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Identification of phenolic compounds and evaluation of biological activities of methanolic extracts obtained from two varieties of longan (*Dimocarpus longan*) peels

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Abstract *Dimocarpus longan* belongs to the Sapindaceae family, which are revealed as cryptic species. In Thailand, *Dimocarpus longan* spp. *longan* var. *longan* such as Edor is the most cultivated. Another *Dimocarpus longan* spp. *longan* var. *obtusus* (*Dimocarpus obtusus*), called Lum-Yai Thao, is found in Eastern Thailand. However, there is still little information on the biological activity of Thao. Hence, this research was designed to identify phenolic compounds and evaluate the biological activities of methanolic extracts obtained from peels of two longan varieties: Edor VS Thao. For the total phenolic compound, no significant differences were observed between Edor and Thao. For antioxidant activity using DPPH, ABTS, and FRAP assays, the analogs showed Edor had more activity than Thao. The disc diffusion test at 5 mg/disc showed that Edor peels extract effectively inhibited the growth of bacteria, but not Thao extracts which were strong against *Propionibacterium acnes.* However, both of them also did not have activity against *Escherichia coli* and *Staphylococcus epidermidis*.For anti-tyrosinase activity, both variety extracts had less effective anti-tyrosinase activity. In addition, anti-inflammatory activity was evaluated by measuring nitric oxide (NO) generated from SNP and using LPS-stimulated RAW 264.7 cells which showed excellent activity.In cytotoxicity using MTT assay, the results demonstrated that the methanolic extract of longan peel from Thao exhibited low cytotoxicity against both L929 and HaCaT. On the other hand, the extract from Edor exhibited cytotoxic activity. These results suggested that Thao peel extracts are different from Edor peel extract. Thao peel extracts showed excellent anti-inflammatory and little cytotoxic activity for L929 and HaCaT cell lines and antibacterial against *P. acnes*. Therefore, Thao peel extracts could be used in preparing products for anti-inflammatory supplements and cosmetics.

Keywords: *Dimocarpus longan*, Phenolic compound, Biological activity

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Introduction

Dimocarpus is a genus belongs to the family of Sapindaceae. There are nine species and six subspecies (spp.) assigned to this genus, of which is the most frequently reported in China. Longan (*Dimocarpus longan*), known as Lam-Yai in Thailand, is subdivided into two subspecies: spp. *malesianus* and spp. *longan*. In addition, there are three varieties (var.): var. *obtusus*, var. *longan*, and var. *longepetiolulatus* from *Dimocarpus longan* spp. *longan* (Santisuk and Larsen, 1999). In Thailand, two varieties (var.); var. *obtusus* and var. *longan* of *Dimocarpus longan* spp. *longan* have been reported. The first variety, *Dimocarpus longan* spp. *longan* var. *longan* has many cultivars such as Edor, Chompoo, Biew Kiew, and E Wai, which are the most well-known and grown commercially for consumption. Another variety, *Dimocarpus longan* spp. *longan* var. *obtusus*, called Lum-Yai Thao or Thao, is found in Eastern Thailand. Thao is mainly grown as ornamental plants rather than commercial as it is not popular with consumers.

Numerous studies have been only focused on the bioactive compounds and biological activities from *Dimocarpus longan* spp. *longan* var. *longan* which used in fruit and health promotion (Yang *et al.,* 2011). Previous investigations have reported that both edible and nonedible parts of longan extracts contain a higher phenolic compounds such as gallic acid, ellagic acid, and corilagin (Rangkadilok *et al.,* 2005; Huang *et al.,* 2012; Liu *et al.,* 2012). The previous research has shown excellent correlation between the phenolic compound and the antioxidant potential of the longan aril, peel and seed extracts (Rangkadilok *et al.,* 2007; Prasad *et al.,* 2009; Liawruangrath, 2011; Keawsa-ard *et al.,* 2011; Yang *et al.,* 2014; Rerk-am *et al.,* 2016; Natungnuy *et al*., 2018). Including some studies by Zhang *et al*., (2018) and Natungnuy *et al*., (2018) where the total phenolic content (TPC) between longan varieties were compared. The extract from the longan seed and peel possessed antibacterial activity such as *Bacillus cereus*, *Micrococcus luteus*, *Staphyloccocus aureus*, *Propionibacterium acnes* (Tseng *et al.,* 2014; Natungnuy *et al.,* 2018; Chollakup *et al.,* 2021) and antifungal activity (Rangkadilok *et al.,* 2012). For tyrosinase inhibition, longan seed and pericarp, or peel extracts, also showed moderate tyrosinase inhibitory activity (Rangkadilok *et al*., 2007; Natungnuy *et al*., 2018). In addition, longan flower, peel, and seed extracts have more anti-inflammatory activity (Kunworarath *et al*., 2016; Huang *et al*., 2012). For cytotoxic activity, longan peel and seed extracts have been reported for both promotion and inhibition of cell proliferation (Natungnuy *et al*., 2018; Lin *et al.,* 2012). For Thao, the evaluation of biological activities of seed extracts was also reported (Nitteranon, 2018; Natungnuy *et al.,* 2018).

