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**Research Article**

# Anti-aging efficacy of Thai red rice callus cosmetic product

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## Abstract

Plant cell culture is an innovative technology to produce bioactive substances and anti-aging agents for cosmetic products. Red rice (*Oryza sativa*) is a special rice cultivar in Thailand that has high procyanidin content in seed coats. In this study, the red rice callus were cultured from rice seeds and extracted with ethanol. The extract contained 0.42 mg GAE/ml for phenolic compounds and 0.21 mg Glu/ml for amino acids. The anti-aging properties of the induced rice callus extract in solution form was evaluated on 28 volunteers, aged 30 – 55 years. The volunteers were separated into 3 groups; placebo (n = 8), 5 % of the induced stem cell extract cream (n = 10), and 5 % of the commercial red rice stem cell extract cream (n = 10). The tested creams were applied on facial skin twice a day for 12 weeks. Skin color, hydration and elasticity were assessed with Mexameter, Corneometer, and Cutometer, respectively. The results showed that anti-aging efficacies of the group treated red rice stem cell extract cream were greater than those of the commercial cream group, which were skin whitening (38.57%, 29.85%), skin moisture (74.62%, 33.48%) and skin elasticity (88.41%, 64.97%), respectively, however, the placebo group remained no change of skin in any parameters. In sensory evaluation, most of volunteers satisfied with product acceptability; appearance, texture, color, odor, moisture, spreadibility, absorption, smoothness, non-greasiness (> 85 %) and also the reduction of visible aging sign (> 90%). The results demonstrate that Thai red rice stem cell extract can be used as an effective anti-aging ingredient for cosmetic products functionalized in skin lightening, hydration and elasticity.

**Keywords**: anti-aging, cosmetics, red rice, rice stem cell

## Introduction

Nowadays, the increased interest in fighting the aging skin has led to increase in the amount of worldwide cosmetic products which focus on anti-aging active ingredients such as antioxidants, vitamins, and bioactive compounds which scavenge free radicals from skin cells, restore skin elasticity and protect skin wrinkle.

Plant is a source of active compounds. However, the use of plants materials has certain limitation due to seasonal harvesting, plant growth rate, microorganism contamination, and pollution. Plant cell culture technology may help to overcome these essential problems in manufacturing cosmetic products (Schürch et al., 2008). Plant stem cells or callus contain a complex matrix of ingredients like salts, acids, phenols, sugars, lipids, proteins and other undefined fractions of compounds which may contribute to a beneficial protecting effect of skin stem cells (Schürch et al., 2008). Cosmetics industry interest the use of stem cells from plant as a source of materials for replenishing the aging body (Draelos, 2012).

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Rice (*Oryza sativa*) consists of many phytochemicals such as γ-oryzanol, vitamin E homologues, and phenolic acids which produce beneficial health properties (Finocchiaro et al., 2007; Yawadio et al., 2007). Red rice is a special rice cultivar in Thailand that contain high procyanidin in the seed coated (Oki et al., 2002). Procyanidins, known as condensed tannins, are polymers of flavan-3-ol monomer units (catechin and epicatechin diastereoisomers) (Gu et al., 2004). Procyanidins have been reported to be high antioxidant, matrix metalloproteinases inhibition (Grimm et al., 2004), tyrosinase inhibition (Momtaz et al., 2008) and effective in protecting against cardiovascular diseases and cancer (Santos-Buelga & Scalbert, 2000).

The anti-aging efficacies of many plant stem cell such as apple, grape, raspberry, tomato have been previously reported. However, it has not been reported the anti-aging potential of Thai red rice stem cell. Therefore, the aim of the present study was to examine the effects of topical application of Thai red rice stem cell on 28 volunteers (aged 30 – 55 years) in comparison to commercial red rice stem cell based on skin color, hydration and elasticity.

## Materials and methods

### Materials

Red rice was cultivated in Chiang Rai, Thailand. Ethanol, folin-ciocalteu reagent, gallic acid, ninhydrin and sodium carbonate were purchased from Sigma-Aldrich Co., USA. 1-Naphthaleneacetic acid (NAA), 2,4-Dichlorophenoxyacetic acid (2,4-D), 6-Benzylaminopurine (BAP), gallant gum and Murashige and Skoog (MS) medium were purchased from PhytoTechnology Laboratories, USA. Commercially available rice stem cell extract was purchased from Water Doctor Company Limited, which extracted from red rice (*Oryza sativa*) callus. The claim of the extract is to provide anti-aging effect.

