Antioxidant activity, anti-tyrosinase activity, and lipstick pigments of *Oryza sativa* Linn. and *Carissa carandas* Linn. extracts

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ABSTRACT

Oryza sativa Linn. and *Carissa carandas* Linn extracts are composed of phenolic and flavonoid contents. *Oryza sativa* Linn. extract had antioxidant activities at 36.07 ± 5.71 mg Trolox (TE)/g dry weight (DW), 26.14 ± 4.43 mg TE/g DW, and 50.08 ± 1.54 mM TE/g DW in 2,2-diphenyl-1-picrylhydradzyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric-reducing antioxidant power (FRAP) assays, respectively. For *C. carandas* Linn., the extract had antioxidant activities at 41.53 ± 1.77 mg TE/g DW, 38.44 ± 4.39 mg TE/ g DW, and 109.18 ± 25.07 mM TE/g DW in DPPH, ABTS, and FRAP assays, respectively. The *O. sativa* Linn. extract could inhibit the tyrosinase at $45.70\% \pm 11.76\%$, which was higher than the *C. carandas* Linn. extract ($36.90\% \pm 5.83\%$) at the same concentration (0.10 mg/ml). All gel lipstick formulas retained antioxidant and anti-tyrosinase activities. In the sensory test, the overall acceptability, color, and odor of gel lipsticks, which had *C. carandas* Linn. extract added, had the higher scores, suggesting a proper pigment in a darker color lipstick. However, the spreadability, flakiness, and color stability of all formulas should be further studied for better lipstick quality.

INTRODUCTION

Color pigments of natural extracts (organic colors) have been of interest for cosmetic applications such as lipsticks and blushes for health and beauty. Weak points in chemical pigments that are composed of heavy metals or have metal contamination have been reported and should be avoided (Azwanida *et al.*, 2015; Borowska and Brzoska, 2015; da Fanca *et al.*, 2015; Mohana *et al.*, 2020). The accumulation of heavy metals can cause melasma (Granstein and Sober, 1981) and several acute and chronic toxic effects, such as gastrointestinal and kidney dysfunction, nervous system disorders, skin lesions, vascular damage, immune system dysfunction, birth defects, and cancer (Balali-Mood et al., 2021).

Many researchers have introduced the application of natural extracts as ingredients in lipstick products to counteract the side effects of chemical pigments, such as cinnamon bark powder, turmeric powder, beetroot powder, pomegranate juice, carrot juice, tomato juice, *Clitoria ternatea*, and *Pandanus amaryllifolius* (Azwanida *et al.*, 2015; Rasheed *et al.*, 2020).

Oryza sativa Linn. (red jasmine rice) and *Carissa carandas* Linn. are widely found in Thailand, and are eaten as local foods (Fig. 1). The extract of *C. carandas* Linn. had the ability of antioxidant activities at $80.75\% \pm 0.02\%$ in the in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In addition, the 100% ethanol extract had anti-tyrosinase activity at $71.84\% \pm 0.00\%$ (Khunchalee and Charoenboon, 2019).

A previous study found that jasmine rice had antioxidant activities (the half maximal inhibitory concentration: IC_{50}) at 314.51 µg/ml in ABTS assay, 145.67 µg/ml in DPPH assay, and 21.26 ± 0.21 µM Fe (II)/µg in FRAP assay (Widowati *et al.*, 2016). Moreover, the extract of rice berries, which was germinated, could inhibit melanin synthesis (Sangsila *et al.*, 2018), indicating the potential of rice extracts in cosmetic applications for skin brightening and rejuvenation. However, the report of the anti-tyrosinase activity of red jasmine rice has not been found.

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Figure 1. Images of (A) *C. carandas* Linn. and (B) *O. sativa* Linn.

The antioxidant and anti-tyrosinase activities above are interesting for use as natural pigments in lipstick, not only for their activities but also for their beautiful colors. Lipsticks containing active compounds that have anti-tyrosinase and antioxidant activities induce rejuvenation and reduce darkness on lips. The ethanol extract of *O. sativa* Linn. has a yellowish-brown color, while the ethanol extract of *C. carandas* Linn. has a dark red color. However, their application as natural pigments in gel lipsticks has not been reported. Also, the stabilities of their antioxidant, anti-tyrosinase activities, and color stability after adding them to gel lipsticks are here determined by *in vitro* study and sensory test.