However, biological activities have not been previously reported on extracts from longan peel of Thao (var. *obtusus*). Therefore, this present research aimed to evaluate the TPC and biological activity of methanolic extracts from two varieties (Thao: var. *obtusus* and Edor: var. *longan*) of longan peel.

Materials and methods

Preparation and extraction plant material

There are three groups of mature longan fruits in the experiment.

Group 1: The fruits were harvested from only the longan tree of var. *longan* (cultivar Edor) which were purchased from the main fruit production areas in Lamphun province, Thailand.

Group 2: The sample of mature longan fruits of var. *obtusus* (Thao1) was collected within a single tree from Rayong province, Thailand.

Group 3: The mix of fruit var. *obtusus* (Thao2) were randomly obtained from the local markets in Chonburi and Rayong provinces, Thailand.

After complete drying, the longan peel powder was then extracted with methanol as a solvent for seven days. The methanolic extracts were evaporated using a rotary evaporator at 40^{o}C.

Evaluation of total phenolic content

The TPC of longan peel extracts was evaluated using Folin–Ciocalteu method according to Soong and Barlow (2004) with some modifications. Briefly, 50 microliters (µL) of the sample at 1,000 microgram/milliliter (µg/mL) were mixed with equal volume of 10% Folin–Ciocalteu solution in a 96-well plate and incubated for 6 minutes in the dark. After that, 100 µL of 7.5% sodium carbonate solution was added and incubated for 30 minutes and then measured at 765 nm. The TPC was expressed as mg of gallic acid equivalents per gram extract (mgGAE/g of extract).

Evaluation of antioxidant capacity

For antioxidant activity using DPPH, ABTS and FRAP assays, the method of DPPH and ABTS modified according Shimada *et al*. (1992) and Re *et al.* (1999) with slight modifications. For DDPH and ABTS assays, trolox has been used as a standard, and expressed in milligrams trolox equivalent/g extract (mgTE/g extract). According to Benzie and Strain

(1996), FRAP assay was undertaken with minor modifications. Ascorbic acid was used to generate the standard curve, and the results were reported as milligrams ascorbic acid equivalent/g extract (mgAAE/g extract).

Evaluation of antibacterial activity

The antibacterial activity was evaluated against six different strains of bacteria, including *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* TISTR 1466, *Staphylococcus epidermidis* ATCC 12228, *Propionibacterium acnes* and *Escherichia coli* ATCC 25922 using paper disc diffusion method tested by the standard CLSI adapted in 2012. Briefly, the purified bacteria culture was swabbed on the surface of Mueller Hinton Agar (MHA). The disc containing 5 mg of the extract was placed on the surface of inoculated agar and incubated for 18-24 hours. Methanol and gentamicin (10 µg/disc) were used as the negative and positive control, respectively. The zone of inhibition was to be measured.

Evaluation of anti-tyrosinase activity

Tyrosinase inhibitory activity was assessed using L-DOPA as substrate described by Masuda *et al*. (2004) and Saewan *et al*. (2011). The final volume of each sample reaction is 200 µL; the phosphate buffer (pH 6.8) was mixed with 20 µL of mushroom tyrosinase solution (25 U/mL) in a 96-well plate and incubated at 25 ℃ for 10 minutes in the dark. Then, 40 µL of L-DOPA (2.5 mM) was added to start the reaction (the control, no sample: A). Likewise, 60 µL of extract solution was mixed with all reaction reagents (the test sample: B) and the other reactions without tyrosinase (the blank of the sample, color of the test sample: C). After 15 minutes of incubation at 25 ℃, the absorbances were measured at 475 nm. Ascorbic acid was used to generate the standard curve, and the results were expressed anti-tyrosinase capacity (mgAAE/g extract). The IC_{50} (50% inhibitory concentration) were calculated by GraphPad prism 8 and %tyrosinase inhibition was calculated as follows the below equation.

