International Journal of Agricultural Technology 2024 Vol. 20(4):1603-1620
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Characteristics of crude extracts from Thai local longan (*Dimocarpus longan* var. *obtusus)* seeds for skin moisturizing serum

Sudprasert, P.[1], Promsomboon, P.[2], Phupiewkham, W.[1] and Promsomboon, S.[1*]

[1]Faculty of Science and Technology, Rajamangala University of Technology Tawan-OK, Bangpra Campus, Bangpra, Sriracha, Chonburi province 20110, Thailand; [2]Faculty of Agriculture and Natural Resources, Rajamangala University of Technology Tawan-OK, Bangpra Campus, Bangpra, Sriracha, Chonburi province 20110, Thailand.

Sudprasert, P., Promsomboon, P., Phupiewkham, W. and Promsomboon, S. (2024). Characteristics of crude extracts from Thai local longan (*Dimocarpus longan* var. *obtusus*) seeds for skin moisturizing serum. International Journal of Agricultural Technology 20(4):1603-1620.

Abstract The crude extract of *Dimocarpus longan* var. *obtusus* (DLO) seeds was obtained through maceration of dried longan seeds using 95% ethanol which exhibited a yield of 13.7% The crude extract demonstrated the highest ability to scavenge free radicals, with an EC_{50} value of 0.35 mg/ml. Furthermore, young and mature DLO seeds were compared for antioxidant activity, revealing that mature seeds possessed greater antioxidant potential with an EC_{50} of 0.15 mg/ml. The total phenolic content was found to be 657 mg/g. The DLO seed ethanol extract displayed noteworthy antioxidant activity through DPPH radical scavenging, ABTS radical scavenging, and ferric reducing/antioxidant power (FRAP) assays. The crude extract also exhibited anti-tyrosinase activity with an IC_{50} of 18.12 mg/ml higher than standard substance as kojic acid with an IC_{50} of 0.12 mg/ml. Cytotoxicity on various cell lines demonstrated the safety of the DLO seed extract. Moreover, the moisturizing serum formula was developed and assessed for qualities including texture, skin absorption, stickiness, moisturizing, lusciousness, and overall preference. Formula 2, which contained 0.6% carbopol ultrez 10, 1% propylene glycol, 2.5% glycerine, 0.13% triethanolamine, and 1% DLO seed crude extract, exhibited the highest satisfaction scores across all categories, with an average liking score of 7.67. The stability of each formula was measured pH at 5, viscosity at 49,120 cP, and it exhibited stability over one year of storage. This research revealed the light on the potential of DLO seeds as a valuable source of bioactive compounds with antioxidant and potential skincare benefits, contributing to the development of innovative cosmetic products.

Keywords: *Dimocarpus longan* var. *obtusus*, Skin serum, Antioxidant, Anti-tyrosinase, Cytotoxic activity

[*] **Corresponding Author:** Promsomboon, S.; **Email:** sutunya_pr@rmutto.ac.th

Introduction

In recent years, the pursuit of natural and effective ingredients for cosmetic formulations has driven extensive research into the bioactivity of plant extracts. *Dimocarpus longan* var. *obtusus* (DLO), a distinctive local Thai variant of longan renowned for its succulent fruit, has garnered attention due to its potential bioactive compounds, positioning it as a compelling candidate for the development of innovative skincare products.

DLO, often known as "Lumyai Thao", is a unique local variant of the longan fruit native to Thailand and other Southeast Asian countries (Lithanatudom *et al.*, 2017). While the more common longan variants have attracted international attention, DLO has gained regional attention for its distinct characteristics and possible bioactivity. The huge size of the seeds combined with a relatively thin seed coat distinguishes DLO (Figure 1). This property is of special importance because of its possible impact on the bioactive chemicals found in seeds. The thin seed coat implies that these seeds may have a larger proportion of bioactive components that are more conveniently extracted for a variety of applications.

Figure 1. *Dimocarpus longan* var. *obtusus* (DLO) seeds

The skincare industry has increasingly embraced the integration of natural antioxidants in products to counteract oxidative stress and the detrimental effects of free radicals on the skin. Additionally, the methanolic extracts from two varieties of *Dimocarpus* longan seeds have been demonstrated to exhibit noteworthy biological activities (Natungnuy *et al*., 2018).

Furthermore, research has unveiled the potential of plant-derived compounds in inhibiting tyrosinase, central glycoprotein enzyme to melanin production, making them valuable for skin-lightening and hyperpigmentation-reducing applications. Deguchi *et al.* (2019) explored the anti-tyrosinase and antioxidative activities of Asana heartwood extract, while Nitteranon (2018) investigated the anti-inflammatory, antioxidant, and quinone reductase inducing effects of DLO seed extract.

The objectives were to investigate the antioxidant properties of DLO seed extract, evaluate its anti-tyrosinase activity, to assess its cytotoxicity, and to develop a skin moisturizing serum formulation incorporating the DLO seed extract, creating a novel product that leverages the extract's beneficial properties for enhanced skin care.

Materials and methods

Preparation and crude extraction from DLO seeds

DLO seeds were classified as young or mature based on the appearance of the longan fruit. Young seeds were collected from small size of longan fruits with a brown-green peel, while mature seeds were obtained from large size and dark brown peel of longan fruit. DLO seeds were thoroughly washed and air-dried before being subjected to drying at 60°C for 6 h. Subsequently, the dried seeds were finely crushed to allow for effective extraction. The crushed seeds were then immersed in 95% ethanol with a ratio of 1:3 (w/v) in the dark chamber at 25°C. After 7 days of immersion, crude extracts were obtained by filtering and evaporating, respectively.