METHODS AND MATERIALS

Materials

Mushroom tyrosinase, DPPH, 3,4-dihydroxyphenylalanine (L-Dopa), quercetin, Trolox (TE), aluminum chloride, ABTS, tripyridyltriazine, and ferric chloride were purchased from Sigma-Aldrich, Darmstadt, Germany. Isododecane, silicone film, dimethicone, nylon 12, silica dimethyl silylate, matte silica powder, polyethylene wax, titanium dioxide, and phenoxyethanol were purchased from Chanjao Longevity Co., Ltd., Bangkok, Thailand. Whatman No.1 was purchased from GE Healthcare UK Limited, Shanghai Amersham, China. Ethanol was purchased from ACL Labscan Limited, Bangkok, Thailand. Sodium acetate, potassium acetate, and potassium persulfate were obtained from Carlo Erba, Cornaredo, Italy. Gallic acid was purchased from Fluka, Buchs, Switzerland. Folin–Ciocalteau reagent was purchased from Loba Chemie, Mumbi, India. Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Himedia, Mumbai, India. Sodium carbonate (Na_2CO_3) was purchased from Kemaus, New South Wales, Australia. Acetic acid was purchased from ACL Labscan Limited, Bangkok, Thailand.

Plant extraction

Ripe fruits (black color) of *C. carandas* Linn. were harvested, as can be seen in Figure 1. Both plant materials (*O. sativa* Linn. and *C. carandas* Linn.) were cleaned using tap water. The materials were chopped into small pieces and dried in a hot air oven at 50°C for 2 days. After that, they were ground to a powder and macerated in 95% ethanol in the plant-ethanol ratio of 1 (g):25 (ml) by stirring every day. After maceration for 1 week at room temperature (28°C–35°C), the materials were filtered

using a Buchner funnel with Whatman No. 1 filter paper. The filtrates were dried by pouring them on a tray, put in a hot air oven at 50°C, and kept at −20°C for approximately 1 month before use.

Gel lipstick preparation

The lipstick base was prepared by mixing isododecane, silicone film, and dimethicone in a beaker A and was stirred by a glass rod and heated at approximately 85°C until clearer for approximately 5 minutes. A beaker B contained nylon 12, silica dimethyl silylate, and matte silica powder was heated at approximately 85°C and was stirred by a glass rod until clearer for approximately 5 minutes. After that, the mixture in beaker B was added to beaker A and stirred by the glass rod until homogeneous. After that, the polyethylene wax was melted in a beaker E at approximately 85°C, and then the mixture of the beaker A and B was quickly added to the beaker E because the wax was quickly changed to be solid at approximately 80°C–83°C and stirred until homogeneous. As shown in Table 1, the lipstick base was mixed by the glass rod with the plant extracts in the ratio of 1:0.3 (lipstick base: plant extract), resulting in five gel lipstick formulas, as detailed in Table 2.

Phenolic content assay

A colorimetric method was used for phenolic content, which was adapted from a previous study (Pekal and Pyrzynska, 2004). Shortly, the extract, gallic acid (a standard

Table 1. Composition of lipstick base used for five gel lipstick formulas.

Composition	g
Isododecane	25
Silicone film	13
Dimethicone	8
Nylon 12	1
Silica Dimethyl Silylate	3
Matte Silica powder	1
Polyethylene wax	1
Total	52

Table 2. Composition of five gel lipstick formulas.

Composition / Formula no.	1	2	3	4	5
Lipstick base (Table 1)	1 g	1 g	1 g	1 g	1 g
Titanium dioxide	0.0025 g	-	0.0025 g	-	-
Rice extract	0.3 g	0.3 g	-	-	0.3 g
C. carandas Linn. extract	-	-	0.3 g	0.3 g	0.3 g
Dimethicone	0.05 g	0.05 g	0.05 g	0.05 g	0.05 g
Phenoxyethanol (0.59%)	0.08 g	0.08 g	0.08 g	0.08 g	0.08 g

phenolic substance in 0.01–0.09 mg/ml), and ethanol (a negative control) in various concentrations were introduced at 150 µl in each microtube. After that, 10% Folin-Ciocalteu reagent, 375 µl, was added to each microtube, following 7.5% Na_2CO_3, 75 µl, which was mixed in each microtube. After incubation in the dark at room temperature for 1 hour, each sample had its absorbance measured at 715 nm using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA). The phenolic content of all extracts was calculated from a gallic acid standard graph.

Flavonoid content

A colorimetric method was adapted from a previous study (Pourmorad et al., 2005) for determining the aluminum chloride content, indicating a flavonoid content. Briefly, 0.3 ml of the extracts, quercetin (a standard substance in 20–120 µg/ml), and ethanol (a negative control) were added to each microtube. Next, 0.10 ml of 10.00% aluminum chloride was mixed in each tube, followed by 1 M potassium acetate 0.10 and 2.8 ml of distilled water additions. After incubation at room temperature for 30 minutes, each sample had its absorbance measured at 415 nm using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA). The flavonoid contents of the extracts were calculated from a standard graph of quercetin.

