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Effect of germination on antioxidant, anti-inflammatory and keratinocyte proliferation of rice

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Abstract

The aim of this study was to investigate the impact of germination on the amount of bioactive compound, antioxidant, anti-inflammatory and keratinocyte proliferation of three rice varieties: one non-pigmented rice (Hom mali 105; HM) and two pigmented rice (Mun pu; MP and Leum phua; LP rice). The phenolic and procyanidin content were evaluated and the antioxidant activities were investigated using FRAP, DPPH and SOD assays. Germinated rice showed a trend for higher amounts of bioactive compounds and antioxidant activities than ungerminated rice. The highest phenolic content was found in germinated Leum phua (LP-G) rice extract (0.11±0.00 mg GAE/ml extract). While germinated Mun pu (MP-G) rice extract showed the highest procyanidin content with 0.13±0.00 mg CE/ml extract. LP-G rice extract had the highest activities with FRAP (0.51±0.01 mg AAE/ml extract) and DPPH (70.97±1.15%) as well as increased enzymatic antioxidant SOD activity (51.40±1.07%). The potential anti-inflammation and cell proliferation promoting of the extracts were investigated on normal adult human primary epidermal keratinocyte cells (NHEK). LP-G extract exhibited the best keratinocyte proliferation promoting (29.29±0.47%). Moreover, germinated rice extracts, especially pigmented rice (MP-G and LP-G) showed anti-inflammation activity by suppressing nitric oxide production. The results revealed that the germinated rice extracts can be used as a natural source of bioactive compound with antioxidant, anti-inflammation and keratinocytes' proliferation properties which high potential for use in cosmetics, functional foods and pharmaceuticals.

Introduction

Free radicals are unpaired electron molecules that can be generated in a wide variety of chemical and biological systems (Halliwell, 2001). At high concentrations they can damage and adversely modify cell components that cause oxidative stress (Halliwell, 2001; Sharma *et al*., 2012), which has been implicated in the pathogenesis of numerous diseases including inflammation, cancer, neurological disorders, atherosclerosis, hypertension, diabetes, asthma and aging (Birben *et al.*, 2012). Excess free radicals can be neutralized by antioxidants such as natural bioactive phytochemicals, phenolic compounds, flavonoids, vitamin C, vitamin E, superoxide dismutase (SOD) (Sen and Chakraborty, 2011).

Keratinocytes are major epidermis cells and play an important role in healing wounds (Pastar *et al*., 2008). Numerous investigations demonstrated that promoted keratinocyte proliferation greatly enhanced the wound healing process (Nasca *et al*., 1999; Braun *et al.*, 2006) and affected skin ageing and, particularly, ageing induced by oxidative stress and chronic inflammation (Jenkins, 2002; Chung *et al*., 2009). One inflammatory reaction, pro-inflammatory cytokine led to expression of nitric oxide synthases (NOS) in many cells and it synthesized large amounts of nitric oxide (NO) that acted as a pro-inflammatory mediator (Sharma *et al*., 2007). Thus, the inhibition of NO level can indicate the anti-inflammation activity.

Rice (*Oryza sativa* L.) is one of the most consumed cereals and has high phytochemical contents such as ferulic acids, anthocyanins, procyanidins, γ-oryzanols, vitamin E homologues and etc. (Nam *et al*., 2006; Chung and Shin, 2007; Yawadio *et al*., 2007; Finocchiaro *et al.*, 2010). These phytochemicals are powerful antioxidants (Ichikawa *et al.*, 2001; Hu *et al.*, 2003) which reduce atherosclerotic plaque formation (Ling *et al.*, 2001), inhibit aldose reductase activity (Yawadio *et al.*, 2007), decrease hyperlipidemia (Guo *et al.*, 2007) and suppress cancer cell proliferation (Nam *et al.*, 2005). In addition, the germination process improved general functionality of rice seed. During this process, the chemical compositions of rice changed dramatically, because the biochemical activity produced essential compounds and energy for the

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formation of seedling (Moongngarm and Saetung, 2010). The level of nutrients and bioactive compounds showed a significant improvement after germination of γ-aminobutyric acid, phenolic acid, ferulic acid, tocotrienols, γ-oryzanol, magnesium, potassium, zinc and dietary fiber (Kayahara *et al*., 2001; Tian *et al*., 2004; Yodpitak *et al.*, 2013). In addition, germination can increase antioxidant activity (Frias *et al*., 2005), which is a potential alternative in preventing diabetes (Imam *et al.*, 2012), cancer (Oh and Oh, 2004; Latifah *et al*., 2010), obesity (Ho *et al*., 2012) and neurodegenerative disease (Ismail *et al*., 2012; Soi-ampornkul *et al*., 2012).

