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Physiological Activities of Several Different Types of Flavonoids

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SUMMARY

For ensuring useful materials including pharmaceutical compounds, it is important to extract and separate the underlying leading compounds from natural products such as plants, and to characterize their structure and pharmacological activity. Kaempferol (1), quercetin (2), quercitrin (3), rutin (4), hesperetin (5), and hesperidin (6) used in this study were flavonoids having a phenylchroman skeleton as the basic structure. Antioxidant ability, skin-whitening effect, and histamine-release suppressing effect were examined as their new physiological activities in this study. As a result, in DPPH radical scavenging effect test, which evaluates activity of antioxidant ability, high scavenging effect (90-96%) was observed in (1), (2), and (4). In skin-whitening effect test, it was clarified that (1) and (2) exert higher tyrosinase inhibition activity (42-60) than that of commercially available arbutin (7.3). In toxicity test using human alveolar epithelial cells (*in vitro*), meanwhile, no difference from control was observe in (3), (4), (5), and (6) at 100 μM concentration, and it was reasoned that there are no considerable toxicity.

Key word：flavonoids, antioxidant, whitening effect, anti-allergic activity, cell toxicity

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INTRODUCTION

Higher plants growing on the Earth are surrounded by many stress factors including physicochemical and biological stresses, and contains functional organic compounds in their living body as a defense function against these environmental stress factors. In recent years, flavonoids, which are secondary metabolic products discriminated as functional components for descriptive purpose in the metabolic pathway, chemical reactions conducted by plants, become the object of attention with clarification of biological regulation functions in plants[1, 2]. The flavonoids are compound having a phenylchroman skeleton (C_6-C_3-C_6) as the basic structure, and produced by the condensation reaction of C_6- unit (A ring) derived from malonyl-CoA in the acetate-malonate pathway and C_6-C_3- unit (B and C rings) derived from coumaric acid in the shikimate pathway. The flavonoids exist as educts or glycosides in the plant kingdom, and it is estimated that more than 2,000 of flavonoids exist[3]. Known physiological activities of flavonoids include protein kinase activity, regulation of cell cycle, inhibition of angiogenesis, immunostimulatory activity, suppression of high-affinity IgE receptor FcεRI expression, and suppression of IgE production (class switching) [4-7].

Such compounds are widely distributed in plants and have phenolic hydroxyl group; and many of them exist as glycoside and used as pigment including dye. In this study, authors evaluated antioxidant ability, skin-whitening effect, histamine-release suppressing effect and toxicity to human alveolar epithelial cells, and clarified a relevance between difference in the chemical structure and the onset of the activities of flavonoids, including (1), (2) and its glycoside forms, (3) and (4); and typical flavanones including (5), and its glycoside form, (6) aiming at application as a compounding ingredient for cosmetic products as a development of new application.

EXPERIMENTAL

Preparation of samples

Orignal commercially supplied (Tokyo Chemical Industry Co., Ltd.) (1), (2), (3), (4), (5), and (6) were used in this experiment.

Physiological activity test

1) DPPH radical scavenging effect test

Test was conducted according to previous report[8], and results were measured with a spectral photometer at 517 nm.

2) Test for inhibitory effect on active oxygen (SOD)

Test was conducted according to previous report[9], and results were measured with a spectral photometer at 560 nm.

3) Tyrosinase inhibition test

Test was conducted according to previous report[10] using tyrosinase and DOPA, and results were measured with spectral a photometer at 475 nm.

4) Test for suppression of histamine release from rat mast cell (*in vitro*)

Using male Wistar rats (14-17 weeks old), suppression of histamine release was measured according to previous report[11,12] with Eicom HPLC system consist of fluorescence (FL Detector GL-7453A; GL Sciences).

