Inhibitory effect of mung bean seed (*Vigna radiata* L.) and time-dependent germinated sprouts extracts on whitening effect

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SUMMARY

In this study, we evaluated the inhibitory effects on melanogenesis of the ethyl acetate and methylene chloride fraction of 80% methanol extracts of mung bean (*Vigna radiata* L.) seeds and sprouts, germinated for specific lengths of time. The ethyl acetate fractions of extracts from mung bean seed and sprouts germinated for 1 d inhibited melanogenesis more effectively than arbutin, a known whitening agent. Moreover, *in vitro* tyrosinase inhibitory activity on mushroom tyrosinase was higher in the ethyl acetate fraction of the extract from mung bean seeds and sprouts germinated for 2 d than in the methylene chloride fraction. The active components of ethyl acetate fraction were identified as the flavones vitexin and isovitexin which are known to have antioxidant and whitening effects. Results indicate that the ethyl acetate fraction of extracts from mung bean seeds and sprouts germinated for 1 d shows excellent whitening effects compared to the methylene chloride fraction and show that the active components were vitexin and isovitexin.

INTRODUCTION

Melanin is one of the key factors in determining the color of human skin. Melanin is biosynthesized by the melanin-producing cells (melanocytes) present in the basal layer of the skin and is transported by keratinocytes through the dendrites, moving to the outer layer of skin during the process of keratinization [1]. Tyrosinase is known as a speed-adjustment factor that catalyzes the initial step in melanin synthesis, and two tyrosinase-related proteins (TRP-1 and TRP-2) regulate melanin synthesis. Synthesized melanin serves a positive function of protecting the skin by suppressing aging and actinic keratosis of the skin caused by UV [6]. However, if excessive melanin is produced or if the function of the skin is reduced by skin aging, melanin can be deposited on the surface of the skin, appearing as spots and freckles; these spots on the skin are induced by toxicity caused by melanin precursors, which can also induce cell death [12]. Therefore, the development of a new whitening agent is necessary to maintain a bright healthy skin [15]. The mung beans used in this study (*Vigna radiata* L.) were one-year-old herbaceous plants; this legume, which originated in India [18]. In addition, mung bean seeds and their sprouts are widely used in China, India, Bangladesh and Southeast Asia [20]

and have been reported to have antitumor [29], antioxidant [30], antidiabetic [31], antimicrobial, anti-inflammatory, and anti-melanocytic effects [22]. In recent years, studies have shown that after germination, mung beans exhibit more obvious biological activities and more plentiful secondary metabolites because relevant biosynthetic enzymes are activated during the initial stages of germination. Thus, germination is thought to improve the nutritional and medicinal qualities of mung beans [22]. In this study, we evaluated the inhibitory effect on melanogenesis of the extracts of mung bean (*V. radiata* L.) seeds and sprouts germinated for specific lengths of time by using mushroom tyrosinase and B16F1 melanoma cells. In addition, we confirmed the changes in contents of the main components of the extracts of mung bean seeds and germinated sprouts that inhibit melanogenesis.

RESULTS AND DISCUSSION

1. Whitening effect of mung bean and sprouts extract

1.1. Melanin synthesis inhibitory activity

α-Melanocyte-stimulating hormone (α-MSH) induces melanogenesis. B16F1 melanoma cells were treated with α-MSH (Sigma, USA, 200 nM), and inhibitory actions were evaluated for fractions (ethyl acetate and methylene chloride) of extracts from mung bean seeds (0 h) and sprouts germinated for 12 h, 1 d, 2 d, 4 d, and 6 d (Figure 1). Melanin contents (%) were 0 h (148.6%), 12 h (102.6%), 1 d (79.6%), 2 d (88.2%), 4 d (123.9%), 6 d (135.6%), and control (α-MSH) (136.1%). The inhibitory effects of melanogenesis were especially apparent in ethyl acetate fractions of mung bean sprouts germinated for 12 h (33.5%), 1 d (56.5%), and 2 d (47.9%). The inhibitory effects on melanogenesis was weakly indicated in methyl chloride fractions germinated for 0 h (134.9%), 12 h (127.3%), 1 d (146.1%), 2 d (133.0%), 4 d (134.7%), and 6 d (134.5%).