DPPH scavenging assay

A colorimetric method was adapted slightly from a previous study (Manosroi et al., 2010). Then, 2 mM DPPH, dissolved in ethanol, 250 µl, was added to each microtube. Moreover, 250 µl of the extracts, the standard substance (TE) in 2–16 µg/ml, and ethanol (a negative control) were added to microtubes and mixed with 250 µl of DPPH solution in each tube. After incubation for 30 minutes, all samples had their absorbance measured at 517 nm using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA). The antioxidant activities of each extract were calculated from a TE standard graph and expressed in terms of TE equivalent antioxidant capacity (TEAC), mg TE/g dry weight (DW).

ABTS scavenging assay

The antioxidant activity of ABTS free radicals was determined using a previous method (Re et al., 1999) with some modifications. A mix of 500 µl, 7.4 mM ABTS dissolved in distilled water and 500 µl of 2.6 mM potassium persulfate dissolved in distilled water was left for approximately 14 hours at room temperature in the dark. Then, the ABTS solution was diluted with 95% ethanol to give an absorbance at 732 nm of approximately 7.2, and it was added to each microtube at 250 µl. Next, the extracts, a positive control (TE) in 1–6 µM, and ethanol (a negative control) were added in microtubes at 250 µl and then were incubated at room temperature and in the dark for 30 minutes. After that, all treatments were measured at the absorbance of 732 nm using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA). The antioxidant activities of each extract were calculated from a standard graph of TE and expressed in terms of TEAC (mg TE/g DW).

FRAP assay

A colorimetric method for ferric-reducing power by Zhang et al. (2014) was slightly modified. The 120 µl of the extracts, TE (a positive control in 20–100 µM), and ethanol (a negative control) were added in microtubes. The 450 µl of FRAP solution [300 mM acetate buffer (pH 3.6): 10 mM tripyridyltriazine: 20 mM ferric chloride in the ratio of 10:1:1] was added and mixed in each tube. After incubation for 30 minutes in the dark, all samples had their absorbance at 593 nm measured using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA) and subtracted from the absorbance of a sample color (a mixture of solvent and plant extracts). After that, the antioxidant activities of each extract were calculated from a standard graph of TE and expressed in terms of TEAC (mg TE/g DW).

Anti-tyrosinase activity

The method of anti-tyrosinase activity was adapted slightly from a previous method (Di Petrillo et al., 2016). To begin with, the 50 µl extracts and 171.25 µl phosphate buffer (pH 6.8, 0.5 mM) were mixed in each microtube and incubated with 3.75 µl tyrosinase enzyme (2,500 units/ml) for 5 minutes. After that, 25 µl L-Dopa (2.5 mM) was treated, mixed, and incubated for 10 minutes. The anti-tyrosinase activity of each sample was determined by measuring absorbance at 495 nm using a spectrophotometer (Thermo Fisher Scientific, Genesys 10-s, USA) and then calculated as a percentage of anti-tyrosinase activity as in the following formula.

$$\text{Anti-tyrosinase (\%)} = \frac{(A-B)}{(C-D)} \times 100,$$

where A represents the plant extract incubating with the mixture described above, B represents a color blank (The mixture described above, but adding a phosphate buffer instead of the enzyme.), C represents a positive control (The mixture described above, but adding ethanol instead of the plant extract.), and D represents a negative control (The mixture described above, but adding ethanol and buffer instead of the plant extract and the enzyme.)

Color stability

In total, 6 g of each lipstick formula was put into a transparent plastic bag (7.00 × 8.70 cm), and then the color stability was measured at 1-, 2-, 3-, and 4-month periods of storage and compared with that the start time. The color meter (Konica Minolta, CR-400, Japan) was calibrated with a standard white tile, as specified by the manufacturer. It was then used to measure L*(lightness), a*(redness), and b*(yellowness) values at the same position three times.

Antioxidant activity and anti-tyrosinase activity of the gel lipsticks

Each lipstick formula was prepared in 95% ethanol to obtain 10 mg/ml and was then centrifuged at 5,000 rpm for 5 minutes. The supernatant was kept at −20°C until required for measuring the antioxidant activities in the DPPH scavenging assay, ABTS scavenging assay, FRAP assay, and anti-tyrosinase activity.

