
190 concentrations of 50 and 100 µg/mL without any cytotoxicity. Arbutin, which is used in  
191 skin-whitening cosmetics, was used as a positive control (Virador, Kobayashi,  
192 Matsunaga & Hearing, 1999). In contrast, the extract of the flesh of the onion did not  
193 lead to melanin inhibition even at concentrations up to 250 and 500 µg/mL. According  
194 to these data, the dried skin extract is more potent than the flesh extract. This result may  
195 be related to the amount of active compound in the dried skin, as shown in Figures 4  
196 and 5. Figure 4 depicts HPLC chromatograms obtained by the injection of 20 µL of  
197 solution which contained methanol extract of the dried skin of *A. cepa* at the  
198 concentration of 1 mg/mL. Based on the methanol extract of the dried skin, the content  
199 of quercetin, isoquercitrin; quercetin 4'-*O*-glucoside; and quercetin 3,4'-*O*-diglucoside

200 were 13.8, 10.3, 6.4 and 8.3%, respectively. We have tried to analyze the flesh extract at  
201 a concentration of 1 mg/mL but each peak of the compounds was too small to be  
202 detected; therefore, we increased the concentration for HPLC analysis. Figure 5 shows  
203 the HPLC chromatogram obtained by the injection of 20  $\mu$ L of solution which contained  
204 methanol extract of the flesh of *A. cepa* at the concentration of 11 mg/mL. Based on the  
205 methanol extract of the flesh, the content of quercetin; isoquercitrin; quercetin  
206 4'-*O*-glucoside, and quercetin 3,4'-*O*-diglucoside were 0.01, 0.59, 0.35 and 0.50%,  
207 respectively. As seen on the Y axis of both figures, the content of quercetin derivatives  
208 in the methanol extract of the dried skin of *A. cepa* was apparently much higher than  
209 that in the extract of the flesh. Also, it was shown that the extract of dried skin contained  
210 quercetin (1) and isoquercitrin (2) dominantly, but in the flesh extract, isoquercitrin (2)  
211 and quercetin 3,4'-*O*-diglucoside (4) were contained abundantly. It was reported that the  
212 content of quercetin, quercetin 4'-*O*-glucoside and quercetin 3,4'-*O*-diglucoside in  
213 different parts of the onion bulb (*Allium cepa* L.) found difference as well as the various  
214 cultivars (Beesk, Perner, Schwarz, George, Kroh, & Rohn, 2010). They found the outer  
215 layer of *A. cepa* content approximately 2.2, 2.7, 1.7% of quercetin, quercetin  
216 4'-*O*-glucoside, and quercetin 3,4'-*O*-diglucoside in the dry weight of outer layer,  
217 respectively. In the inner parts, they reported that the content of quercetin, quercetin

218 4'-*O*-glucoside, and quercetin 3,4'-*O*-diglucoside was 0, 0.53 and 0.72% in the dry  
219 weight of inner parts, respectively. In our opinion, the difference of quercetin and its  
220 derivatives content should affect the difference in their melanin inhibitory activity as  
221 depicted in Figures 2 and 3.

### 222 3.2. Isolated compounds

223 The biologically-guided fractionation of methanol extracts of *A. cepa* on B16  
224 melanoma cells led us to focus on the active fraction of the dried skin extract. As a  
225 result of fractionation, Fr. 3 was identified as one of the active compounds as well as Fr.  
226 15. Thus, NMR assignment was performed to elucidate the structure of these fractions  
227 by comparison with previous data (Alfonso & Kapetanidis, 1994; Tanabe, Ogawa,  
228 Tesaki & Watanabe, 1997). It was revealed that Fr. 3 and Fr. 15 were **1** (69.9 mg, 4.9 %  
229 yield in the extract) and **3** (84.7 mg, 6.1 % yield in the extract), respectively.