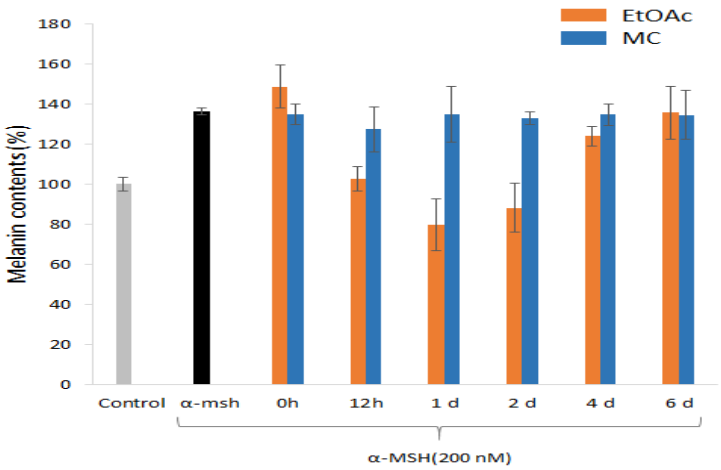

Figure 1. Melanogenesis inhibitory effect of the ethyl acetate (EtOAc) and methylene chloride (MC) fractions of mung bean (*Vigna radiata* L.) seed (0 h) and sprout extracts (12 h, 1 d, 2 d, 4 d and 6 d)

1.2. *In vitro* tyrosinase inhibitory activity

In this study, we compared the inhibitory activity of the fractions from the mung bean seed and sprout extracts on *in vitro* tyrosinase activity (Figure 2). The inhibition of *in vitro* tyrosinase activity was evaluated and compared using the same concentration of tyrosinase as the concentration of each fraction (1,000 μg/mL). Inhibition of tyrosinase activity by the ethyl acetate fraction of extracts of mung bean sprouts according to the germination time was 0 h (24.0%), 12 h (31.5%), 1 d (35.9%), 2 d (70.5%), 4 d (38.7%), and 6 d (30.2%), respectively. At 2 d (germination time), the ethyl acetate fraction of the mung bean sprout extracts, in particular, showed inhibition of tyrosinase activity. Furthermore, the inhibition of tyrosinase activity of the methylene chloride fraction of the mung bean sprout extract was 0 h (31.4%), 12 h (34.6%), 1 d (26.1%), 2 d (31.4%), 4 d (27.7%), and 6 d (24.6%), respectively. The methylene chloride fraction of extracts from mung bean sprouts germinated for 12 h showed only a slight inhibition of tyrosinase activity. These results indicate that mung bean seed and sprout extracts do not directly inhibit tyrosinase at the evaluated concentrations, which are much higher that the concentrations found in the melanin in the cells.