Crude extract was partitioned using chloroform as a solvent with a ratio of 1:2 (w/v), two distinct layers of extracts were obtained (water layer extract and chloroform layer extract). The water layer extract and chloroform layer extract were then extracted with ethyl acetate and hexane, respectively. The water layer extract produced two sub-extracts: a water layer extract and an ethyl acetate layer extract. The chloroform layer extract was then subjected to extraction with hexane and 90% methanol. Finally, each obtained extract underwent volume reduction using a rotary evaporator.

The effect of aging of DLO seeds on antioxidant activity was studied. The young and mature DLO seeds were extracted and compared the antioxidant activity which was assessed by the DPPH radical scavenging assay, with modifications based on the method proposed (Masuda *et al.*, 1999; Tachibana *et al.*, 2001). One gram of the crude extract was dissolved in 100 ml of 70% ethanol, then diluted at concentrations of 0.1, 0.5, 1, 2, 3, 4, and 5 mg/ml, yielding a total

volume of 30 µl. Subsequently, 0.05 mM DPPH was dissolved in 3 ml of 70% methanol, then added to each diluted extract tube and mixed immediately. The antioxidant activity was measured at an absorbance of 517 nm. The percentage of DPPH antioxidant activity was then calculated using the formula as follows:

$$\%\ AA = \frac{[A_S - (A_{se} - A_e)]}{\mathrm{A}_s} \times 100$$

Where, $\%\ AA$ is antioxidant activity or DPPH scavenged (%), A_S, A_e and A_{se} is absorbance of DPPH solution, extract, and DPPH solution and extract, respectively, at the wavelength of 517 nm.

Total phenolic content in the DLO seed extract was determined using the Folin-Ciocalteu reagent method (Kähkönen *et al.*, 1999; Soong and Barlow, 2004). Standard solutions of phenolic were prepared using gallic acid. Dilutions were made to obtain concentrations of 0, 50, 100, 250, and 500 mg/l, using propylene glycol as a solvent. After that, 1 ml of the standard gallic acid solution and 5 ml of the Folin-Ciocalteu reagent were added to a 100 ml volumetric flask containing 60 ml of distilled water, well mixed, and incubated for 30 min. Then, 15 ml of a 7.5% sodium carbonate solution was added and mixed. The volume was adjusted to 100 ml with distilled water. After 1 h of incubation, the absorbance was measured at an absorbance of 765 nm using a spectrophotometer.

Quantification of antioxidant activities

DPPH radical scavenging activity (DPPH assay) was determined by preparation of the samples at concentrations of 20-250 µg/ml. Then, 1 ml of 0.1 mM DPPH solution (in 95% ethanol) was added to 1 ml of each sample, well-mixed, and incubated for 30 min. The absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The BHT, α-tocopherol, and ascorbic acid were used as standards with measured in triplicate of each sample. The DPPH assay was calculated as followed by equation below:

$$\%\ DPPH\ assay = \frac{(A_0 - A_1)}{\mathrm{A}_0} \times 100$$

Where, A_0 is absorbance of the control and A_1 is absorbance of the sample. The value of % radical scavenging activity was reported as an IC_{50} value, which represents the concentration of the test sample that causes a 50% reduction in DPPH free radicals.

ABTS radical scavenging activity (ABTS assay) occurred from the oxidation reaction of ABTS with potassium persulfate (Re *et al.*, 1999). A 7 mM of ABTS solution was mixed with a 2.45 mM of potassium persulfate and kept in the dark at room temperature. After 16 h, 2 ml of ABTS solution were added to diluted samples, well-mixed and incubated for 6 min. The antioxidant activity

was measured by spectrophotometer with an absorbance of 734 nm. The experiment was performed in triplicate. The BHT, α-tocopherol, and vitamin C were used as standard. The ABTS radical scavenging activity was calculated as follows:

$$\%\ ABTS\ assay = \frac{(A_0 - A_1)}{\mathrm{A_0}} \times\ 100$$

Where, A_0 is absorbance of the control and A_1 is absorbance of the sample. The values of % ABTS radical scavenging activity was reported as IC_{50} value. The IC_{50} value represents the concentration of the test compound that causes a 50% reduction in ABTS free radicals.

Ferric reducing antioxidant power activity (FRAP assay) was obtained (Benzie and Strain, 1996). FRAP reagent was prepared by mixing acetate buffer, 20 µM $FeCl_3 \cdot 6H_2O$, TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), and distilled water with a ratio of 10:1:1:1.2. After that, 180 µl of distilled water and 1.8 ml of FRAP regent were added to tube contained 60 µl of samples and incubated at 37°C for 4 min. Antioxidant activities were determined using spectrophotometer at 595 nm. The results were reported as µmol of Fe (II)/1 g of the sample.

Anti-tyrosinase activity

Anti-tyrosinase activity was performed (Kubo *et al.*, 2000; Saewan *et al.*, 2011). The mixture was prepared by mixing 1 ml of 2.5 mM L-DOPA with 1.8 ml of 0.1 M sodium phosphate buffer (pH 6.8), then incubated for 10 min. A 0.1 ml of sample and 0.1 ml of tyrosinase (138 units) were immediately added and continuously incubated for 10 min. The reaction was analyzed by measuring the absorbance of dopachrome using a spectrophotometer at a wavelength of 475 nm. The percentage of tyrosinase inhibition was calculated using the following formula:

$$\%\ Tyrosinase\ inhibition = \frac{[\mathrm{A} - (\mathrm{B} - \mathrm{C})]}{\mathrm{A}} \times\ 100$$

Where, A is absorbance of the control sample (without the test substance), B is absorbance of the test sample (with tyrosinase), and C is absorbance of the test sample (without tyrosinase). The % tyrosinase inhibition results at various concentrations of the test substance were plotted on a calibration graph to calculate the IC_{50} value. Where the IC_{50} is the test substance concentration that inhibits the tyrosinase enzyme by 50%.

