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Antioxidant activity in mung bean sprouts and safety of extracts for cosmetic use

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Synopsis

The antioxidant activity of mung bean sprouts was studied in this research. Active ingredients in different lengths of mung bean sprouts were extracted with water. Concentrations of the main proteins and polyphenols were determined. Antioxidizing capacities of the extracts were measured *in vitro* using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test, ferric reducing antioxidant power method, and chelation abilities of ferrous ion. The safety of the extracts was determined using the red blood cell (RBC) test, chick chorioallantoic membrane (CAM) assay, and human skin patch test. Results show that DPPH radical scavenging rates at different shoot lengths were all greater than 85%, while the total antioxidant capacity of the extracts reached more than 4.0 and the chelation abilities of first-day sprout extract is nearly 80%, indicating that mung bean sprouts have excellent anti-oxygenic property. Results of RBC (hemolysis ratio), CAM (vascular morphological), and human skin patch tests (changed subjects) illustrated extracts of mung bean sprouts are safe and can be used as additives in antiaging cosmetic products.

INTRODUCTION

The mung bean (*Vigna radiata* L.) is a legume used as medicine and food. Mung bean sprouts are made by soaking the seeds. Doctors of traditional Chinese medicine consider mung bean sprouts to be cool and sweet, and use them to clear heat, dredge meridians, detoxify the kidney, and correct diuresis. Modern pharmacological studies show that mung bean sprouts can treat tumors, restrain bacteria, treat acne, and so on (1–3). In the process of sprouting mung beans, not only is a large proportion of the original nutritional value of the mung bean seeds retained, but also because of a series of metabolic activities, the content of some active substances increases significantly. Compared with mung bean

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seeds, mung bean sprouts contain more potential antioxidant active substances such as polyphenol and protein (4–7).

Increasing environmental pollution overexposes the body to ultraviolet and other pollutants, thereby inducing it to produce too many free radicals, such as super oxygen anion and hydroxy radical. Poor living habits and work stress also increase free radicals in the modern human body. As people age, free radicals gradually accumulate. Excess fat and free radicals can lead to biomacromolecule crosslinking polymerization and lipofuscin accumulation in organ tissue. Organ tissue cells are damaged and reduced, eventually leading to the aging of the body (8–10). This research is devoted to the antioxidizing capacities of mung bean sprouts and the safety of their extracts for potential use as additives in cosmetics.

RESULTS AND DISCUSSION

CONCENTRATIONS OF POLYPHENOLS AND PROTEINS IN EXTRACTS

The concentrations of polyphenols and proteins in water extracts of mung bean sprouts are shown in Figures 1 and 2. Each sample was measured three times and the mean value was recorded as the detection result.

In the active ingredients of mung bean sprouts, the protein content is greater than that of phenolic acids. Compared with mung bean seed (0 day), the total content of phenolic acids in sprouts 3 days after germination was significantly increased. Morphological analysis shows that the most rapid growth is on the third day of this process, which involves significant bioconversion of related enzymes. The protein content of mung bean sprouts is the highest between 0 and 8 days after germination. The components that play a major role in the antioxidant properties of mung bean sprouts remain to be elucidated.

ANTIOXIDANT ACTIVITY DETERMINATION

It was found in early experiments that the optimal pH of mung bean sprouts extract is 6.6, which is the best condition for antioxidant and chelating activities. Proteins in

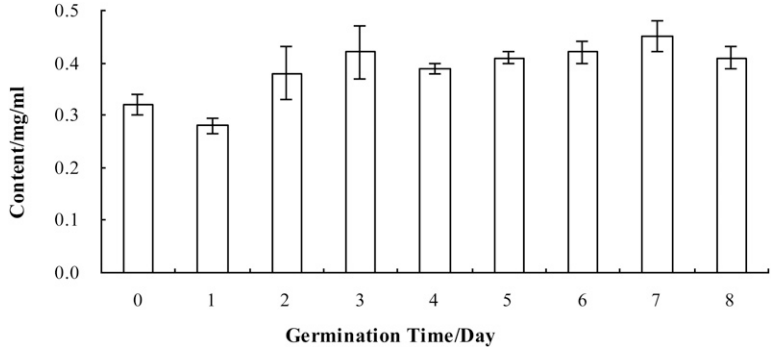

Figure 1. Concentration (mg/ml) of polyphenols in mung bean sprouts germinated from 0 to 8 days (n = 3).

