

Biological activity and its related compounds of Red Jasmine rice extracts linked to normal fibroblast viability for cosmetic product

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ABSTRACT

Red jasmine rice is recognized as a healthy food with high phenolic compounds. These compounds present antibacterial and anti-free radical properties. Moreover, colored rice exhibits a biological activity against anticancer. Objectives of this study are 1) exploring a biological screening and cell viability of 70% ethanol and aqueous extracts of red jasmine rice, 2) investigating cytotoxicity to fibroblast NIH3T3 (IC80) that is one hundred cells were found cell viability 80 cells. Red jasmine rice extracts were dried and transformed into a powder using the freeze-drying method. The extracts were treated with fibroblast NIH3T3 for MTT. The highest of IC50 of red jasmine rice extract to scavenge the DPPH and ABTS radicals was found in ethanol extract (53.20±7.37 and 64.17±5.76, respectively). The experiment showed that the ethanol and aqueous extract of red rice did not show cytotoxicity to fibroblast NIH3T3 (IC80). The extracts of red rice show the biological screening of anti-oxidation with total phenolic compounds and flavonoid contents. Moreover, it does not modify the physical properties of the cream formula. It can be concluded that the red rice extract is highly promising for the value addition.

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1. Introduction

Red jasmine rice is well-known and has grown in Thailand for more than 10 years. One of the major characteristics of this cultivar is having a high anti-oxidant compound¹. Moreover, other properties of red rice such as anticancer², antimicrobial activity³, and reduced glucose response in healthy people⁴ were studied. Therefore, the biological screening such as DPPH, ABTS assays and total phenolic, total flavonoid compounds is the most important for selecting a plant for a further study. Previous studies showed that rice was related to developing herbal medicines as a key to global health and presented some aspects of the possible future role of chemistry in traditional medicine⁵. Thai rice has more varieties reported for biological effect in various aspects, especially in colored rice provided by phenolic acid, flavonoids, and anthocyanins (Cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin chloride)⁶. Colored rice can modulate skin for anti-aging with its Oryzanol and phenolic compounds such as a proanthocyanidin^{7, 8} and anthocyanin⁹. The proanthocyanidin from colored rice affects the skin anti-aging via improving a matrix metalloproteinase 2 (MMP2) degradation equally to collagenase degradation^{10,11} and inhibit mitogen-activated protein kinases (MAPK) induced UVB-irradiation¹². Another phenolic compound with anti-aging properties in colored rice is anthocyanin⁹.

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Red jasmine rice is used to relieve illness in traditional Thai medicine, especially in the North of Thailand. Investigating a biological screening effect, anti-oxidant activity, and related compounds in red jasmine rice should therefore be explored. Moreover, this study aims to find an effective extract compound that can modulate NIH3T3 fibroblast which leads to developing the extracts to promote cell viability. Red jasmine rice extract will be used as an ingredient of cosmetic products and then analyzed for cream stability.

2. Results and Discussion

2.1. Red jasmine rice crude extracts against ABTS and DPPH radicals and their total phenolic (TP) and total flavonoid (TF) contents