Cytotoxicity analysis

The cytotoxic property of DLO seeds extracted was investigated on human cell lines with an MTT assay. Cells such as fibroblast cell, Wi-38, obtained from

lung cells. and five cancer cells, BT474, Chago-KI breast cancer cells, Hep-G2 lung cancer cells, Kato-III liver cancer cells, gastric cancer cells, and SW620 colon cancer cells were cultured in a tissue culture flask with RMPI 1640 medium supplemented with 5% fetal calf serum (FCS) in a CO_2 incubator with 5% CO_2 at 37°C for 3 days. Subsequently, the cells were passaged using 0.05% trypsin mixed with 0.01% EDTA. 5 x 10^3 cells/ml were seeded in a 96-well plate with RMPI 1640 medium containing 200 µl of 5% FCS. The cells were incubated at 37°C for 1 day. The DLO seed extract was prepared by dilution with DMSO at concentrations of 0.01, 0.1, 1, 10, and 100 g/ml, then 200 µl of the prepared extract was added to the cells and incubated in the CO_2 incubator for 3 days. The extract solution in the wells was removed, and 100 µl of RMPI 1640 medium without FCS was added to each well, followed by the addition of 10 µl of 5 mg/ml MTT solution [3-(4,5-Dimethylthiazol-2-yl)-2,5 Diphenyl Tetrazolium Bromide]. The cells were incubated in the CO_2 incubator at 37°C for 4 h, and then the solution was discarded. 150 µl of DMSO and 25 µl of 0.1 M glycine (pH 10.5) were added. The plate was shaken for 10 min and the absorbance measured at 540 nm. The calculation for cell survival (PS) is as follows:

$$\% PS = \frac{A_{test}}{A_{control}} \times 100$$

Where, *%PS* = percentage of viable cells, A_{test} = absorbance value of the well containing the extract, and $A_{control}$ = absorbance value of the well containing DMSO.

Development of skin moisturizing serum

The ten serum formulas which were produced adopting several different gelling agents that were carbopol ultrez 10, carbopol ultrez 21, and water, with gel concentrations of 0.2, 0.6, and 1.2%. Each formulars were performed and swelling with water. After that, 1% propylene glycol, 2.5% glycerine (moisturizer), triethanolamine, and preservatives 0.8% were added in each formulars, respectively. The gel base serum products from all formulas were examined the satisfaction test of 30 volunteers. The formula that garnered the most positive feedback was a prototype serum formula that was adjusted for excellent physical characteristics and high potential.

The formula with the highest satisfaction scores was chosen to be applied to the development of skin care serum compositions. The 1% DLO seed crude extract was added to the mixture, along with a moisturizer, emulsifiers, and preservatives. The formula with the greatest satisfaction score from the selected formulas were used to assess product attributes in terms of chemical, physical,

and biological properties by pH, viscosity, stability, total microbial count, microorganisms index, and shelf life.

Statistical analysis

The values represented the mean of at least two duplicate studies. Duncan's multiple comparison test was used to examine whether there was a significant difference between treatments.

Results

Crude extract from DLO seeds

The crude extract of DLO seeds was obtained by crushed dried longan seeds in macerating of 95% ethanol with the ratio 1:3 w/v at room temperature for 7 days. It was partitioned with chloroform solvent and water and extracted with various polarity solvents which were hexane, ethyl acetate, and 90% methanol. It was found that the percentage yield of the crude extract was 13.7%. The extract was divided into four parts which were the hexane, ethyl acetate, methanol and water, then they were evaporated. The crude extract was obtained in thick and brownish. The antioxidant activity was determined from crude extract.

The crude extracts through partition with six various solvents, including 95% ethanol, 90% methanol, water, chloroform, ethyl acetate, and hexane, were investigated. The DPPH radical scavenging test was estimated to the antioxidant activity (% AA) and the half-maximal effective concentration (EC_{50}) in mg/ml. It was found that the extract using 95% ethanol had the highest ability to decrease free radicals, with an EC_{50} value of 0.35 mg/ml. Moreover, 80 mg/ml of extract from 95% ethanol had a higher % AA than the other solvents (Table 1, Figure 2 and 3).