In this study, the change of phenolic and procyanidin contents were evaluated amongst the ungerminated and germinated rice seed extracts of a non-pigmented rice variety (Hom mali 105, HM) and two pigmented rice varieties (one red rice; Mun pu, MP and one black rice; Leum phua, LP). Their antioxidant capacities were determined both non-enzymatic (ferric reducing power and DPPH radical scavenging) and enzymatic antioxidant (superoxide dismutase activity). Their anti-inflammation activity was determined by nitric oxide assay. Moreover, their promoting skin cell growth was investigated by using primary human keratinocyte cells.

Materials and Methods

Materials and chemicals

The rice samples in this study were collected in Northern Thailand. There were three rice varieties including Hom mali 105 (HM, non-pigmented rice) and Mun pu rice (MP, red rice) from Chiang Rai Province and Leum phua rice (LP, black rice) from Chiang Mai Province, Thailand. Ethanol, sulfuric acid and trichloroacetic acid were purchased from Merck, Germany. 1,1-diphenyl-2-picrylhydrazyl (DPPH), catechin, folin-ciocalteu reagent, gallic acid, lipopolysaccharide (LPS), phehenylmethanesulfonyl, sodium carbonate, Tris/HCl, triton x-100, vanillin and SOD assay Kit-WST were purchased from Sigma-Aldrich Co., USA. Ferric chloride and potassium ferricyanide were purchased from Fisher scientific, USA. Dimethyl sufoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Bio Basic Inc., Canada. Fetal bovine serum, normal adult human primary epidermal keratinocyte cells (NHEK), medium 154 (sterile liquid medium prepared with 200 μM calcium chloride for the culture of human epidermal keratinocytes), penicillin streptomycin solution, phosphate buffered saline (PBS) and typsin/EDTA solution were purchased from Gibco, USA.

Germination of rice samples

Rice seeds were germinated according to method of Tung and Serrano (Tung and Serrano, 2011). Briefly, dehusked rice seeds (HM, MP and LP) were surfaced-sterilized with 70% Ethanol. The rice seed samples were placed on three layers of moist filter paper (3 ml distilled water) in Petri dish, three replicates. The Petri dishes were incubated at room temperature and maintain moisten condition until almost rice seeds were germinated radicles over 1 mm (about 3 days). The germinated rice seed were collected for extraction.

Extraction of rice samples

Our preliminary experiment have been studied the effect of extracting solvents on bioactive compounds of pigmented rice samples (MP and ND). Various polarity solvents including acetone, distilled water, ethanol and propylene glycol were used as extracting solvents. The results showed that ethanol was the highest total phenolic and procyanidin contents. Therefore, ethanol was chosen as extraction solvent in this study. A portion (1 g) of the rice seed samples (ungerminated and germinated rice) were extracted with 10 ml of ethanol with sonication for two hours. Then, the extracted solutions were filtered with Whatman® filter paper no.1 and stored at 4°C until analyzed.

Determination of total phenolic content (TPC)

The total phenolic content was analyzed using the Folin-Ciocalteu assay (Gajula *et al.*, 2009). Briefly, the extracts (20 μl) were mixed with 50 μl of deionized water, 20 μl of Folin-Ciocalteu reagent and 125 μl of 7% sodium carbonate. The mixtures were subsequently incubated at room temperature for 90 minutes. The absorbance was measured at 750 nm using a microplate reader (Biochrom, USA). The results expressed as gallic acid equivalents (mg GAE/ml extract).

Determination of total procyanidin content (TCC)

The total procyanidin content was quantified using the Vanillin assay (Nakamura *et al*., 2003). In brief, the extracts (20 μl) were mixed with 100 μl of 1% vanillin in 75% sulfuric acid. The mixture was incubated for 15 minutes at room temperature and the absorbance was determined at 500 nm by using the microplate reader (Biochrom, USA). A standard curve for TCC was developed using catechin as a standard compound. The results were expressed as catechin equivalent (mg CE/ml extract).