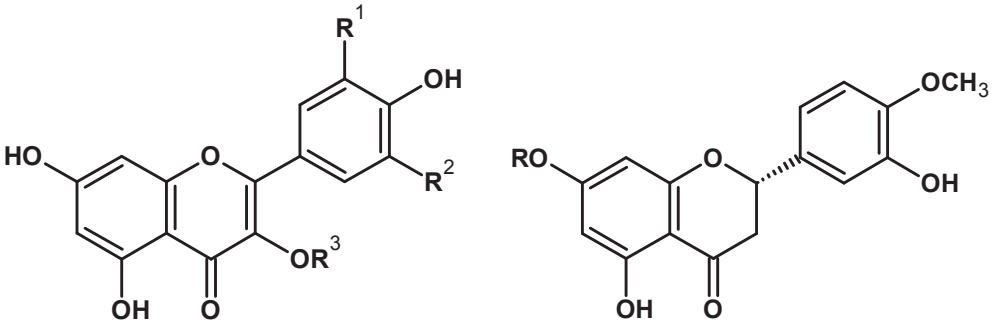
5) Toxicity test using human alveolar epithelial cells

Toxicity test using human alveolar epithelial cell A549

was conducted with a method to measure ATP detected by mitochondrial respiratory response of living cells[13].

RESULTS AND DISCUSSION

Compound (1) is classified as flavonoids and widely distributed in plants such as buckthorn (*Rhamnus japonica*), Geranium herb (*Geranium thunbergii*) as educts or glycosides[14,15]. Also, it has been reported that (2) and its glycoside (3), which present in plants such as *Houttuynia cordata* and cotton plant, have a diuretic effect and others[16-19]. Compound (4) contained in plants including buckwheat is applied to prevention of internal bleeding and treatment of hypertension as an anti-capillary permeability drug[20-22]. On the other hand, (5) and its glycoside (6), which are typical flavanones, are contained in the skin of citrus fruits, and are known to have anti-capillary permeability effect[23-26]. These 6 compounds that are widely distributed in plants as regular components and its pharmacological effects have been demonstrated through the ages as mentioned above were examined for new physiological activities such as antioxidant activities (tests for DPPH radical scavenging effect and inhibitory effect on active oxygen (SOD)) and a skin-whitening effect (tyrosinase inhibition test) in this study. Acoordingly, suppression of histamine release from rat mast cell (*in vitro*) and toxicity test using human alveolar epithelial cell were also conducted to examine their safety.



(1) ~ (4)

R^1=R^2=R^3=H (1)
R^1=R^3=H, R^2=OH (2)
R^1=H, R^2=OH, R^3=rhamnosyl (3)
R^1=H, R^2=OH, R^3=rhamnoglucosyl (4)

(5) ~ (6)

R=H (5)
R=rutinosyl (6)

Fig.1 Chemical Structures of Compounds (1) ~ (6).

At first, tests for DPPH radical scavenging effect and inhibitory effect on active oxygen were conducted for antioxidant activity of each compound. The result is shown in Table 1. In DPPH radical scavenging effect test, it was confirmed that radical scavenging rate of (1), (2), (3) and (4) was 88% and higher and was equivalent to effect of commercially available α-tocopherol or ascorbic acid. SC_{50} value of these flavonoids was also confirmed to have approximated value to control drug. As a result of examination of relevance between the chemical

structure and the onset of the activities, the basic structure of flavonoid in all these compounds have only hydroxyl groups in the B ling; therefore, it was assumed that the hydroxyl groups of the B ling were involved in the onset of the activities. In addition, we successfully obtained good values in test for inhibitory effect on active oxygen.

Then, we examined whether these flavonoids can inhibit tyrosinase activity that functions at the early stage of eumelanin or pheomelanin production causing blotches and freckles. To evaluate function of the flavonoids as a skin-whitening agent, tyrosinase inhibition test with tyrosinase enzyme derived from mushroom was conducted by looking at production of dopachrome using tyrosine and DOPA as substrates. As the result shown in Table 2, the effect as a skin-whitening agent was observed in (1) and (2) with substrate, tyrosine or DOPA, indicated values (42-60) greater than that of commercially available arbutin (7.3) demonstrating good skin-whitening effect. In examination of relevance between the chemical structure and the onset of the activities, we considered that these results were obtained due to effect such as scavenger or elimination of radical oxygen because compounds with flavonoid skeleton have phenolic hydroxyl groups that can donate hydrogen radical or electron.