Table 1. EC_{50} and % antioxidant activity (% AA) of DLO seed extracts in various solvents

Solvent	EC_{50} (mg/ml)	% AA of extract concentration 80 mg/ml
95% ethanol	0.35	90.00
90% methanol	0.45	83.10
Water	0.70	60.00
Chloroform	0.65	61.40
Ethyl acetate	0.60	64.80
Hexane	0.65	63.00

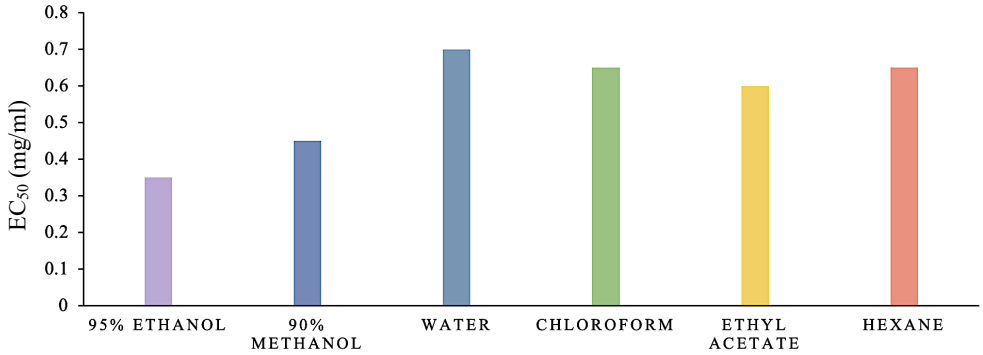

Figure 2. EC_{50} of DLO seed extracts in various solvents

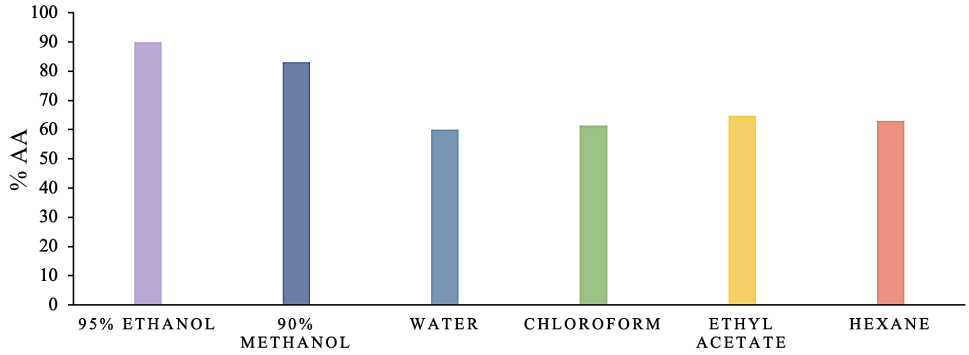

Figure 3. % AA of crude extract at bconcentration of 80 mg/ml of DLO seed extracts in various solvents

The young and mature DLO seeds were compared the antioxidant activity to explain the effect of aging DLO seeds. Their crude extracts by 95% ethanol maceration were evaluated using the DPPH radical scavenging assay. The EC_{50} and % AA of mature DLO seeds were 0.15 mg/ml and 90.00%, respectively (Table 2). The crude extract of the mature DLO seed was greater antioxidant activity than mature DLO seed. Therefore, the aging DLO seeds affected to the active compound.

Table 2. EC_{50} and % antioxidant activity (% AA) of DLO seed extracts in various aging seeds

Aging seed	EC_{50} (mg/ml)	% AA of extract concentration 30 mg/ml
Young seed	0.35	41.60
Mature seed	0.15	90.00

The total phenolic compounds in the extract of DLO seed with the highest antioxidant activity was evaluated using the Folin-Ciocalteu technique. In which phenolic interacted with the Folin reagent to form blue substance that absorb at 765 nm. Gallic acid was applied to plot the calibration graph as a representation of phenolic compounds (Figure 4). The best antioxidant activity was discovered in a 95% ethanol extract of DLO seeds, which included total phenolic compounds comparable to gallic acid at 657 mg/g extract.

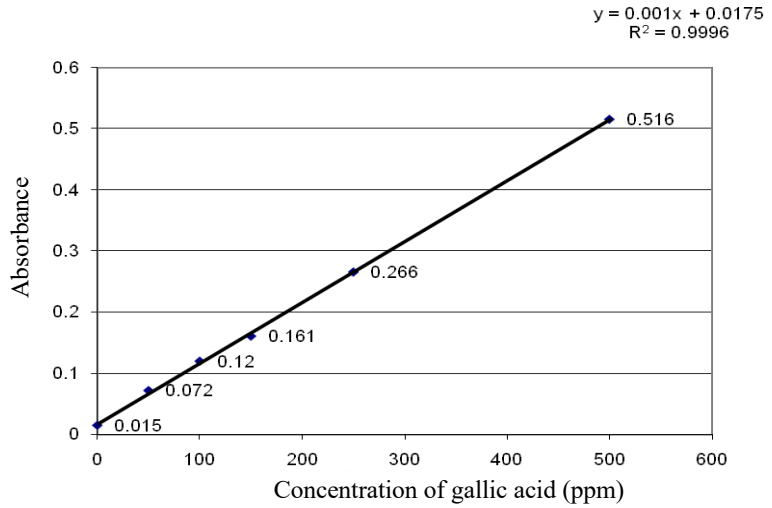

Figure 4. The calibration graph of total phenolic compounds

Antioxidant activity of DLO crude extract

The antioxidant activity of ethanol crude extract from DLO seeds was analyzed by DPPH radical scavenging assay, ABTS radical scavenging activity, and Ferric reducing/antioxidant power (FRAP) assay. For DPPH assay, the IC_{50} of DLO seed from ethanol extraction was found to be lower than that of BHT, indicating that DLO seed ethanol extract exhibited greater antioxidant activity than BHT by DPPH assay. When examined by ABTS assay and the FRAP value assay, the antioxidant activity of DLO seed extracts was lower than that of ascorbic acid and α-tocopherol. The greatest antioxidant was ascorbic acid, α-tocopherol and BHT, respectively. The findings were identical for all three approaches (Table 3).