Ferric reducing power assay (FRAP)

The ferric reducing power was determined according to the ferric reducing power method (Takashi and Toshihiro, 2009). The extracts (25 µl) were mixed with 50 µl of 1% potassium ferricyanide and stood for 60 minutes at room temperature. Trichloro acetic acid (25 µl) and deionized water (75 µl) were added and the absorbance was measured at 700 nm using the microplate reader (Biochrom, USA) as absorbance 1 (A1). Then, 25 µl of 0.1% ferric chloride was added and the absorbance measured at 700 nm again as absorbance 2 (A2). The optical density (OD) of sample was calculated using the following equation:

$$OD = (A2 - A1)_{sample} - (A2 - A1)_{control}$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample. The reducing power activity was determined using the ascorbic acids calibration curve. The results were expressed as ascorbic acids equivalent (mg AAE/ml extract).

DPPH radical scavenging activity assay (DPPH)

The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was determined according to Rangkadilok *et al.* (2007). The reaction mixture containing 5 µl of pigmented rice extract and 195 µl of 0.1 mM of DPPH solution was incubated at room temperature for 30 minutes. Absorbance was measured at 515 nm using the microplate reader (Biochrom, USA). The scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample.

Cell culture

Human primary epidermal keratinocyte cells (NHEK) were cultured in medium 154 supplemented with 10% fetal bovine serum and 1% penicillin streptomycin solution. Cells were cultured until reaching 90 % confluence before being used for the next analysis. Our preliminary study of cytotoxicity of the extracts on NHEK was performed by MTT assay. The concentration of the extract that causes a 50% reduction in NHEK viability was determined and found to be 0.125 mg/ml (data not shown). This concentration of the extract samples was chosen for conducting the analysis of superoxide dismutase, nitric oxide inhibition and keratinocytes' proliferation promoting activities.

Superoxide dismutase activity

NHEK (2,000 cells/well) were incubated at 37°C in a 5% CO_2 humidified incubator for 24 hours. Then, the cells were fed with 100 µl of sample solution to obtain final concentration at 0.125 mg/ml and incubated for 24 hours. Then, cells were harvested with 0.05% typsin solution. Cells were lysed with lysis buffer (20 mM Tris/HCl, 0.5 mM phehenylmethanesulfonyl and 0.2% triton x-100) and centrifuged at 3,000 rpm for 15 minutes. The SOD activity in supernatants was measured by using SOD assay Kit-WST (Sigma-Aldrich, USA). Briefly, the 20 µl of supernatant was added with 200 µl of WST working solution and 20 µl of enzyme working solution. Then, the mixture was incubated at 37°C for 20 minutes and measured absorbance at 450 nm using a microplate reader (Biochrom, USA). The SOD activity was calculated using the following equation:

$$\text{SOD activity (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample.

Nitric oxide assay

NHEK (2,000 cells/well) were supplemented with 100 µl of sample solution (final concentration of 0.125 mg/ml) before being stimulated with 1 µg/ml lipopolysaccharide (LPS) and incubated for 24 hours. The nitric oxide production was assessed using Griess reagent system (Promega, USA). In brief, the culture medium (50 µl) was combined with 50 µl of sulfanilamide solution and 50 µl of 0.1% N-1-napthylethylenediamine dihydrochloride solution. Then, the mixture was incubated for 5 minutes at room temperature and measured absorbance at 540 nm using a microplate reader (Biochrom, USA). The amount of nitrite in the samples was determined using a sodium nitrite standard curve and the percentage of nitric oxide inhibition was calculated as follows:

$$\text{Inhibition of nitric oxide (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample.

Keratinocytes' proliferation promoting

Keratinocytes' proliferation promoting was determined by modified method of Takahashi *et al.* (2009). The sample solutions were added to NHEK (2,000 cells/well), which the concentration of the extract in the mixture was 0.125 mg/ml and incubated for 72 hours at 37°C in a 5% CO_2 humidified incubator. Then, the culture medium was removed. The 50 µl of filtered sterilized MTT solution (0.1 mg/ml) was added and incubated for 4 hours. At the end of incubation, 100 µl of dimethylsulfoxide was added and incubated for a further 30 minutes. The absorbance was measured at 570 nm by using the microplate reader (Biochrom, USA). The keratinocyte proliferation was calculated according to the following equation:

$$\text{Keratinocytes' proliferation (\%)} = [(A_{sample} - A_{control})/ A_{control})] \times 100$$

Where, $A_{control}$ is the absorbance of the control (without extract) and A_{sample} is the absorbance in the presence of the test sample.