### Preparation of red rice callus extract

The sterilized red rice seed was inoculated on agar solidified basal MS medium supplemented with 3 % sucrose, 0.2 % gellant gum, 2 mg/l of 2,4-D, 1 mg/l of NAA, and 1 mg/l of BAP. The pH of medium was adjusted to 5.8. The red rice was incubated in the controlled temperature conditions (25±2°C). The callus formation was observed and collected after incubation for 21 days. The harvested callus (1 g) was extracted with (10 ml) ethanol under sonicator for two hours. The extract was filtered with Whatman® no.1 filter paper and stored at 4°C until analysis.

### Total phenolic content

Total phenolic content was analyzed using the Folin-Ciocalteu assay (Vichit & Saewan, 2015). Briefly, the extract (20 µl) was mixed with 50 µl of deionized water, 20 µl of Folin-Ciocalteu reagent and 125 µl of 7 % sodium carbonate. The mixture was incubated for 60 minutes at room temperature. The absorbance was measured at 750 nm using a microplate reader. The result was determined using gallic acid standard solution and expressed as gallic acid equivalents (mg GAE/ml extract).

### Total amino acid content

Total amino acids content was determined by using the Ninhydrin assay (Friedman, 2004). The 200 µl of extract was mixed with 1 % ninhydrin solution and incubated at 80°C for 10 minutes. Absorbance was measured at 570 nm and then calculated using glutamic acid standard solution. The result was expressed as glutamic acid equivalents (mg Glu/ml extract).

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### Preparation of testing cream

Oil-in-water (O/W) emulsion cream (placebo) was formulated. Briefly, aqueous and oily phase were heated to 75°C. After heating, aqueous phase was then added to the oily phase with continuous stirring until complete homogenization by using homogenizer (IKA Werke, Germany). Finally, emulsion was cooled to room temperature. The testing cream was prepared by incorporation of 5 % of Thai red rice stem cell extract (F1) or commercial rice stem cell extract (F2) which was the maximum recommended dose for the commercial rice stem cell extract.

### Skin irritation test

The protocol was approved by the Ethics Committee of Mae Fah Luang University (REH-60031). Each volunteer signed a written informed consent before participating in the clinical study which explained the type of study, the procedures to be followed, the general nature of the materials being tested, and any known or anticipated adverse reactions that might result from participation.

Twenty-eight healthy volunteers (aged 30 – 55 years) were subjected to the occlusive single patch test. Finn chambers (8 mm) were used for observation of skin irritation for a period of 24 h on the volar forearms of volunteers. Skin irritation severity was graded over arrange of 0 – 4. Data collected were used to calculate mean irritation index (M.I.I). A M.I.I value < 0.2 was classified as non-irritation.

### Clinical anti-aging effects

Volunteers were randomized to one of three treatment groups. Group 1 received placebo cream. Group 2 received cream containing 5 % of Thai red rice stem cell extract. Group 3 received cream containing 5 % of commercially rice stem cell extract. Volunteers were instructed to apply 0.5 mg of the tested cream on facial skin twice daily, in the morning and before bedtime. Modification of the facial skin-care habits and concomitant use of other skin care products were not permitted during the study.

All skin measurements were conducted under controlled temperature (20 ± 5°C) and humidity (50 ± 10%) conditions. Volunteers washed their face with an assigned facial cleanser and allowed to acclimate to the room conditions for at least 30 min prior to measurements. Melanin content was determined with Mexameter. Skin moisture was measured by Corneometer. Skin elasticity was evaluated the net elasticity of the skin without viscous deformation by using Cutometer. The values of each volunteer were recorded and calculated as the percentage of change by following equation:

$$\% \text{ Change} = [(X_t - X_0)/X_0] \times 100 \quad (1)$$

Where $X_0$ is the initial value which measured before application (week 0) and $X_t$ is the value that measured during application (Fox et al., 2014).

### Sensory study

The products were sensory evaluated by all volunteers at the end of experiment. Each volunteer was asked to answer the questionnaire about the cream texture and self-assesment for anti-aging results which divided into 5 scores, as follows: (1) dislike extremely, (2) dislike, (3) neither like nor dislike, (4) like, and (5) like extremely (Herndon et al., 2014). Then, the

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total number of volunteer who favorable responsed to the product properties (score 4 – 5) was calculated as preference percentage of each treatment group by using following equation:

Preference (%) = (Number of preferable response volunteer/ Total volunteer) x 100 (2)

**Statistical analysis**

All measurements were performed in triplicate. The obtained data were statistically analyzed using the SPSS program version 11.5 for Window (SPSS Inc, Chicago, IL, USA) and the differences were considered significant when $p<0.05$. For clinical trial, paired sample t-test was used to determine the skin improvement during sample application compared with initial condition (week 0) and * indicated significant differences ($p<0.05$).