$$\%\text{Tyrosinase inhibition} = \left[\frac{(A)-(B-C)}{(A)}\right] \text{x}100$$

A is the absorbance value of the control, without the extract; B is the absorbance value of the test sample with tyrosinase; and C is the absorbance value of the blank of sample, without tyrosinase.

Evaluation of anti-inflammatory activity

Anti-inflammatory activities were evaluated by measuring nitric oxide (NO) both generated from sodium nitroprusside (SNP) and using lipopolysaccharide (LPS) – activated RAW 264.7 cells. For in vitro nitric

oxide radical scavenging assay, nitric oxide radical generated from SNP was measured using the Griess reaction according to Aktas *et al*. (2013). Briefly, the reaction mixture (200 µL) containing SNP (10 mM) in phosphate-buffered saline (pH 7.3), without (the control, no sample: A) or with (the test sample: B) the plant extract at different concentrations (62.5 – 500 µg/mL) and treated simultaneously with different concentrations of the samples without SNP (the blank of the sample, color of the test sample: C) were incubated at 25˚C for 30 minutes in a 96-well plate. After that, the reaction mixture was mixed with an equal volume of freshly prepared Griess reagent. Gallic acid was used as a positive control. Alternatively, nitric oxide (NO) production by RAW 264.7 macrophages was determined by measuring the accumulation of nitrite, which was slightly modified from Buapool *et al*., (2013). RAW 264.7 cells were cultured at 37 ℃ in the 5% CO_2 incubator in RPMI 1640 containing gentamicin and 10% FBS and were sub-cultured weekly using a cell scraper. After that, the cells were seeded at a density of 1.5×10^5 cells per well into a 96-well plate and incubated overnight. The cells were stimulated with 1 mg/mL lipopolysaccharide (LPS) alone (the control: A) and treated simultaneously with different concentrations (62.5-200 µg/mL) of the samples dissolved in DMSO (the test sample: B) or 62.5 – 200 µg/mL of indomethacin which used as a positive control as well as the other reactions without LPS (the blank of sample: C). After 24 hours, equal volume of supernatant from each well and Griess reagent were mixed into a new 96-well plate.

The absorbance of the mixture was measured at 540 nm after 10 minutes of incubation. The % nitric oxide inhibition was calculated as follows:

$$\%\text{Nitric oxide inhibition} = \left[\frac{(A)-(B-C)}{(A)}\right] \text{x}100$$

A is the absorbance value of the control, without the extract; B is the absorbance value of the test sample; and C is the absorbance value of the blank of sample, without SNP or LPS.

Evaluation of cytotoxic activity

The mouse fibroblast cell line (L929) and human epidermal keratinocyte cell line (HaCaT) were cultured and evaluated for cytotoxic activity using MTT assay as described by Poeaim *et al.* (2017). The cells were seeded into a sterile 96-well plate at a density of 1×10^5 cells/well and incubated for 24 hours. Then, the extract was added to the cells (1000 µg/mL) and further incubated for 48 hours before performing MTT assay. DMSO and mitomycin C were included as a negative and positive controls, respectively. Following incubation, 50 microliters of 2 mg/mL of MTT were added to each well and incubated at 37 ℃ for 4 hours. The medium

was then gently removed, and 100 µL of DMSO: ethanol (1:1 v/v) was added to dissolve the formazan crystals. The amount of solubilizing the formazan was determined at 570 nm. For background absorbance, each test should contain control wells (untreated cells) and blank wells (without cells). All values are final after subtraction of background absorbance. The percentage of cell viability was calculated as follows:

$$\%\text{Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \text{x}100$$

Statistical analysis

All experiments were performed in triplicate; data are expressed as means ± standard deviation (SD) using SPSS version 25 statistical software for A one-way analysis of variance (ANOVA).