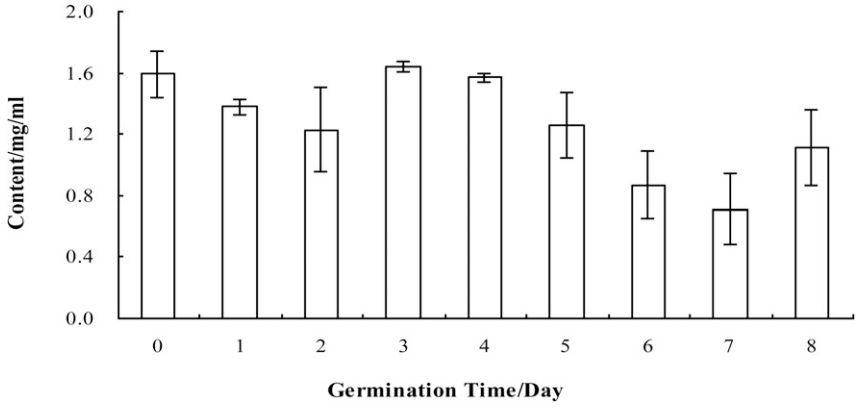

Figure 2. Concentration (mg/ml) of proteins in mung bean sprouts germinated from 0 to 8 days (n = 3).

extract will precipitate under acidic conditions (pH = 3.0–5.0), which cause a decrease in antioxidant activity. In neutral or acidic conditions, polyphenols has better activity and stability. Therefore, the suitable pH condition of mung bean sprouts extract is between 6 and 7, and our research is working on this condition.

Results of 1,1-diphenyl-2-picrylhydrazyl radical scavenging. The antioxidizing capacities of mung bean sprout extracts (total soluble solid content: 200 mg/ml) are shown in Figure 3.

Rates of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging of mung bean extracts (200 mg/ml) between 0 and 8 days after germination were all greater than 85%, close to the activity of vitamin C. Compared with mung bean seeds, extracts of sprouts had higher DPPH radical scavenging capacity. Analysis of variance showed significant differences in the antioxidant activity between seeds and sprouts, indicating that bud length did influence the antioxidant activity, possibly because the budding process

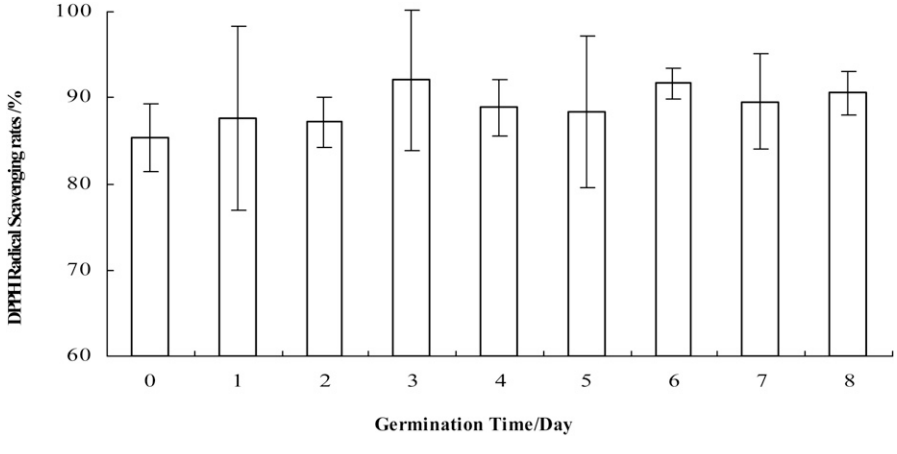

Figure 3. DPPH radical scavenging rates (%) of extracts obtained from different germination times of mung bean sprouts (n = 3).

increases the antioxidizing activity of certain smaller components. So, sprout extracts have good potential for use in food and cosmetic products as a new antioxidant, which is natural, pro-environment, and the production process relatively much simpler.

Results of total antioxidant capacity. The experiment measuring the standard curve is $y = 0.23x + 0.0101$ ($R^2 = 0.9886$, x: concentration of $FeSO_4$ (mM); y:absorption value). The total antioxidant capacity of mung bean extracts (200 mg/ml) from 0 to 8 days after germination was examined by the ferric reducing antioxidant power (FRAP) method.