In this study, red jasmine rice was collected from Lamphun Province. Each total phenolic contents of extracted (TP) were analyzed and expressed as milligram gallic acid equivalent per gram extract (mg GE/g of ext). It was found that the ethanol and aqueous crude extracts had similar TP, which was equal to 30.58 ± 1.19 and 31.76 ± 1.06 of gallic acid, respectively. The differences were not considered significant at the level of $p < 0.05$ (*) to ascorbic acid and Trolox of ABTS and DPPH assays except the red jasmine rice extract by water. The total flavonoid content (TF) was determined by aluminium chloride colorimetric assay and expressed as catechin equivalent per gram extract (mg CE/g of ext). A high level of TF was also found in both ethanol and aqueous crude extracts. The TF in this extract was equal to 24.07 ± 3.28 and 24.25 ± 1.41 mg CE/g of ext. The anthocyanin contents were found in red jasmine rice extract in water extract (W) and ethanol extract (EtOH). The highest anthocyanin content was 43.33 ± 0.82 mg/L was found in water extraction (W). The anti-oxidant activities of red jasmine rice crude extracts were measured by determining their abilities to scavenge DPPH and ABTS radicals. As shown in **Table 1**, the anti-oxidant activities of red jasmine rice extract at 0-200 mg/ml were found to inhibit the ABTS-radical and DPPH-radical in a dose-dependent manner when compared with Trolox as a positive control and ascorbic acid, respectively. Red jasmine rice extract in the EtOH at 200 mg/ml displayed the highest inhibitory effect when it could inhibit ABTS and DPPH radical, followed by Water extract at the same concentration levels. This finding is well agreed with the evaluation of total phenolic contents and total flavonoid contents of each solvent extracts (see **Table 1**). Both of two solvent extracts of red jasmine rice were correlated to anti-oxidation against ABTS and DPPH radicals. The previous study showed that the extract of red jasmine rice by 70% ethanol performed biological activity to cancer cells at 20 mg/ml¹. Moreover, the red jasmine rice extract showed inhibitory effect to DPPH radical (IC_{50}) at 25 mg/ml at $p < 0.001$ and was able to ⁷. This result is quite similar to our finding that the concentration of red jasmine rice extracted by EtOH and W were able to scavenge free radicals.

Table 1. IC_{50} of the red jasmine rice extracts against ABTS and DPPH radicals and their total phenolic (TP), total flavonoid (TF), and Anthocyanin contents.

| Extract | IC_{50} (μ g/ml) | | TP (mg GE/g of ext) | TF (mg CE/g of ext) | Anthocyanin content (mg/L) |
|------------------------------|-------------------------|---------------------|---------------------------|---------------------------|----------------------------------|
| | ABTS assay | DPPH assay | | | |
| Positive control | | | | | |
| Ascorbic acid | 53.48 ± 3.28 | 38.94 ± 3.32 | - | - | - |
| Trolox | 8.95 ± 1.07 | 6.31 ± 1.73 | - | - | - |
| Red jasmine rice extracts | | | | | |
| - EtOH | 64.17 ± 5.76 | 53.20 ± 7.37 | 30.58 ± 1.19 | 24.07 ± 3.28 | 21.21 ± 4.72 |
| - Water | $183.65 \pm 4.02^*$ | $138.86 \pm 8.20^*$ | 31.76 ± 1.06 | 24.25 ± 1.41 | 43.33 ± 0.82 |

Note: The present value of red jasmine rice crude extracts against ABTS and DPPH radicals and their total phenolic (TP) and total flavonoid (TF) contents are presented as mean \pm SD. The differences were considered significant at the level of $p < 0.05$ (*).

Red jasmine rice extract has been reported to possess various bioactive compounds such as flavonoids and polyphenols, related to its potent anti-oxidative activities^{3, 6}. The active W extracts has been linked to the solvent polarity that can extract different fractions of polar/nonpolar constituents out of the plant¹³. The polysaccharides extract from rice brans found in this area also showed the anti-oxidant and antimicrobial activities³. The preliminary study found that the proanthocyanidin extract from color rice showed the potency to scavenging of free radical and skin anti-aging^{1, 7, 9}. On the other hand, the extract could modulate the biochemical metabolism due to the anti-oxidant effect and anti-oxidant compounds. The phenolic compounds and flavonoids had the potential to modulate collagen degradation via matrixmetalloproteinase-2 (MMP2)⁸. Our study shows that similar anti-oxidant properties and anti-oxidant compounds of red jasmine rice extracts belonged to previous study^{1, 8, 11}.

2.2. Cell viability assay by MTT test

The present study characterized the cell viability effect of red jasmine rice extract on NIH3T3 fibroblast cells by conducting an MTT assay. Cells were treated with various concentrations of extract for 24 h. The viability of cells was moderate with the extracts (see **Fig. 1**). Red rice extracted with EtOH and Water had no effect on NIH3T3 fibroblast cells

hence the Water extract at the concentration of 400 $\mu\text{g/ml}$. The EtOH and Water extracted from red rice did not show the cytotoxicity to fibroblast NIH3T3 (IC_{80}).