Sensory test

The sensory test has been approved by the human research ethics committee of Rajamankala University of Technology of Isan (HEC-01-65-028), the Helsinki Declaration, the Belmont Report, CIOMS guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). Twenty female participants (19–41 years old) were informed how to test the gel lipsticks and their benefits before research participation (Esposito and Kirilov, 2021). All participants had all the gel lipsticks smeared on their forearms in an area of approximately 1.00×2.00 cm at room temperature ($25°C \pm 2°C$) and answered the questions with a nine-point hedonic scale.

Statistical analysis

All experiments were performed in triplicate, and their data were expressed as means ± standard deviation (SD). The statistical analysis was performed by Statistical software (SPSS for Windows, version 23, SPSS Inc., Chicago, IL, USA) was used to analyze the data. The differences in phenolic contents, flavonoid contents, antioxidant activities (ABTS, DPPH, FRAP), and anti-tyrosinase activities between the plant extracts were analyzed by an independent sample t-test, $p < 0.05$. The differences in antioxidant activities, colors, and anti-tyrosinase activities among the lipstick extracts were analyzed by one-way analysis of variance (ANOVA), Duncan, $p < 0.05$. The sensory test was statistically analyzed using the one-way ANOVA in randomized complete block design (RCBD) with Duncan, $p < 0.05$.

RESULTS AND DISCUSSION

Phenolic and flavonoid content assay

Phenolic compounds consist of aromatic rings and hydroxy groups. They were found in plants to defend against ultraviolet, pathogens, and other predators (Balasundram *et al.*, 2006; Shah *et al.*, 2018). They have diverse bioactivities such as antioxidants, anti-collagenase, anti-tyrosinase, and anti-elastase. They can scavenge free radicals by hydrogen atom transfer, transfer of a single electron, sequential proton loss electron transfer, and chelation of transition metals (Zeb, 2020). Also, they can inhibit melanin synthesis such as vitamin E analogs, including TE and coumarin derivatives (Kamei *et al.*, 2009; Lu *et al.*, 2023). Therefore, many pharmaceutical products are composed of phenolic compounds as active compounds for their beneficial effects on health.

The phenolic contents of *C. carandas* Linn. and *O. sativa* Linn. extracts were almost equal (not significant), which were 1.14 ± 0.14 and 1.09 ± 0.05 mg gallic equivalent (GAE)/g DW, respectively (Table 3). However, the phenolic content of *C. carandas* Linn., which is commonly found in Thailand and was harvested in Nakhon Ratchasima province, had lower phenolic content than those in previous studies (Khunchalee and Charoenboon, 2019; Pewlong *et al.*, 2014; Thitilertdecha and Pintathong, 2022), which reported the phenolic content of *C. carandas* Linn. were 20.20 ± 0.00 mg GAE/g DW (100% ethanol extraction), 4.67 ± 0.59 mg GAE/g (40% ethanol extraction), 16.18 ± 2.21 mg GAE/g by absolute

Table 3. Antioxidant activities and anti-tyrosinase activities of *O. sativa* Linn. and *C. carandas* Linn. extracts.

Biological activities	*C. caranda* Linn.	*O. sativa* Linn.
Phenolic content (mg GAE/g DW)	1.14 ± 0.14^a	1.09 ± 0.05^a
Flavonoid content (mg QE/g DW)	2.39 ± 0.33^a	0.65 ± 0.39^b
DPPH: TEAC (mg TE/g DW)	41.53 ± 1.77^a	36.07 ± 5.71^a
ABTS: TEAC (mg TE/g DW)	38.44 ± 4.39^a	26.14 ± 4.43^b
FRAP: TEAC (mM TE/g DW)	109.18 ± 25.07^a	50.08 ± 1.54^b
% anti-tyrosinase (0.1 mg/ml)	36.90 ± 5.83^a	45.70 ± 11.76^a

Data are given as means ± SD, $n = 3$. The different letter superscripts indicate significant differences at $p < 0.05$ in rows using independent sample t-test.

ethanol extraction. Also, the phenolic content of *O. sativa* Linn. extract of this study was lower than those of previous reports, which were approximately 81.91 mg GAE/g by 95% ethanol extraction (Suantai *et al.*, 2022), 3.4119 mg GAE/g DW by 80% ethanol extraction (Kammapana, 2023), $1,018 \pm 0.095$ mg GAE/g by 70% ethanol extraction (Thitipramote *et al.*, 2016). The different planting areas and solvent extractions may be the reason for the lower phenolic content.

