Table 1: Experimental matrix, percent yields, and colors of the Thai Riceberry rice extracts

Run	Extracting condition		Percent yield (% w/w)	Extract color
	pH value	Spray-drying temperature (°C)		
R1	2	170	8.36±1.93	Deep reddish
R2	5.5	170	8.40±0.89	Light purple
R3	2	150	7.9±1.08	Deep reddish
R4	5.5	150	11.03±1.32	Light purple

citric acid (2% w/w) was a pH-adjusting agent. The percent yield and antioxidant activities of each extract were then evaluated and analyzed for determining the correlation.

DPPH assay

DPPH assay was performed following the modified method of Poomanee *et al*., [10]. The reaction mixture, containing extract solution and DPPH methanolic solution, was incubated at room temperature in the dark for 30 min. The absorbance was then determined at 517 nm. Free radical scavenging abilities of the extracts were expressed as 50% inhibitory concentration (IC_{50} value).

ABTS assay

ABTS radical scavenging properties of the extracts were also evaluated by the method of Poomanee *et al*. with some modification and expressed as Trolox equivalent antioxidant capacity and IC_{50} value [10]. The extract aqueous solutions were mixed with diluted ABTS solution in the ratio of 1:100 and incubated at room temperature for 6 min. The absorbance at 734 nm was measured.

The inhibitory effect on linoleic acid peroxidation

Linoleic acid peroxidation assay was carried out for determining the inhibitory effects of the extracts against lipid peroxidation following the method of Poomanee *et al*. and expressed as IC_{50} value [10]. To measure the amount of peroxyl radicals, ferric thiocyanate method was performed as the method of Poomanee *et al*. [11].

Reducing power

The reducing powers of the extracts were determined using ferric reducing antioxidant power (FRAP) assay and expressed as FRAP value and EC_1 value. FRAP reagent (2 ml), consisting of 10 mM TPTZ in 40 mM HCl solution, 300 mM Acetate buffer, and 20 mM $FeCl_3 \bullet 6H_2O$, was added with the extract aqueous solution (1 ml) and left for 30 min in the dark. The absorbance of each concentration at 593 nm was measured.

Anti-tyrosinase activity

Anti-tyrosinase activities of the extract representing an ability of depigmentation were evaluated through L-tyrosine pathway by the modified method of Poomanee *et al*. [11]. The effects were reported as IC_{50} value comparing to those of positive controls, including kojic acid, alpha-arbutin, and l-ascorbic acid. The absorbance of each concentration at 490 nm was measured.

TPC

Folin–ciocalteu's method was performed for measuring the TPCs of the extract offering equivalent concentration to that of Gallic acid equivalent. The absorbance at 765 nm was then measured [10].

Evaluation of the extract's stability profile

The extract, of which the greatest biological activities presented, was further evaluated for its stability profile under several storage conditions. The extract aqueous solution (0.5%) was stored at 4°C, room temperature with light, as well as without light, 45°C for 30 d and six cycles of heating-cooling (HC) condition (1 cycle: 4°C for 48 h and 45°C for 48 h). After storage, antioxidant and anti-tyrosinase activities were evaluated by ABTS and anti-tyrosinase assays, respectively, and compared to those of the initial. The alterations of color and pH value were also observed.

Statistical analysis

All experiments were done in triplicates, and all results were expressed as Mean ± standard deviation (S.D.). SPSS statistic 17.0 software was performed for statistically analyzing the results of antioxidant, anti-tyrosinase assays, and TPCs through using one-way ANOVA with multiple comparison test of Tukey. In the case of stability testing, a dependent t-test was carried out. p-value less than 0.05 ($p<0.05$) was considered as a statistical significance.

RESULTS AND DISCUSSION

Thai organic Riceberry broken rice, grown in Lampang Province, was extracted and evaluated for its anti-aging property in this study. Deionized water served as an extracting solvent herein since it is considered as the safest solvent, which potentially extracts polyphenols from plants [12]. The percent yield of each extract (as shown in Table 1) was not significantly different from the others. The difference in colors of the extracts was observed, which was following the pH value. At pH 2.0, deep reddish extract powder was obtained, while at pH 5.5, the extracted color was light purple.

Reactive oxygen species (ROS) or free radicals are ubiquitously considered as one of the important initiators of aging processes [13-15]. By virtue of ROS, oxidative stress, lipid peroxidation, inflammation, and DNA-base alteration are potentiated, which eventually generates several skin aging signs [16]. In this study, we, thus, evaluated free radical scavenging efficacies, reducing power, and inhibitory effects against lipid peroxidation of the extracts for representing its antioxidant and firstly establishing its anti-aging property. Table 2 showed that Riceberry extracts presented free radical scavenging effect and inhibitory effect against lipid peroxidation. Among all extracts, R3, by which the method of the spray-drying temperature of 150°C and pH 2.0 extracted, exhibited the greatest antioxidant activities. The results of DPPH and ABTS assays were also corresponding to those of linoleic acid peroxidation assay.