Table 3. IC_{50} of DLO seed extract compared to standard substances with various methods

Solvent	DPPH assay IC_{50} (mg/ml)	ABTS assay IC_{50} (mg/ml)	FRAP value µmol Fe^{2+}/g
DLO seed extract	0.088[c]	0.588[d]	3138.18[c]
Ascorbic acid	0.005[a]	0.201[a]	12164.95[a]
Alpha tocopherol	0.012[b]	0.495[c]	5229.62[b]
BHT	0.129[d]	0.454[b]	3010.52[d]
Average	0.65	0.435	5885.82

[a,b,c,d,e,f]: Values with different superscripts within the same column are significantly different (p<0.05).

Anti-tyrosinase activity

The tyrosinase inhibition of DLO seed extract was considered from the IC_{50}, which is the quantity of the test ingredient that inhibits tyrosinase at 50% well, was determined. The standard substance, kojic acid exhibited an IC_{50} of 0.121 mg/ml, while DLO seed extract had an IC_{50} of 18.123 mg/ml (Table 4).

Table 4. Anti-tyrosinase capacity of DLO seed extract and kojic acid

Aging seeds	Tyrosinase IC_{50} (mg/ml)
DLO seed extract	18.123
Kojic acid	0.121
Average	9.122

Cytotoxic activity

The DLO seed crude extract was examined for cytotoxicity on human cell line using the MTT Assay. Varying concentration of extract have been mixed into cell cultures. The cell viability (% cell survival) of mixed and unmixed cells was then evaluated. The experiment was carried out on one type of fibroblast cell, Wi-38, obtained from lung cells. and five cancer cells, BT474, Chago-KI breast cancer cells, Hep-G2 lung cancer cells, Kato-III liver cancer cells, gastric cancer cells, and SW620 colon cancer cells (Table 5).

Skin moisturizing serum

The prototype serum formula was investigated from ten formulars with three gelling agents such as carbopol ultrez 10, carbopol ultrez 21, and aqua, at concentrations of 0.2, 0.6, and 1.2%, respectively. Water was used to inflate the gel, followed by 1% propylene glycol, 2.5% glycerine (moisturizer), 1-3 ml triethanolamine, and 0.8% preservative. After that, 30 volunteers were surveyed

to determine their level of satisfaction in order to identify the most appropriate formulation for use as a prototype serum gel base formulation. The formula for a serum was subsequently developed by combining 1% DLO seed crude extract with a skin moisturizer, solubilizer, preservative and emulsifier.

Table 5. % cell survival of cell cultures mixed with various concentration of DLO seed crude extract

Concentration (g/ml)	% cell survival						
	BT474	Chago-KI	Hep-G2	Kato-III	SW620	Wi-38	Average
100	75	78	86	71	104	108	87.00
10	91	90	83	81	104	109	93.00
1	92	90	91	81	93	109	92.67
0.1	94	91	90	84	96	106	93.50
0.01	95	95	93	85	97	103	94.67
0.001	92	95	94	89	98	102	95.00
0.0001	96	100	96	90	98	104	97.33
Average	90.71	91.29	90.43	83.00	98.57	105.86	93.31

The satisfaction test was carried out to determine the formula with which the samples were most satisfied by selecting the formula with the greatest satisfaction score from the selected formulas and testing the chemical, physical, and biological aspects of the product by measuring physical and chemical properties, pH, viscosity, stability, total microbial count, microorganisms index, and shelf life. Formula 2 exhibited the highest score of 6.5 out of 9 for skin penetration qualities, 6.7 for stickiness of the gel base serum texture (the gel was less sticky on the skin), and 6.7 for overall preference (Table 6 and Figure 5). Across all score, Formula 2 had an average satisfaction score of 6.7. Therefore, it was a prototype serum formula which was appropriated for developing to serum product.

The moisturizing serum product was developed relying on Formula 2 including 0.6% carbopol ultrez 10, 1% propylene glycol, and 2.5% glycerine, and 0.13% triethanolamine. Then, the formula was adjusted by adding 1% DLO seed crude extract and mixed with moisturizer, solubilizer, emulsifier, and preservatives which were 0.8%. The two formulas were generated by various concentrations of adding agents, which were the moisturizer and emulsifier. As a comparison, the commercial serum product was used. All three products were examined for sample satisfaction with their characteristics such as texture, skin absorption, stickiness, moisturizing, luscious, and overall preference. Formula 2 received the highest satisfaction score of all samples on every category of serum characteristics (Table 6, Figure 7). The average like score was 7.67, indicating a

modest level of liking. The satisfaction rating was greater than that of Formula 3, a commercially available product.

Table 6. Satisfaction evaluation of serum formulas for prototype

Formula	Texture	Skin absorption	Stickiness	Overall preference	Average
1	6.4^{abc}	6.1^{ab}	6.3^{ab}	6.0^{a}	6.20
2	6.7^{ab}	6.5^{a}	6.7^{a}	6.7^{a}	6.65
3	6.0^{bc}	5.8^{ab}	6.0^{abc}	5.9^{a}	5.93
4	6.0^{bc}	6.2^{ab}	6.6^{a}	6.5^{a}	6.33
5	6.2^{bc}	6.0^{ab}	6.4^{ab}	6.4^{a}	6.25
6	5.5^{cd}	5.5^{abc}	5.4^{bcd}	5.8^{a}	5.55
7	4.3^{e}	4.3^{d}	4.3^{ef}	4.7^{b}	4.40
8	5.6^{cd}	5.2^{bcd}	4.9^{de}	5.7^{a}	5.35
9	4.9^{de}	4.6^{cd}	3.9^{f}	4.6^{b}	4.50
10	7.3^{a}	6.3^{ab}	6.3^{ab}	6.7^{a}	6.65
Control	2.1^{f}	2.0^{e}	5.1^{cde}	4.1^{b}	3.33

^{a,b,c,d,e,f}: Values with different superscripts within the same column are significantly different (p<0.05).