### 230 3.3. Anti melanogenesis properties

231 The anti melanogenesis effects of isolated compounds **1** and **3** (Figure 1) from the  
232 dried skin of *A. cepa* were determined by using B16 melanoma cells. Table 1 depicted  
233 the IC<sub>50</sub> values for the melanin biosynthesis inhibition of quercetin and its derivatives.  
234 The activities of the tested compounds, in terms of IC<sub>50</sub> values, decreased in the  
235 following order: **1** (<50 μM), **3** (100–150 μM), **4**, **5**, **2** and **6** (>150 μM). Notably,

236 isolated compounds **1** and **3** from *A. cepa*, were found to be more-potent inhibitors of  
237 melanin formation in B16 melanoma cells than the positive control, arbutin (198  $\mu$ M). It  
238 should be noted that the opposite results were reported previously, namely that **1**  
239 enhanced melanogenesis in human melanoma cells and normal epidermal melanocyte  
240 (Nagata, Takekoshi, Takeyama, Homma, & Osamura, 2004). Kubo, Nitoda, & Nihei  
241 (2007) also reported that **1** enhanced the total melanin content in B16 melanoma cells.  
242 Recently, it was reported that **1** suppressed melanin formation in B16 melanoma cells  
243 with decreased intracellular tyrosinase activity and its protein expression (Fujii & Saito,  
244 2009). The reason for the difference of the effect of **1** on melanin production in cells  
245 remains unclear.

246 Based on our analysis, the IC<sub>50</sub> of **1** to inhibit melanin formation was 26.5  $\mu$ M with  
247 88% cell viability, while that of **3** was 130.6  $\mu$ M with 82% cell viability. The attachment  
248 of a glucoside moiety in quercetin tended to reduce the ability of quercetin to inhibit  
249 melanin formation in B16 melanoma cells. These results led us to evaluate other  
250 quercetin derivatives such as **2**, **4**, **5**, and **6** (Figure 1) in B16 melanoma cells.  
251 Compounds **2**, **5** and **6**, which represent different glycosyl moieties (glucoside,  
252 rhamnoside, and galactoside) at C-3, displayed no inhibition of melanin formation.  
253 Concerning this result, a previous report showed that quercetin 3-*O*-L-arabinofuranoside

254 did not suppress melanin formation (Fujii & Saito, 2009). These results revealed that the  
255 attachment of a glycosyl moiety at the C-3 position may decrease the ability of  
256 quercetin to reduce the melanin formation. We concluded that the hydroxyl group at the  
257 C-3 position is essential to the inhibition of melanin formation in B16 melanoma cells.  
258 To our knowledge, this is the first report showing that the dried skin of *A. cepa* and its  
259 isolated compound, **3**, showed the ability to act as a potent skin-whitening agent by  
260 inhibiting the melanin formation in B16 melanoma cells similarly to arbutin, which was  
261 used as a positive control. Therefore, further experiments are needed to determine the  
262 exact mechanism of this compound.

### 263 *3.4. Antioxidant properties*

264 Skin is a major candidate for and target of oxidative stress caused by reactive  
265 species (RS), including reactive oxygen species and reactive nitrogen species. RS are  
266 major and significant contributors to skin hyperpigmentation and skin aging (Kim,  
267 Kang, & Yokozawa, 2008). It has been generally believed that agents having  
268 antioxidant activity show anti-aging, whitening, and anti-inflammatory activities (Choi,  
269 Song, Hur & Sim, 2008). Since compounds **1** and **3** have shown promising results as  
270 whitening agents using B16 melanoma cells, we conducted an antioxidant assay  
271 (ORAC) in order to determine their ability to counteract oxidative stress from UV



272 radiation. Table 2 shows the ORAC value results of compounds **1** – **6**, which were 7.64,  
273 8.65, 4.82, 4.32, 8.17 and 9.34  $\mu\text{mol TE}/\mu\text{mol}$ , respectively. Compounds **1**, **2**, **5** and **6**  
274 showed more potent antioxidant activity than **3** and **4**. In the ORAC assay, quercetin  
275 showed an ORAC value similar to that reported by Kohri et al., (2009). However, we  
276 did not find any previous report of an ORAC assay for **3**. Based on the results in Table 1,  
277 it was revealed that 4'-glycosylation of quercetin decreased its ability as an antioxidant  
278 as represented by ORAC values.