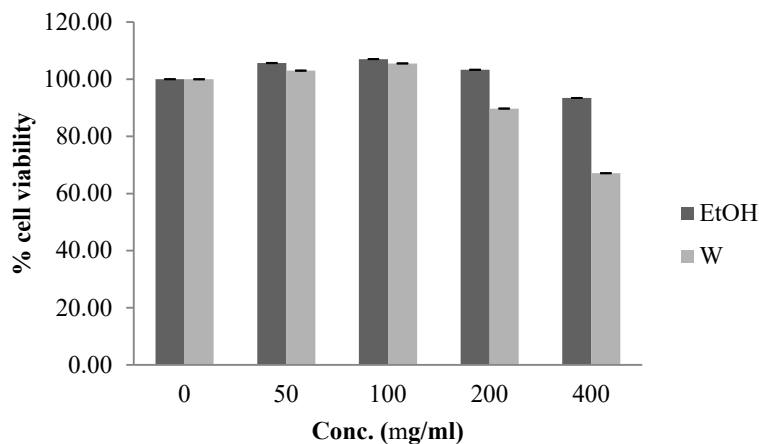


Fig. 1. The NIH3T3 fibroblast cell viability to red jasmine rice extracts was tested by MTT assay. The extracts had no toxicity to fibroblast cells. The red jasmine rice extract can maintain the fibroblast cell.

According to the NIH3T3 fibroblast cell viability, the EtOH extract contributed a better performance more than the W extract so that they have many researches in red jasmine rice extracted using ethanol. Results expressed no harmful effect to fibroblast cells in the presence of UVB exposure and then it has a sunscreen property to UVB^{7,12}. Generally, the common feature of UV-absorbing secondary metabolites is the presence of aromatic or conjugated bond structures found in plants with phenolic or flavonoid substances. These molecules are one of the most effective UV radiation absorbers^{14,15}. An earlier study by Parzonko and Kiss (2019) showed that cosmetic formulation with herbal extracts protects human fibroblast against UVA radiation *in vitro*¹⁶. Moreover, the formulation with plant extracts could maintain the moisture of dry skin¹⁷. The photoprotective capacities of plant extracts such as polyphenols have been demonstrated in previous studies^{18,19}. Moreover, the plant extracts that showed anti-oxidant activities with anti-oxidant compounds can protect against the range of UVB²⁰. Indeed, using a similar *in vitro* UV method, three sunscreen emulsions with ethyl acetate plant extracts (10 %wt.) were tested *in vitro*²¹, and the authors obtained an SPF value of 26.61 ± 0.10 . Therefore, it could be recommended that red jasmine rice extracts have a potential to be a new ingredient in cosmetic products.

2.3 Physical evaluation of formulated cream

The physical evaluation such as color, homogeneity, phase separation, thermal stability, pH, and viscosity of formulated cream was performed (see **Table 2**). The difference to the base cream (F-1) was not shown and the same ranges of pH and viscosity to F-1 were observed. The pH of cream was determined to examine the possible side effects due to acidic or alkaline pH, which can lead to skin irritation and influence the rate of hydration of polymer. In general, the cream should have pH 6-9²².

Table 2. Physical evaluation of formulated cream.

| Physical evaluation | F-1 | F-2 | F-3 | F-4 | F-5 |
|----------------------|-----------|-----------|-----------|-----------|-----------|
| Color | No change | No change | No change | No change | No change |
| Homogeneity | Yes | Yes | Yes | Yes | Yes |
| Oil phase separation | No | No | No | No | No |
| Liquidify separation | No | No | No | No | No |
| Thermal stability | Stability | Stability | Stability | Stability | Stability |
| pH | 7.0 | 6.9 | 6.9 | 6.9 | 6.9 |
| Viscosity (cP) | 28500 | 31007 | 31084 | 31259 | 31519 |

All formulations had increasing viscosity values after storage in freeze-thaw condition. All samples were oil-in-water creams; hence, their water contents might lose at fluctuated temperatures. Therefore, the suggested storage condition for these products should be at a constant temperature. The formulations with suitable viscosity could provide more adhesiveness and spreading efficiency. No phase separation and change in color, as well as odor, were observed in all samples after the stability test; however, they seemed to be more viscous. It is observed from the results that the given formulations are relatively stable at accelerated temperature and humidity. The formulated cream with red jasmine rice extracts did not show the physical changes when compared to the cream base. According to these results, the red jasmine extracted by ethanol is a good natural resource for ingredients in a cosmetic product.

