Evaluation of Antioxidant and Anti-Aging Activities of Thai Rice Germ Extracts

Leakkhaing Taing[1], Jittima Tomacha[2], Piya Prajumwongs[2], Nisana Namwat[2,3], Poramate Klanrit[2,3], Arporn Wangwiwatsin[2,3], Anchalee Techasen[2], Hasaya Dokduang[4], Bundit Promraksa[5], Nattha Muangritdech[2] and Watcharin Loilome[2,3,*]

[1]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Cholangiocarcinoma Research Institute, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Department of System Biosciences and Computational Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[4]Faculty of Medicine, Mahasarakham University, Mahasarakham 44150, Thailand
[5]Regional Medical Sciences Center 2 Phitsanulok, Department of Medical Sciences, Ministry of Public Health, Phitsanulok 65000, Thailand

(*Corresponding author's e-mail: watclo@kku.ac.th)

Abstract

Aging in humans is a natural process characterized by the accumulation of oxygen-derived free radicals, leading to cellular and tissue damage and aging-related diseases. Bioactive compounds from plants, known for their antioxidant properties, can mitigate the effects of aging by scavenging these free radicals. The present study aimed to explore the antioxidant and anti-aging properties of commercial rice germ extracts. Fifteen types of extensively commercial rice germ (RG) products from Thailand were coded as RG1 - RG15. These products were assessed for phenolic content and antioxidant activity. The rice germ with the highest phenolic content and antioxidant activity was further investigated for anti-aging properties and endogenous antioxidant activities (SOD, CAT and GPx). Additionally, protein expressions related to longevity were analyzed using western blot analysis. Among the 15 RG products, RG1 exhibited the highest total phenolic content (21.07 ± 0.54 mg GAE/g dry weight) and significant antioxidant activities in ORAC, FRAP and DPPH scavenging assays, showing approximately 748.22 ± 20.30 µmol Trolox/g dry weight, 3.82 ± 0.10 mg GAE/g dry weight and 75.10 ± 0.19 % inhibition of free radicals, respectively, compared to RG2 - RG15. Consequently, RG1 was selected for further anti-aging and *in vitro* studies. The anti-aging assays demonstrated that rice germ extract significantly inhibited collagenase, elastase and hyaluronidase in a dose-dependent manner (62.5 - 500 µg/mL). The extract also enhanced endogenous antioxidant activities (SOD, CAT and GPx) in H_2O_2-treated cells in a dose-dependent manner. Moreover, RG1 extract induced the expression of longevity-related proteins such as AMPK, FoxO and SIRT, while reducing the expression of mTOR and Akt proteins. Rice germ extract has high phenolic content and provides antioxidant and antiaging activities. In addition, rice germ can inhibit aging-related enzymes via inducing endogenous antioxidants and the longevity-related protein expression.

Keywords: Phytochemicals, Rice germ extract, Antioxidant, Anti-aging, Longevity

Introduction

Aging is the biological process of becoming older, characterized by a progressive decline in physiological function and an increased susceptibility to age-related diseases. It is a complex phenomenon influenced by various genetic, environmental and lifestyle factors. As individuals age, they experience changes at the cellular, tissue and organ levels, leading to a gradual deterioration in overall health and functional capacity [1]. Longevity refers to the duration or lifespan that a living organism can survive, which is distinct from the

biological aging process. Previous studies have reported an association aging-related diseases and the lifespan of organisms. The longevity of an organism is influenced by functional genes and the molecular mechanisms of aging process [2]. Previous studies have reported an association aging-related diseases and the lifespan of organisms. The longevity of an organism is influenced by functional genes and the molecular mechanisms of aging [3]. Generally, aging, which shortens lifespan, can be attributed to metabolic changes, obesity, type 2 diabetes, neurodegenerative factors and decreased mitochondrial activity. The widely accepted theory that aging is primarily caused by reactive free radical species suggests these radicals damage biomolecules such as DNA, proteins and lipids, accelerating aging and causing phenotypic changes [4]. Therefore, delaying the aging process by balancing free radical levels induced by oxidative stress is essential for prolonging lifespan. Intracellular antioxidants act as scavengers of these free radicals, slowing mutation and cell senescence rates. However, intracellular antioxidants decrease while free radical species increase with age. Consequently, supplementing with exogenous antioxidants is necessary to maintain this balance. Research on antioxidants, particularly secondary metabolites from plants, has confirmed that active phytochemical compounds can enhance health and extend lifespan. Therefore, exploring supplementary nutrition from natural products is of great interest, as their components may help eliminate aging factors and extend lifespan [5].

Phytochemicals, which are bioactive compounds found in plants, have garnered attention for their diverse potential uses, particularly due to their antioxidant properties believed to enhance health benefits like slowing aging and promoting overall well-being. Besides their nutritional value, there's a growing interest in incorporating phytochemicals into pharmaceutical research [6]. Given that aging is influenced by genetic, lifestyle, and environmental factors, the study of phytochemical extraction relating to longevity is caught up the interest [7]. Consequently, there's a significant focus on selecting foods supplemented with these compounds to enhance human health. The purpose of using such supplements is to improve health, prevent diseases or aid in rehabilitation. These supplements can be sourced from plant-based materials such as fruits,

vegetables, grains and even parts like peels, roots or embryos [8]. Among sources of those supplements, rice as the major daily dietary, is broadly used since the past to present. Rice contained vital phytochemical compounds grouped in phenolic, flavonoids, terpenoids, alkaloids and others. These metabolic compounds benefit for plant growing and eliminating the insect or microbial. Rice also contains antioxidant, anticytotoxicity and anti-inflammatory properties which can be used to improve well-being properties [9-11]. In the essential part of rice, rice germ, also referred to as rice embryo, is the small component of rice that is often separated during processing. Despite its small size compared to the rest of the rice grain, research has revealed the presence of numerous phytochemical compounds in the rice germ. Additionally, it has been found to possess potent antioxidant properties, surpassing other parts of the rice grain in this regard [12,13]. The rice germ extract has shown significant antioxidant capacity, highlighting the importance of the biological properties present in the extract [13]. Several studies focusing on the antioxidant and anti-inflammatory properties of rice germ and bran have demonstrated their ability to scavenge free radicals and exhibit a strong correlation with anti-inflammatory effects as found in *in vitro* [14] and *in vivo* [15-17] experiments. In the context of medical research, Chaiyasut *et al.* [15] revealed that supplementation with germinated Thai black rice extract has shown promising results in diabetic rats. It exhibited antioxidant properties and improved antioxidant enzyme levels in the treated rats. Furthermore, germinated Thai black rice extract led to reduce blood glucose, cholesterol and triglyceride levels. The germinated rice germ also demonstrated a positive impact on insulin resistance and glucose tolerance in the diabetic rats. Furthermore, rice germ is rich in potential secondary metabolites which have been recognized for their antioxidant, anti-inflammatory and their role as functional dietary nutrients that promote animal health [12,13] such as gamma-oryzanol [18-21], alpha-tocopherol [22-24] and gammaaminobutyric acid (GABA) [25,26]. Given its potential to enhance endogenous enzymatic activity and act as a free radical scavenger, rice germ is considered a suitable dietary supplementation option for daily consumption. It not only helps protect biomolecules from damage but also aids in disease prevention.

