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Antibacterial and anti-tyrosinase activities of the methanolic extracts from leaves of *Tectona grandis*

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Abstract The antibacterial and anti-tyrosinase activities of methanolic extracts from fresh and fallen leaves of *Tectona grandis* (Teak) was studied. Fresh and fallen of teak leaves were collected from Thongphaphum and Phitsanulok silviculture research station. Antibacterial activity was determined by disc diffusion method with concentration at 500 µg/disc. Fresh and fallen of teak leaves extracts showed a good activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. In the other hand, fresh teak leaves inhibited growth of *Propionibacterium acnes* better than fallen teak leaves. The anti-tyrosinase activity was determined by Dopachrome method with concentration at 1500 µg/ml, inhibitory power of tyrosinase enzyme from fresh and fallen leaves extracts value were detected in the percentage between 35.45%-73.65%.

Keywords: Antibacterial, Anti-tyrosinase, *Tectona grandis*, Teak

Introduction

The plants have been used as medicine for a long time and apply effectively for to develop a natural product. *Tectona grandis* or Teak is a plant in the Verbenaceae family found most in South-East Asian countries, including Thailand, Myanmar and Indonesia. The extracts from different parts of *T. grandis* can used as the medical properties such as hair growth activity, antioxidant activity, cytotoxic, anti-inflammatory, anti-hyperglycemic and diuretic (Nidavani and Am, 2014).

In the previous reports that the methanolic extracts from leaves of *T. grandis* with tetracycline and essential oils had the antibacterial activity. (Purushotham *et al.*, 2010; Aboaba *et al.*, 2013). The antibiotic is powerful medicines that resistant bacterial infection, the development of new drugs from plants for the antibacterial activity (Cushnie and Lamb, 2005; Farjana *et al.*, 2014; Wikaningtyas and Sukandar, 2016).

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Malanin is pigment produced by melanocyte in skin, the factors that stimulate the melanin product, including ultraviolet radiation, sunlight, etc. The action of ultraviolet radiation light stimulating the tyrosinase enzyme. The tyrosinase enzyme is involved in the oxidation and reduction process in the epidermis for controlling the production of melanin, this enzyme responsible for color change to browning (Stacey *et al.,* 2016). In the previous reports the antioxidant and anti-tyrosinase activities indicate for inhibition of melanin (Sun *et al.,* 2017; Haliloglu *et al.,* 2017). Whereas, the anti-tyrosinase activity of *T. grandis* not have been reported.

In addition, have reported a comparison of biological activity age of leaves from the first stage and second stage of *Olea europaea* extracts and the ethanol extract from young and old leaves extracts of *Aronia melanocarpa* antioxidant activity (Brahmi *et al.,* 2013; Do Thi and Hwang 2014). While most of the literature in fresh leaves of *T. grandis*. The purpose of this study was compared antibacterial and anti-tyrosinase activity of methanolic extracts from fresh and fallen leaves of *Tectona grandis* (Teak).

Materials and methods

Plant material and extraction

The fresh and fallen leaves of 20 samples from 5 Plus tree are Chaingmai (CM), Phrae (PH), Sukhothai (ST), Lampang (LP) and Khonkaen (KK) of *T. grandis* were collected from Thongphaphum and Phitsanulok silviculture research station, Thailand showed in Figure 1. The sample of *T. grandis* leaves were blean to powder (50 g) after that soaked in methanol (250 ml) in dark room at room temperature for 7 days. Finally, the solvent was evaporating by rotary vacuum evaporator and the crude extracts keep to study.

Antibacterial activity

The antibacterial activity was performed by disc diffusion method were checked against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propionibacterium acnes* was modified by Tendencia (2004). Bacterial were culture in Mueller-Hinton Broth, MHB (2 ml) and incubated at 37 ℃ for 18-24 hours. Adjust turbidity by normal saline solution (0.85%) and determine by spectrophotometry the optical absorbance to 0.08-0.13 at 625 nm. Then, swab culture on Mueller-Hinton Agar (MHA) and dry the surface for 3-5 minutes. The crude extracts were dissolved in methanol and solutions were dropped on paper disc (500 µg/disc), place paper disc on MHA and incubated for 18-24

hours. After that measure the diameter of inhibition zone and gentamicin was used as a positive control and methanol was used as a negative control.