## Results and discussion

**Preparation of red rice callus extract**

In this study, auxins (2,4-D and NAA) and cytokinins (BAP) were used to induce red rice callus on agar solidified MS medium supplementing with sugar and gellant gum. The induced red rice callus appeared as yellow compact texture (Figure 1). The growth percentage was calculated based on the weight of rice seed and showed that stem cells were developed in range of 385 %.

Phenolic compounds and amino acids are two main groups of anti-aging agents in cosmetic products (Ganceviciene et al., 2012). Phenolic compounds are antioxidants which eliminate the causes of skin aging and skin damage, including wounds and burns. Moreover, phenolic compounds also help prevent or attenuate the progression of certain skin disorders such as wrinkles and acne (Dzialo et al., 2016). Amino acids are cell regulators which have the ability to stimulate collagen synthesis and activate dermal metabolism through topical application (Lupo & Cole, 2007). In addition, amino acids have the ability to stimulate collagen synthesis and restore dermal collagen impaired by UV irradiation (Lupo & Cole, 2007; Murakami et al., 2012).

In this study, the total phenolic and amino acid contents in the induced callus and the commercial extracts were determined by using the Folin-Ciocalteu and Ninhydrin assays (Friedman, 2004), respectively. The induced stem cell extract contained total phenolic content with 0.42 mg GAE/ml and total amino acid content with 0.21 mg Glu/ml, while, the total phenolic and amino acid of the commercial extract were 0.37 mg GAE/ml and 0.22 mg Glu/ml, respectively.

**Figure 1.** Appearance of red rice callus and ethanol extract.

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### Skin irritation test

Skin irritation was evaluated by using single application closed patch test. The result showed that all volunteer were no any irritation or side effect. Then, the M.I.I value was calculated and showed that M.I.I value of Thai red rice stem cell and commercially rice stem cell was 0 and 0.1, respectively. Thus, the products were classified as non-irritant.

### Clinical anti-aging effects

The clinical anti-aging effects of red rice stem cell cream were evaluated on facial skin of 28 volunteers compared with placebo and commercial rice stem cell cream for 12 weeks. The evaluation of wrinkles in the eye area by naked eye observation and photograph showed that placebo cream were no any change, while, the creams contained the induced stem cell (F1) and commercial extract (F2) showed reduction of fine wrinkles (Figure 2). The anti-aging efficacy was assessed for melanin content, skin moisture and elasticity. Then, the percentage change of all volunteers was calculated and statistical analyze was determined by comparing with week 0.

Skin moisture relate with stratum corneum water content and has an important role in skin function such as cell proliferation, skin hydration, and anti-inflammation process. The product which can improve moisture of skin can prevent skin dryness and alterations resulting from aging. The skin moisture was determined by using Corneometer. According to Figure 3, the percentage change of skin moisture of the group applied formula F2 was significant increased in week 2 and continuously increased to 33.48 % at week 12. The group treated formula F1 had caused an increase in skin moisture in all volunteers since first week of application (32.23 %) and enhanced skin moisture to 74.62 % at the end of application. The skin moisture effect may be due to the constituent of amino acid which are the main component of the natural moisturizing factor (NMF), helping maintain hydration in corneocyte and stratum corneum (Verdier-Sévrain & Bonté, 2007).

Melanin is a skin pigment which effect to the appearance of skin color. Therefore, determination of melanin content can imply the darker and lighter skin. The melanin content was determined by using Mexameter. The result showed that melanin content of volunteers who applied placebo were no any change values, while, volunteers who applied formulas F1 and F2 were continuously decreased. In overall, at week 12, the percentage change of melanin content was calculated and showed that no statistically significant difference ($p < 0.05$) observed in placebo group. The change of melanin content was significantly decreased by 38.57 and 29.85 % for F1 and F2, respectively (Figure 4).