279 The potency of **1** and **3** as antioxidants might be related to the presence of hydroxyl  
280 groups in the form of a catechol moiety as reported by Cao, Soffic, & Price (1997).  
281 Moreover, the relationship between flavonoid structure and antioxidant activity has been  
282 studied, and it has been found that 3',4'-dihydroxy substitution in the B ring, as in  
283 quercetin, increases the antioxidant activity substantially compared with a  
284 mono-hydroxy substituent, as in kaempferol (Rice-Evans, Miller, Bolwell, BramLey, &  
285 Pridham, 1995; Cotelle, Bernier, Catteau, Pommery, Wallet, & Gaydou, 1996).

286

#### 287 **4. Conclusion**

288 Our study demonstrated that the melanin inhibition ability of quercetin derivatives  
289 with glycosyl moieties at C-3 is reduced. Also, our results showed that quercetin

290 derivatives with a glycosyl moiety at C-4' reduced its antioxidant activity. In addition,  
291 compounds **1** and **3** from the dried skin of *A. cepa*, are promising compounds that could  
292 be useful for treating hyperpigmentation as skin-whitening agents and as antioxidants.  
293 However, it should be noted that safety is a primary consideration for its practical use in  
294 humans.

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297

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391 **Legend of Figures**

392 **Figure 1.** The structure of quercetin and its derivatives.

393 **Figure 2.** Effect of the methanol extracts of the dried skin of *A. cepa* on B16 melanoma  
394 cells [Arbutin : 100 µg/mL]. Each column represents the mean ± SD of three  
395 independents test (Student's *t*-test). Significantly different from the control  
396 value : P<0.05 (\*), P<0.01 (\*\*).

397 **Figure 3.** Effect of the methanol extracts of the flesh of *A. cepa* on B16 melanoma cells.  
398 Each column represents the mean ± SD of three independents test (Student's  
399 *t*-test). Significantly different from the control value : P<0.05 (\*), P<0.01  
400 (\*\*).

401 **Figure 4.** HPLC analysis of the methanol extracts of dried skin of *A. cepa* [1. Quercetin,  
402 2. Isoquercitrin, 3. Quercetin 4'-*O*-glucoside, 4. Quercetin  
403 3,4'-*O*-diglucoside, Flow rate : 1 mL/min of MeOH (60): Water (40); Sample  
404 concentration : 1 mg/mL, Wave length 250 nm, Injection volume: 20 µL]

405 **Figure 5.** HPLC analysis of the methanol extract of flesh of *A. cepa* [1. Quercetin, 2.  
406 Isoquercitrin, 3. Quercetin 4'-*O*-glucoside, 4. Quercetin 3,4'-*O*-diglucoside  
407 Flow rate : 1 mL/min of MeOH (60): Water (40); Sample concentration : 11  
408 mg/mL, Wave length 250 nm, Injection volume: 20µL]



409 **Legend of Tables**

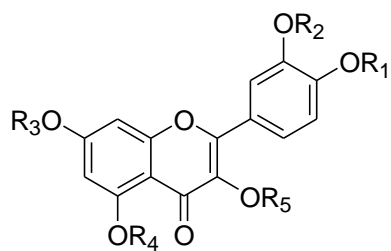
410 **Table 1.** Effect of quercetin and its derivatives on B16 melanoma cells (n = 3)

411 **Table 2.** Effect of quercetin and its derivatives on ORAC assay (n = 3)

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- 1:  $R_1 = R_2 = R_3 = R_4 = R_5 = H$
- 2:  $R_1 = R_2 = R_3 = R_4 = H, R_5 = \text{glc}$
- 3:  $R_1 = \text{glc}, R_2 = R_3 = R_4 = R_5 = H$
- 4:  $R_1 = \text{glc}, R_2 = R_3 = R_4 = H, R_5 = \text{glc}$
- 5:  $R_1 = R_2 = R_3 = R_4 = H, R_5 = \text{rha}$
- 6:  $R_1 = R_2 = R_3 = R_4 = H, R_5 = \text{gal}$