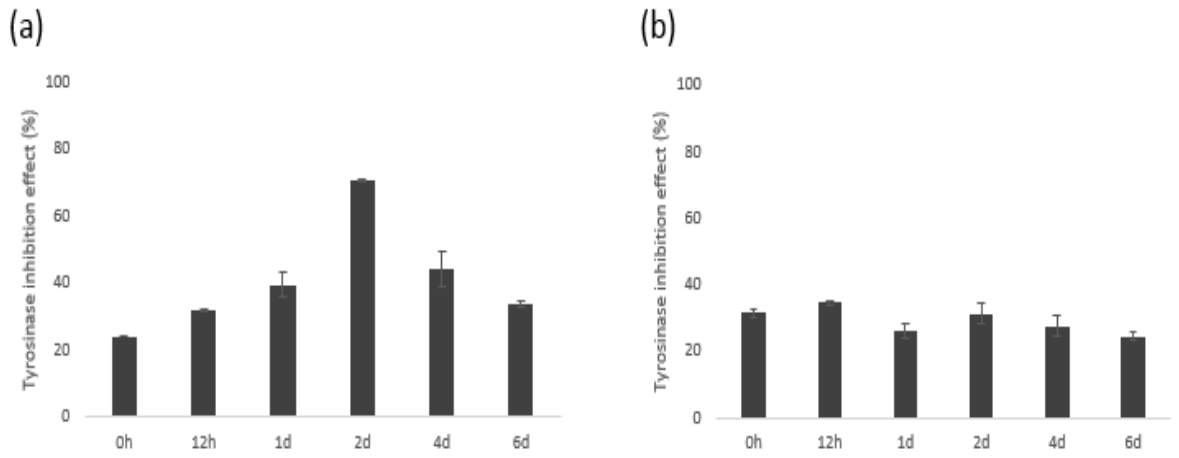

Figure 2. *In vitro* inhibition of mushroom tyrosinase by ethyl acetate (EtOAc) (a) and methylene chloride (MC) fractions (b) of mung bean (*Vigna radiata* L.) seed (0 h) and sprout extracts (12 h, 1 d, 2 d, 4 d, and 6 d).

2. Composition and content analysis of extracts of mung bean seeds and sprouts

2.1. TLC analysis

The TLC chromatogram of the ethyl acetate fraction of the extract from mung bean sprouts germinated for 2 days shows the highest content of melanin in the cell (Figure 3). In the ethyl acetate fraction, standard materials (vitexin and isovitexin) that are known to be main components of mung

bean were identified by TLC to confirm that these components were present. The ethyl acetate fraction of the extracts from mung bean sprouts germinated for 2 d separated into eight bands under mobile phase conditions that can separate flavonoid glycosides. After identifying the respective bands as VRS-1 to VRS-8 from the bottom of the TLC, the experiment was performed. The two bands that were clearly separate were the focus of this research. Using reference materials for comparison, VRS-1 (with an Rf value of 0.36)is isovitexin, and VRS-2 (Rf: 0.51) is vitexin.