Statistical analysis

All measurements were performed in triplicate. SPSS program version 21 (SPSS Inc, Chicago, IL, USA) was used for all statistical analyses in this study. The differences were considered significant when $P < 0.05$. The comparison of mean values between germinated rice varieties were analyzed by using One Way Analysis of Variance (ANOVA). The mean values of ungerminated and germinated rice seed of each rice varieties were analyzed by paired-samples t-test.

Results and Discussion

Total phenolic content

The bioactivities of phenolic compounds have been widely studied and confirmed to be beneficial to humans. They have potential for antioxidant, antibacterial, antiviral, anti-inflammatory and anti-allergenic activities and reduce the risk of cancer, heart disease and diabetes (Yao *et al.*, 2004; Cevallos-Casals and Cisneros-Zevallos, 2010). A previous study reported that the major phenolic compounds in ungerminated rice were ferulic acid and p-coumaric acid, while the most abundant phenolic in germinated rice was ferulic acid and sinapinic acid (Tian *et al*., 2004).

In this study, TPC was determined by following a modified Folin-Ciocalteu reagent method and the results were expressed as gallic acid equivalents (Figure 1). The highest TPC was germinated Leum phua (LP-G) with 110.44 µg GAE/ml extract followed by germinated Mun pu (MP-G) with 56.06 µg GAE/ml extract). Results showed that pigmented rice extract had significantly higher TPC than non-pigmented rice extract. Pigmented rice (MP and LP) had an average of 43.68 and 83.25 µg GAE/ml extract for ungerminated and germinated rice extracts, respectively. Whereas, non-pigmented rice (HM) had an average of 21.55 and 29.39 µg GAE/ml extract for ungerminated and germinated rice extracts, respectively. This finding supports a previous study about phenolic compounds in rice being mainly associated with the pericarp color (Tian *et al*., 2004; Zhou *et al*., 2004; Walter and Marchesan, 2011).

Moreover, the germination process significantly increased TPC with germinated MP and LP rice extracts increasing 2 times and 1.8 times, respectively. Germinated Hom mali (HM-G) extract slightly increased TPC about 1.5 times, compared to its ungerminated rice extract. The TPC increasing in germinated rice may have been due to the decomposition of the cell wall during germination, which affected both soluble and insoluble phenolics. Tian *et al.* (2004) reported that sinapinic acid increased nearly 10 times and insoluble phenolics (ferulic acid and p-coumaric acid) increased about 1-2 times after germination of brown rice samples.

Total procyanidin content

Procyanidins are a subclass of phenolics found in commonly consumed foods that have attracted increasing attention due to their potential health benefits (Hammerstone *et al*., 2000). They have potent antioxidant, antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic and vasodilatory actions (Fine, 2000).

In this study, TCC was determined by using a vanillin assay and the results were expressed as catechin equivalents (Figure 1). Ungerminated rice extracts had an average of 88.10 - 112.00 µg CE/ml extract, while germinated rice extracts had an average of 73.73 - 128.26 µg CE/ml extract. MP rice extracts had a significantly higher TCC than other extract samples in both ungerminated and germinated rice extracts. The correlation between TPC and TCC of MP rice extracts had a very high positive correlation ($r = 0.964$), which indicated that MP rice extracts had procyanidin as the major phenolic compounds. These results are in agreement with the study of Oki (2002) which reported that red rice had higher procyanidin than other colored rice.

However, the germination of MP rice had a slightly enhanced TCC (about 1.1 times). HM-G