Results

Total phenolic compound

This study revealed both methanolic extracts from two varieties (Thao: var. *obtusus* and Edor: var. *longan*) of longan peels presented of phenolic compound. The TPC of the Thao1 and Thao2 were 84.73±3.01 and 65.57±2.32 mg GAE/g extract, respectively. In comparison, the methanolic extract of Edor had TPC of 67.56±4.93 mg GAE/g extract. The TPC of Thao1 peel extracts was significantly ($p < 0.05$) higher than the Thao2 and Edor extracts. Alternatively, there was no significant difference between Thao2 and Edor peels extract ($p > 0.05$) (Figure 1).

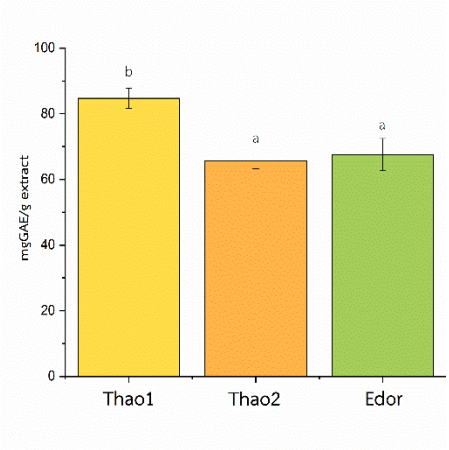

Figure 1. The TPC of the methanolic extracts from longan peels by the Folin-Ciocalteu method

Antioxidant capacity

Antioxidant activities of the methanolic extract from longan peel were evaluated using DPPH, ABTS and FRAP assays. The results showed that longan peel exhibited radical scavenging and reducing activity. The antioxidant capacity of methanolic extract from Edor (*var. longan*) revealed high antioxidant activities than Thao (*var. obtusus*), significantly different for ABTS and FRAP assays ($p < 0.05$). For DPPH assay, Thao1 and Thao2 were 279.83±0.17 and 376.30±0.87 mgTE/g extract, respectively. At the same time, the methanolic extract of Edor had 194.71±0.66 mgTE/g extract. The antioxidant capacity of Thao2 extract was also significantly ($p < 0.05$) higher than the Thao1 extract. On the other hand, it was found that the Thao1, Thao2 and Edor extracts from longan peels have antioxidant activity with the value 85.59±0.75, 93.64±0.71 and 200.78±2.36 mgAAE/g extract, respectively which presented in Table 1.

Table 1. Antioxidant activity of the methanolic extracts from longan peels

The methanolic extracts from longan peels	Antioxidant capacity		
	DPPH (mgTE/g extract)	**ABTS (mgTE/g extract)**	**FRAP (mgAAE/g extract)**
Thao1	279.83^{b}±0.17	80.79^{b}±0.54	85.59^{c}±0.75
Thao2	376.30^{a}±0.87	84.03^{b}±0.89	93.64^{b}±0.71
Edor	194.71^{c}±0.66	94.04^{a}±0.06	200.78^{a}±2.36

The data are expressed as mean±SD, the letters a-c within the same column indicate the statistical significant at $p<0.05$

Antibacterial activity

The antibacterial activity of the methanolic extract from longan peel was evaluated using a disc diffusion method. Two varieties (Thao: *var. obtusus* and Edor: *var. longan*) were tested against 5 gram-positive including *B. subtilis*, *S. aureus*, *M. luteus*, *S. Epidermidis,* and *P. acnes* and only 1 gram-negative bacteria *(E. coli)*. At 24 hours, the inhibition zones were measured using a digital caliper and expressed in millimeters (mm.). These extracts were tested at concentrations at 5 mg/disc against *B. subtilis*, *S. aureus*, *M. Luteus*, and *P. acnes* (Figure 2). *Staphylococcus epidermidis* and *Escherichia coli* were not inhibited by any of the extracts which there were no inhibition zones observed (data not shown). Gentamicin (10 μg/disc) that used as positive control showed inhibition diameters ranging from 22.31±0.12 to 27.44±2.79 mm. There was no significant difference between the inhibition zone of Thao1, Thao2, and Edor peels extract against *B. subtilis* ($p > 0.05$) (Table 2).

