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Biological activities of the methanolic extracts from two varieties of *Dimocarpus longan* seeds

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Abstract *Dimocarpus longan* belongs to Sapindaceae which two varieties are found in Thailand. In addition to commercial longan (*D. longan* ssp. *longan* var. *longan*), in the eastern part of Thailand found another type of flora longan called Thao (*D. longan* ssp. *longan* var. *obtusus*). The total phenolic content and various biological activities was investigated including antioxidant, antibacterial, anti-tyrosinase and cytotoxic activities of the methanolic extracts from two varieties of these longan seeds. The results showed that the most activity of Edor (var. *longan*) seed extract presented higher biological activities than Thao (var. *obtusus*) seed extract. The total phenolic content by Folin-Ciocalteu method related to antioxidant activity in DPPH, ABTS and FRAP assays. In antibacterial activity using disc diffusion method both varieties of extracts (5 mg/disc) inhibited *Staphylococcus aureus*, *Micrococcus luteus* and *Bacillus subtilis*. However, in anti-tyrosinase activity by the dopachrome method Thao revealed similar activity to the commercial longan cultivar Edor with 50% inhibitory concentration (IC_{50}) values of 504.73 and 527.04 µg/ml, respectively. In cytotoxic activity using MTT assay with L929 (Mouse fibroblast cell line), Thao seed extracts were non-cytotoxic activity and stimulated skin cell lines, while Edor showed low cytotoxic activity. Therefore, their bioactive compounds should be studied further for developing longan seed extracts to cosmetic and pharmaceutical products in the future.

Keywords: Antibacterial activity, Antioxidant activity, Anti-tyrosinase activity, Cytotoxic activity, *Dimocarpus longan*

Introduction

Longan (*Dimocarpus longan* Lour.) belongs to the Sapindaceae family, this edible fruit widely found in Southeast Asia. Longan that general found belongs to subspecies (ssp.) *longan* and its own varieties are var. *longan*, var. *obtusus* and var. *longepetiolutatus* (Lithanatudom *et al*., 2017). From which

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two varieties are found in Thailand. First is *D. longan* ssp. *longan* var. *longan*, this is the commercial longan that general found and has many cultivars such as Edor, Baidom, BiewKhiew and Chompoo (Jaroenkit, 2015). Second variety is *D. longan* ssp. *longan* var. *obtusus* that called Thao and found in the eastern part of Thailand (*e.g.* Chonburi and Rayong provinces). Currently, Thao is mostly grown as ornamental plants rather than the commercial as well as it is not popular with consumers due to the small-sized fruit, thin pulp and large seeds.

Longan seed, approximately 16-40% by weight of the whole fresh fruit are agricultural wastes that can be used as an alternative source of pharmaceutical supplements and natural antioxidants (Yang *et al*., 2011). In the general reports, longan seed extracts have been presented polyphenolic compounds such as corilagin, ellagic acid and gallic acid that displays higher than pulp and peel extracts (Rangkadilok *et al*., 2005). Besides, dried longan seed extracts of cultivar Edor exhibited antioxidant and anti-tyrosinase activities (Rangkadilok *et al*., 2007). In addition, the ethanolic extract from longan seed against leukemic cell lines K562 and HL60 in cytotoxic activity (Ampasavate *et al*., 2010). For antimicrobial activity, the methanolic extract from longan seed can inhibit *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Candida albicans* (Sudjaroen, 2013).

From the past reports focus on the extract from commercial longan seed (var. *longan*) for biological activities. There have been few studies of extract from Thao (var. *obtusus*). Therefore, the aim of this research is to examine the total phenolic content and various biological activities of the methanolic extracts from two varieties of these longan seeds for the antioxidant, antibacterial, anti-tyrosinase and cytotoxic activities.

Materials and methods

Preparation and extraction plant material

Three samples of mature longan fruits of var. *obtusus* (Thao1, Thao2 and Thao3) were collected from Chonburi province. As well as one sample of var. *longan* (cultivar Edor) was purchased from a local market in Bangkok, Thailand. After that longan seeds were separated and dried in hot air oven at 40^{o}C. Then seeds were homogenized to a powder using an electric grinder. The longan seed powder was macerated with methanol for a week. The extract solution was filtered and evaporated using rotatory evaporator.