The total antioxidant capacity of mung bean extracts (200 mg/ml) from days 0 to 8 was examined by aFRAP test. As Figure 4 shows, the total antioxidant capacities of the extracts from 2 and 6 days after germination are the highest, about 7.47 mmol/kg. The total antioxidant capacity of the samples from other days are lower than these two days, but compared with mung bean seed, mung bean sprouts have higher total antioxidant capacity.

The FRAP assay showed that extracts from 2 and 6 days after germination had a high ferric reducing antioxidant capacity. We also observed a positive and significant correlation between the concentrations of polyphenols in water extracts of mung bean sprouts and FRAP results ($R^2 = 0.989$ and $p = 0.020$). As section on "Concentrations of Polyphenols and Proteins in Extracts" shows, the concentrations of polyphenols 2–3 days and 6–7 days after germination are higher than that on the other days. A correlation observed between FRAP and the concentrations of polyphenols in mung bean sprouts was also reported by Huang *et al.* (11), owing to the ability of polyphenols to act as hydrogen donors.

Results of chelation abilities of ferrous ion. Chelation abilities of ferrous ion of mung bean extracts (200 mg/ml) from 0 to 8 days after germination are shown in Figure 5.

Antioxidants can quench reactive oxygen, accept free radicals, or chelate metal ions. Through these and other various ways of preventing free radical chain reactions, they can eliminate damage to the organism by the oxidation of the material, such as lipid peroxidation. Therefore, the chelating ability of Fe(II) is also commonly used to evaluate

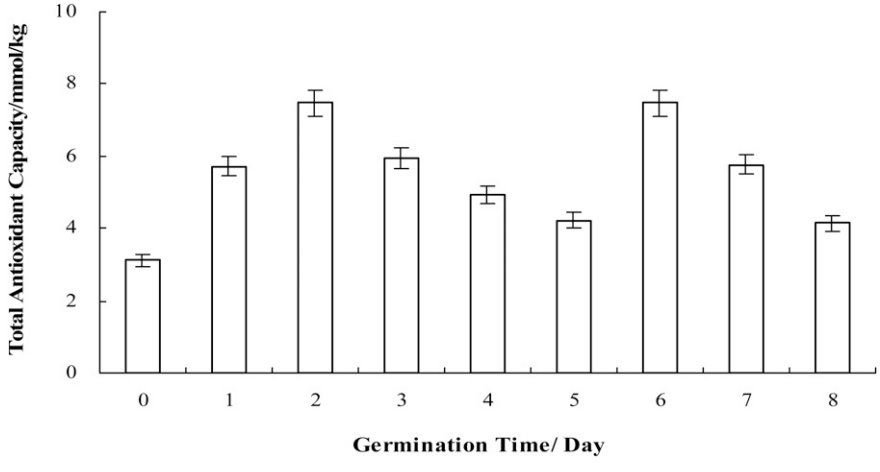

Figure 4. Total antioxidant capacity (mmol/kg) of extracts obtained from different germination times of mung bean sprouts (n = 3).

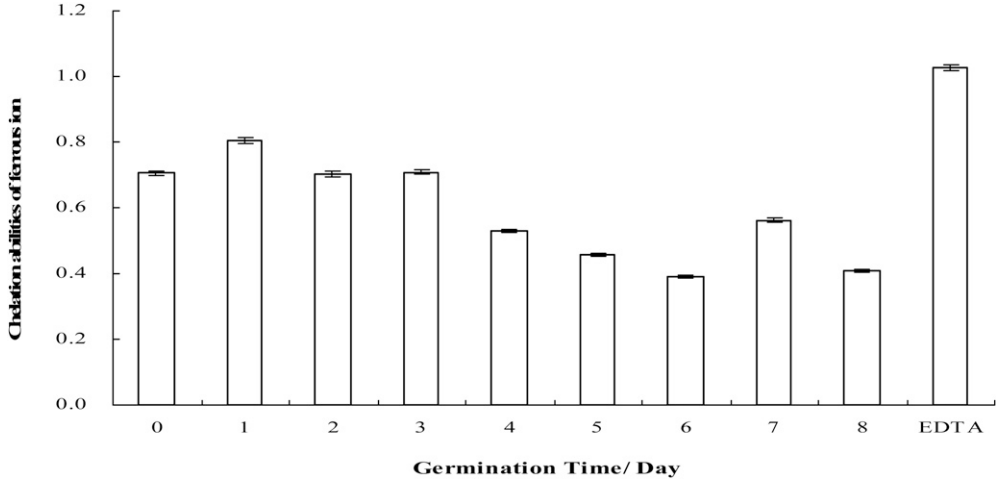

Figure 5. Chelation abilities of ferrous ion rates (%) of extracts obtained from different germination times of mung bean sprouts (n = 3).

antioxidant oxidation resistance. Antioxidant capacity is proportional to chelating ability (12).