Despite its promising attributes, the anti-aging properties of rice germ extract and its molecular mechanisms related to longevity remain underexplored. This study investigates the antioxidant capacities of 15 different varieties of Thai rice germ extracts and identifies the variety with the highest activity for further analysis. The highlights of this research are the identification of a high-antioxidant rice germ extract, its potential anti-aging effects and the exploration of molecular mechanisms that may contribute to longevity. By focusing on these extracts, this research aims to elucidate their potential anti-aging effects and the underlying mechanisms contributing to longevity, offering insights into the broader applications of rice germ in health promotion and disease prevention.

Materials and methods
Rice germ collection/Preparation

The fifteen commercial rice germ products were online purchased and the supplements contained in each rice germ products are indicated in supplementary data, respectively. Fifty g of rice germ powder was boiled with 1,000 mL of distilled water at 93 °C. The mixture was stirred using a magnetic stirrer for 5 min. The rice germ extract was then filtered through filter paper (Merck, Cytiva Life Science, UK) to remove any residues. The filtered extract was subsequently evaporated and lyophilized using a freeze dryer (Labconco/FreeZone 4.5, Labconco Corporation, USA) to obtain a concentrated water extract. The dried rice germ extract was stored at −20 °C for use in further experiments (**Figure 1**).
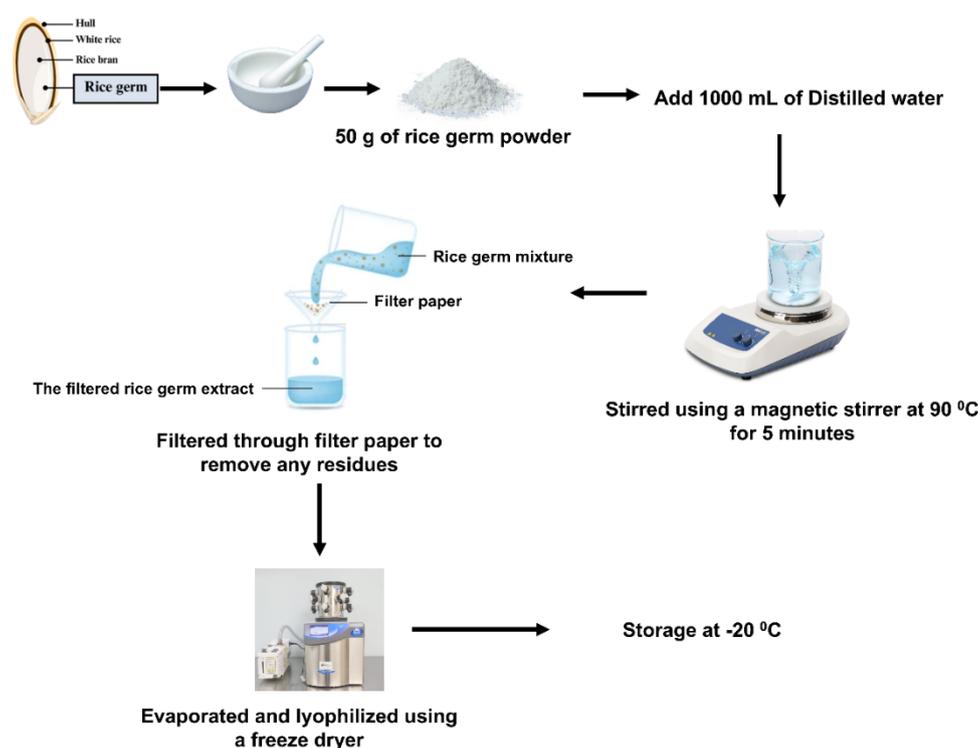
Figure 1 Preparation and extraction of rice germ extract.

Total phenolics content

The total phenolic content of rice germ extracts was determined by the Folin-Ciocalteu method. The experiment was adapted from a previous study [27,28]. Briefly, 20 μL of rice germ were mixed with 40 μL of 10 % (w/v) Folin-Ciocalteu reagent (Sigma-Aldrich, St Louis, MO, USA) and incubated with light protection for 30 min. Then, 160 μL of 700 mM Na_2CO_4 was added to the mixture and incubated for 2 h at room temperature with the light protection. Subsequently, the mixture was measured absorbance utilizing a microplate reader at 765 nm against a standard graph for gallic acid (Sigma-Aldrich, St Louis, MO, USA). Total phenolic contents were presented as micrograms of gallic acid equivalents per mg of dry weight (weight) (μg GAE/mg).

Ferric reducing antioxidant power (FRAP) assay

To evaluate total antioxidant activity in rice germ extracts, FRAP method was performed using modified methods of Benzie and Strain [27]; Promraksa *et al.* [28]. Briefly, Fresh FRAP reagent was prepared by mixing 300 mM sodium acetate buffer pH 3.6, 10 mM 2,4,6-Tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, St Louis, MO, USA) in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ at a ratio of 10:1:1. Then 100 µL of the rice germ extract were mixed with 100 µL of FRAP reagent, and incubated at room temperature for 5 min. The absorbance at 593 nm was measured. The standard gallic acid was performed as a calibration curve. Antioxidant activity was calculated as milligrams gallic acid equivalent per g dry weight (µg GAE/mg dry weight).

Radical scavenging activity using DPPH assay

The DPPH radical scavenging ability method was modified from previous studies of Benzie and Strain [27]; Promraksa *et al.* [28]. In a brief, the 80 µL of rice germ extract were mixed with 120 µL of 200 µM DPPH reagent (Sigma-Aldrich, St Louis, MO, USA) into a 96-well plate, and gently shaking for 2 min. Then the mixture was incubated at room temperature for 30 min with light protection. After reaction of the DPPH free radical with the extract, a microplate reader (Tecan, Switzerland) was applied absorbance at 517 nm to measure the DPPH radical scavenging ability. The measurement of the free radical scavenging was done in triplicate and the calculation was performed [27,28].

Oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity was applied to obtain the capacity of rice germ antioxidants that can inhibit the oxygen free radical species. The ORAC assay was performed according to a previous method by Cao and Prior [29]. The fluorescein solution (Sigma Aldrich, St Louis, MO, USA) was prepared with 10 mM potassium phosphate buffer (pH 7.4) to a final concentration of 10 nM. Afterward, 25 µL of sample solution and 150 µL of fluorescein solution were added to the black bottom 96 well plate, which was kept at room temperature for 15 min followed by 25 µL of 240 mM 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (Thermo Fisher Scientific, UK). The absorption measurements were recorded every 2 min for 2 h with 538 nm as the emission wavelength and 485 nm as the excitation wavelength. Trolox (Sigma-Aldrich, St Louis, MO, USA) was performed as standard for calibration curve. Results were expressed as micromole of Trolox equivalent (TE) per g of dry weight (µmol TE/g dry weight).

Collagenase inhibition assay

The collagenase inhibitory assay was performed followed the steps in collagenase activity assay kit (Sigma-Aldrich, St Louis, MO, USA), as previously described [30,31]. Briefly, 2 µL of different concentration of rice germ extracts and 12 µL of DI water (blank) were added into 96 well plate. Then 10 µL of 0.35 µ/mL collagenase solution were added into each tested well, except the negative control wells (blank). Mixture was incubated at 37 °C for 15 min and then, the reaction was started by adding 100 µL of the reaction buffer (containing the collagenase buffer 60 µL and 40 µL of collagenase substrate, FALFPA,) into each well. The kinetic reaction was measured immediately at 345 nm for 15 min and the percent collagenase activity inhibition was calculated.

Elastase inhibition assay

The procedure of that elastase inhibitory assay was performed by modifying according to the manufacturer's instructions (Sigma-Aldrich, St Louis, MO, USA) and previous studies by Thring *et al.* [30]; Widowati *et al.* [31]. A mixture of 20 µL of various concentration of rice germ extracts and 200 µL of 1 mM N-suc-(Ala)3-nitroanilide (SANA), which was prepared in 0.1 M Tris-HCl buffer pH 8.0, was added into 96 well plate. The mixed solutions were vortexed and pre-incubated for 10 min at room temperature. Then 20 µL of elastase from porcine pancreas (Sigma 45124, USA) (0.5 mU/mL in the cool aquades) was added into mixture. Finally, mixture solutions were incubated at 25 °C for 10 min. Optical density was detected at 410 nm and percent elastase inhibition activity was calculated.

Hyaluronidase inhibition assay

Hyaluronidase inhibitory assay was determined by modifying of Sigma Aldrich and previous study [30,31]. Twenty-five µL of various concentration of rice germ extracts were pre-incubated with 3 µL of bovine

hyaluronidase (H3506, Sigma-Aldrich, St Louis, MO, USA) for 10 min at 37 °C. Then 12 μL phosphate buffer (300 mM, pH 5.35) was added and incubated for 10 min at 37 °C. Afterward 10 μL hyaluronic acid substrate (Sigma H5542, USA) was added and incubated for 45 min at 37 °C. Decomposition reaction of hyaluronic acid was inhibited after adding 100 μL acidic albumin acid. Mixture solution was incubated at room temperature for 10 min, then absorbance was measured at 600 nm wavelengths. Then, the percent hyaluronidase inhibition activity was calculated.

Cell line and culture condition

A human dermal fibroblasts (HDFs) cell line was obtained from the American Type Culture Collection (ATCC). Fibroblast cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Wako Pure Chemical Industries, Ltd., Osaka, Japan) supplemented with 10 % fetal bovine serum (FBS) (HyClone Laboratories, Inc., Logan, UT, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Thermo Fisher Scientific, Grand Island, NY, USA). Cells were incubated in a humidified incubator at 37 °C and 5 % CO_2. At approximately 70 - 80 % confluence, cells were detached from the culture flask using 0.25 % w/v trypsin/EDTA and processed according to the particular assays.

Endogenous antioxidant assays

To determine endogenous antioxidants including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), the measurement methods were modified according to the manufacturer's instructions. Briefly, approximately 2×10^6 fibroblast cells were incubated at optimal concentration of rice germ extract for 24 h. Then cells were treated with H_2O_2 for 2 h. After treatment, fibroblast cells were gathered by trypsinization and centrifuged at 4 °C, 15,000 rpm for 15 min. Thereafter, the collected cells were lysed using the RIPA cell lysis buffer and centrifuged at 4 °C, 15,000 rpm for 5 min. Finally, supernatant of cell lysate was collected and performed to determine endogenous antioxidant activities by measuring SOD (ab65354, Abcam, United Kingdom), GPx (ab102530, Abcam, United Kingdom) and CAT (ab83464, Abcam, United Kingdom) using the commercial kits.

Antibodies

Antibodies to β-actin, sirtuin (SIRT), AMP-activated protein kinase (AMPK), Forkhead box O3 (FoxO3), the mechanistic target of rapamycin (mTOR), Protein kinase B (PKB) or also known as Akt, were purchased from Abcam, United Kingdom. HRP-linked goat-anti-rabbit IgG (7074) and horse-anti-mouse IgG (7076) were purchased from Cell Signaling Technology (Danvers, MA, USA), respectively.

Western blot analysis

Fibroblast cells were treated with various concentrations of rice germ extracts and incubated for 48 h. The viable cells were gathered after treatment and then washed by phosphate buffer (cold buffer) saline followed by the lysis buffer, basically used RIPA lysis buffer (0.1 % SDS, 0.5 % sodium deoxycholate, Tris-HCl, pH 7.4 and 1 % Tween-20) composing protease inhibitor cocktail (Nacalai Tesque, Tokyo, Japan). After that, protein concentrations were determined using the bicinchoninic acid (BCA) protein assay (Thermo Science, Rockford, IL). Ten μg of protein were separated by 10 % (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then proteins on the gel were transferred onto a PVDF membrane (GEHealthcare Japan, Tokyo, Japan) by western blotting assay. The PVDF membrane was probed with primary antibodies including rabbit antiFoxO1 Ab (1:1,000), mouse-anti-SITR1 Ab (1:1,000), mouse-anti-AMPK Ab (1:1,000) and rabbit-anti mTOR Ab (1:1,000) and mouse anti-actin Ab (1:10,000). Horseradish peroxidase conjugated secondary antibodies were further incubated. Targeted proteins were detected using of Enhanced Chemiluminescent reagents (ELC) and protein band were visualized and captured with an ImageQuantTM Image (GE Healthcare UK, Ltd., Uk). The β -actin antibody was used as an internal loading control. Band intensity of protein was determined by ImageJ software.

Statistical analysis

All of the experiments were performed in 3 independent replications. Data were presented as mean ± standard deviation. Statistical comparisons between groups were tested using the student's T- test. The data were analyzed using GraphPad Prism® 7.0 software (GraphPad software, Inc., San Diego, CA, USA) and

SPSS 23 software (SPSS, Chicago, IL, USA). A probability level of *p*-value < 0.05 was used and considered statistically significant.