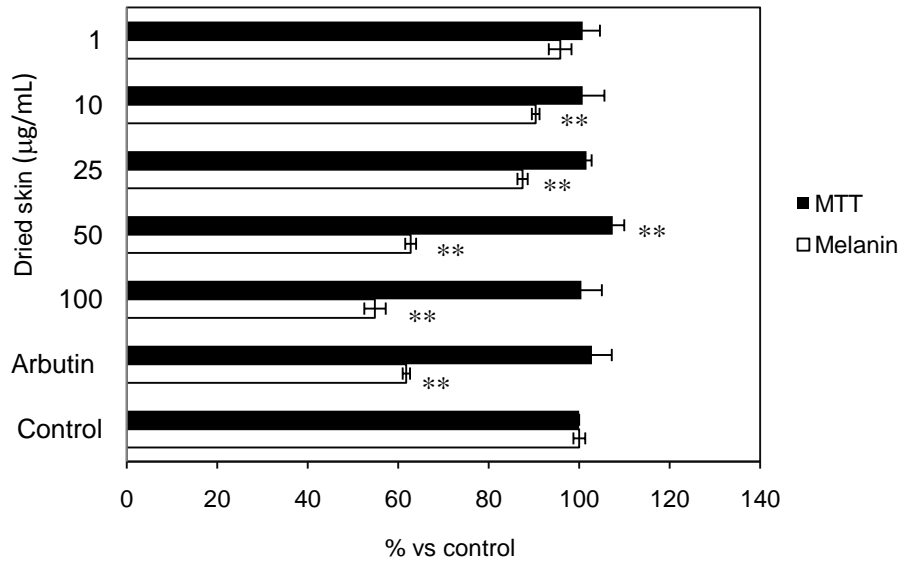
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**Figure 1.**

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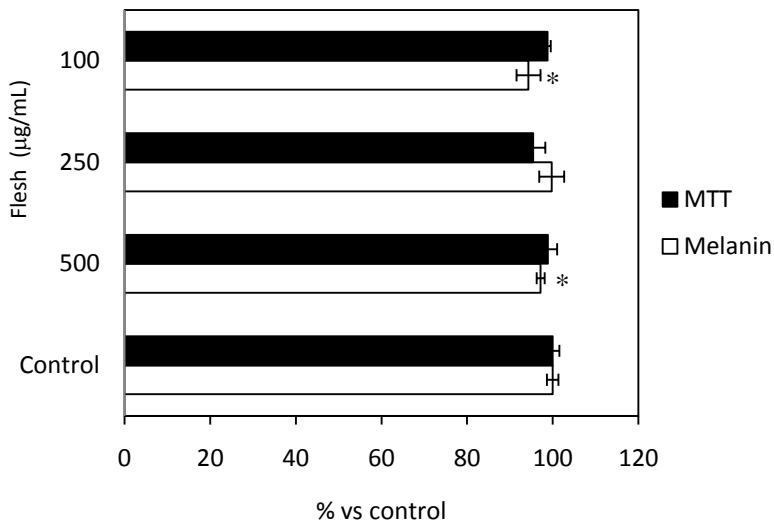
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420 **Figure 2.**

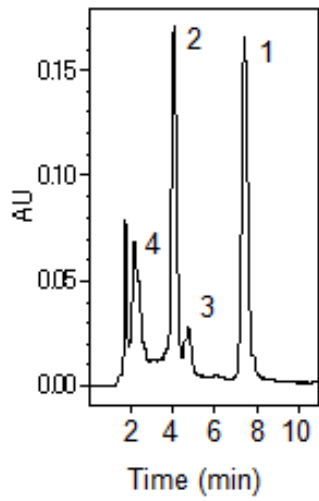
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424 **Figure 3.**

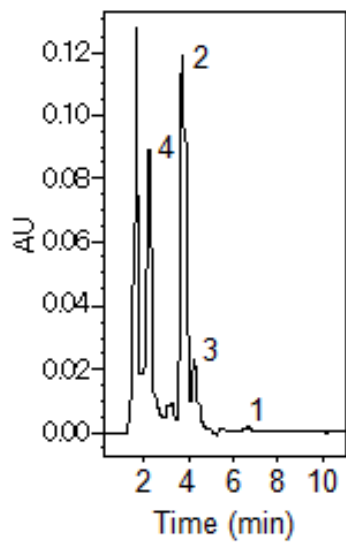


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426 **Figure 4.**

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430 **Figure 5.**

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