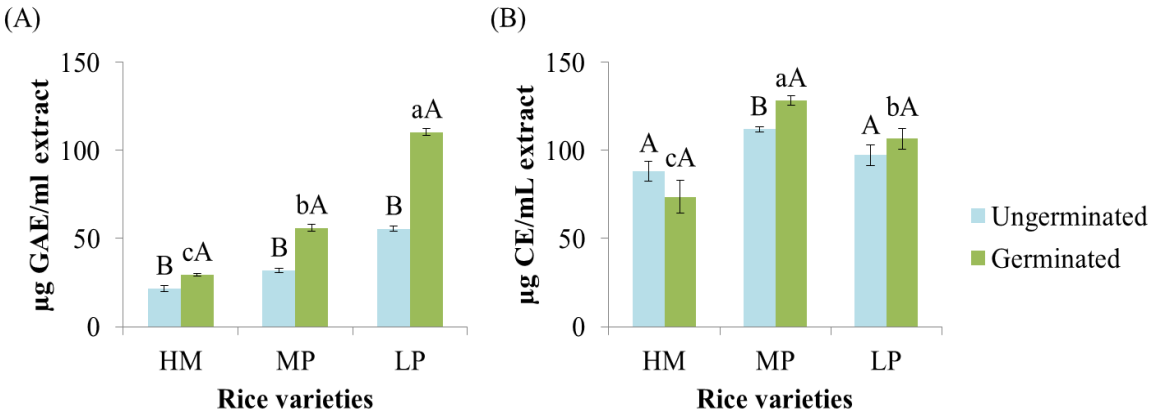

Figure 1. Total phenolic (A) and procyanidin (B) contents of ungerminated and germinated rice. Values are presented as means ± SD (n = 3). Data with different upper-case letters indicate significant differences ($p < 0.05$) between ungerminated and germinated rice of the same varieties, while those with different lower-case letters indicate significant differences ($p < 0.05$) between germinated rice varieties.

extract had a slightly decreased TCC, while LP-G extract did not show a significantly higher TCC than its ungerminated rice extracts. These results indicated that the germination process was not enhanced the procyanidin content.

Antioxidant activities by the FRAP and DPPH methods

The antioxidant activities of the extracts were measured by two different methods: ferric reducing power (FRAP) and DPPH radical scavenging activity assay (DPPH). Both assays were utilized in plant activity screening, presumably on the assumption that a combination of the data would provide a better description of antioxidant activity than data obtained from a single assay (Clarke *et al*., 2013). The FRAP method is based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}), whereas the DPPH method provide on a hydrogen atom donating ability (Alam *et al*., 2013).

As shown in Figure 2, the highest FRAP and DPPH were LP-G (514.13 µg AAE/ml extract and 70.97%). It also showed that the pigmented rice extracts displayed good antioxidant activities in both FRAP and DPPH methods. As reported in numerous previous studies, pigmented rice had higher antioxidant activities than non-pigmented rice which could be identified by high phenolic content (Nam *et al*., 2005; Nam *et al*., 2006; Jun *et al*., 2012; Lum and Chong, 2012; Saikia *et al*., 2012).

The antioxidant activities of germinated rice extracts were higher than those of the ungerminated rice extracts. It was found that germination significantly increased FRAP levels by about 2.0 times in HM and LP rice and 1.3 times in MP rice. Meanwhile, the levels of DPPH were significantly increased about 1.6 times in HM rice and 1.2 times in MP and LP rice. This result is in agreement with Mohd Esa *et al.* (2013) who reported that germinated rice has higher antioxidant activities than ungerminated rice.

Moreover, the correlation of TPC and antioxidant activities showed a high positive correlation with FRAP ($r = 0.986$) and DPPH ($r = 0.835$). Thus, the increased phenolic content in germinated rice has potentially higher antioxidant activities. While the correlation between TCC and antioxidant activities showed a very low correlation.

Antioxidant enzyme activity by superoxide dismutase activity method

An imbalance between free radical production and antioxidant levels leads to oxidative stress as observed with the lowered activity of the antioxidant enzymes such as superoxide dismutase (SOD) (Mohd Esa *et al*., 2013). SOD is a primary antioxidant enzyme which protects organisms against the toxic effect of superoxide radicals (O_2^-) by catalyzing their desmutation to hydrogen peroxide (H_2O_2) and oxygen (O_2) (Pieme *et al*., 2010).

The SOD activity of the extracts was shown in Figure 2. The highest SOD activity was germinated rice extracts of pigmented rice, LP (51.40%) and MP (33.57%). This finding which was in agreement with Chiang *et al.* (2006) showed black rice extract had significantly higher SOD activity than non-pigmented rice extract.