Evaluation of total phenolic content

Folin-Ciocalteu method was performed to evaluate the total phenolic content of longan seed extracts according to Soong and Barlow (2004) with few modifications. Fifty µl of samples (1000 µg/ml) and Folin-Ciocalteu reagent were mixed in 96-well plate and kept for 6 minutes in the dark. After that added 100 µl of 7.5% sodium carbonate solution, incubated for 30 minutes then measured the absorbance at 765 nm using a microplate reader. The total phenolic content was calculated using gallic acid as a standard compound in milligrams gallic acid equivalent/g extract (mgGAE/g extract).

Quantification of antioxidant activities

Three assays of free radical scavenging activities including DPPH, ABTS and FRAP assays were used for quantification of antioxidant activity. The method of DPPH and ABTS assays according to Shimada *et al*. (1992) and Re *et al*. (1999) with slight modifications. Both of DPPH and ABTS assays, trolox was used as a standard and the results were expressed as antioxidant capacity in milligrams trolox equivalent/g extract (mgTE/g extract). In FRAP assay following Benzie and Strain (1996) with few modifications, the result was compared with ascorbic acid as a standard and expressed in FRAP values as milligrams ascorbic acid equivalent/g extract (mgAAE/g extract).

Anti-tyrosinase activity

The dopachrome method was performed to investigate anti-tyrosinase activity according to Masuda *et al*. (2004) with few modifications. Briefly, control group (A) containing phosphate buffer (pH 6.8), tyrosinase (25 U/ml) and L-DOPA (2.5 mM) while the blank of control group (B) without tyrosinase and sample. The sample group (C) containing phosphate buffer, tyrosinase, sample and L-DOPA. The blank of sample group (D) was without tyrosinase. The mixture of each groups was incubated for 30 minutes at 25 ℃. After that, the samples were measured the absorbance at 475 nm and calculated percentage of tyrosinase inhibition follow below equation. The result expressed in anti-tyrosinase capacity (mgAAE/g extract). The IC_{50} (50% inhibitory concentration) values were determined by GraphPad prism 5.

$$\% \text{ Tyrosinase inhibition} = [(A-B)-(C-D)/ (A-B)] \times 100$$

Antibacterial activity

The paper disc diffusion method was used for antibacterial activity using five strains of bacteria including *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341, *Staphyloccocus aureus* TISTR 1466 and *Staphylococcus epidermidis* ATCC 12228 follow the protocol of CLSI (2012). For experiment, all stains were swabed on Mueller Hinton Agar (MHA). Preparing the disc, the extract was dissolved with methanol and dropped (5 mg/disc) on the disc with diameter 6 mm. Then, the disc was placed on the surface of MHA medium, incubated at 37 ℃ for 16-18 hours and measured the inhibition zone reporting in millimeters (mm). Gentamycin (10 µg/disc) was used as positive control and methanol as negative control.

Cell culture and cytotoxic activity

The normal cell line (Mouse fibroblast cell line: L929) was cultured in RPMI-1640 medium in the 5% CO_2 incubator at 37 ℃. MTT assay was applied to test cytotoxicity according to Poeaim *et al*. (2017) with few modifications. For the experiment, 100 µl of cell line were added in 96-well plate then incubated in the CO_2 incubator for 48 hours. After that 100 µl of extracts (1000 µg/ml) were treated with cell line for 20 hours. The DMSO used as negative and mitomycin C used as positive control. After that 50 µl of MTT solution was added and incubated for 4 hours. Then removed the supernatant and dissolved formazan crystal by 100 µl of DMSO: ethanol (1:1 v/v) and measured the absorbance at 570 nm. The percentage of cell viability was calculated following below equation.

$$\% \text{ Cell viability} = \frac{\text{OD treated}}{\text{OD control}} \times 100$$

Where as Control = cells without extracts
Treated = cells with extracts

Statistical analysis

The data are represented a mean±standard deviation (SD) of the experiments for three replications (n=3). Duncan's Multiple Range Test of SPSS statistics software (version 23.0) was used to analyze intergroup differences, $p<0.05$ was considered to be statistically significant.