Besides, reducing the power of the extracts, evaluated by FRAP assay, presented similarly to their antioxidant properties. A higher FRAP value indicates the greater capacity to reduce ferric (Fe^{3+}) into ferrous (Fe^{2+}), whereas a lower EC_1 value means a lower concentration that can produce an equivalent reducing effect to that of 1 mM $FeSO_4$. Therefore, R3 showed the highest reducing power among all extracts, as shown in Fig. 1.

Furthermore, skin hyperpigmentation, causing melasma and pigmentation disorders in the elderly, is partially accelerated in the presence of free radicals since $NO^{\bullet}$ stimulates tyrosinase and tyrosinase-related protein 1, which are the main components for melanin production [13]. Four Riceberry rice extracts additionally exerted an anti-tyrosinase effect through the L-tyrosine pathway (Fig. 2). At a concentration of 3.125 mg/ml, R3 likewise showed the strongest effect. However, the IC_{50} value of R1, R2, R3, and R4 was 2.42±0.24, 2.30±0.06, 2.13±0.07, and 3.33±0.17 mg/ml, respectively, which were not significantly different between R1, R2, and R3. Our study firstly reported the IC_{50} value of riceberry rice extract against tyrosinase enzyme. Meanwhile, Teeranachaideekul *et al*. reported that hydroethanolic extract of riceberry rice exert weak anti-tyrosinase effect [4].

Phenolic compounds play a crucial role in a variety of beneficial effects, especially the antioxidant properties of the natural compounds [12,17]. Within pericarp of the purple rice, anthocyanins, and anthocyanidins (aglycone molecules), which were regarded as flavonoid derivatives, considered as one of the polyphenols, were thought to be fundamental constituents [6,7]. In a consequence of hydrogen donation, most of the polyphenols can neutralize free radicals as well as stop the lipid chain peroxidation [12]. Fig. 3 illustrated that R3 contained the highest TPC, which were in correspondence with the results of antioxidant, anti-tyrosinase effects, and reducing power. Moreover, TPCs of Riceberry rice extracts, reported in our study, were approximately 2-fold higher than the study of Luang-In *et al*. [5]. It is worth noting

Table 2: Antioxidant activities of the Thai riceberry broken rice extracts

Samples	DPPH assay	ABTS assay		Lipid peroxidation
	IC_{50} value (mg/ml)	IC_{50} value (µg/ml)	TEAC (mg Trolox/ g extract)	IC_{50} value (mg/ml)
R1	0.49±0.07[a]	100.52±8.10[b]	32.29±0.52[a,c]	0.58±0.07[b,c]
R2	0.56±0.05[a]	120.87±1.18[c]	31.81±1.63[a,c]	1.14±0.10[b]
R3	0.38±0.05[a]	70.06±5.37[a]	33.39±2.09[a]	0.14±0.01[a]
R4	0.53±0.01[a]	113.76±8.37[b,c]	28.81±1.23[c]	0.50±0.04[c]
Standards	(µg/ml)	(µg/ml)	(mg Trolox/g standard)	(mg/ml)
Trolox	5.37±0.69	-	-	0.09±0.02
L-ascorbic acid	29.93±0.39	2.26±0.54	838.38±60.62	0.12±0.04

Superscripts [(a,b,c)]Indicate significant differences between groups using One-way ANOVA with multiple comparison test of Tukey ($p<0.05$). ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, TEAC: Trolox equivalent antioxidant capacity