The loss of skin elasticity is a main problem of aging, thus the active ingredient which can improve skin elasticity are interest to use as anti-aging active. The skin elasticity was determined by using Cutometer. The skin elasticity of each volunteer showed a little change values in placebo group, while, formulas F1 and F2 were increased skin elasticity evidently. After 12 weeks of application, formula F1 enhanced skin elasticity to 88.41 % which was significantly more than formula F2 (64.97 %) (Figure 5).

The induced stem cell extract showed higher anti-aging efficacy than the commercial exract may be due to it contain higher phenolic compounds which inhibit tyrosinase (the key enzyme responsible for melanin production) synthesis, dopachrome formation and melanogenesis (Chang, 2009; Couteau & Coiffard, 2016; Kim & Uyama, 2005). Moreover, phenolic compounds demonstrate the ability to promote the enzymatic inhibition of specific proteinases such as collagenase and elastase which can be useful to prevent loss of skin elasticity and thus skin sagging (Melzig et al., 2001; Silva et al., 2017; Thring et al., 2009).

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In overall, the induced stem cell and the commercial stem cell extracts were found to be an effective ingredient for use as anti-aging ingredient in cosmetic formulation due to its ability to reduce the melanin content and enhance of skin moisture and elasticity.


**Figure 2.** Photographs of skin appearance at week 0 and week 12.

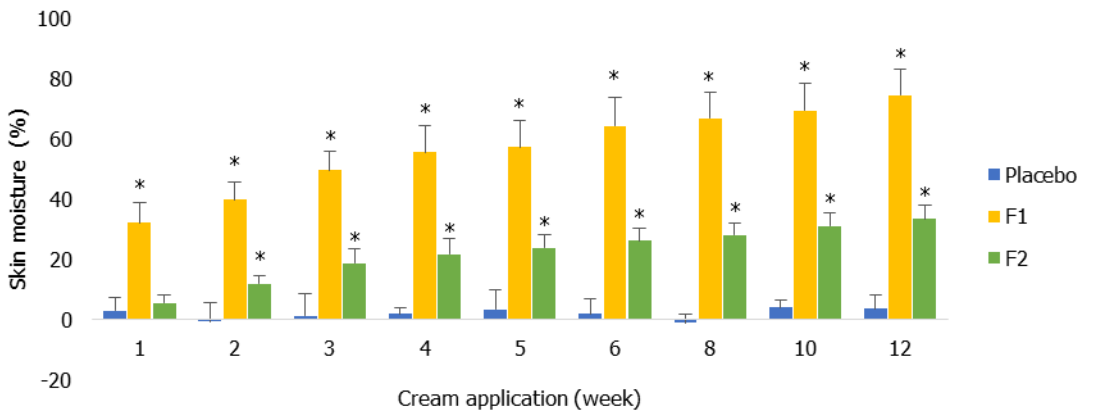


**Figure 3.** The change of skin moisture during experiment. * indicate significant differences ($p < 0.05$) between the skin property during experiment and baseline (week 0).

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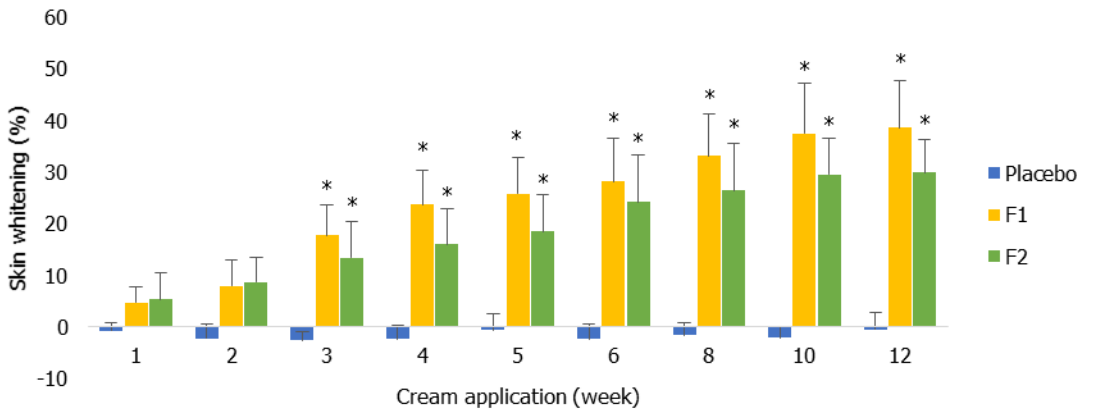


**Figure 4.** The change of skin whitening during experiment. * indicate significant differences ($p < 0.05$) between the skin property during experiment and baseline (week 0).