Table 1 DPPH Radical Scavenging Assay and SOD Activity Assay of (1)～(6).

Compound	DPPH Radical Scavenging Assay		SOD Activity Assay
	Scavenging rate (%)[a)]	SC_{50}[b)]	Inhibition Rate (%)[c)]
(1)	95.7	12.0	45.6
(2)	93.5	7.0	38.8
(3)	88.1	11.0	21.4
(4)	90.5	13.0	11.2
(5)	11.1	>400	3.9
(6)	14.5	>400	24.1
α-Tocopherol	97.6	9.0	8.0
Ascorbic acid	98.9	7.0	15.8

a) Concentration 0.1 mM.　b) 50% Scavenging Concentration (mM.)
c) Concentration 0.04 mM.

Table 2　Tyrosinase inhibition Assay of (1) ~ (6)

Compound	Tyrosinase inhibition Assay	
	L-Tyrosine[a)]	*L*-DOPA[a)]
(1)	42.8	5.9
(2)	59.8	35.0
(3)	-	N.D.
(4)	-	N.D.
(5)	-	N.D.
(6)	-	N.D.[b)]
Ascrobric acid	7.3	1.2

a) Concentration 0.1 mM.　b) N.D.: Not Detected.

Meanwhile, there are findings that in melanocyte, melanin production is stimulated by activation of tyrosinase enzyme with released histamine causing allergic symptoms. In other words, it is expected that suppression of histamine release could suppress melanin production that causes blotches and freckle. Therefore, we conducted test for suppression of histamine release from rat peritoneal mast cells in anticipation of application of skin-whitening agents with antiallergic effect. Results are shown in Figs. 2 and 3. In the first screening, good histamine-release suppression effect was observed in (1), (2), (4), and (6). And in the second screening, histamine-release suppression effect was observed in (1) and (2) in rat peritoneal mast cells stimulated with Compound 48/80. Especially, potent histamine-release suppression effect was observed in (2).

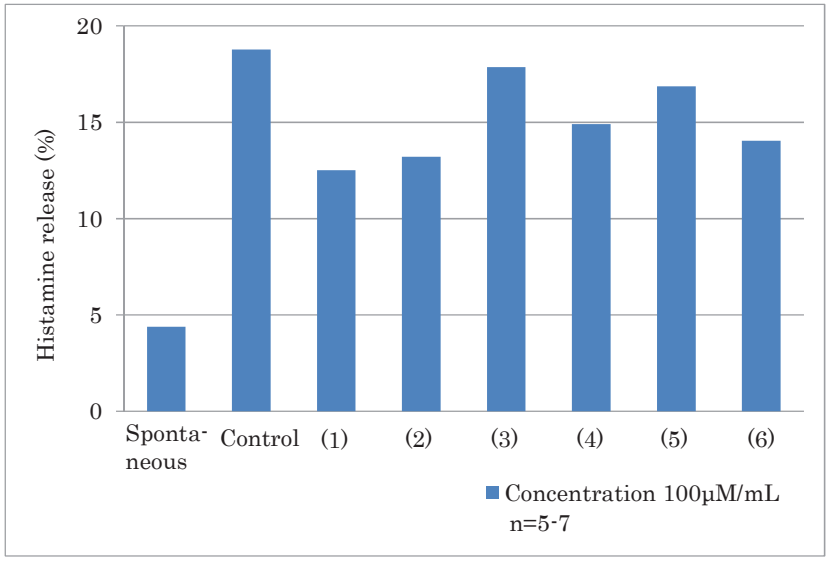


Fig.2 Histamine isolation inhibit Assay of (1)～(6).