The ability of mung bean sprout extracts (200 mg/ml) obtained 0–8 days after germination to chelate ferrous ions was examined using ferrozine. Compared with mung bean seeds, mung bean first-day sprouts have higher chelation capacity. The chelation abilities of the first-day extract is the highest, which is nearly 80%, probably because antioxidants are activated in the initial germination process, which enhances the inducing activity of the related enzymes (13). However, the chelation abilities of mung bean extracts decreased noticeably during the process of germination. A possible cause is that the activity of the antioxidant was suppressed in the later period of budding.

SAFETY

Results of red blood cell test. At different postgermination times, we did the red blood cell (RBC) test, the results of which are shown in Figure 6.

The rates of hemolysis of mung bean sprout extract for 0–8 days after germination were all lower than 10%, less than the 20% excitant limit according to current regulations. Results showed that the extracts from mung bean sprouts without stimulation can be used as potential cosmetic additives.

Results of the chorioallantoic membrane assay. The chorioallantoic membrane (CAM) assay results are shown in Figure 7, where the chick embryos of the positive control groups all died after 48 h while the embryos of the sample groups all survived.

Morphological observation results show that in the sample groups, there were no hyperemia or exudation in capillary ending, no visual change of capillary network, no change of vessel, and morphological structure was clear. Compared with the negative control group, mung bean sprout extracts were safe without irritation.

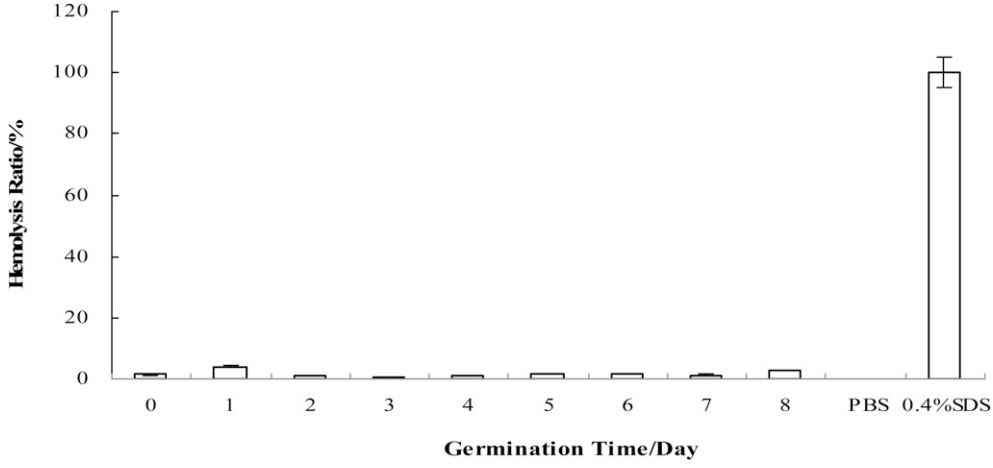

Figure 6. Hemolysis ratios during different periods of mung bean sprout.

Human skin patch test results. The results of the human skin patch test are shown in Table I.

Results show that no change, even light erythema, was observed on the skin of those 32 subjects, indicating that mung bean sprout extracts from 0 to 8 days after germination are safe to human skin.

In conclusion, all safety evaluation results mentioned above indicate that the mung bean sprout extracts are safe to use in products intended for application on human skin.

