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Radical scavenging, antioxidant and melanogenesis stimulating activities of diiferent species of rice (*Oryza sativa L.*) extracts for hair treatment formulation

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Keywords: Radical scavenging assay, Antioxidant activities, Melanogenesis stimulating activities, Melanocyte proliferation, Hair treatment

Objectives: The goal of the present study was to evaluate the radical scavenging, antioxidant and melanogenesis stimulating activities of different species of rice *(Oryza sativa L.)* extracts i.e., Sri-Nin, Tabtim Lanna, Hom-Nin, Rice Berry, Dang Sungyod and Kum-Doy-Moo-Ser for hair treatment formulation.
Methods: The radical scavenging and antioxidant activities of different species of rice (*Oryza sativa L.*) extracts were assessed by using DPPH radical scavenging assay and ferric ions reducing antioxidant power assay (FRAP), while the melanogenesis stimulating activity of ethanolic extracts were determined by using the stimulation of tyrosinase enzyme activity and melanocyte proliferation. The stimulation of tyrosinase enzyme was tested by tyrosinase activity assay, and the melanocyte proliferation of these extracts at different concentrations (10 - 500 µg/ml) was evaluated using mouse melanoma cells (B16F10) by MTT assay.
Results: For the DPPH radical scavenging activity, both ethanolic extracts of Gum-Doy-Moo-Ser and Rice Beery had an effective DPPH radical scavenging activity with EC_{50} about 140.00 ± 1.73 µg/ml and 169.67 ± 2.51 µg/ml. For the determination of ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of all extracts, Hom-Nin and Gum-Doy-Moo-Ser showed the strongest value of FRAP and the value were 1996.67 ± 3.67 and 1933.06 ± 3.37 FeFug/ml respectively. From the results of stimulating activity of tyrosinase enzyme, the ethanolic extract of Rice Berry had the highest stimulating activity (91.8 ± 0.10 %), while Sri-Nin extract showed the only lowest stimulating activity (32.59 ± 1.17 %). Among all tests, the extract of Rice Berry (500 µg/ml) showed the strongest stimulating on melanocyte proliferation with the proliferation index (P.I.) at 1.3 ± 0.02, but the extract of Gum-Doy-Moo-Ser had no effect on the stimulating activity of melanocyte proliferation with the proliferation index (P.I.) about 1.0 ± 0.02.
Conclusion: The effect of various species of rice (*Oryza sativa L.*) extracts had the high antioxidant, and it was able to stimulate the melanogenesis activity, providing the increase in potential value added of Thai rice with different species. It could beneficially apply for hair treatment formulation in cosmetic products.

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Introduction

Natural hair color, which is based on the complex genetic control, is dependent on amount and classification of melanin. It is produced by the follicular melanocytes which derived from the neural crest cells located in the hair follicles and produces the content of melanins by the melanogenesis pathway. Melanogenesis, a complicated pigment biosynthesis, involves the oxidative reaction of tyrosine to be either brown/black eumelanin or yellow/red pheomelanin, depending on the cysteine or glutathione existing. These produced melanins are packed into granules known as melanosomes and then transferred to the cortical keratinocytes. The onset of white hair in Thailand are at the late 30 years old and in the mid of 40 years old [1-3]. Currently, there is no treatment procedure for this condition and the masking of white hairs with hair dye is popularly used. Nevertheless, the hair masking with hair dye has been reported to have the numerous harmful effects, such as dermatitis, hair loss and cancer [4]. Many researchers have investigated the low toxic compounds which can induce the melanogenesis pathway. For the development of gray hair prevention agent, a screening program was carried out to find a potential stimulant of melanogenesis from the natural resources by using cultured murine B16 melanoma cells with theophylline as a reference drug [2, 5-7]. It was shown that, theophylline could enhance the pigmentation in cultured murine B_{16} melanoma cells without any effects on cell proliferation [2, 5-7]. Moreover, Somvong *et al.* investigated antioxidant and melanogenesis stimulating activity of Thai traditional medicinal plant extracts included aqueous, ethyl acetate, methanol and hexane extracts of *Tiliacora triandra*, *Centella asiatica*, *Clitoria ternatea*, *Morus alba* and *Pueraria mirifica.* The ethyl acetate extract of T. triandra had the strongest stimulating

activity with % stimulation of 94.34. The study on melanocyte proliferation of these extracts showed that methanol extract of *C. ternatea* and aqueous extract of *T. triandra* had strong stimulating activities with proliferation index (P.I.) of 1.7 and 1.6, respectively [1]. The goal of present study was to evaluate the radical scavenging and melanogenesis stimulating activities of different species of rice *(Oryza sativa L.)* extracts for hair treatment formulation.

Methods

Materials : 2,2-diphenyl-1-picrylthydrazyl (DPPH) (Sigma Aldrich, USA), Ascorbic acid, α-tocopherol (Nam Siang Co., Ltd., Bangkok, Thailand) were used. All herbs were harvested from Agricultural and Food Technology Department, Thailand Institute of Scientific and Technological Research.