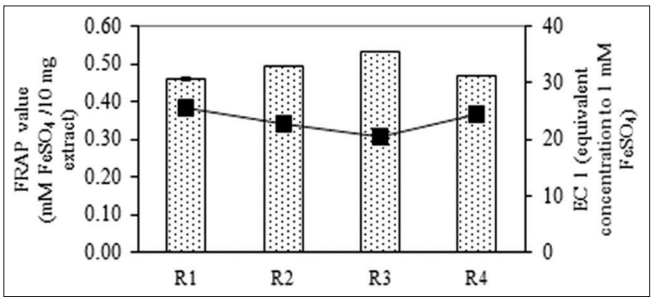

Fig. 1: Reducing power of the Thai Riceberry rice extracts

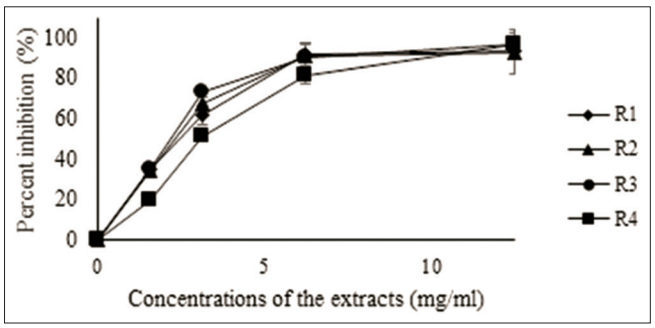

Fig. 2: Anti-tyrosinase effects of the Thai Riceberry rice extracts

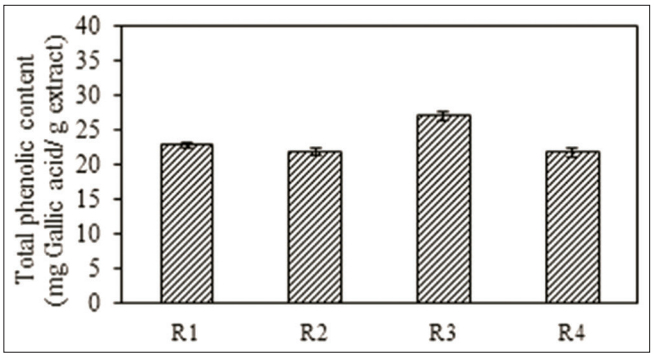

Fig. 3: Total phenolic contents of the Thai Riceberry rice extracts

that the extracting condition had a noticeable impact on the amount of anthocyanins and the biological effects of the extracts. At higher temperature (170°C), polyphenols tend to be degraded rather than at lower temperatures. Besides, acylation under acidic conditions could enhance the stability of the anthocyanin due to the molecular change from non-acylated anthocyanin into acylated anthocyanin [18]. In our study, citric acid served as an acylating agent due to its non-toxic manner [19]. Interestingly, acylation might additionally have an impact on the biological activities of the anthocyanins. The most adequate

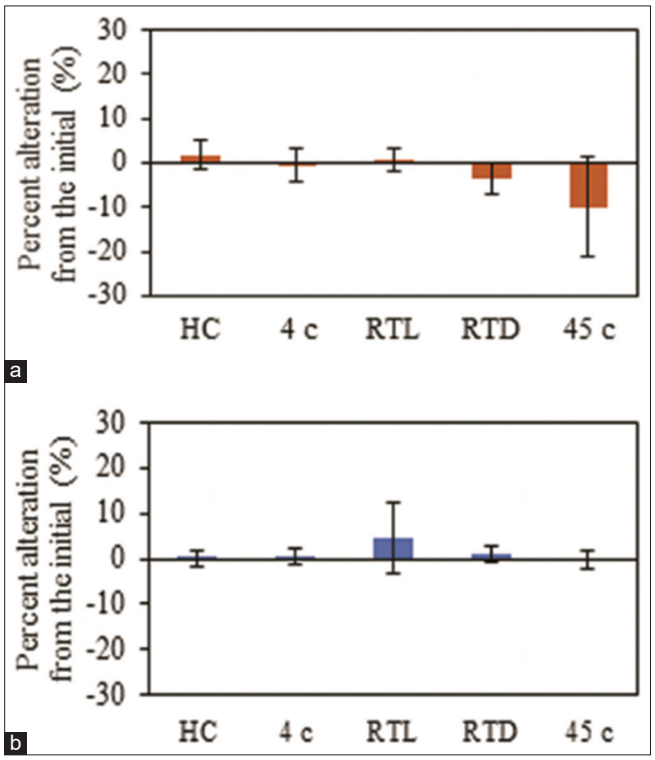

Fig. 4: Percent alteration of (a) 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid radical scavenging effect and (b) anti-tyrosinase effect of the extract (R3) compared to those of the initial

extracting condition was therefore using a spray-drying temperature of 150°C and a pH value of 2.0. As a consequence, R3 was chosen for further determining the stability profile.

After storage in various conditions, antioxidant and anti-tyrosinase activities of R3 did not show significantly different from those of the initial (Fig. 4a and b). However, according to (Fig. 4a), the highest reduction in ABTS radical scavenging effect was shown after storage at 45°C. In addition, after storage at 45°C and HC, the obvious fade color was observed. As a result, the extract should be stored in low-temperature conditions to avoid degradation.

CONCLUSION

The aqueous extracts of Thai organic broken Riceberry rice, grown in Lampang Province, exhibited notable antioxidant, and anti-tyrosinase properties, correlating to their polyphenol contents. Therefore, this extract could be a promising agent for attenuating skin aging. Nevertheless, to prevent degradation, the extracts should be not stored under high temperatures.