Results

Total phenolic content

The total phenolic content of longan seed extracts using gallic acid as a standard phenolic compound, the calibration curve showed y = 0.0117x with r^2 value of 0.9928. The result of this study revealed both of the methanolic extracts from two varieties of longan seeds presented of phenolic compound. The methanolic extracts from var. *obtusus* Thao1, Thao2 and Thao3 presented the total phenolic content of 83.01±2.75**,** 103.96±6.36 and 61.15±1.56 mgGAE/g extract**,** respectively. Thao seed extracts showed a lower phenolic content than the Edor seed extract that contained 140.06±6.84 mgGAE/g extract (Figure 1).

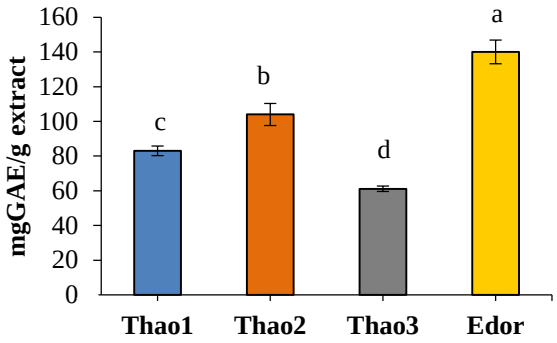


Figure 1. The total phenolic content of the methanolic extracts from longan seeds by Folin- Ciocalteu method. Letters a-d each column indicate the statistical significant at $p<0.05$

Antioxidant activities

Three assays of free radical scavenging activities including DPPH, ABTS and FRAP were used to estimate antioxidant activity. The antioxidant capacity of longan seed extracts were calculated and showed both varieties of the methanolic extracts presented antioxidant activity. Edor extract (var. *longan*) showed the highest antioxidant capacity with the values of 338.95±5.48, 184.01±4.60 mgTE/g extract and 81.42±0.87 mgAAE/g extract in DPPH, ABTS and FRAP assays, respectively. For DPPH, ABTS and FRAP assays, Thao2 presented higher antioxidant activity than Thao1 and Thao3 with the values of 136.80±1.61, 158.71±4.20 mgTE/g extract and 69.68±3.07 mgAAE/g extract (Table 1).

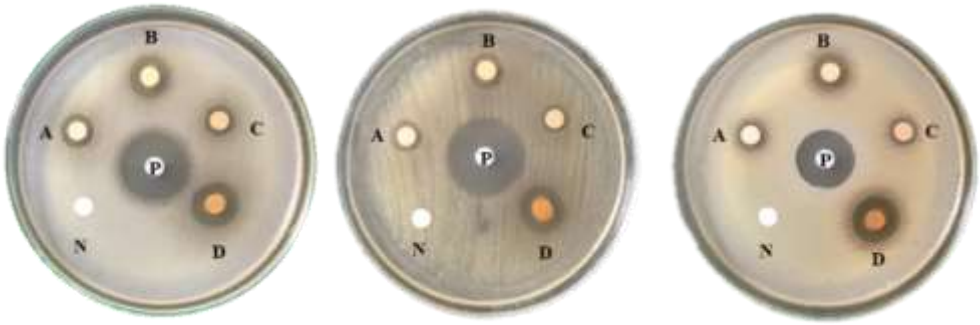
Table 1. Antioxidant capacity of the methanolic extracts from longan seeds

The methanolic extracts from longan seeds	Antioxidant capacity		
	DPPH assay (mgTE/g extract)	ABTS assay (mgTE/g extract)	FRAP assay (mgAAE/g extract)
Thao1	133.00^{c} ±4.04	150.59^{c} ±9.86	39.18^{c} ±1.94
Thao2	136.80^{b} ±1.61	158.71^{b} ±4.20	69.68^{b} ±3.07
Thao3	66.13^{d} ±2.41	139.51^{d} ±2.29	36.13^{d} ±2.85
Edor	338.95^{a} ±5.48	184.01^{a} ±4.60	81.42^{a} ±0.87