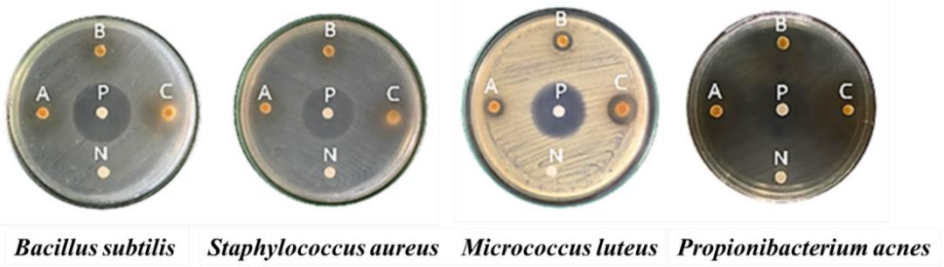

Figure 2. Inhibition zone against *Bacillus subtilis*, *Staphylococus aureus*, *Micrococcus luteus* and *Propionibacterium acnes* using methanolic extracts from longan peels (A: Thao2, B: Thao1, C: Edor, N: negative control and P: positive control)

Table 2. The inhibition zone of methanolic extracts from longan peels and gentamicin using the disc diffusion method

The methanolic extracts from longan peels (5 mg/disc)	Inhibition zone (mm)			
	B. subtilis	***M. luteus***	***S. aureus***	***P. acnes***
Thao1	$16.76^{a}\pm0.25$	$9.27^{b}\pm0.72$	$7.34^{b}\pm0.19$	$25.95^{a}\pm0.71$
Thao2	$16.98^{a}\pm0.62$	$9.35^{b}\pm0.82$	$7.29^{b}\pm0.13$	$24.99^{a}\pm1.88$
Edor	$17.43^{a}\pm1.25$	$15.09^{a}\pm0.86$	$9.54^{a}\pm0.05$	$22.97^{b}\pm1.08$
Gentamicin (10 µg/disc) (positive control)	27.37 ± 0.38	26.60 ± 0.52	22.31 ± 0.12	27.44 ± 2.79

The data are expressed as mean±SD, the letters a-b within the same column indicate the statistical significant at $p<0.05$

Anti-tyrosinase activity

The anti-tyrosinase activity of the methanolic extracts of longan peel was examined by the dopachrome method at the concentration range of 5 to 15 mg/mL. Anti-tyrosinase capacity and IC_{50} values of the methanolic extracts from longan peel are presented in Table 3. The methanolic extract from Edor (*var. longan*) revealed high anti-tyrosinase activity than Thao (*var. obtusus*), significantly different for both anti-tyrosinase capacity and IC_{50} ($p < 0.05$). However, both varieties extracts showed a more excellent IC_{50} value than the previous report.

Table 3. Anti-tyrosinase capacity and IC_{50} values of the methanolic extracts from longan peel**s**

The methanolic extracts from longan peels	Anti-tyrosinase capacity (mgAAE/g extract)	IC_{50} (µg/mL)
Thao1	$264.75^{b}\pm0.015$	9277.96
Thao2	$278.90^{b}\pm0.043$	9013.96
Edor	$381.92^{a}\pm0.008$	4124.91

The data are expressed as mean±SD, the letters a-b within the same column indicate the statistical significant at $p<0.05$

Anti-inflammatory

This study evaluated the anti-inflammation activity of the methanolic extracts of longan peel range from 62.5 to 1000 µg/mL concentration. The results expressed in % nitric oxide inhibition and IC_{50} are shown in Table 4. At 1000 µg/mL, the longan extracts exhibited intense scavenging activity having a % nitric oxide inhibition values between 78.98 and 89.76, which noticeably showed no significant differences between the two different varieties. The Thao1 and Thao2 extracts were caused by the most potent inhibitory activity having IC_{50} values of 82.30 and 92.70 µg/mL, respectively.

For nitric oxide - LPS – activated RAW 264.7 cells, this study evaluated the anti-inflammation activity of the methanolic extracts of longan peel from two varieties in the range of 6.25 - 200.00 µg/mL. At 200 µg/mL, the results showed that all extracts significantly decreased NO production in RAW 264.7 cells having a % nitric oxide inhibition value of 90.00. The IC_{50} of anti-inflammation activity of Thao1, Thao2, and Edor extracts of longan peels were in the range of 7.60 - 9.74 µg/mL (Table 4).