3. Conclusions

Red jasmine rice extract possesses various bioactive compounds such as flavonoids and polyphenols, related to its potent anti-oxidative activities. On the other hand, the extract could modulate the biochemical metabolism as a result of the anti-oxidant effect and anti-oxidant compounds. The red jasmine rice extract in ethanol fraction also showed a sunscreen property to UVB. The formulated cream with the red jasmine rice extract did not show the physical changes when compared to the cream base. Proanthocyanidins claimed as the active compound were found in the red jasmine rice extract. Therefore, ethanol red jasmine rice extract is a good natural resource for sunscreen cosmetic products.

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4. Experimental

4.1 Chemicals and materials

The red jasmine rice was collected from agricultural land in Lamphun province located in the North of Thailand and was verified following the Plant List (<http://www.theplantlist.org>). Red jasmine rice leaves were cleaned and dried at 40°C. The dried samples were kept at -20°C until being used. All chemical reagents for this research were an analytical grade. 2,2-Diphenyl-1-picryl hydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), catechin hydrate, and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Gallic acid was obtained from Fluka (Buchs, Switzerland). Folin-Ciocalteu phenol reagent was purchased from Merck (Darmstadt, Germany). Sodium hydroxide was obtained from Fisher Chemical (Mumbai, India). Potassium chloride, ferric chloride, potassium acetate, potassium persulfate, aluminum chloride hydrated, and methanol were purchased from Ajax Finechem (Auckland, New Zealand). Hexane was obtained from Macron Fine Chemicals (PA, USA). Ethanol 99.9% was purchased from QRĒC (New Zealand). Double-distilled and deionized water was applied for a preparation step of all solutions.

4.2 Preparation of ethanolic crude extract

One kilogram of dried samples was grounded into powder and macerated in 4L of 80% (v/v) ethanol and deionized water for 24h at room temperature. The extraction was performed twice under the same conditions. Chlorophyll was removed by the charcoal adsorption method. Then, the chlorophyll-free extracts were filtered through Whatman's No.1 filter paper, and the solvent was removed using a vacuum rotary evaporator at room temperature. The concentrated aqueous portion was lyophilized into a powder and further experiment.

4.3 Evaluation of anti-oxidant activities from crude extract

Two methods measured the free radical scavenging activity of rice bury crude extract. The DPPH inhibition assay and the ABTS inhibition assay were performed and slightly modified as described by previous studies^{13,22}. With treatments of various extract concentrations, the decrease in absorbance was measured at 517 nm for the DPPH assay, and 735 nm for the ABTS assay, and the percentage of inhibition and IC₅₀ value were also reported.

4.4 Determination of total phenolic and total flavonoid contents from crude extract

Total phenolic content (TP) and total flavonoid content (TF) were determined using the Folin-Ciocalteu assay and aluminium chloride colorimetric assay, respectively, as was described by previous studies with minor modifications¹³. Quantification was expressed as milligram gallic acid equivalent per gram extract (mg GE/g of extracts) for TP and milligram catechin equivalent per gram extract (mg CE/g of extracts) for TF. Three replications were performed for each experiment. The extract that gave the highest anti-oxidant activities and anti-oxidant compounds will be monitored for the evaluation of the formulation.