Results and discussion

   Total phenolic contents

   Total phenolic content (TPC) in 15 commercial rice germ extracts were shown in **Figure 2**. The result showed that rice germ extracts contained significantly different amount of total phenolic compounds. The RG1 extract had the highest total phenolic content, with a value of 21.07 ± 0.54 mg GAE/g dry weight, followed by the RG10 extract, which had 18.66 ± 0.89 mg GAE/g dry weight. The lowest total phenolic compounds were found in RG13, followed by RG15, RG14, RG9 and RG7, with values of 4.58 ± 0.13, 5.22 ± 0.15, 5.41 ± 0.36, 5.62 ± 0.39 and 6.69 ± 0.43 mg GAE/g dry weight, respectively.
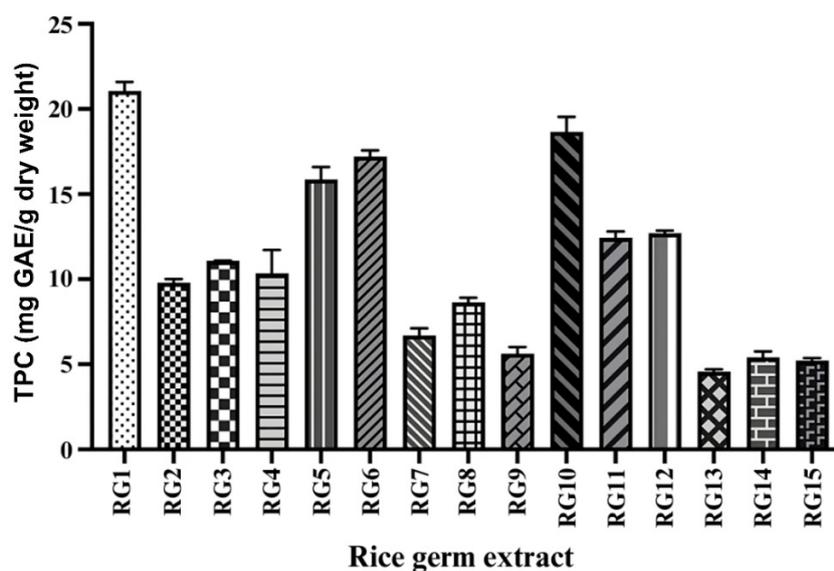
Figure 2 Total phenolic contents of 15 commercial rice germ extracts.

   Antioxidant activity of rice germ extracts

   To evaluate the antioxidant capacity, ferric ion reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and DPPH radical scavenging analysis were performed. The results were shown in **Table 1**. In FRAP analysis, the highest value was RG10, followed by RG1 and RG12 with a value of 3.95 ± 0.80, 3.82 ± 0.10 and 3.71 ± 0.03 mg GAE/g dry weight, respectively. For ORAC analysis, RG1 had the high value of oxygen radical absorbance capacity to 748.22 ± 20.30 μmol Trolox/g dry weight, followed by RG10 and RG12 having values in order of 746.25 ± 28.67 and 744.91 ± 38.98 μmol Trolox/g dry weight. In addition, DPPH radical scavenging ability showed that RG1 demonstrated the greatest inhibition of the DPPH radical by 75.10 ± 0.19 %, subsequently, RG12 and RG2 having a value of free radical inhibition with 73.77 ± 0.30 and 72.07 ± 0.37 %, respectively. In terms of antioxidant assessment, RG1 exhibited the highest activity compared to other rice extracts. The correlation between higher TPC levels and increased antioxidant activity is supported by Parikh and Patel [32], who suggested that plants with high phenolic content tend to have significant antioxidant activities. This apparent discrepancy between TPC levels and antioxidant activities can be explained by factors beyond the phenolic content alone. Previous studies, such as those by Gülçin *et al.* [33] have indicated that antioxidant activities may also be influenced by compounds other than phenolics. Furthermore, the chemical structure of phenolic compounds, particularly the number and position of hydroxyl groups, plays a crucial role in determining their antioxidant properties. Such structural attributes can enhance or reduce the ability of phenolic compounds to scavenge free radicals, contributing to the observed variations in activity. Additionally, the methods used to measure antioxidant activity may influence the results, as different assays target distinct

mechanisms of antioxidant action [34]. Despite these variations, the health benefits of phenolic compounds as antioxidants are well-documented. In this study, RG1, which had the highest phenolic content, was selected for experiments related to longevity, highlighting the potential of phenolic compounds with specific structural features in promoting health benefits.

**Table 1** Antioxidant activities of 15 commercial rice germ extracts.

No.	RG code	FRAP assay (mg GAE/g dry weight)	ORAC analysis (µmol Trolox/g dry weight)	% DPPH inhibition
1	RG1	$3.82 \pm 0.10^*$	748.22 ± 20.30	$75.10 \pm 0.19^*$
2	RG2	3.11 ± 0.06	656.76 ± 40.15	72.07 ± 0.37
3	RG3	3.22 ± 0.15	593.22 ± 12.01	70.80 ± 0.19
4	RG4	2.96 ± 0.10	682.49 ± 48.21	44.85 ± 0.49
5	RG5	2.88 ± 0.15	733.13 ± 14.23	21.46 ± 1.74
6	RG6	2.73 ± 0.06	700.96 ± 39.98	51.00 ± 1.18
7	RG7	2.13 ± 0.10	443.49 ± 0.80	48.87 ± 0.20
8	RG8	2.68 ± 0.09	508.69 ± 42.83	58.36 ± 0.84
9	RG9	1.89 ± 0.09	331.88 ± 8.62	45.12 ± 3.00
10	RG10	3.95 ± 0.80	$746.25 \pm 28.67^*$	66.36 ± 0.84
11	RG11	3.28 ± 0.20	695.69 ± 11.17	47.85 ± 1.78
12	RG12	3.71 ± 0.03	744.91 ± 38.98	73.77 ± 0.30
13	RG13	1.63 ± 0.01	305.45 ± 26.84	35.78 ± 0.64
14	RG14	1.78 ± 0.13	411.04 ± 19.93	43.07 ± 0.60
15	RG15	1.85 ± 0.09	347.99 ± 26.95	44.36 ± 1.27

Note: Data are expressed as mean ± SD. Data with different superscripts (*) was the highest value.