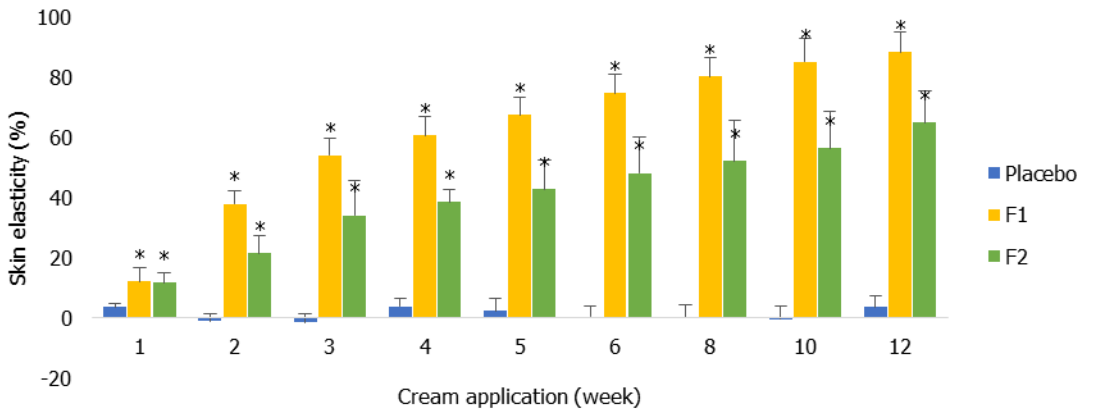


**Figure 5**. The change of skin elasticity during experiment. * indicate significant differences ($p < 0.05$) between the skin property during experiment and baseline (week 0).

## Sensory study

The satisfaction of using placebo, Thai rice stem cell and commercial extracts creams were evaluated. The result showed that all volunteer groups prefer appearance of their product at similar level (90 %) in texture, skin absorption, smoothness, non-greasiness, and overall. The color, odor, and moisture of all products were little difference (Figure 6a). The efficacy preference of product was evaluated after application for 12 weeks. Volunteers who applied rice stem cell cream were more preferred the anti-aging efficacy of the product in skin whitening, moisture, smoothness, elasticity, firmness, wrinkle and dark spot reduction than the group obtained the commercial extract cream (Figure 6b).

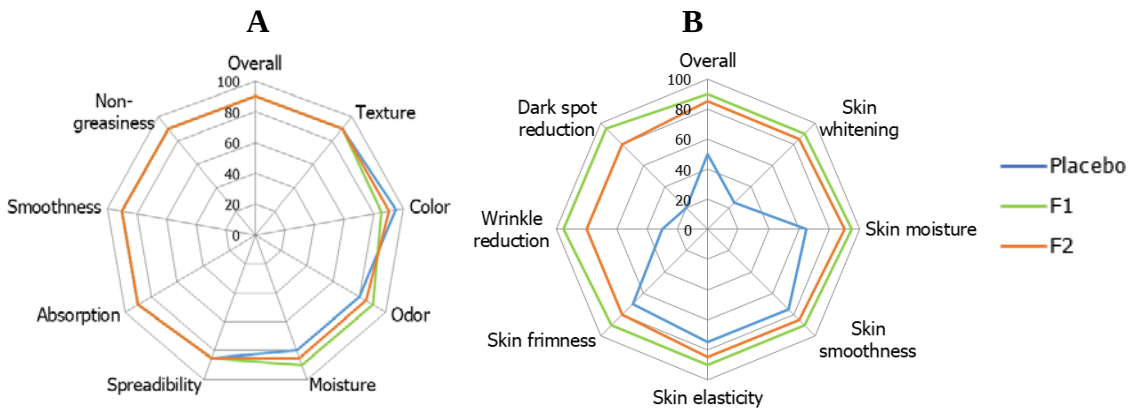


**Figure 6.** Sensory study of products with percentage of subject who extreamly like and like (A) texture and (B) efficacy.

## Conclusion

The data from this study show that topical application of the product containing 5% Thai red rice stem cell extract offers anti-aging benefits. Statistical improvements were indicated in its ability to reduce the melanin content and enhance of skin moisture and elasticity of aging skin against baseline. Thus, red rice stem cell extract is an effective ingredient for use as anti-aging ingredient in cosmetic formulation.

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