4.5 Total monomeric anthocyanin content

The total monomeric anthocyanin content (TAC) was measured by pH differential method²³. Briefly, 0.3 ml of the extract was put in to 2.7 ml of different buffer solutions including KCl buffer (0.025 M, pH = 1.0) and sodium acetate buffer (0.4 M, pH = 4.5). The obtained solutions were measured absorbance at 510 and 700 nm. Total anthocyanin in form of cyanidin-glucoside (Cyd-3-glu) was calculated using the following equation: TAC (mg/g) = $(A \times MW \times \text{dilution factor} \times 1000) / \epsilon \times L$

where: $A = (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 1.0}} - (A_{510 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH 4.5}}$,

MW for cyd-3-glu = 449.2 g mol⁻¹, ϵ = 26900 molar extinction coefficient in M⁻¹ cm⁻¹ for cyd-3-glu, Dilution factor = 10

4.6 Cell viability assay

Cell line. NIH3T3 fibroblast cells were maintained in DMEM, 100 U/ml penicillin, and 100 mg/ml streptomycin plus 10% FBS. The culture was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO₂ at 37 °C.

Cell viability assay. NIH3T3 fibroblast cells (1.0×10⁴ cells/well) were plated in 96-well plates and cultured in DMEM with 10% FBS. After being cultured for 24 h, the various concentrations of red jasmine rice extracted with EtOH or W (0-400 µg/ml) were loaded and incubated for 24 h. At the end of treatment, 15 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added and it was then incubated for 4 h. The MTT formazan was dissolved with dimethyl sulfoxide (DMSO), and absorbance was measured using a microplate reader at 570 nm with a reference wavelength of 630 nm.

4.7 Evaluation of formulation

Physical parameters. The appearance, color, and homogeneity of each formulated cream (see **Table 3**) are determined.

Table 3. Formula for development of photo protective cream formulations

| Ingredients | F-1(g) | F-2 (g) | F-3 (g) | F-4 (g) | F-5 (g) |
|-------------------------------|--------|---------|---------|---------|---------|
| Red jasmine rice extract | 0.000 | 0.125 | 0.250 | 0.500 | 1.000 |
| Cetostearyl alcohol | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 |
| Stearic acid | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 |
| PEG-200 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 |
| Cetyl alcohol | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Phenoxyethanol | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Carbopol ultrez 21 | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
| Disodium EDTA | Qs. | Qs. | Qs. | Qs. | Qs. |
| Triethanolamine | 0.500 | 0.500 | 0.500 | 0.500 | 0.500 |
| Distilled water qs. to 100 mg | | | | | |

The formulated cream procedure was following:

Step 1: Aqueous phase

Part 1. Disodium EDTA, phenoxyethanol, and extracts were weighed accurately and dissolved in some the distilled water.

Part 2. The remain distilled water was dispersed with carbopol ultrez 21 while heating up to 70°C to swelling using a homogenizer.

Step 2: Oil phase

Stearic acid, cetyl alcohol, and cetostearyl alcohol were weighed accurately, mixed, and heated to 70°C. Then mix oil phase to part 2 of aqueous phase at 70°C with continuous stirring for 30 min till it was homogenized and uniformed. Part 1 of the aqueous phase was added to the formulated cream when it cools down to 40°C. The formulated cream will be used for further experiments.

For physical parameters were followed by Donglikar and Deore method with minor change²².

Thermal stability. The formulated cream was tested at 60-70% RH and 37.0 ± 1.0°C room. The thermal cycle was freeze-thaw (0°C to -4°C and room temperature) for 4 cycles. To pass the test, there should not be separation oil or liquidity in the cream.

pH determination. Formulated cream might have a variety of pH, mostly ranging from 5 to 9. In general, it has a pH 6 to 9. The formulated creams were diluted to 10% dilution with distilled water. The ranging pH of mixtures was determined with a pH meter.

Viscosity. Viscosities of creams were measured by the Brookfield viscometer (Applied Scientific Instruments Co., Ltd., Dial reading viscometer, Thailand). The right spindle was selected (spindle no. 4) for the given product then the operating condition was set up. Then the viscosity was measured directly at 6 rpm speed by keeping the torque constant.

4.8 Statistical analysis

Each experiment was performed in triplicate. All values are presented as a mean value (mean ± SD). The statistically significant differences between the means of the samples were calculated by one-way ANOVA. The differences were considered as significant at a level of $p < 0.05$ (*).

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