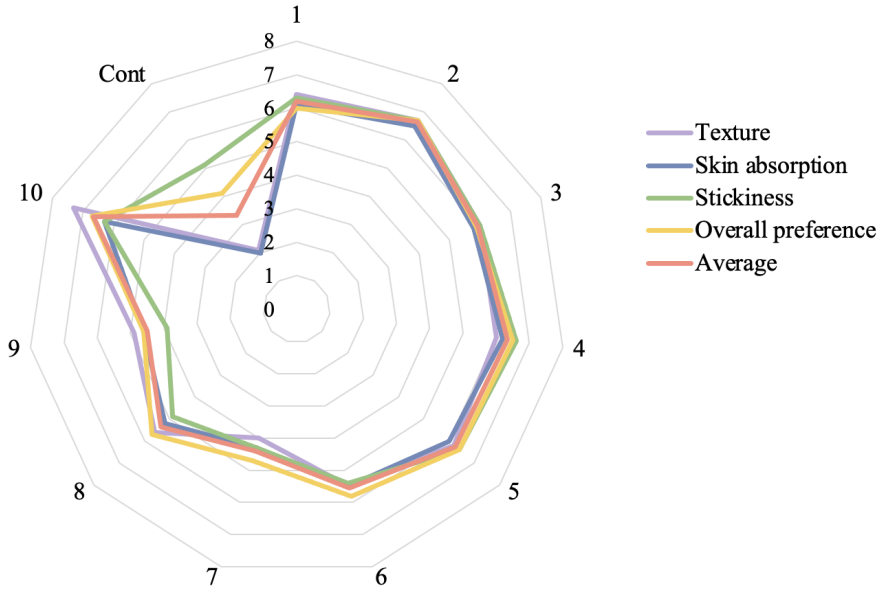

Figure 5. Graph of satisfaction evaluation of serum formulas for prototype

Formula 2 serum product were tested for chemical properties. It was investigated that the pH was measured at 5. The physical properties were

measured by the viscosity at 49,120 cP. The microbial property was determined that microorganisms were not contaminated by yeast and mold, and were not found to be *Staphylococcus aureus*. The product was stable and did not separate during storage at room temperature for a period of a year.

Table 7. Satisfaction evaluation of serum formulas for moisturizing serum product

Serum	Texture	Skin absorption	Stickiness	Moisturizing	Luscious	Overall preference	Average
Formula 1	7.1[a]	6.3[a]	6.6[a]	7.0[a]	7.3[ab]	6.6[a]	6.82
Formula 2	7.8[a]	7.2[a]	7.6[a]	7.9[a]	7.9[a]	7.6[a]	7.67
Commercial serum	7.3[a]	6.2[a]	6.2[a]	6.8[a]	6.4[a]	6.6[a]	6.58

[a,b]: Values with different superscripts within the same column are significantly different (p<0.05).

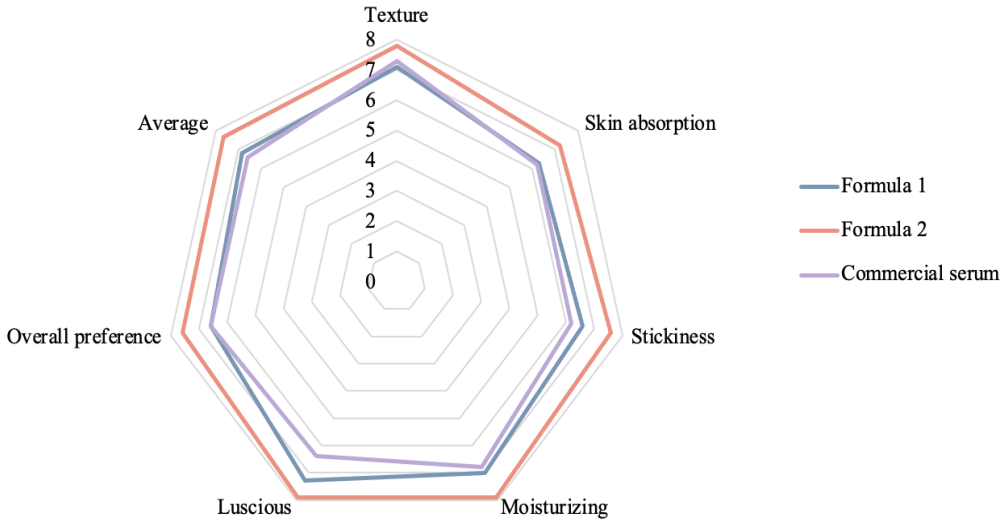

Figure 6. Graph of satisfaction evaluation of serum formulas for moisturizing serum product

Discussion

The antioxidant ability of various solvent-extracted DLO seed crude extracts corresponded to EC_{50} and % AA. The crude extract by 95% ethanol was the most effective in reducing free radicals. Besides, the results agreed with the report on longan's antioxidant properties that associated with the other species of

longan. For example, the antioxidant activity of Edor longan dried seed extract was comparable with that of Japanese green tea extract when determined using the DPPH radical scavenging assay (Rangkadilok *et al.*, 2007).