Flavonoid compounds, a polyphenolic secondary metabolite, are composed of 15 carbon atoms arranged in three rings such as C6–C3–C6. The differences in the structure of each subgroup are partly attributed to the pattern and degree of hydroxylation, prenylation, glycosylation, or methoxylation, such as quercetin, catechin, naringenin (Dai and Mumper, 2010; D'Archivio et al., 2007). Many flavonoids have antioxidant activity, such as quercetin and catechin (Bernatoniene and Kopustinskiene, 2018; Xu *et al.*, 2019). Some flavonoids can inhibit melanin synthesis, such as quercetin and catechin. Quercetin is bound to tyrosinase by hydrophobic interaction resulting in a conformational change and malfunction (Fan *et al.*, 2017).

Both extracts used in this study consisted of flavonoid compounds. The flavonoid content of *C. carandas* Linn. was significantly higher than that of the *O. sativa* Linn. extract, which was 2.39 ± 0.33 and 0.65 ± 0.39 mg quercetin equivalent (QE)/g DW, respectively (Table 3). However, many previous studies reported the flavonoid content of *C. carandas* Linn., which was approximately 43.75 mg catechin equivalent (CE)/g extract (Nanasombat *et al.*, 2019). Moreover, the flavonoid content of other Thai rice (Sang-Yod, Mun-Poo, Hom-Nin, Rice Berry, and Luem-Pua) was in the range of 86–341 mg CE/g extract (Teeranachaideekul *et al.*, 2018). However, the active compound contents in the previous reports cannot be compared with the results of this study because of the different units and solvent extraction.

DPPH scavenging assay

Both extracts and the gel lipsticks containing the extracts were evaluated for antioxidant activities by DPPH assay. DPPH radicals are purple and stable in ethanol. The decrease in purple color indicates antioxidant activity. The antioxidant activities of the *C. carandas* Linn. and *O. sativa* Linn. extracts

were 41.53 ± 1.77 and 36.07 ± 5.71 mg TE/g DW, respectively (Table 3). Previous studies have reported the antioxidant activity of *C. carandas* Linn. extract at $80.75\% \pm 0.02\%$ by 100% ethanol extraction (Khunchalee and Charoenboon, 2019), 0.56 ± 0.05 mg/ml (IC_{50}) by 95% ethanol extraction (Sudjaroen *et al*., 2021), and 16.86 ± 2.31 mg TE/g by absolute ethanol extraction (Thitilertdecha and Pintathong, 2022). In addition, *O. sativa* Linn. extract had DPPH antioxidant activity (IC_{50}) at 314.51 µg/ml (Widowati *et al*., 2016), 0.3477 mM TE/g DW by 80% ethanol extraction (Kammapana, 2023), and 7,528 mg TE/g by 70% ethanol extraction (Thitipramote *et al*., 2016). However, the antioxidant activities in the previous reports cannot be compared with the results of this study because of the different units and solvent extraction.

Moreover, the DPPH antioxidant activities of all gel lipstick formulas (Table 4) at 10 mg/ml had antioxidant activities in the range of 5.45 ± 0.28 to 6.84 ± 0.13 mg TE/g DW and antioxidant percentages in the range of $70.00\% \pm 3.35\%$ to $86.40\% \pm 0.14\%$. The highest antioxidant activity was the gel lipstick 2, which used the *O. sativa* Linn. extract and titanium dioxide as a brown and white pigment, respectively, while the lowest antioxidant activity was the gel lipstick 4, which used *C. carandas* Linn. extract as a red pigment. The results suggest beneficial effects such as rejuvenation and antiaging on the lips.

ABTS scavenging assay

The extracts and the gel lipsticks containing the extracts were evaluated for antioxidant activities by ABTS assay. ABTS are free radicals and are blue in water or ethanol. The antioxidant ability was obtained by measuring the reduction of blue color. The antioxidant activities of the *C. carandas* Linn. and *O. sativa* Linn. extracts were 38.44 ± 4.39 and 26.14 ± 4.43 mg TE/g DW, respectively (Table 3). In addition, previous studies have reported the antioxidant activities in ABTS of *C. carandas* Linn. (100% ethanol extraction) at approximately $61.72\% \pm 0.00\%$ (Khunchalee and Charoenboon, 2019), 131.8 ± 3.9 µM/g by 95% ethanol extraction (Neimkhum *et al*., 2021), and 19.67 ± 0.07 mg TE/g DW by water extraction (Jiangseubchatveera *et al*., 2021). Also, previous studies have reported the antioxidant activity in ABTS of *O. sativa* Linn. at approximately 189.45 ± 11.58 mg TE/g by 95% ethanol extraction (Suantai *et al*., 2022) and 84,476 mg TE/g by 70%

ethanol extraction (Thitipramote *et al*., 2016). The reason why the activity in this study was lower than that in the previous report of Suantai *et al*. (2022) is possibly due to the different environmental conditions. However, the antioxidant activities in other previous reports cannot be compared with the results of this study because of the different units and extraction processes.