Interestingly, the germination could significantly increase SOD activity about 3.0 times higher than that of ungerminated HM and LP rice extracts and 1.3 times higher than that of ungerminated MP rice extract. These results showed that the germination

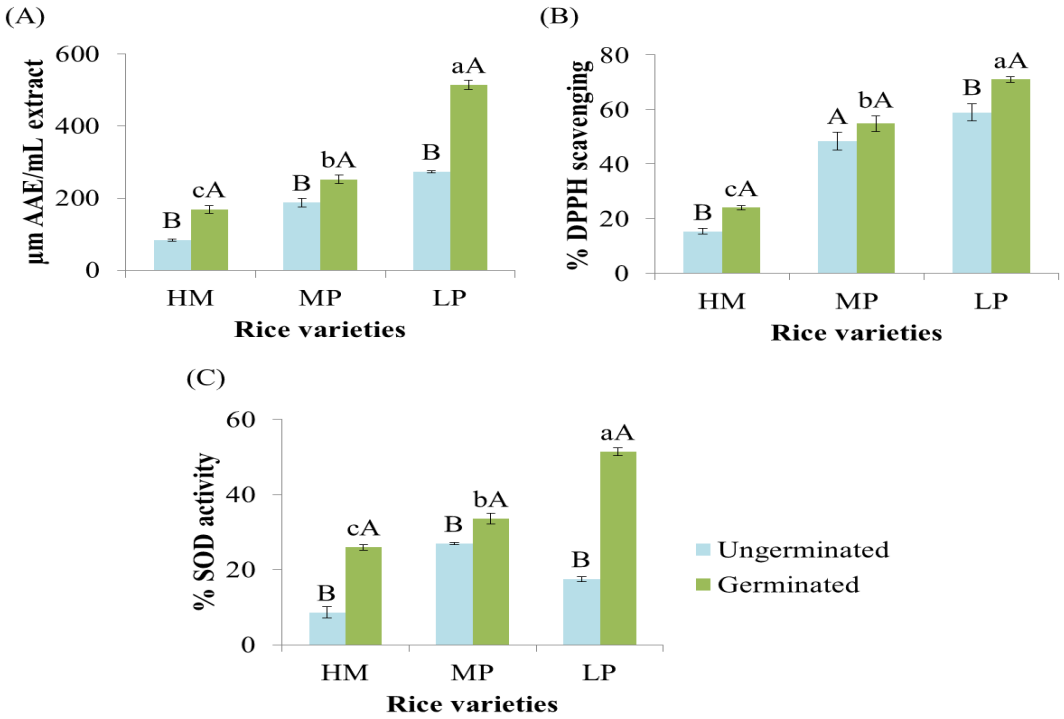

Figure 2. Ferric reducing power (A), DPPH radical scavenging activity (B), and superoxide dismutase activity (C) of ungerminated and germinated rice. Values are presented as means ± SD (n = 3). Data with different upper-case letters indicate significant differences ($p < 0.05$) between ungerminated and germinated rice of the same varieties, while those with different lower-case letters indicate significant differences ($p < 0.05$) between germinated rice varieties.

process enhanced SOD activity of rice. In addition, the high positive correlation between SOD and other antioxidant assays (r = 0.870 and 0.719 with FRAP and DPPH, respectively) was established. Also, there was a high positive correlation between TPC and SOD (r = 0.844), indicating that phenolic compounds may be the major chemical constituent that provide SOD activity.

Nitric oxide assay

Nitric oxide (NO) is involved in various types of inflammatory disorders by acting as an anti-inflammatory agent under normal physiological conditions. On the other hand, the overproduction of NO is considered a pro-inflammatory mediator and under abnormal conditions induces inflammation (Debnath *et al*., 2013). In this study, anti-inflammation activity of the extracts was determined by using Griess reagent.

The results of NO inhibition of the extracts were shown in Figure 3. Pigmented rice extracts had significantly higher NO inhibition than non-pigmented rice extracts. The highest NO inhibition was LP-G (64.96%) and MP-G (63.06%), followed by HM-G (45.31%) extracts. While NO inhibition of ungerminated HM, MP and LP extracts was 24.18, 36.38 and 31.47%, respectively. It can be seen that germination significantly increased NO inhibition levels nearly two times more than ungerminated rice. According to these results, germinated pigmented rice appeared to be potent as an anti-inflammatory agent. The phytochemical compounds in rice have been reported as having anti-inflammation action such as γ-oryzanols (Saenjum *et al*., 2012), tricin (Shalini *et al*., 2012), triterpene alcohols and sterols (Akihisa *et al*., 2000). The anti-inflammation activity of germinated rice may be due to the presence of these compounds which could lead to investigating the molecular mechanisms of anti-inflammatory action in a further study.

Keratinocytes' proliferation promoting

Keratinocytes are the major cell type in the epidermis and have a critical role in the complex process of wound healing (Pastar *et al*., 2008). This is characterized by three orderly but overlapping phases: the inflammation phase, the cell proliferation phase and the remodeling phase (Diegelmann and Evans, 2004). The keratinocytes' proliferation promoting activity is used to prove it is a source of material for wound healing and anti-aging mechanisms.