Preparation of crude ethanol extracts: The different species of rice (*Oryza sativa L.)* extracts e.g. Sri-Nin, Tabtim Lanna, Hom-Nin, Rice Berry, Dang Sungyod and Kum-Doy-Moo-Ser. Initially, 500 g of herb powders was accurately weighed, mixed with 70% ethanol for 4 nights, filtered through Whatman paper No. 41 and rinsed in the same solvent. The solvent was removed under reduced pressure using a rotary evaporator (Heidolph, Hei-VAP Precision) at 45 ^{0}C.

Determination of DPPH radical scavenging activity: The effect of pigment extracts on the DPPH radical scavenging activity was adapted from Somvong & Prasitpuriprecha [1] and Kriengsak *et al* [9]. First, 50 µg of extract was accurately weighed and then dissolved in 1 ml of 20% DMSO. After that, the solution of extract (50 µg/ml) was mixed with 100 µM DPPH in absolute methanol and the solution was adjusted to the final volume about 2000 µl. The sample was incubated at 37 ^{0}C for 20 min and then the absorbance of solution was evaluated by using a microplate reader (Sunrise, Tecan Co., Austria) at 517 nm. The absorbance of sample and control was calculated on the effective concentration at 50% (EC_{50}) according to the following equation: EC_{50}= [(Abs. control – Abs. sample)/ Abs. control] X100. The sample was performed in triplicate.

Determination of Ferric ions (Fe3+) reducing antioxidant power assay (FRAP): Frist, a solution of ferric chloride solution (20 mM) was prepared in acetate buffer (300 mM, pH 3.6) and TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine, 10 mM)) solution. These solutions were vortexed thoroughly and incubated in dark. Second, 1 mg/mL of sample was dissolved in 20 % DMSO and was mixed with FRAP reagent and distilled water. After that, the sample solution was adjusted the final volume (2,040 µl) with distilled water. The absorbance value of the reaction mixture was recorded at 593 nm. The relative antioxidant activity (FRAP value) of sample was calculated using a standard curve of Ferrous sulfate ($FeSO_4$).

Determination of stimulation of tyrosinase enzyme activity: The stimulation of tyrosinase enzyme activity was tested by tyrosinase activity assay adapted from Somvong & Prasitpuriprecha [1], Choi *et al* [8] and Jeon *et al* [10]. First, the sample was diluted with distilled water at 50 mg/ml. Second, the diluted extract was mixed with tyrosinase enzyme (100 µg/ml) in phosphate buffer pH 6.8 and then incubated at 37 ^{0}C for 10 min. After that, the samples was added with 2 mM tyrosine and then incubated at 37 ^{0}C for 40 min. After incubation, the absorbance of solution was determined by using a microplate reader (Sunrise, Tecan Co., Austria) at 450 nm. The tyrosinase stimulation was calculated according to the following equation: % stimulation = [(A-B)/A] x 100 where A was the absorbance of extract solution, and B was the absorbance of control. The sample was performed in triplicate.

Determination of stimulation of melanocyte proliferation using mouse melanoma cells (*B16F10*): The stimulation of melanocyte proliferation was tested by MTT assay with mouse melanoma cells (B16F10) using the method of Somvong & Prasitpuriprecha [1], Matsuda *et al* [2], Itoh *et al* [6] and Jung *et al* [7]. Frist, mouse melanoma cells ($B_{16}F_{10}$) were seeded at the density of 1.5 x 10^4 cells/well in 96-well plates and incubated at 37 ^{0}C for 24 h under 5% CO_2 atmosphere for cell adhesion. Second, the cell was diluted with completed medium DMEM at concentration in the range of 500- 10 µg/ml. After that, 100 µl of different concentrations of all extracts were added in the 96 well plate system and then incubated at 37 ^{0}C for 72 h. After incubation, 50 µl of MTT in PBS at 1 mg/ml was added to the medium in each well and incubated for 4 - 6 h. Medium and MTT were then removed from the well and solubilized with 100 µl of DMSO. The absorbances of all samples were assessed using a microplate reader (Sunrise, Tecan Co., Austria) at 570 nm. The proliferation index (P.I.) was calculated according to the following equation: proliferation index (P.I.) = [Mean absorbance of sample well x 100] /Mean absorbance of control. The sample was performed in triplicate.

Results and Discussion

DPPH radical scavenging activity: The DPPH radical scavenging activity had been widely used in the model system to investigate the scavenging activity of several natural compounds such as phenolic compounds, anthocyanins, or crude extracts of plants. DPPH radical was scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H•. The color changed from purple to yellow after reduction, which could be quantified by its decrease of absorbance at wavelength 517 nm. The radical scavenging activity of the different species of rice (*Oryza sativa L.*) extracts e.g. Sri-Nin, Tabtim Lanna, Hom-Nin, Rice Berry, Dang Sungyod and Kum-Doy-Moo-Ser using the DPPH coloring method was displayed in Table 1.The result indicated that both ethanolic extracts of Gum-Doy-Moo-Ser and Rice Beery had an effective DPPH scavenging activity with the EC_{50} about 140.00 ± 1.73 and 169.67 ± 2.51 µg/ml, respectively, while the extract of Dang Sungyod showed the lowest efficacy on the DPPH radical scavenging activity and the EC_{50} value was 327.5 ± 0.61 µg/ml.
Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP): For the determination of Ferric ions (Fe^{3+}) reducing antioxidant