Moreover, the ABTS antioxidant activities of all gel lipstick formulas (Table 4) at 10 mg/ml had antioxidant activities in the range of 2.61 ± 0.49 to 3.92 ± 0.14 mg TE/g DW and antioxidant percentages in the range of $58.95\% \pm 9.06\%$ to $83.27\% \pm 2.61\%$. The highest antioxidant activity was the gel lipstick 2, which used only the *O. sativa* Linn. extract as a brown pigment. In contrast, the lowest antioxidant activity was the gel lipstick 1, which used *O. sativa* Linn. extract and titanium dioxide as a brown and white pigment. The results indicate that the other lipstick components had no major effects on the antioxidant activity of the lipsticks and confirmed the potential of the extracts for anti-aging activity.

FRAP assay

The FRAP of the extracts also determined the hydrogen electron transfer by FRAP; the results are shown in Table 3. The antioxidant activity of the *C. carandas* Linn. (109.18 ± 25.07 mM TE/g DW) was significantly higher than that of the *O. sativa* Linn. extract (50.08 ± 1.54 mM TE/g DW). However, previous studies have reported that the *O. sativa* Linn. extract had IC_{50} at 21.26 µM Fe(II)/µg by 70% ethanol extraction (Widowati *et al*., 2016) and 3,292.46 g $FeSO_4$/g by 95% ethanol extraction (Suantai *et al*., 2022) and 10,792 mg TE/g by 70% ethanol extraction (Thitipramote *et al*., 2016). For *C. carandas* Linn., previous studies have shown the extract had 2,085.23 g $FeSO_4$/g by 100% ethanol extraction (Khunchalee and Charoenboon, 2019) and 198.50 ± 5.6 µM $FeSO_4$/g by 95% ethanol extraction (Neimkhum *et al*., 2021). However, the antioxidant activities in the previous reports cannot be compared with the results of this study because of the different units and solvent extractions.

Also, the FRAP antioxidant activities of all gel lipstick formulas (Table 4) at 10 mg/ml, had antioxidant activities in the range of 9.68 ± 0.19 to 26.87 ± 0.27 M TE/g DW. The highest antioxidant activity was the gel lipstick 4, which used

Table 4. Antioxidant activities and anti-tyrosinase activities of all lipstick formulas added with *O. sativa* Linn. and *C. carandas* Linn. extracts added.

Biological activities	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
% DPPH inhibition	82.80 ± 0.13^a	86.40 ± 0.14^b	$83.60 \pm 0.59^{a,b}$	70.00 ± 3.35^c	$85.00 \pm 1.04^{a,b}$
DPPH: TEAC (mg TE/g DW)	6.54 ± 0.01^a	6.84 ± 0.13^b	$6.60 \pm 0.05^{a,b}$	5.45 ± 0.28^c	$6.71 \pm 0.09^{a,b}$
% ABTS inhibition	58.95 ± 9.06^a	83.27 ± 2.61^b	73.33 ± 10.98^b	80.65 ± 6.25^b	$70.72 \pm 3.87^{a,b}$
ABTS: TEAC (mg TE/g DW)	2.61 ± 0.49^a	3.92 ± 0.14^b	3.39 ± 0.59^b	3.78 ± 0.34^b	$3.24 \pm 0.21^{a,b}$
FRAP: TEAC (M TE/g DW)	9.68 ± 0.19^a	23.96 ± 1.41^b	26.75 ± 0.55^c	26.87 ± 0.27^c	23.18 ± 0.54^b
% anti-tyrosinase (10 mg/ml)	70.19 ± 8.90^a	60.84 ± 12.44^a	57.67 ± 9.16^a	20.99 ± 3.70^b	56.79 ± 20.47^a

Data are given as means ± SD, $n = 3$. The different letter superscripts indicate significant differences at $p < 0.05$ in rows using one-way ANOVA, Duncan.

the *C. carandas* Linn. extract as a red pigment, while the lowest antioxidant activity was the gel lipstick 1, which used the *O. sativa* Linn. extract and titanium dioxide as brown and white pigments.