EXPERIMENTAL

REAGENTS AND MATERIALS

Mung beans (*V.radiatus* L.) (Beijing, China); DPPH radical, Folin–Ciocalteu reagent, AR (Sigma-Aldrich Co., Ltd, St. Louis, MO) and Coomassie Brilliant Blue, AR (Biodee

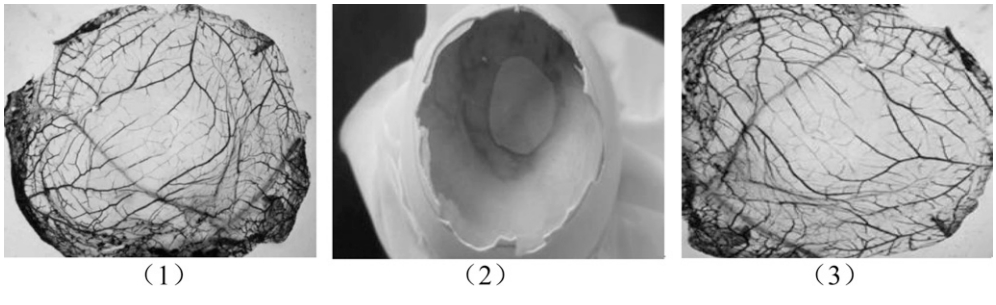

Figure 7. Photographs from CAM assay taken during vascular morphological observation of chick embryos: (A) negative control (0.9% normal saline), no hyperemia, no exudation, clear morphological structure and profile; (B) positive control (0.4% SDS), obvious hyperemia, obvious fading, hemoglobin degeneration; (C) mung bean sprout extract (10 mg/ml), slight hyperemia, no exudation, clear morphological structure and profile, no change.

Table I
Results of Human Skin Patch Test

Group	Time (h)	No change (0)	Light erythema (1)	Erythema, infiltration, papules (2)	Edematous erythema or papules (3)	Significant erythema with pimples or blisters (4)
Negative control	0.5	32	0	0	0	0
	24	32	0	0	0	0
	48	32	0	0	0	0
Mung bean Sprouts (10 mg/ml)	0.5	32	0	0	0	0
	24	32	0	0	0	0
	48	32	0	0	0	0

Biotechnology Co., Ltd., Beijing, China); FRAP work liquid (Beyotime Institute of Biotechnology, Shanghai, China); Chick embryos (Merial Vital Laboratory Animal Technology Co.. Ltd., Beijing, China); Ferrozine (3-(2-pyridyl)-5, 6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt), AR (Sigma-Aldrich, Poznań, Poland); Ferrous sulfate ($FeSO_4 \cdot 7H_2O$), AR (Beyotime Institute of Biotechnology, Shanghai, China); Ethylene diamine tetraacetic acid (EDTA), AR (Beijing Chemical Reagent Company, Beijing, China). Other reagents were bought from Beijing Chemical works.

SAMPLE PREPARATION

Whole, unbroken mung beans without insect damage or mildew were chosen as the experimental raw materials. Each group consisted of 12 g mung bean seeds, which were budded. The mung bean seeds were washed in water and disinfected by 0.1% sodium hypochlorite solution. Three-times-deionized water (W/V) was put in 26 constant-temperature incubators to soak the mung bean seeds for 16 h, until the mung bean shells burst to bud. To sprout the mung beans, they were put in petri dishes (Φ = 150 mm) that had been sterilized by 75% alcohol, with two layers of filter paper as a germination bed (14). The germinating seeds were kept moist with sterile water and incubated in the incubator without light at 26°C. Assays were performed daily for the next 8 days.

EXTRACTION AND ANALYSIS OF COMPOSITION

Every 4 g of sprout pieces (1.0 g, 0–8 days, respectively) with 20 ml water was kept at 50°C for 2 h and centrifuged at 5000 rpm for 5 min. The supernatant of the sample was used for the analysis of composition.

Concentrations of polyphenols in the extracts of mung bean sprouts were measured by the Folin–Ciocalteu colorimetry (14). The Folin–Ciocalteu reagent was prepared by diluting the commercial reagent concentrate in a 1:4 ratio with water. The supernatant of the sample (0.3 ml), the Folin–Ciocalteu reagent (1.0 ml), 20% Na_2CO_3 (3.0 ml), and water (5.7 ml) were added in order and then kept in the dark for 2 h. Then the samples were

shaken thoroughly and the absorbance was measured at 765 nm using a microplate reader. The standard curve was constructed using gallic acid.

Total proteins were measured using aluminum nitrate colorimetric assay developed by Qu *et al*. (15), in which 1.0 ml of extracts of mung bean sprouts were mixed with 5.0 ml of the configured Coomassie Brilliant Blue G-250 for 10 min. Then the samples were mixed and the absorbance was measured at 595 nm using a microplate reader. The standard curve was constructed using bovine serum albumin.