The keratinocytes' proliferation promoting of the extracts was shown in Figure 3. LP-G was the highest keratinocytes' proliferation with 29.29%, followed by MP-G (21.20%) and HM-G (19.69%) extracts. Whereas, keratinocytes' proliferation of

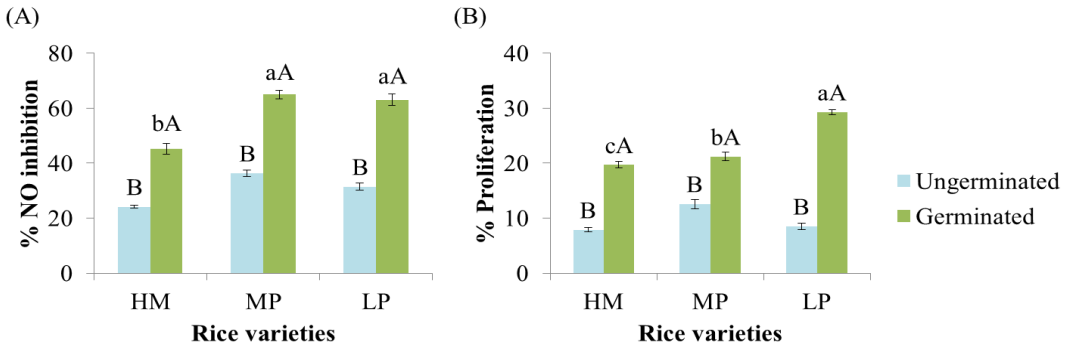

Figure 3. The anti-inflammation activity (A) and keratinocytes' proliferation promoting (B) of ungerminated and germinated rice. Values are presented as means ± SD (n = 3). Data with different upper-case letters indicate significant differences ($p < 0.05$) between ungerminated and germinated rice of the same varieties, while those with different lower-case letters indicate significant differences ($p < 0.05$) between germinated rice varieties.

ungerminated HM, MP and LP extracts was 7.87, 12.61 and 8.52%, respectively. In this study, it was demonstrated that both ungerminated and germinated rice seeds can promote the growth of keratinocytes. Ungerminated extracts showed no significant difference in keratinocytes' proliferation between pigmented rice and non-pigmented rice. Whereas, germination of pigmented rice extracts were significantly higher in keratinocytes' proliferation than germination of non-pigmented rice extract.

Moreover, the germinated rice extracts had a significantly higher promoting effect than ungerminated rice extract. Especially interesting is that germination could increase keratinocytes' proliferation in HM, MP and LP rice about 2.5, 1.7 and 3.4 times more, respectively, than their ungerminated rice extracts. There was a very high positive correlation between TPC and keratinocytes' proliferation among the germinated rice extracts ($r = 0.973$); in contrast, the correlation between TPC and keratinocytes' proliferation among the ungerminated rice extracts was very low ($r = -0.100$). These results suggested that the germination process produced some non-phenolic bioactive compounds which had an effect on the proliferation of keratinocyte cells. Many studies reported that the germination of rice could induce the formation of bioactive compounds such as γ-oryzanols, tocopherol, tocotrienol and gamma-aminobutyric acid (GABA) (Ng *et al*., 2013; Wu *et al*., 2013; Lin *et al.*, 2015; Cho and Lim, 2016) which these compounds may be contribute to keratinocytes' proliferation.

Conclusion

Rice is a source of nutrition with beneficial health properties. Pigmented rice, especially, contains high concentrations of phenolic compounds. The germination process can increase phenolic and activities in all rice samples. LP-G extract had the highest TPC, antioxidant activities, FRAP, DPPH and SOD assays and keratinocytes' proliferation promoting. Whereas MP-G extract had the highest TCC and NO inhibition. Pigmented rice extracts (MP and LP) had significantly higher bioactives, antioxidant and anti-inflammatory activities than non-pigmented rice (HM). Overall, the results showed that germination of pigmented rice extract enhanced antioxidant, anti-inflammatory and keratinocytes' promoting activities. Thus, the germinated rice extracts, especially pigmented rice, were a natural source with potential for use as an active ingredient in cosmetics, functional foods and pharmaceuticals. Further research is necessary to explore the exact anti-inflammatory mechanisms and efficacy on skin cells of germination of pigmented rice extract.

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