Anti-aging Assays: Collagenase, elastase and hyaluronidase inhibitions

Given the antioxidant properties linked to anti-aging, RG1 was further analyzed for its inhibition of aging-related enzymes, namely collagenase, elastase and hyaluronidase. The concentrations of rice germ extract including 6.25, 125, 250 and 500 µg/mL were conducted in the experiments. The percentage inhibitions of rice germ extract were shown in a dose-dependent manner to inhibit 3 enzymes. At the highest concentration (500 µg/mL) showed strong inhibitory activities to collagenase, elastase and hyaluronidase enzymes with 45.4 ± 1.26, 40.19 ± 1.88 and 21.70 ± 2.15 %, respectively (**Figure 3**).
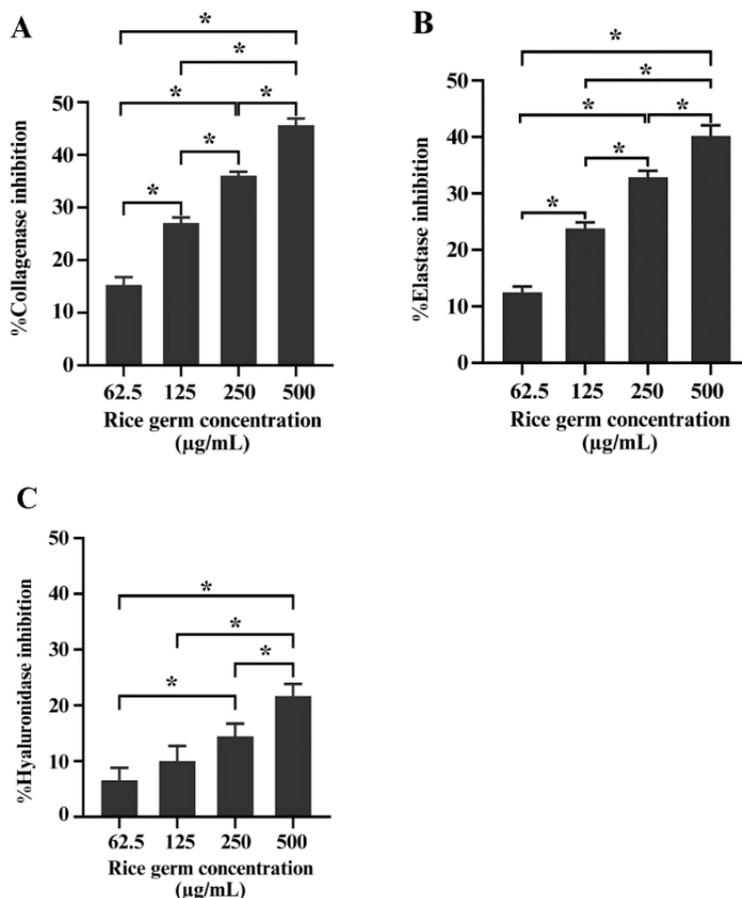
Figure 3 The anti-aging assay; (A) collagenase, (B) elastase, and (C) hyaluronidase inhibitions.

Anti-aging tests with different concentrations of RG1 extract showed that the higher the RG1 extract added, the higher the inhibition of enzyme activity related to aging, namely collagenase, elastase and hyaluronidase. A previous study using rice extract demonstrated similar results, although the inhibition levels of collagenase, elastase and hyaluronidase (IC50 at 816.78, 107.51 and 203.13 µg/mL, respectively) were lower compared to the present study where the highest concentration of RG1 extract (500 µg/mL) resulted in inhibition percentages of 45.64, 40.19 and 21.70 %, respectively. In antioxidant assessments using the DPPH assay, the IC50 value of rice extract was 314.51 µg/mL, whereas the RG1 extract at 26.4 µg/mL exhibited a DPPH inhibition value of 75.10 %. This suggests that RG1 extract may have greater anti-aging potential than rice extract, possibly due to its antioxidant activities. Concordant with previous study by Jiratchayamaethasakul *et al.* [35] on 22 plant extracts found varying percentages of anti-aging enzyme inhibition, with some extracts showing no capacity to inhibit these enzymes. The study suggested that plants with high DPPH scavenging activity, such as Rosa rugosa, might be more effective in inhibiting aging-related enzymes than those with low DPPH activity. This supports the finding that RG1 extract, with its high DPPH scavenging activity, can significantly inhibit aging-associated enzymes.

We further investigated whether the rice germ extract, which had high antioxidant ability could provide anti-aging activities by determining the endogenous antioxidants such as SOD, CAT and GPx and longevity-related proteins such mTOR, Akt, FoxO, Sirtuin and AMPK, respectively.

Rice germ extract induced antioxidant mediated via endogenous enzymes

This study aims to evaluate whether rice germ extract can activate endogenous antioxidant enzymes. The result revealed that rice germ extract could significantly induce SOD, CAT and GPx actives in a dose-dependent manner when compared their vehicle

control (**Figure 4**). Generally, reactive oxygen species (ROS) are generated as by-products of various intracellular redox processes. The endogenous enzymes including SOD, CAT and GPxs play a crucial role in directly eliminating the oxidative stress caused by ROS [36]. The trigger of the external stimuli could provide the activities of endogenous antioxidants [37,38]. The present study showed that the RG1 extract exhibited the dose-dependent manner of rice germ extract effect on the endogenous antioxidants, SOD, CAT and GPx

induction. This result is concordant with previous studies. They found that different types of rice extracts - white, brown and germinated - yielded varying results in the expression of endogenous antioxidants. Germinated rice extracts showed higher levels of SOD, CAT and GPx compared to white and brown rice extracts. This suggests that the rice germ in germinated rice might activate or enhance endogenous antioxidant activities [39,40].
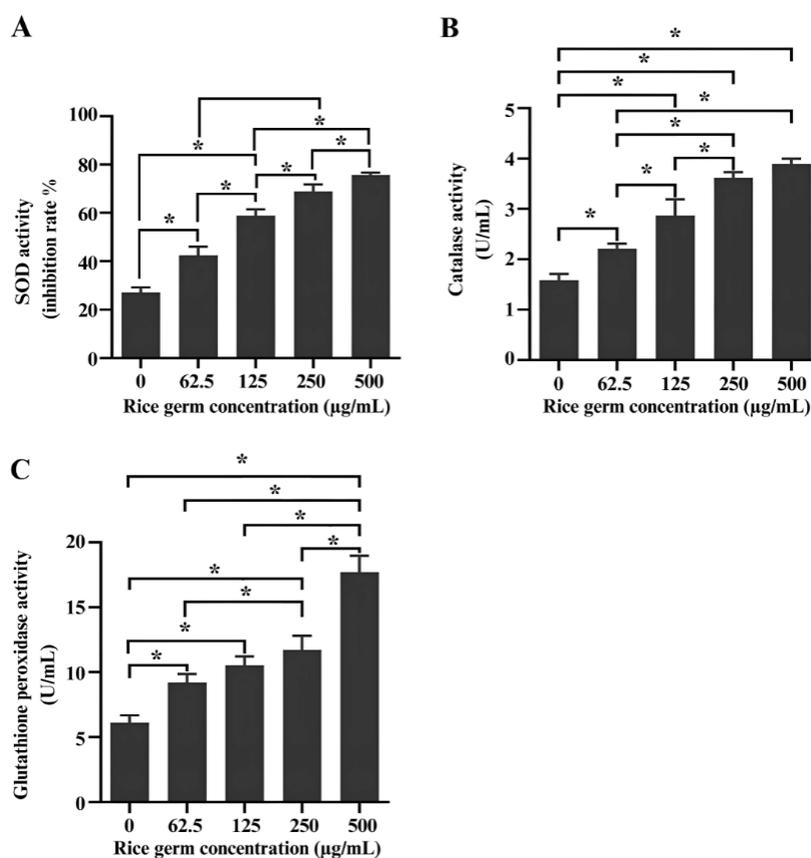