Anti-tyrosinase activity

The activity in tyrosinase inhibition is one of the favorite bioactivities for cosmeceuticals in Asia. The anti-tyrosinase activities of the *C. carandas* Linn. and *O. sativa* Linn. extracts were 36.90% ± 5.83% and 45.70% ± 11.76%, respectively (Table 3). The anti-tyrosinase activity of the *C. carandas* Linn. was consistent with that of previous studies, in which the anti-tyrosinase activity was 71.84% ± 0.00% by 100% ethanol extraction (Khunchalee and Charoenboon, 2019). The *C. carandas* Linn. extract at 1 mg/ml inhibited tyrosinase activity at 6.55% ± 0.16% by water extraction (Jiangseubchatveera *et al.*, 2021). For *O. sativa* Linn., the anti-tyrosinase activity corresponds to a previous study (Kanlayavattanakul *et al.*, 2016), which reported that white jasmine rice extracted by a different extract method could inhibit melanogenesis via tyrosinase inhibition at 61.66% ± 0.12%. In addition, the activity remained after adding extracts into gel lipsticks (20.99% ± 3.70% to 70.19% ± 8.90%), as can be seen in Table 4, indicating the potential as cosmeceutical lipsticks.

Sensory test

The colors of the gel lipsticks in each formula (Table 2) were observed by eye and the color meter (Fig. 2). In eye observation (Fig. 3), the color of gel lipstick formula 1, which had *O. sativa* Linn. extract and titanium dioxide added as pigments, was yellow. The color of gel lipstick formula 2, which had *O. sativa* Linn. extract added as a pigment, was brown. The color of gel lipstick formula 3, which had *C. carandas* Linn. extract and titanium dioxide added as pigments, was pink. The color of gel lipstick formula 4, which had *C. carandas* Linn. extract added as a pigment, was red. The color of gel lipstick formula 5, which had *C. carandas* Linn. extract and *O. sativa* Linn. Extract, added as pigments was brown-red. The parameters, which are absorption, color, odor, spreadability, and overall acceptability, were determined by 20 women participants, and the results are shown in Figure 4. In color parameter, lipstick formulas 3, 4, and 5, which were not significantly different, had the higher scores. Formulas 1 and 2 were not significantly different and had lower scores than others. In the odor parameter, the formulas that had the highest scores were formulas 4 and 5 (not significantly different), followed by formulas 2 and 3 (not significantly different). The lowest score is formula 1. In the overall acceptability parameter, the highest scores which were not significantly different were formulas 3, 4, and 5, followed by formulas 1, 2, and 3 (not significantly different). The lipstick which had *C. carandas* Linn. extract added had a higher score than those lipsticks which had *O. sativa* Linn. extract added in color, odor, and overall acceptability scores, indicating the greater flavor of *C. carandas* Linn in dark color lipsticks. Also, the *O. sativa* Linn. extract is suggested as a pigment for tint lipsticks (light color). The spreadability and absorption scores of all lipstick formulas were not significantly different. However, the spreadability and flakiness of all formulas should be further developed.

Color stability

All lipstick formulas were tested for color stability by measuring the color at the start time compared with that of the storage times (4 months), as can be seen in Figure 2. Comparing their storage times, the yellowness of all formulas was slightly increased, but the redness and lightness of all formulas decreased. The redness of all formulas significantly
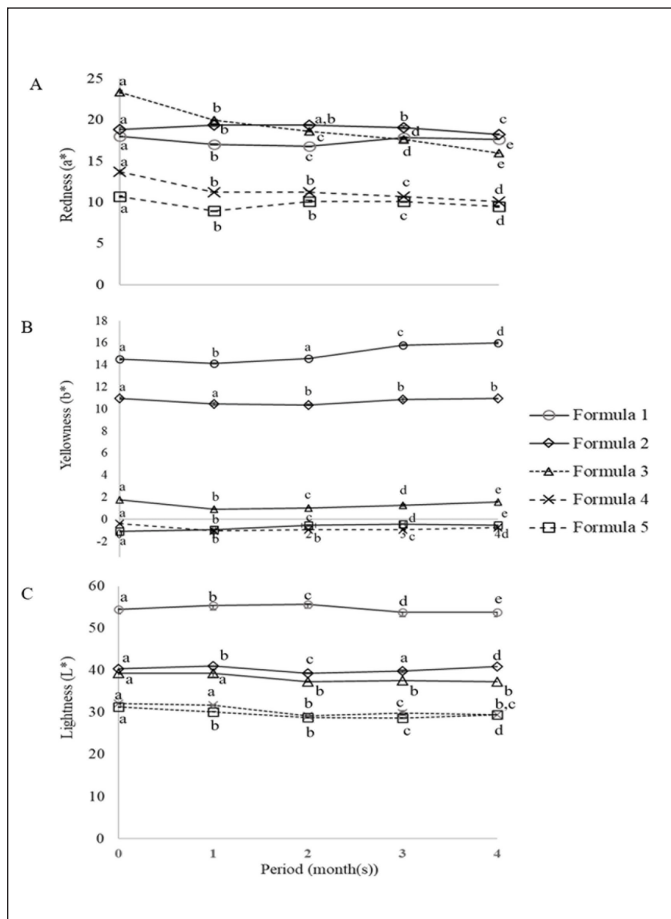