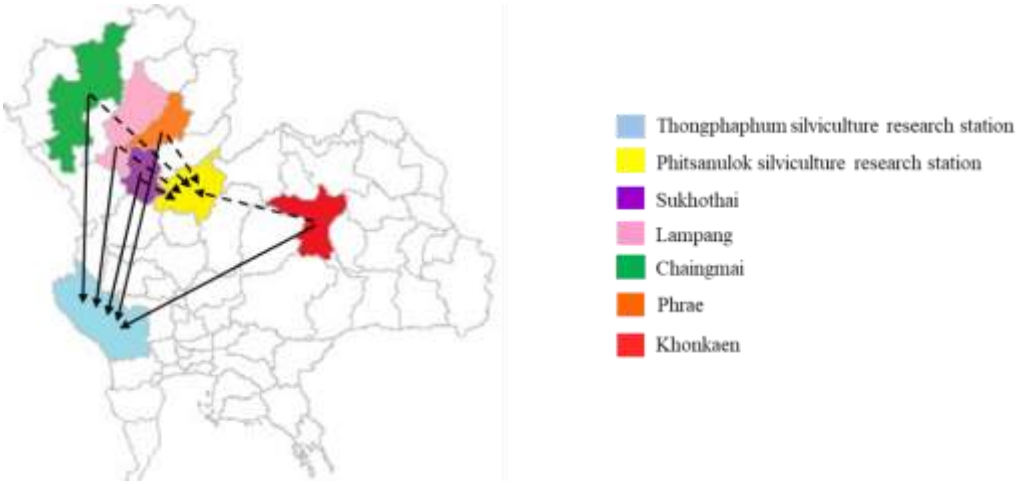


Figure 1. Different location, where the plus trees of *T. grandis* was collected from Thongphaphum and Phitsanulok silviculture research station, Thailand

Anti-tyrosinase activity

In the present study**,** the anti-tyrosinase activity follow the protocol to Chang and Tseng (2006) with few modifications. 20 µl of tyrosinase enzyme (25 Unit/ml) mixed with 80 µl of phosphate buffer (pH 6.8) and added 60 µl of the sample (1500 µg/ml), added into 96 well plate was incubated at 25 ℃ in the dark room for 10 minutes. After added 40 µl of of L-Dopachrome (2.5 mM) and incubated in the dark room for 20 minutes. The Dopachrome was checked in the absorbance at 475 nm with microplate reader. Inhibition the percentage of tyrosinase activity was calculated according to the equation.

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

A is the absorbance at 475 nm with control, B is the absorbance at 475 nm with blank control, C is the the absorbance at 475 nm with sample and D is the absorbance at 475 nm with blank sample.

Statistical Analysis

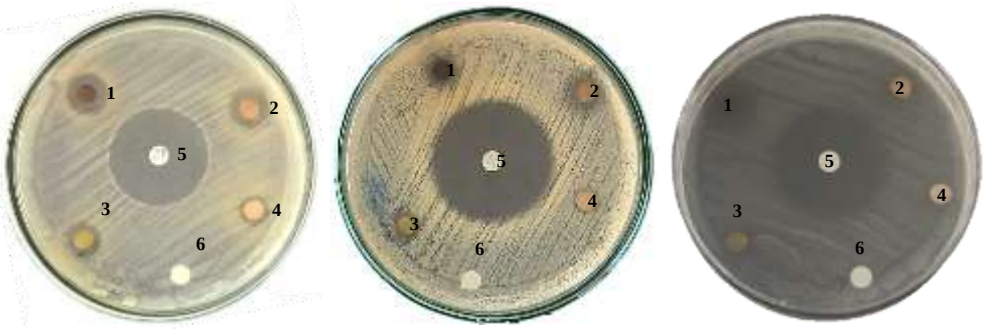
For all the experiments, the results of antibacterial and anti-tyrosinase activites are presented as mean values ± standard deviation (SD) using IBM

SPSS Statistics version 25.0. The differences between the samples were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test at $P < 0.05$ were considered significantly.