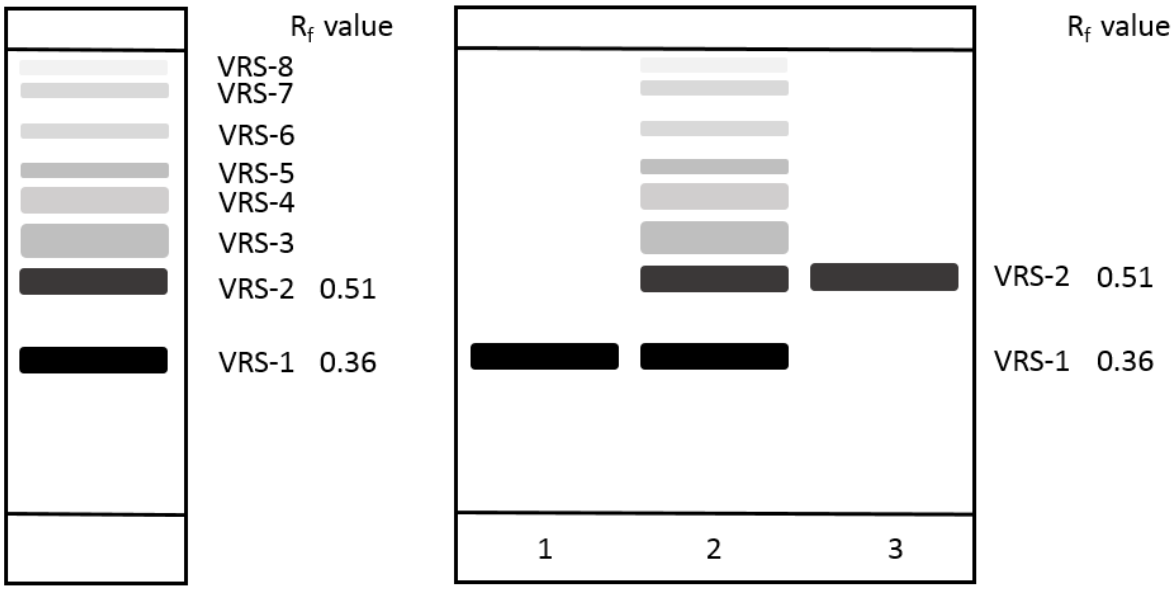

Figure 3. TLC analysis. Eluent system: ethyl acetate : chloroform : formic acid : water = 8 : 1 : 1 : 1 (v/v). 1, isovitexin standard; 2, ethyl acetate fraction from *V. radiata* L. extract; 3, vitexin standard.

2.2. HPLC analysis

The peaks for vitexin (37.063 min) and isovitexin (41.241 min) were confirmed with HPLC analysis (Figure 4). In addition, the HPLC chromatogram of the ethyl acetate fraction of extracts from mung bean sprouts germinated for 1 d, which showed a superior whitening effect, is shown in Figure 5. Experimental results use a reference material to demonstrate that peak 1 and peak 2 are in the same positions as vitexin and isovitexin, respectively. These two materials were confirmed to have the highest intensity.