Figure 2. Color stabilities (redness, yellowness, and lightness) of five lipstick formulas. The different letters (a, b, c, d, and e) in the set for each period represent significant differences by one-way ANOVA (Duncan).
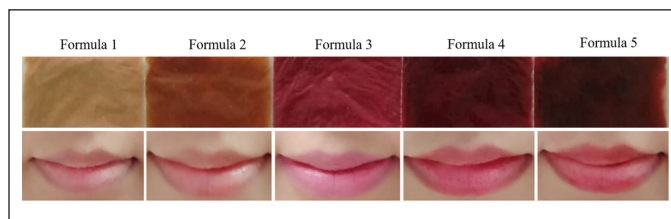

Figure 3. Lipstick colors examined by eye for five formulas. The lipstick formula 1 had *O. sativa* Linn. extract and titanium dioxide added as pigments. The lipstick formula 2 had *O. sativa* Linn. extract added as a pigment. The lipstick formula 3 had *C. carandas* Linn. extract and titanium dioxide added as pigments. The color lipstick formula 4 had *C. carandas* Linn. extract added as a pigment. The lipstick formula 5 had *C. carandas* Linn. extract and *O. sativa* Linn. extract added as pigments.
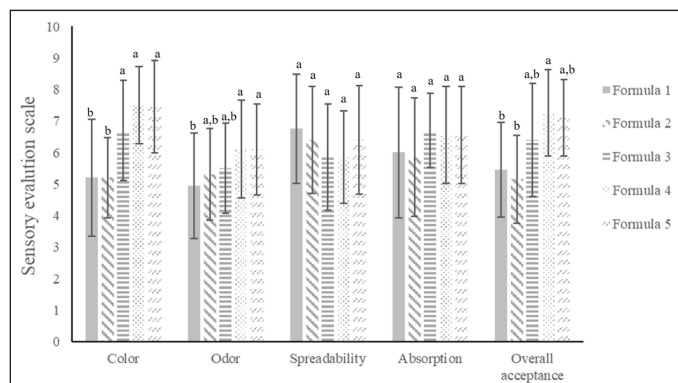
Figure 4. Sensory scores for odor, color, spreadability, absorption, and overall acceptability of each lipstick. The different letters (a, b, c, d, and e) in the columns for each characteristic represent significant differences by using one-way ANOVA in RCBD with Duncan, $p < 0.05$.

decreased after one month of storage, corresponding to the results measured by the color meter. Similarly, the yellowness trend of all formulas increased slightly but significantly since the 1-month storage. Also, the lightness trend of formulas 1, 2, and 5 decreased slightly but significantly since the 1-month of storage. However, the lightness trend of formulas 3 and 4 decreased slightly but significantly since the 2-month storage. Normally, natural pigments are less stable than synthetic pigments because of microbial contaminations, light, and heat (Azwanida *et al.*, 2015; da Fanca *et al.*, 2015; Mohana *et al.*, 2020).

The color instability of these lipsticks suggests that perhaps the percentage of a preservative should be higher, but no more than 1.00%. Therefore, the color stability of the lipsticks using the *C. carandas* Linn. extract as a red pigment should be further studied for more stability.

CONCLUSION

The antioxidant and anti-tyrosinase activities of *O. sativa* Linn. and *C. carandas* Linn. extracts remain active after adding into gel lipstick as pigments, indicating that they may reduce wrinkles and melanin on lips. Also, in the sensory test, the lipstick with *C. carandas* Linn. extract added had a higher score than those lipsticks with *O. sativa* Linn. extract added in terms of color, odor, and overall acceptability scores. *Oryza sativa* Linn. extract was suitable for tint lipsticks because of its light color. *Carissa carandas* Linn. extract was appropriate for use to darken lipsticks as a pigment because of its darker red color. However, the spreadability, flakiness, and color stability of all lipsticks should be developed for better quality.

AUTHOR CONTRIBUTIONS

The authors confirm their contribution to the article as follows: study conception and design: Thitiporn Anunthawan was responsible for data collection, analysis, and interpretation of results. Thitiporn Anunthawan and Thipwarin Rimlumduan contributed to the draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVALS

The sensory test has been approved for ethics by the human research ethics committee of Rajamankala University of Technology of Isan (HEC-01-65-028), the Declaration of Helsinki, the Belmont Report, CIOMS guideline, and the ICH-GCP.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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