Figure 4 Quantification of endogenous enzyme actives in fibroblast cell line treated or not with germ rice extract exposed to H_2O_2, (A) SOD activity, (B) catalase activity, and (C) GPx activity.

Antioxidative effect of rice germ extract induced antiaging via longevity-related proteins

Following the antioxidant and anti-aging experiments with rice germ extract, the expression of longevity-related proteins was analyzed to validate the molecular mechanism of anti-aging through antioxidant activity using western blot analysis. SIRT, AMPK FoxO3, mTOR and Akt were set of proteins which related to cellular longevity. Firstly, Fibroblast cells

were pre-incubated with rice germ extract, followed by treated with H_2O_2. The western blot result revealed that the expression of SIRT, AMPK and FoxO3 were increased when fibroblast treated increase concentration of rice germ extract (0 - 500 µg/mL), while mTOR and Akt were decreased expression (**Figure 5**). This result could be explained by caloric restriction (CR) and nutrient-responsive pathways to regulate longevity signaling [41,42].
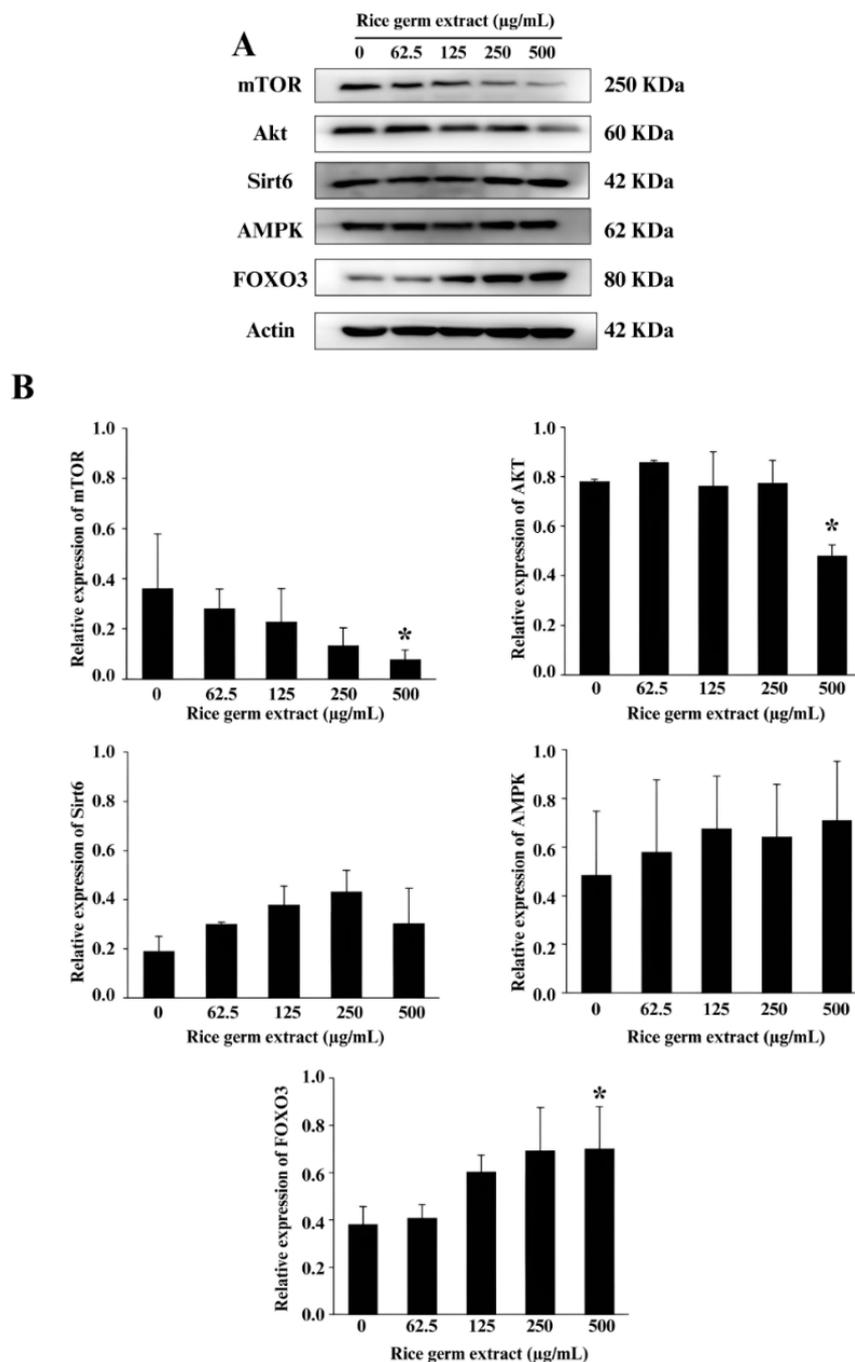
Figure 5 The expression of longevity-related proteins after treated with rice germ extract in H_2O_2-treated cells; (A) western blot result and (B) relative expression of longevity-related proteins.

Caloric restriction (CR) is known to extend lifespan by modulating several cellular pathways that influence longevity. CR activates key nutrient sensors, including sirtuins (SIRTs) and AMPK, while downregulating mTOR. These proteins help regulate stress responses, autophagy and inflammation, promoting cellular resilience and longevity [43]. SIRTs, NAD^+-dependent deacetylases, influence insulin regulation, fat storage, glucose metabolism and inflammation, notably by inhibiting NF-κB, a mediator of inflammation. AMPK, activated under nutrient scarcity, inhibits mTOR, which otherwise drives anabolic processes. Studies with rapamycin, an mTOR inhibitor, show extended lifespan and reduced age-related weight gain in animal models [44].