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

Antibacterial activity

Antibacterial activity of the methanolic extracts from two varieties of longan seeds was done using *B. subtilis*, *M. luteus*, *S. aureus* and *S. epidermidis* as gram-positive bacteria and *E. coli* as gram-negtive bacteria. The result revealed both varieties of longan seed extracts (5 mg/disc) presented antibacterial activity with *B. subtilis*, *M. luteus* and *S. aureus* as shown in Figure 2. However, no inhibit *S. epidermidis* and *E. coli*. Among Thao extracts, Thao2 showed the highest activity with the inhibition zone of 11.30±0.59 and 10.28±0.81 mm for *M. luteus* and *S. aureus*, respectively. However, Edor seed extract (11.46±2.76 and 13.80±1.10 mm) showed higher antibacterial activity than Thao seed extracts except for *B. subtilis* (Table 2).



B. subtilis *M. luteus* *S. aureus*

Figure 2. Antibacterial activity of the methanolic extracts from longan seeds by disc diffusion method. A: Thao1, B: Thao2, C: Thao3, D: Edor, N: negative control and P: positive control

Table 2. The inhibition zone of the methanolic extracts from longan seeds (5 mg/disc) and gentamicin (10 µg/disc)

The methanolic extracts from longan seeds	Inhibition zone (mm)		
	B. subtilis	***M. luteus***	***S. aureus***
Thao1	11.61^{b} ±0.80	10.31^{c} ±0.40	9.11^{c} ±0.62
Thao2	13.52^{a} ±0.71	11.30^{b} ±0.59	10.28^{b} ±0.81
Thao3	9.72^{c} ±0.18	8.97^{d} ±0.14	7.91^{d} ±0.51
Edor	14.50^{a} ±0.77	11.46^{a} ±2.76	13.80^{a} ±1.10
Gentamicin	22.35±0.41	25.37±0.89	18.77±0.41

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

Anti-tyrosinase activity

Both varieties of longan seed extracts presented anti-tyrosinase activity by dopachrome method in a concentration-dependent manner. In addition, the IC_{50} values and anti-tyrosinase capacity of extracts presented Thao2 and Edor same activity with the anti-tyrosinase capacity of 182.00±4.75 and 181.09±0.54 mgAAE/g extract, respectively (Table 3).

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

The methanolic extracts from longan seeds	Anti-tyrosinase capacity (mgAAE/g extract)	IC_{50} (µg/ml)
Thao1	161.29^{b} ±3.10	776.57
Thao2	182.00^{a} ±4.75	504.82
Thao3	168.41^{b} ±8.70	623.87
Edor	181.09^{a} ±0.54	527.05

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

Cytotoxic activity

The methanolic extracts from two varieties of longan seeds at 1000 µg/ml also tested with L929 cell line for cytotoxic activity by MTT assay. The percentage of cell viability of Thao1, Thao2 and Thao3 showed the values of 122.64±0.11, 113.40±0.05 and 112.09±0.13%, respectively while Edor seed extract showed cell viability of 87.57±0.01% as shown in Figure 3.

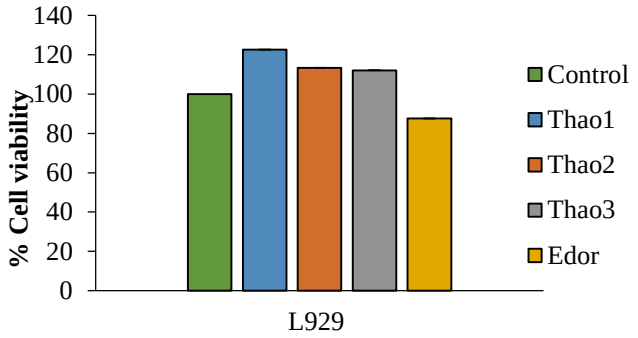


Figure 3. The percentage of cell viability of L929 cell line after tested with the methanolic extracts from longan seeds