Table 4. Anti-inflammation by nitric oxide inhibitory assay of the methanolic extracts from longan peels

The methanolic extracts from longan peels	**NO - generated from SNP**		**NO - LPS-activated RAW 264.7 cells**	
	%Nitric oxide inhibition	IC_{50} (µg/mL)	%Nitric oxide inhibition	IC_{50} (µg/mL)
Thao1	83.57^{a}±6.03	82.30	89.48^{a}±3.44	7.60
Thao2	87.83^{a}±3.15	92.70	89.68^{a}±3.27	8.77
Edor	78.98^{a}±4.46	116.73	89.76^{a}±1.69	9.74

The data are expressed as mean±SD, the letters a within the same column indicate the statistical significant at $p<0.05$

Cytotoxic activity

In this study, the mouse fibroblast cell line (L929) and human epidermal keratinocyte cell line (HaCaT) were used to evaluate the cytotoxicity effect of extracts.The preliminary screening for cytotoxic activity of methanolic extract of longan peel from 2 cultivars at concentration 1,000 µg/mL was estimated by MTT assay. The percentage of cell viability was calculated after exposure to the extracts for 24 hours of incubation. The cytotoxic activity of those extracts on the viability of two

cell lines is presented in Figure 3. The methanolic extract from Edor exhibited cytotoxic effects in both L929 and HaCaT cell lines with of 28.83±0.01 and 16.57±0.02 % cell viability, respectively. On the other hand, the methanolic extract from peel of Thao1 and Thao2 exhibited low cytotoxicity against L929 cell line that showed cell viability of 81.36±0.01 and 77.86±0.04 %; while in HaCaT cell line, it showed cell viability of 90.95±0.01 and 78.32±0.03%, respectively. There were no significant differences in percentage cell viability between two samples of Thao as well.

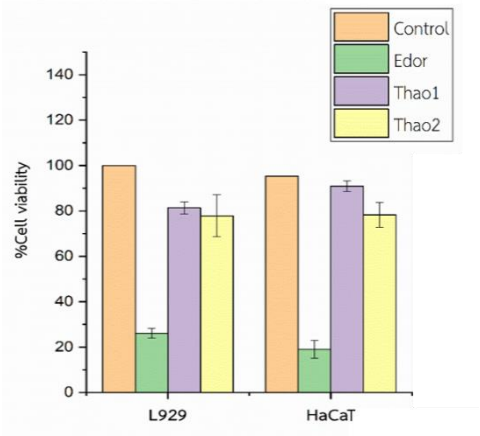

Figure 3. The percentage of cell viability of L929 and HaCaT after exposure to the methanolic extract of longan peels from two cultivars at concentration 1,000 µg/mL for 24 hours of incubation

Discussion

Numerous research have been reported that both edible and nonedible parts of *Dimocarpus longan* spp. *longan* var. *longan* such as extracts of Edor, Chuliang, and Shixia varities contain higher phenolic compounds; including those compounds and their derivatives that were shown in many biological activities. For *Dimocarpus longan* spp. *longan* var. *obtusus*, commonly called Lum-Yai Thao or Thao, the evaluation of biological activities of seed extracts has been previously reported in a few studies. On the other hand, the bioactive profile of peel has not yet been reported. Hence, this present research was to evaluate the TPC and biological activity of methanolic extracts of longan peel from Thao and Edor. For the TPC, no significant differences were observed between Edor and Thao. Furthermore, previous studies have reported that both edible and nonedible parts of longan extracts contain higher phenolic compounds such as gallic acid, corilagin, and ellagic acid (Rangkadilok et al., 2005; Yang *et al*., 2011; Huang *et al*., 2012; Liu *et al*., 2012). Studies of Zhang *et al*.

(2018) and Natungnuy *et al*. (2018) had as well compared the TPC between the longan variety. However, TPC has not been previously reported for var. *obtusus* peel extract. This study indicated that peel of var. *obtusus* had the TPC. Several studies have demonstrated that the number of phenolic compounds in plants can be highly affected by intrinsic and external factors such as the growing area of plants, climatic conditions, genetic background, phenological stages, extraction method, and standard of calibration. More previous studies have also concluded that there was a significant linear correlation between total phenolic content and antioxidant potential. For antioxidant activity, evaluated by DPPH, ABTS and FRAP assays, the result of the present study indicated that cultivar Edor peel extract showed more antioxidant activity comparable to other cultivars such as Thao. However, there were noticeably no significant differences between the two samples of Thao. These results agree with previous reports that the extract from the longan peel, or pericarp, and seed exhibited antioxidant activities (Prasad *et al*., 2009, 2010; Liawruangrath, 2011; Lin *et al*., 2012; Yang *et al*., 2014; Natungnuy *et al*., 2018).Therefore, longan peel could be good candidate for natural antioxidants.