Under nutrient-rich conditions, insulin/IGF-1 signaling activates pathways like Akt, which stimulates mTOR. Inhibiting mTOR with rapamycin in models like Drosophila and mice extends lifespan by slowing cellular growth and inflammation [45,46]. RG1 extract treatment in fibroblasts reduced Akt and mTOR levels, suggesting it may extend cellular lifespan through similar mechanisms. Additionally, FOXO proteins, which are involved in aging and longevity [47,48], and SIRT1 has been shown to extend the lifespan of mice on a standard diet compared to those on standard or high fat diets alone, likely due to delayed aging and SIRT activation [49]. In the present study, fibroblasts treated with varying concentrations of rice germ extract showed increased expression of FoxO3, Sirt6 and AMPK at higher concentrations. This implies that rice germ extract might enhance fibroblast lifespan.

Conclusions

In summary, rice germ extract shows promising anti-aging potential through its antioxidant properties, inhibition of aging-associated enzymes and modulation of longevity-related pathways. It's diverse health benefits make it a valuable candidate for further research and development as a dietary supplement. Future research should start with preclinical animal studies to evaluate safety, efficacy and optimal dosage. Upon successful outcomes, clinical trials in humans should follow to assess long-term safety and effectiveness. Additionally, investigating the synergistic effects of rice germ extract with other antioxidants could further enhance its potential benefits.

Acknowledgements

References

[1] T Flatt. A new definition of aging? *Frontiers in Genetics* 2012; **3**, 148.

[2] RB McDonald and RC Ruhe. Aging and longevity: Why knowing the difference is important to nutrition research. *Nutrients* 2011; **3(3)**, 274-282.

[3] M Niso-Santano, RA Gonzalez-Polo, M Paredes-Barquero, JM Fuentes and M Aschner. Natural products in the promotion of healthspan and longevity. *Clinical Pharmacology and Translational Medicine* 2019; **3(1)**, 149-151.

[4] C Forni, F Facchiano, M Bartoli, S Pieretti, A Facchiano, D D'Arcangelo, S Norelli, G Valle, R Nisini, S Beninati, C Tabolacci and RN Jadeja. Beneficial role of phytochemicals on oxidative stress and age-related diseases. *BioMed Research International* 2019; **2019(1)**, 8748253.

[5] AJ Ding, SQ Zheng, XB Huang, TK Xing, GS Wu, HY Sun, SH Qi and HR Luo. Current perspective in the discovery of anti-aging agents from natural products. *Natural Products and Bioprospecting* 2017; **7(5)**, 335-404.

[6] A Gadnayak and B Dehury. *Phytochemicals: Recent trends in food, pharmacy, and biotechnology. In*: S Pati, T Sarkar and D Lahiri (Eds.). Recent frontiers of phytochemicals. Elsevier, Amsterdam, Netherlands, 2023, p. 85-93.

[7] RCG Correa, RM Peralta, CWI Haminiuk, GM Maciel, A Bracht and I Ferreira. New phytochemicals as potential human anti-aging compounds: Reality, promise, and challenges. *Critical Reviews in Food Science and Nutrition* 2018; **58(6)**, 942-957.

[8] M Timm, LC Offringa, BJ van Klinken and J Slavin. Beyond insoluble dietary fiber: Bioactive compounds in plant foods. *Nutrients* 2023; **15(19)**, 4138.

[9] C Thepthanee, CC Liu, HS Yu, HS Huang, CH Yen, YH Li, MR Lee and ET Liaw. Evaluation of phytochemical contents and *in vitro* antioxidant, anti-inflammatory, and anticancer activities of black rice leaf (*Oryza sativa* L.) extract and its fractions. *Foods* 2021; **10(12)**, 2987.

[10] N Saji, N Francis, LJ Schwarz, CL Blanchard and AB Santhakumar. The antioxidant and anti-inflammatory properties of rice bran phenolic extracts. *Foods* 2020; **9(6)**, 829.

[11] T Pattananandecha, S Apichai, S Sirilun, J Julsrigival, K Sawangrat, F Ogata, N Kawasaki, B Sirithunyalug and C Saenjum. Anthocyanin

profile, antioxidant, anti-inflammatory, and antimicrobial against foodborne pathogens activities of purple rice cultivars in Northern Thailand. *Molecules* 2021; **26(17)**, 5234.

[12] A Moongngarm, N Daomukda and S Khumpika. Chemical compositions, phytochemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. *APCBEE Procedia* 2012; **2**, 73-79.

[13] SS Smuda, SM Mohsen, K Olsen and MH Aly. Bioactive compounds and antioxidant activities of some cereal milling by-products. *Journal of food science and technology* 2018; **55(3)**, 1134-1142.

[14] S Mapoung, W Semmarath, P Arjsri, P Thippraphan, Srisawad K, Umsumarng S, K Phromnoi, S Jamjod, C Prom-u-Thai and P Dejkriengkraikul. Comparative analysis of bioactive-phytochemical characteristics, antioxidants activities, and anti-inflammatory properties of selected black rice germ and bran (*Oryza sativa* L.) varieties. *European Food Research and Technology* 2023; **249(2)**, 451-464.

[15] C Chaiyasut, BS Sivamaruthi, N Pengkumsri, W Keapai, P Kesika, Saelee M, P Tojing, S Sirilun, K Chaiyasut, S Peerajan and N Lailerd. Germinated Thai black rice extract protects experimental diabetic rats from oxidative stress and other diabetes-related consequences. *Pharmaceuticals* 2016; **10(1)**, 3.

[16] SF Pasaribu, B Wiboworini and LR Kartikasari. Effect of germinated black rice krisna extract on fasting blood glucose and body weight in diabetes mellitus rats. *International Journal of Nutrition Sciences* 2021; **6(4)**, 194-200.

[17] P Aimvijarn, W Payuhakrit, N Charoenchon, S Okada and P Suwannalert. Riceberry rice germination and UVB radiation enhance protocatechuic acid and vanillic acid to reduce cellular oxidative stress and suppress B16F10 melanogenesis relating to F-actin rearrangement. *Plants* 2023; **12(3)**, 484.

[18] S Yu, ZT Nehus, TM Badger and N Fang. Quantification of vitamin E and γ-oryzanol components in rice germ and bran. *Journal of Agricultural and Food Chemistry* 2007; **55(18)**, 7308-7313.

[19] YH Cho, SY Lim, A Rehman, M Farooq and DJ Lee. Characterization and quantification of γ-oryzanol in Korean rice landraces. *Journal of Cereal Science* 2019; **88**, 150-156.

[20] P Klongpityapong, R Supabphol and A Supabphol. Antioxidant effects of gamma-oryzanol on human prostate cancer cells. *Asian Pacific Journal of Cancer Prevention* 2013; **14(9)**, 5421-5425.

[21] K Srikaeo. *Organic rice bran oils in health*. *In*: RR Watson, VR Preedy and S Zibadi (Eds.). Wheat and rice in disease prevention and health. Academic Press, San Diego, 2014, p. 453-465.