However, the EC_{50} of DLO seed extract examined its free radical's efficiency that is lower than other plant extracts, such as turmeric crude extract by acetone, which has an EC_{50} value of 0.235 mg/ml. This might be because the young seed of the longan affects to the low ability to decrease free radicals. Therefore, the antioxidant activity depends not only on the species of plant but also on the extraction process, extraction solvent system, appropriate time, and plant growing period (Maisuthisakul and Pongsawatmanit, 2004).

The antioxidant activity of DLO seed extract was compared with butylated hydroxytoluene (BHT), as antioxidant standard. The BHT standard had a DPPH (EC_{50}) of 0.01 mg/ml, which it was comparable to DLO seed extract. It has been demonstrated that the extract from DLO seeds has the potential to bind DPPH free radicals in similarly to the BHT However, the free radical binding capacity should also be compared to that of other standard substances, in units of equivalent weight as standard substances.

Phenolic compounds are very significant active ingredients that found in many kinds of organism such as vegetables, fruits, and plants due to its antioxidant activity (Pokorny and Schmidt, 2001). Therefore, the total phenolic can be indicated the antioxidant ability. The extract with a high phenolic compound has better antioxidant activity than the extract with a low phenolic compound. These results revealed that the phenolic compounds concentration of DLO seed extract was greater than that of mangosteen peels, mango peels and rambutan peel (Chaiwarit *et al.*, 2021) and was greater than blueberries (Sanchez-Ballesta *et al.*, 2023). Because of its high phenolic compounds and remaining antioxidant activity, DLO seed extract is suitable for development as an ingredient in cosmetic products.

However, in the DPPH and ABTS assays for antioxidant activity, the IC_{50} of DLO seed extract was similar to that of the three standard compounds, which it was described that seed extract had antioxidant activity comparable to that of standard compounds. It was consisted of a mechanism for eliminating free radicals by binding with free radicals. Because of the of the antioxidants analysis by DPPH radical scavenging assay investigates the efficiency of antioxidants in binding to free radicals DPPH (2,2-diphenyl-1-picrylhdrazyl). For the ABTS radical scavenging activity test, the antioxidant activity is evaluated by binding to free radicals (ABTS radical cation: $ABTS^{\bullet+}$), while the Ferric reducing/antioxidant power (FRAP) technique determines the efficiency or reducing ability or electron donating of antioxidants, which performs free radicals to stable radicals, therefore terminating the free radical chain reaction.

In addition, there have also been reports of antioxidant activity of other natural compounds; for example, turmeric extracted with methanol had an EC_{50} DPPH value of 8.04 mg/ml (Thaikert and Paisooksantivatana, 2009), while walnut extract had an EC_{50} DPPH value of 0.11 mg/ml (Fernández-Agulló *et al.*, 2020). Therefore, DLO seed extract is a potential natural product with antioxidant activity that might be used in cosmetic and health food applications.

Although Kojic acid inhibits tyrosinase activity more than DLO seed extract. This is attributed to the crude extract may have a lower impact than the standard substance, which is pure substance (Deguchi *et al.*, 2019). Kojic acid standard substance used as a comparator is a substance produced from *Aspergillus* and *Penicillium* fungi, which has properties to inhibit the activity of the enzyme tyrosinase by acting as a chelating agent to bind to cu^{2+} at the active site of the enzyme and trapping free radicals. Substances that inhibit tyrosinase activity are taken advantage of as active compounds in cosmetic treatments which contribute to brighten the skin (Tengamnuay *et al.*, 2006).

DLO crude extract had antioxidant activity and inhibits tyrosinase activity, which catalyzes melanin synthesis. As a result, it could potentially be applied as an ingredient in cosmetic products to cleanse the skin and postpone the aging of skin cells. Despite considering that the influence was less than the standard. Its qualities will improve if it is purified. As a consequence, this approach it was decided to investigate the cytotoxicity of the extracts to cells.

Ethanol-extracted of DLO seed was non-toxic to all cell types tested. When the extract was added to the cell culture medium, it tended to have a maximum concentration of 100 g/ml in cultivated cancer cells isolated from the breast, lung, liver, and stomach. The cell viability of cancer cell or human cell are decreased by concentration dependent manner. Besides, DLO seed extract is likely to increase the proliferation of normal cells separated from the lungs and colon cancer cells. Cell viability was shown to be greater when high concentration extract was mixed with cell culture medium than when low concentration extract was adopted. The results were related to cytotoxic activity, which seed extracts were non-cytotoxic activity (Natungnuy *et al*., 2018; Nitteranon, 2018).

This led to the formulation of a skin moisturizing serum. Formula 2, containing 1% DLO seed crude extract, exhibited high satisfaction scores across various attributes including texture, skin absorption, stickiness, moisturizing, lusciousness, and overall preference. The developed serum product demonstrated favorable chemical, physical, and microbial properties and maintained stability over a one-year storage period.

This research is explored the distinctive characteristics of crude extracts from locally sourced Thai DLO seeds, particularly their potential for creating skin moisturizing serums. By assessing their antioxidant capacity, anti-tyrosinase

activities, and moisturizing effects, this study adds valuable insights to the growing field of natural skincare ingredients. The results suggest that DLO seed extracts could be successfully incorporated into innovative cosmetic products that meet modern consumers' desires for safe, effective, and stable skincare solutions.

The findings highlighted the potential of *Dimocarpus longan* var. *obtusus* seed extract as a natural ingredient for skin care products, particularly in moisturizing serums. Further studies could explore the extract's performance in real-world applications and its interactions with other cosmetic ingredients.