Discussion

D. longan were used as traditional medicine for a long time, fruits of longan can relief of swelling and neural pain in traditional Chinese medicine. In the past reports found, each part of longan extracts including pulp, seed and peel were used to study bioactive compounds and their biological activities. Especially in longan seeds, the rich source of polyphenolic compounds such as gallic acid, corilagin, and ellagic acid that higher than pulp and peel (Rangkadilok *et al*., 2005). However, the most reports focus on the extracts from commercial longan (var. *longan*) that popular for consumer in various cultivars. In this study another variety of longan called Thao belong to var. *obtusus* also used to study their biological activities.

According to the results of this study, the methanolic extracts from two varieties of longan seeds contained the phenolic compounds. Edor seed extract of var. *longan* has the total phenolic content of 140.06±6.84 mgGAE/g extract similar to result from Liu *et al*. (2012) that study the total phenolic content of leave and bark extracts from longan with the values of 132.47 and 140 mgGAE/ g extract. The total phenolic content of Thao seed extracts (var. *obtusus*) in this study range from 61.15-103.96 mgGAE/g extract. However, it hasn't been reported for the total phenolic content from seed extracts of var. *obtusus*. Moreover, Edor seed extract presented the highest both of total phenolic content and antioxidant activity. The results of total phenolic content of each extracts in this study are associated with antioxidant activity that related to Hossain and Shah (2015) found the positive relationship between amount of phenolic compounds and antioxidant activity of the extracts. For antibacterial activity, both varieties of longan seed extracts at concentration of 5 mg/disc was effective to inhibit *S. aureus*, *M. luteus*, and *B. subtilis* similar to Sudjaroen (2012) found the methanolic extracts from longan seeds (10 mg/ml) can inhibit *S. aureus* like the result of this study.

Furthermore, both varieties of longan seed extracts showed anti-tyrosinase activity especially for all sample of Thao seed extract showed IC_{50} values of 504.82-776.57 µg/ml that higher activity than the commercial longan seed extract of Rangkadilok *et al*. (2007) with IC_{50} values of 2.9-3.2 mg/ml. Therefore, Thao seed extract should be further study of their bioactive compound and could be developing for anti-tyrosinase products in the future. Normally, the extracts have to test for preliminary cytotoxicity with normal cell lines before develop to cosmetic products. In this study normal skin cell line of mouse (L929) was used to test for cytotoxic activity by MTT assay. The result showed Edor seed extract presented percentage of cell viability lower than control with the value of 87.57 % that revealed low cytotoxicity with normal skin cell line. All sample of Thao seed extracts showed increase percentage of cell viability rang from 112.09-122.64 % that meaning Thao seed extracts are non-cytotoxic and also stimulate cell division. This result similar to Ampasavate *et al*. (2010) that showed the ethanolic extract from longan seeds was non-cytotoxic activity and also can stimulate normal human leukemic cell lines (PBMCs).

It concluded that the biological activities of the methanolic extracts from two varieties of longan seeds showed that both of Thao (var. *obtusus*) and the commercial longan cultivar Edor (var. *longan*) extracts presented phenolic compound and demonstrated antioxidant activity in DPPH, ABTS and FRAP assays. In addition, both of extracts showed anti-tyrosinase activity by dopachrome method. Moreover, the results of total phenolic content of each extracts are related to antioxidant and anti-tyrosinase activities. Furthermore, both of varieties can inhibit *B. subtilis*, *M. luteus* and *S. aureus* in antibacterial activity. Edor seed extract (var. *longan*) presented low cytotoxicity while Thao seed extract of var. *obtusus* revealed non-cytotoxic and also stimulate normal mouse skin cell line with low concentration. Therefore, the flora longan "Thao" of var. *obtusus* that larger seed than the commercial longan is an alternative source of natural bioactive compounds that presented various biological activities should be promoted and needed further study to identify the constituent of their compounds to develop Thao seed extracts for cosmetic and medicinal applications in the future.

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