[22] MJ Cloarec, GM Perdriset, FA Lamberdiere, JF Colas-Belcour, JP Sauzieres, HN Neufeld and U Goldbourt. Alpha-tocopherol: Effect on plasma lipoproteins in hypercholesterolemic patients. *Israel Journal of Medical Sciences* 1987; **23(8)**, 869-872.

[23] D Mafra, FR Santos, JC Lobo, D de Mattos Grosso, AL Barreira, LGC Velarde, DSP Abdalla and M Leite. Alpha-tocopherol supplementation decreases electronegative low-density lipoprotein concentration (LDL ($-$)) in haemodialysis patients. *Nephrology Dialysis Transplantation* 2009; **24(5)**, 1587-1592.

[24] I Sadowska-Bartosz and G Bartosz. Effect of antioxidants supplementation on aging and longevity. *BioMed Research International* 2014; **2014(1)**, 404680.

[25] V Warunee, T Patcharee, S Vipa, W Ladda and L Wang. Effects of water soaking on gamma-aminobutyric acid (GABA) in germ of different Thai rice varieties. *Agriculture and Natural Resources* 2005; **39(3)**, 411-415.

[26] M Koubaa, F Delbecq, S Roohinejad and K Mallikarjunan. *Gamma-aminobutyric acid*. *In*: L Melton, F Shahidi and P Varelis (Eds.). Encyclopedia of food chemistry. Academic Press, Oxford, London, 2019, p. 528-534.

[27] IFF Benzie and JJ Strain. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry* 1996; **239(1)**, 70-76.

[28] B Promraksa, J Phetcharaburanin, N Namwat, A Techasen, P Boonsiri and W Loilome. Evaluation of anticancer potential of Thai medicinal herb extracts against cholangiocarcinoma cell lines. *PloS One* 2019; **14(5)**, e0216721.

[29] G Cao and RL Prior. Measurement of oxygen radical absorbance capacity in biological samples. *Methods in Eenzymology* 1999; **299**, 50-62.

[30] TSA Thring, P Hili and DP Naughton. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine* 2009; **9**, 27.

[31] W Widowati, H Herdiman, M Afni, E Afifah, H Kusuma, H Nufus, S Arumwardana and D Rihibiha. Antioxidant and anti-aging assays of *Oryza sativa* extracts, vanillin and coumaric acid. *Journal of Natural Remedies* 2016; **16(3)**, 88-99.

[32] B Parikh and VH Patel. Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses. *Journal of Food Science and Technology* 2018; **55(4)**, 1499-1507.

[33] İ Gülçin, AC Gören, P Taslimi, SH Alwasel, O Kılıc and E Bursal. Anticholinergic, antidiabetic and antioxidant activities of Anatolian pennyroyal (*Mentha pulegium*)-analysis of its polyphenol contents by LC-MS/MS. *Biocatalysis and Agricultural Biotechnology* 2020; **23**, 101441.

[34] TM Djordjevic, SS Šiler-Marinkovic and SI Dimitrijevic-Brankovic. Antioxidant activity and total phenolic content in some cereals and legumes. *International Journal of Food Properties* 2011; **14(1)**, 175-184.

[35] C Jiratchayamaethasakul, Y Ding, O Hwang, ST Im, Y Jang, SW Myung, JM Lee, HS Kim, SC Ko and SH Lee. *In vitro* screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals. *Fisheries and Aquatic Sciences* 2020; **23**, 6.

[36] C Vives-Bauza, A Starkov and E Garcia-Arumi. Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. *Methods in Cell Biology* 2007; **80**, 379-393.

[37] O Nakchat, N Nalinratana, D Meksuriyen and S Pongsamart. Tamarind seed coat extract restores reactive oxygen species through attenuation of glutathione level and antioxidant enzyme expression in human skin fibroblasts in response to oxidative stress. *Asian Pacific Journal of Tropical Biomedicine* 2014; **4(5)**, 379-385.

[38] BT Tung, E Rodriguez-Bies, HN Thanh, H Le-Thi-Thu, P Navas, VM Sanchez and G López-Lluch. Organ and tissue-dependent effect of resveratrol and exercise on antioxidant defenses of old mice. *Aging Clinical and Experimental Research* 2015; **27(6)**, 775-783.

[39] NM Esa, KKA Kadir, Z Amom and A Azlan. Antioxidant activity of white rice, brown rice and germinated brown rice (*in vivo* and *in vitro*) and the effects on lipid peroxidation and liver enzymes in hyperlipidaemic rabbits. *Food Chemistry* 2013; **141(2)**, 1306-1312.

[40] F Wu, N Yang, A Touré, Z Jin and X Xu. Germinated brown rice and its role in human health. *Critical Reviews in Food Science and Nutrition* 2013; **53(5)**, 451-463.

[41] N Barzilai, DM Huffman, RH Muzumdar and A Bartke. The critical role of metabolic pathways in aging. *Diabetes* 2012; **61(6)**, 1315-1322.

[42] H Pan and T Finkel. Key proteins and pathways that regulate lifespan. *Journal of Biological Chemistry* 2017; **292(16)**, 6452-6460.

[43] M Yu, H Zhang, B Wang, Y Zhang, X Zheng, B Shao, Q Zhuge and K Jin. Key signaling pathways in aging and potential interventions for healthy aging. *Cells* 2021; **10(3)**, 660.

[44] JH Jessica, JHR Stéphane, I Ben-Sahra and DM Brendan. A growing role for mTOR in promoting anabolic metabolism. *Biochemical Society Transactions* 2013; **41(4)**, 906-912.

[45] P Kapahi, BM Zid, T Harper, D Koslover, V Sapin and S Benzer. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. *Current Biology* 2004; **14(10)**, 885-890.

[46] RA Miller, DE Harrison, CM Astle, E Fernandez, K Flurkey, M Han, MA Javors, X Li, NL Nadon, JF Nelson, S Pletcher, AB Salmon, ZD Sharp, S van Roekel, L Winkleman and R Strong. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* 2014; **13(3)**, 468-477.

[47] X Sun, WD Chen and YD Wang. DAF-16/FOXO transcription factor in aging and longevity. *Frontiers in Pharmacology* 2017; **8**, 548.

[48] S Du and H Zheng. Role of FoxO transcription factors in aging and age-related metabolic and

neurodegenerative diseases. *Cell & Bioscience* 2021; **11**, 188.

[49] JM Sarah, A Martin-Montalvo, MM Evi, HP Hector, MW Theresa, G Abulwerdi, RK Minor, GP Vlasuk, JL Ellis, DA Sinclair, J Dawson, DB Allison, Y Zhang, KG Becker, M Bernier and RD Cabo. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Reports* 2014; **6(5)**, 836-843.