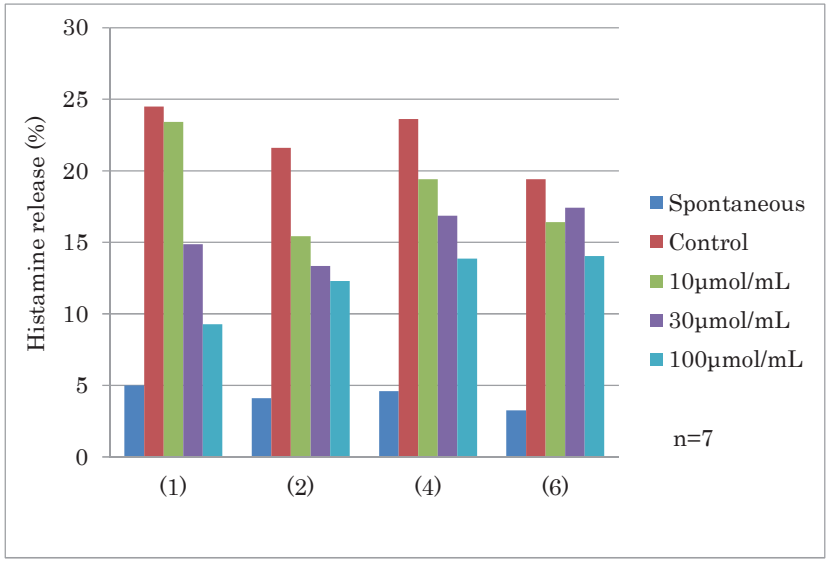


Fig.3 Histamine isolation inhibit Assay of (1),(2),(4) and (6).

Finally, for cytotoxicity, *in vitro* toxicity test using human alveolar epithelial cells was conducted using an inflammation model in the lung with rapidly advancing inflammation that is a system that artificially produces IL-8 by stimulating alveolar epithelial cells A 549 with inflammatory protein IL-1ß. A result is shown in Fig. 4.

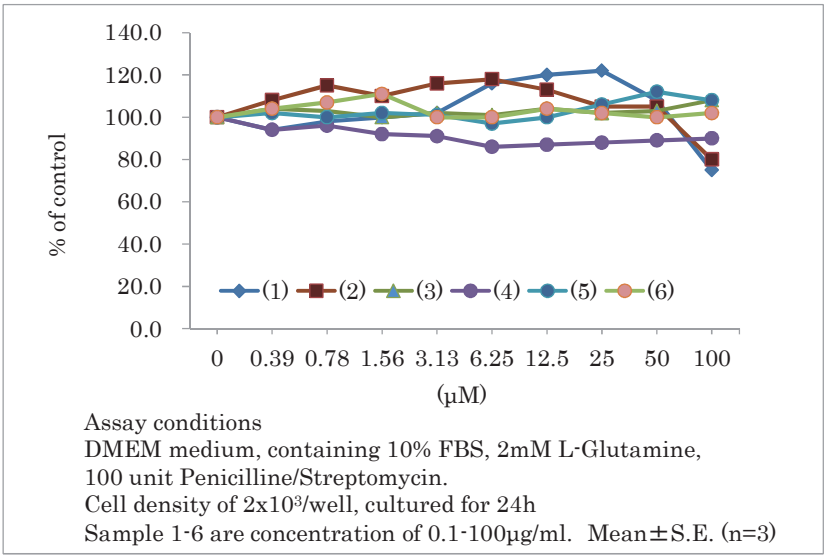


Fig.4　Cytotoxicity Assay of (1)～(6).

At 100μM concentration, a weak toxicity was observed in (1) and (2). Especially, about 30% cell inhibition was observed in (1) at most as compared with control. On the other hand, no difference from control was observe in other (3), (4), (5), and (6) up to 100μM concentration; therefore, it was reasoned that there are no considerable toxicity.

As mentioned above, it was confirmed that compounds with flavonoid skeleton demonstrate multifunctional antioxidant abilities such as scavenger or elimination of radical oxygen because the compounds with flavonoid skeleton have phenolic hydroxyl groups that can donate hydrogen radical or electron. DPPH radical scavenging effect was also confirmed in flavonoids that the basic structure B ring has only hydroxyl groups. We believe that flavonoid compounds are highly potential to be applied as a compounding ingredient for sophisticated skin-whitening cosmetics because of improvement in separation and synthesis efficiencies of flavonoid compounds due to a recent significant advance in analytical technology.

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