Acknowledgements

This study was funded by a research grant from Thailand Science Research and Innovation (TSRI). We would like to express our gratitude to the Faculty of Science and Technology, the Faculty of Agriculture and Natural Resources, and the Institute of Research and Development at Rajamangala University of Technology Tawan-OK in Chonburi Province, Thailand, for their involvement and facilities assistance.

References

Benzie, I. F. F. and Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry, 239:70-76.

Chaiwarit, T., Kantrong, N., Sommano, S. R., Rachtanapun, P., Junmahasathien, T., Kumpugdee-Vollrath, K. and Jantrawut, P. (2021). Extraction of Tropical Fruit Peels and Development of HPMC Film Containing the Extracts as an Active Antibacterial Packaging Material. Molecules, 26.

Deguchi, T., Tamai, A., Asahara, K., Miyamoto, K., Miyamoto, A., Nomura, M., Kawata-Tominaga, T., Yoshioka, Y. and Murata, K. (2019). Anti-Tyrosinase and Anti-Oxidative Activities by Asana: The Heartwood of *Pterocarpus marsupium*. Natural Product Communications, 14.

Fernández-Agulló, A., Castro-Iglesias, A., Freire, M. S. and González-Álvarez, J. (2020). Optimization of the Extraction of Bioactive Compounds from Walnut (*Juglans major 209 x Juglans regia*) Leaves: Antioxidant Capacity and Phenolic Profile. Antioxidants, 9.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J., Pihlaja, K., Kujala, T. S. and Heinonen, M. (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry 47:3954-3962.

Kubo, I., Kinst-Hori, I., Chaudhuri, S.K., Kubo, Y., Sánchez, Y. and Ogura, T. (2000). Flavonols from *Heterotheca inuloides*: Tyrosinase Inhibitory Activity and Structural Criteria. Bioorganic and Medicinal Chemistry, 8:1749-1755.

Lithanatudom, S. K., Chaowasku, T., Nantarat, N., Jaroenkit, T. Smith, S. R. and Lithanatudom, P. (2017). A First Phylogeny of the Genus *Dimocarpus* and Suggestions for Revision of Some Taxa Based on Molecular and Morphological Evidence. Scientific Reports, 7.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T., Andoh, T., Shinohara, A. and Nakata, M. (1999). Evaluation of the Antioxidant Activity of Environmental Plants: Activity of the Leaf Extracts from Seashore Plants. Journal of Agricultural and Food Chemistry, 47:1749-1754.

Natungnuy, K., Chareonsap, P. P. and Poeaim, S. (2018). Biological Activities of The Methanolic Extracts from Two Varieties of *Dimocarpus longan* Seeds. International Journal of Agricultural Technology, 14:1505-1514.

Nitteranon, V. (2018). Anti-Inflammatory, Antioxidant and Quinone Reductase Inducing Effects of Lumyai Thao (*Dimocarpus longan* var. *obtusus*) seed extract. Journal of Food Science and Agricultural Technology, 4:29-35.

Maisuthisakul, P. and Pongsawatmanit, R. (2004). Effect of Sample Preparation Methods and Extraction Time on Yield and Antioxidant Activity from Kradonbok (*Careya sphaerica* Roxb.) Leaves. Agriculture and Natural Resources, 38:8-14.

Pokorny, J. and Schmidt, S. (2001). Antioxidants in Food: Practical Applications. Antioxidants in Foods, pp.331-354.

Rangkadilok, N., Sitthimonchai, S., Worasuttayangkurn, L., Mahidol, C., Ruchirawat, M. and Satayavivad., J. (2007). Evaluation of Free Radical Scavenging and Anti-Tyrosinase Activities of Standardized Longan Fruit Extract. Food and Chemical Toxicology, 45:328-336.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, R. (1999). Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. Free Radical Biology and Medicine, 26:1231-1237.

Saewan, N., Koysomboon, S. and Chantrapromma, K. (2011). Anti-Tyrosinase and Anti-Cancer Activities of Flavonoids from *Blumea balsamifera* DC. Article in Journal of Medicinal Plants Research, 5:1018-1025.

Sanchez-Ballesta, M. T., Marti-Anders, C., Álvarez, M. D., Escribano, M. I., Merodio, C. and Romero, I. (2023). Are the Blueberries We Buy Good Quality? Comparative Study of Berries Purchased from Different Outlets. Foods, 12.

Soong, Y. Y. and Barlow, P. J. (2004). Antioxidant Activity and Phenolic Content of Selected Fruit Seeds. Food Chemistry, 88:411-417.

Tachibana, Y., Kikuzaki, H., Lajis, N. H. and Nakatani, N. (2001). Antioxidative Activity of Carbazoles from *Murraya koenigii* Leaves. Journal of Agricultural and Food Chemistry, 49:5589-5594.

Tengamnuay, P., Pengrungruangwong, K., Pheansri, I. and Likhitwitayawuid, K. (2006). *Artocarpus lakoocha* Heartwood Extract as a Novel Cosmetic Ingredient: Evaluation of the *in vitro* Anti-Tyrosinase and *in vivo* Skin Whitening Activities. International Journal of Cosmetic Science, 28:269-276.

Thaikert, R. and Paisooksantivatana, Y. (2009). Variation of Total Curcuminoids Content, Antioxidant Activity and Genetic Diversity in Turmeric (*Curcuma longa* L.) Collections. Kasetsart Journal Natural Science, 43.

(Received: 2 November 2023, Revised: 3 July 2024, Accepted: 6 July 2024)