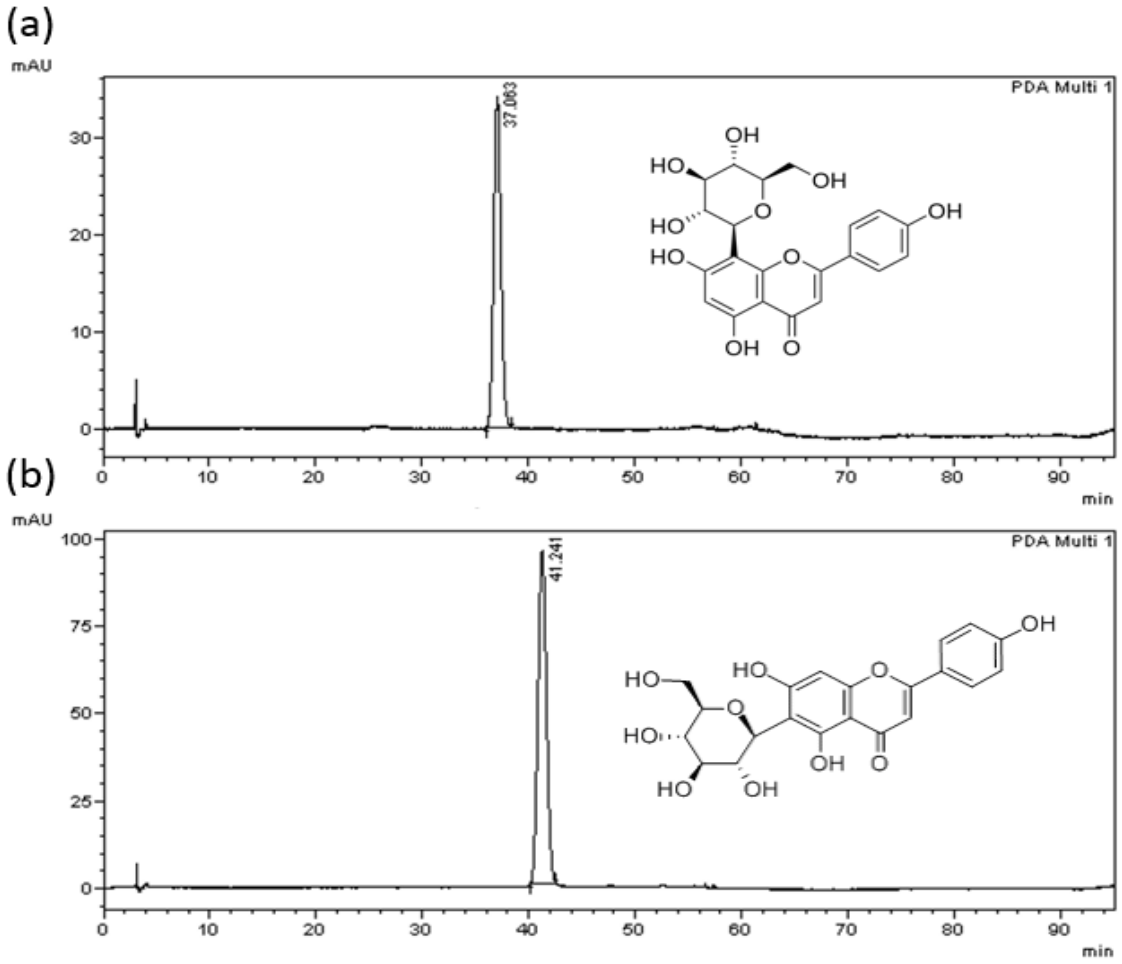

Figure 4. HPLC profile of vitexin (a) and isovitexin (b).

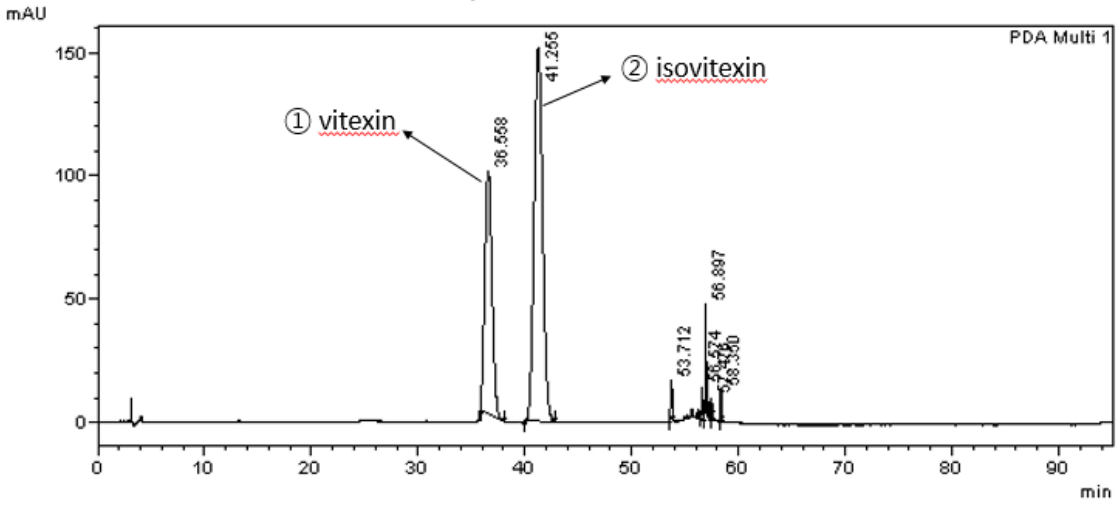

Figure 5. HPLC chromatogram of the ethyl acetate fraction from *Vigna radiata* L. sprout extract (1 d) at λ = 335 nm.

2.3. Content analysis

HPLC analysis was performed to determine the presence of vitexin and isovitexin in the ethyl acetate fraction of extracts from the mung bean sprouts germinated for 1 d. The HPLC chromatogram of the ethyl acetate fraction of this extract is shown in Figure 5. According to germination time, the contents of vitexin were 23.0%, 36.7%, 37.9%, 20.5%, 3.7%, and 2.9% and of isovitexin were 4.1%, 7.5%, 8.5%, 2.9%, 1.6%, and 1.4% at 0, 0.5, 1, 2, 4, and 6 d, respectively. The ethyl acetate fraction of the extract from mung bean sprouts germinated for 1 d, which showed a superior whitening activity earlier in the intracellular inhibition of melanin biosynthesis, contained high concentrations of vitexin and isovitexin (Table 3).