ANTIOXIDANT ACTIVITY

Scavenging of DPPH radicals. DPPH radical scavenging rates in the extracts were measured by the DPPH method according to Li *et al.* (16). Seed pieces (1.0 g, 0–8 days, respectively) with 5.0 ml water were extracted by ultrasound for 30 min and centrifuged at 5,000 rpm for 5.0 min. For each sample, an aliquot of 0.5 ml at different concentrations was added to 1.0 ml DPPH solution. Methanol was used as a blank solution. Ascorbic acid was used as the positive control. The decrease in absorbance was measured at 517 nm.

Total antioxidant capacity assay. The total antioxidant capacity of the extracts was measured by the FRAP method. We analyzed our results using a similar method to that used by Wang *et al.* (17). In 96-well plates of each detection hole, we added 180 µl FRAP working liquid. In the blank control hole we added 5 µl distilled water. We then added 5 µl of $FeSO_4$ standard solution at concentrations of 0.15, 0.3, 0.6, 0.9, 1.2, and 1.5 mM to the standard curve detection hole. We took 5 µl cultivated bean sprout samples extracted from 0 to 8 days after germination and added these to the sample detection hole in proper sequence, each group of three parallels, in addition to 0.15–1.5 mM Trolox reagent as the positive control. Gently shaking it, we then incubated it at 37°C for about 5–7 min, and then the decrease in absorbance was measured at 593 nm. Finally, according to the standard curve, we calculated the total antioxidant capacity of the sample.

Chelation abilities of ferrous ion. Fe(II) chelation activity of water extract from mung beans and their sprouts (*V. radiata*) was examined using ferrozine (18). Solutions (0.1 ml) were mixed with 0.1 mL of 1.0 mM $FeSO_4{\cdot}7H_2O$, and then 0.1 ml of 5 mM ferrozine and 2.7 ml deionized water were added. Then the reaction mixture was left for 10 min at room temperature and absorbance at 562 nm was measured. The positive control was prepared in the same way, but EDTA was added instead of the sample extract. Deionized water was selected as a blank control.

The percentage of Fe(II) bound was calculated according to the following formula: chelating capability = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$.

SAFETY DETERMINATION

Red blood cell test. Potential irritation by mung bean sprout extracts (200 mg/ml) was detected using the RBC test according to the method of Xue *et al*. (19–20). 0.4% SDS and PBS were used as positive control and negative control, respectively. The hemolysis ratio was given by the equation $H\ (\%) = 100\% \times (OD_{530\ nm\ (sample)} - OD_{530\ nm\ (negative\ control)})/(OD_{530\ nm\ (positive\ control)} - OD_{530\ nm\ (negative\ control)})$.

Chicken chorioallantoic membrane assay. Potential irritation of mung bean sprout extracts was detected using the chicken CAM assay according to the methods of Wang *et al*. (21) and Bi *et al.* (22). 0.4% SDS and 0.9% normal saline were used as positive control and negative control, respectively. Morphological changes were observed and data were analyzed using SPSS 17.0 software.

Human skin patch test. According to the method of Zheng *et al.* (23), skin toxicity of mung bean sprout extracts (200 mg/ml) was detected with the human skin patch test. Thirty-two subjects (23 females and 9 males, 20–30 years old) were chosen; extracts were applied on their arms for 24 h. Then results were classified into five grades according to the procedure set out in Hygienic Standard for Cosmetics (24).

STATISTICAL METHODS

The analyses of the data were done using the IBM SPSS Statistics v17.0 statistical package (IBM Corporation, New York, NY). The experimental data were subjected to χ^2 tests, with $p < 0.05$ as a significant difference.

CONCLUSIONS

Mung bean sprout extracts have good antioxidant property. Compared with mung bean seeds, the antioxidant capacity of sprout extracts has improved significantly after budding for 4 days. Later, the antioxidant capacity shows a trend of fluctuations. After budding for 8 days, the DPPH removal effect is the best. Mung bean sprout extracts have remarkable Fe(II) chelation activity. The effectiveness of the chelation of the iron ions tested remains at high level during the beginning of the germination (0–4 days). Safety tests show that extracts of mung bean sprouts from 0 to 8 days after germination are safe and nonirritating to human skin. Therefore, mung bean sprout extracts can be used as potential antioxidant additive to cosmetics for a wide range of applications.

ACKNOWLEDGMENT

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