Results

Antibacterial activity

The antibacterial activity from fresh and fallen leaves extracts by disc diffusion method against *S. aureus*, *S. epidermidis* and *P. acnes* with concentration at 500 µg/disc. The diameter of inhibition zone showed that fresh (13.90±0.91mm) and fallen (13.45±0.70 mm) leaves extracts from Phrae, fresh leaves (12.45±0.30 mm) from Sukhothai, fresh leaves from Chaingmai (11.23±0.79 mm) and fallen (11.68±0.73 mm) leaves from Lampang at Thongphaphum silviculture research station good activity against *S. aureus*. Fresh (13.11±0.51 mm) and fallen (14.31±0.6 mm) leaves extract from Phrae, fallen (13.78±0.51 mm) leaves extract from Lampang and fallen (11.62±0.43 mm) leaves extract from Khonkaen at Thongphaphum silviculture research station inhibited growth of *S. epidermidis*. Fresh (14.01±0.22 mm) leaves extract at Thongphaphum silviculture research station, fallen (13.32±0.82 mm) leaves extract from Lampang at Pitsanulok, silviculture research station, fresh leaves extract from Sukhothai at Thongphaphum silviculture research station inhibited growth of *P. acnes* reported in Table 1 and Figure 2.



S.aureus *S. epidermidis* *P. acnes*

Figure 2. Inhibition zone of *S. aureus*, *S. epidermidis* and *P. acnes* of *T. grandis* leaves extract from Phrae (1) Fresh leaves (2) fallen leaves at Thongphaphum silviculture research station, (3) Fresh leaves (4) fallen leaves at Phitsanulok silviculture research, (5) Positive control is gentamicin and (6) Negative control is methanol

Table 1. Inhibition zone of the methanolic extracts from leaves of *T. grandis*

Bacterial strains	Location	Age of leaves	Mean diameter of inhibition zone (mm) at concentration 500 µg/disc				
			CM	PH	ST	LP	KK
s. aureus	Thongphaphum	F	11.23±0.79[bc]	13.90±0.91[a]	12.45±0.30[ab]	-	-
		D	9.09±1.38[ef]	13.45±0.70[a]	11.26±0.25[cd]	11.68±0.73[c]	-
	Phitsanulok	F	10.26±0.69[de]	9.80±0.67[ef]	-	-	-
		D	8.99±0.12[ef]	9.87±0.59[ef]	-	8.45±0.22[f]	-
	Gentamicin		26.54±0.36	28.42±1.03	27.01±0.30	28.18±0.45	28.07±0.31
s. epidermidis	Thongphaphum	F	-	13.11±0.51[ab]	9.94±0.54[d]	-	-
		D	-	14.31±0.61[a]	9.79±0.01[d]	13.78±0.51[a]	11.62±0.43[c]
	Phitsanulok	F	-	-	-	-	-
		D	-	-	-	-	-
	Gentamicin		31.40±1.64	32.93±0.20	32.62±0.18	31.35±0.63	31.41±0.96
P. acnes	Thongphaphum	F	9.82±0.15[d]	14.01±0.22[a]	12.11±0.05[c]	-	-
		D	-	-	10.51±0.91[d]	-	-
	Phitsanulok	F	-	-	8.76±1.83[e]	-	-
		D	7.88±0.27[e]	-	-	13.32±0.82[b]	-
	Gentamicin		29.44±0.65	30.59±0.27	28.24±0.55	28.52±1.49	29.41±0.48