Table 3. Content of vitexin and isovitexin according to each germination

Germintation (d)	Vitexin (%)	Isovitexin (%)
0	23.0	4.1
0.5	36.7	7.5
1	**37.9**	**8.5**
2	20.5	2.9
4	3.7	1.6
6	2.9	1.4

2.4. Liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) analysis

Along with the earlier TLC and HPLC analysis, LC/ESI-MS/MS was used as indicated below for the structural analysis of VRS-1 (expected as apigenin-6-C-glucoside (isovitexin)), VRS-2 (expected as apigenin-8-C-glucoside (vitexin)):

VRS-1: R.T 17.8 min; UV, 239, 269, 335 nm; MS, 431.1 $[M-H]^-$; MS/MS $[M-H]^-$, 413.1 $[(M-H)-18]^-$, 341.2 $[(M-H)-90]^-$, 311.2 $[(M-H)-120]^-$.

VRS-2: R.T 16.2 min; UV, 239, 268, 335 nm; MS, 431.1 $[M-H]^-$; MS/MS $[M-H]^-$, 341.1 $[(M-H)-90]^-$, 311.1 $[(M-H)-120]^-$.

As a result, a molecular ion $[M-H]^-$ was shown in *m/z* 431.1 (VRS-1) and *m/z* 431.1 (VRS-2) at negative ion mode (data not shown). Therefore, VRS-1 and VRS-2 of the extract of the mung bean sprouts were identified as apigenin-6-C-glucoside (isovitexin), apigenin-8-C-glucoside (vitexin), respectively.

3. Discussion

The whitening effects of the ethyl acetate and methylene chloride fractions of the extracts of mung bean seeds and sprouts germinated for specific lengths of time (0 h, 12 h, 1 d, 2 d, 4 d, and 6 d) were estimated. In melanogenesis, the inhibitory effects of the ethyl acetate fractions of extracts from germinated mung bean sprouts (12 h, 1 d, and 2 d) on B16F1 were 33.5%, 56.5%, and 47.9%,

respectively. The development of B16F1 melanoma cells were inhibited during melanogenesis, as indicated, by the ethyl acetate fractions of extracts from mung bean sprouts germinated for 12 h (33.5%), 1 d (56.5%), and 2 d (47.9%). The *in vitro* tyrosinase inhibitory activity evaluated at the same concentration (1,000 μg/mL) of each fraction showed a different tendency in the inhibition of B16F1 melanoma cells during melanogenesis. The ethyl acetate fraction of the extracts from mung bean sprouts germinated for 2 d shows that tyrosinase is inhibited, the methylene chloride fraction of extracts from mung bean sprouts germinated for 12 h showed only slight inhibition of tyrosinase activity. Based on these results, the main components of the ethyl acetate fraction of the extracts of mung bean sprouts germinated for 1 d that have the most active whitening effect were identified as vitexin and isovitexin by using TLC, HPLC, and LC/ESI-MS/MS. The contents of vitexin and isovitexin showed increases at 12 h and 1 d; however, at 2 d, 4 d, and 6 d, the contents showed decreases. During the initial germination of the mung bean, the mung bean hull exists with the mung bean, and during germination, the mung bean hull is displaced. Similar effects were indicated in melanogenesis in the B16F1 melanoma cell. However, as the germination time increased from 4 d to 6 d, the contents of the vitexin and isovitexin decreased accordingly, and whitening activity was reduced. Through this study, we confirmed that mung bean sprout extract has a whitening activity and that vitexin and isovitexin are the main components involved in this activity. In order to increase the whitening activity of the mung bean sprout extract, the germination time and the choice of extraction solvent are important.

ACKNOWLEDGEMENT

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