The disc diffusion test at 5 mg/disc showed that Edor and Thao peel extracts effectively inhibited bacterial growth for antibacterial activity evaluation. However, both of them also did not have activity against *E. coli* and *S. epidermidis*. Edor extract was found to be more effective against *S. aureus* and *M. luteus* than Thao peel extracts. Alternatively, Thao peel extracts were more effective than the Edor extract against *P. acnes*. These results corresponded with previous report that longan seed extract have antibacterial activities against *S. aureus, B. cereus,* and *M. luteus* (Tseng *et al*., 2014; Natungnuy *et al*., 2018). In addition, the rice straw paper coated with longan peel extracts showed antibacterial activity against *S. aureus* and *B. cereus* (Chollakup *et al*., 2021). Moreover, *P. acne* was susceptible to longan seed extract (Tseng *et al*., 2014). Accordingly, this study has been the first to report on the antibacterial activity against *P. acne* of peel methanolic extract from longan.

For anti-tyrosinase activity, tyrosinase is the main enzyme responsible for browning fruits and vegetables and pigmentation of human skin. Therefore, it is in great need of developing novel tyrosinase inhibitors from natural resources. Longan seed and pericarp, or peel extracts, also showed moderate tyrosinase inhibitory activity (Rangkadilok *et al*., 2007; Natungnuy *et al*., 2018). In this study, both cultivars extracts had less effective anti-tyrosinase activity with IC_{50} values of about 4.0 mg/mL for cultivar Edor, and 9.0 mg/mL for Thao. Consequently, the extracts had less effective anti-tyrosinase activity when compared to the previous report (IC_{50}

values of 2.9 to 3.2 mg/mL) (Rangkadilok *et al*., 2007) indicating that it affects the storage time, drying methods, and processing conditions of extraction.

In addition, anti-inflammatory activity was evaluated by measuring nitric oxide (NO) generated from SNP and using LPS-activated RAW 264.7 cells. In this study, the methanolic extracts of both Thao and Edor varieties of longan peel have high potential as an anti-inflammatory agent. These results agree with Kunworarath *et al*. (2016) and Nitteranon (2018) who reported that the extract from the longan flower and seed exhibited anti-inflammatory. Huang *et al*. (2012) similarly studied extract from longan peels that displayed the best anti-inflammatory activity. However, the present study revealed that both varieties of longan peel extracts exhibit anti-inflammatory activity with a lower IC_{50} value than the previous report.

In cytotoxic activity using MTT assay, the results of this study demonstrated that the methanolic extract of longan peel from Thao variety at 1000 µg/mL concentration exhibited low cytotoxicity against both L929 and HaCaT. On the other hand, the extract from Edor exhibited cytotoxic activity. Some research have reported that both ethanol and ethyl acetate extracts from seed of longan showed percentages of cell viability above 90% (Tseng *et al*., 2014). Those results agree with previously report that the methanolic extracts of both Thao and Edor of longan seed were shown to promote cell division by increasing the percentage of cell viability of the L929 cell line (Natungnuy *et al*., 2018). On the contrary**,** the longan flower extract can also induce apoptosis in colorectal cancer cells (Lin *et al*., 2012). To date, longan peel extract of Thao variety has not been reported for cytotoxic activity. The results demonstrated in this study that the methanolic extract of longan peel from *Thao* variety did not show toxicity on both cell lines after incubation for 24 hours.

These results hence suggested that the variety Thao peel extracts are different from cultivars Edor peel extract. The cultivar Thao peel extracts showed excellent anti-inflammatory and still non-cytotoxic for L929 and HaCaT cell lines with cell viability higher than 80% and antibacterial against *P. acnes*. Furthermore, there were no significant differences in the results obtained with two samples of Thao variety - Thao1 was collected from the same longan tree while Thao2 was collected from different longan trees, in different places. Therefore, the Thao peel extracts should be further studied and developed, as they could be used in preparing products for anti-inflammatory supplements, cosmetics, and pharmaceutical in the future.

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