power (FRAP) of all extracts, Hom-Nin and Gum-Doy-Moo-Ser showed the strongest value of FRAP and the values were 1996.67±3.67 and 1933.06±3.37 FeFug/ml respectively. Nevertheless, the extract of Tabtim Lanna exhibited the significantly (p<0.05) lowest values of FRAP (1522.22 ± 4.39 FeFug/ml). The high value of FRAP demonstrated the high antioxidant of samples according to the ferric ions (Fe^{3+}) – errous ions (Fe^{2+}) transformation. The results on reducing power demonstrate the electron donor properties of active ingredient from extract thereby neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging.

Table 1. Radical scavenging and melanogenesis stimulating activities of different species of rice (*Oryza sativa L.*) extracts

Different species of rice (Oryza sativa L.) extracts	DPPH Radical scavenging (EC_{50}, µg/ml)	FRAP value (FeFug/ml)	Tyrosinase stimulation (%)
1.Sri-Nin	276.00 ± 2.89	1613.89 ± 3.58	32.59 ± 1.17
2.Tabtim Lanna	189.67 ± 0.93	1522.22 ± 4.39	91.38 ± 0.43
3.Hom-Nin	197.33 ± 0.58	1996.67 ± 3.67	88.13 ± 0.25
4.Rice Berry	169.67 ± 2.51	1643.89 ± 6.03	91.80 ± 0.10
5.Dang Sungyod	327.5 ± 0.61	1676.67 ± 5.18	88.09 ± 0.26
6.Kum-Doy-Moo-Ser	140.00 ± 1.73	1933.06 ± 3.37	86.98 ± 0.17

Stimulation of tyrosinase enzyme activity: From the stimulating activity of tyrosinase enzyme of all rice extracts with various species at 50 mg/ml, the ethanolic extract of Rice Berry had the strongest stimulating activity (91.80 ± 0.10 %), whereas the extract of Sri-Nin showed the lowest stimulating activity (32.59 ± 1.17 %) as illustrated in Table1. ***Stimulation of melanocyte proliferation using mouse melanoma cells (B16F10):*** The melanocyte proliferation of different species of rice (*Oryza sativa L.)* extracts at various concentrations (10-500µg/ml) was shown in Figure 1. The results indicated that the increased concentrations of all extracts resulted in the increased melanocyte proliferation with the proliferation index (P.I.) in the range of 1.0 – 1.3. Among all tested extracts, the extract of Rice Berry showed highest stimulating on melanocyte proliferation at all concentrations and the proliferation index (P.I.) was in the range of 1.1 – 1.3 at concentrations about 50 – 500 µg/ml. In opposite result, the extract of Kum-Doy-Moo-Ser had no effect on the stimulating activity of melanocyte proliferation, because, the proliferation index (P.I.) was about 1.0 of all concentrations with the same sample without active ingredient. The proliferation index (P.I.) was higher than 1.0, indicating the increase in potential stimulation on the melanogenesis pathway.

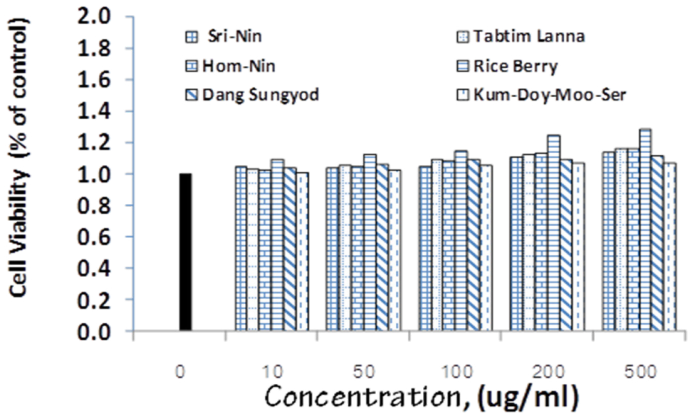

Figure 1. Melanocyte proliferation of different species of rice (Oryza sativa L.) extracts at various concentrations (10 - 500 µg/ml) (*, vs control p<0.05)

Conclusion

In conclusion, both ethanolic extracts of Gum-Doy-Moo-Ser and Rice Berry had an effective DPPH radical scavenging activity, while both extracts of Hom-Nin and Gum-Doy-Moo-Ser showed the strong value of ferric ions (Fe^{3+}) reducing antioxidant power (FRAP). From the stimulating of melanogenesis activity, the ethanolic extract of Rice Berry had the only highest stimulating activity between tyrosinase enzyme and melanocyte proliferation. Therefore, the effect of various species of rice (*Oryza sativa L.)* extracts had the high antioxidant, and it was able to stimulate the melanogenesis activity, providing the increase in potential value added of Thai rice with different species. It could beneficially apply for hair treatment formulation in cosmetic products.

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