(F) fresh leaves extract, (D) fallen leaves extract, (CM) Chaingmai , (PH) Phrae , (ST) Sukhothai, (LP) Lanmpang, (KK) Khonkaen and (-) not inhibition zone

[a-f] Values followed by the same letters in the same column are not significantly different according to the Duncan's Multiple Range Test at $P < 0.05$

Anti-tyrosinase activity

The effect of *T. grandis* leaves extracts on tyrosinase activity by Dopachrome method was used ascorbic acid as standard. The results showed that percentage of inhibition of tyrosinase with concentration at 1.5 mg/ml. Fresh and fallen leaves from Chaingmai, Sukhothai of plus tree from Thonhphaphum and Pitsanulok silviculture research station inhibition tyrosinase enzyme greater than fifty percentage the values between 68.79%-73.65% and 57.22%-59.85%, respectively showed in Table 2 and Figure 3.

Table 2. The effect of *T. grandis* leaves extract on tyrosinase activity

Location	Age of leaves	% Inhibition of tyrosinase				
		CM	PH	ST	LP	KK
Thongphaphum	F	68.97 ±2.83^{ab}	42.02 ±3.41^{f}	57.22 ±3.98^{cde}	53.77 ±1.34^{de}	35.45 ± 1.86^{g}
	D	71.66 ±3.25^{ab}	49.68 ±1.52^{ef}	53.39 ±5.45^{de}	63.41 ±1.06^{bc}	37.32 ±4.32^{g}
Pitsanulok	F	71.73 ±3.53^{ab}	41.09 ±1.13^{g}	56.14 ±0.95^{cde}	59.47 ±2.98^{cd}	40.12 ±0.93^{g}
	D	73.65 ±0.81^{a}	56.54 ±3.43^{cde}	59.85 ±0.72^{cd}	37.63 ±3.46^{g}	37.01 ±1.87^{g}

(F) fresh leaves extract, (D) fallen leaves extract, (CM) Chaingmai, (PH) Phrae, (ST) Sukhothai, (LP) Lanmpang, (KK) Khonkaen

[a-g] Values followed by the same letters in the same column are not significantly different according to the Duncan's Multiple Range Test at $P < 0.05$

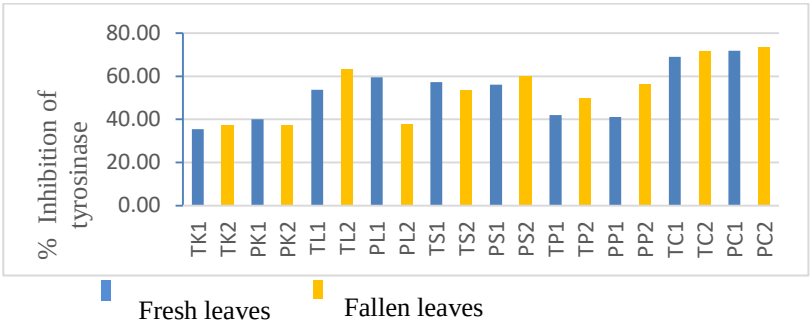


Figure 3. Percentage of Inhibition of tyrosinase activity from *T. grandis* leaves extract (T) Thongphaphum silviculture research station, (P) Pitsanulok silviculture research station, (K) Khonkaen, (L) Lampang, (S) Sukhothai, (P) Phrae, (C) Chaingmai, (1) Fresh leaves, (2) Fallen leaves

Discussion

The study focused on the comparison the methanolic extracts of fresh and fallen leaves from *T. grandis*, the results showed that against *S. aureus*, *S. epidermidis* and *P. acnes* similar to the report that the methanolic extracts of leaves from *T. grandis* had a good activity against *S. aureus* (14.00 mm) by disc diffusion method concentration at 500 µg/disc (Krishna and Nari, 2010). In addition, reported that essential oil extract of *T. grandis* against gram negative and gram positive as *Escherichia coli* (29.00 mm) and *S. epidermidis* (20.00 mm) (Aboaba *et al.*, 2013).

Plant and herbs that presence of bioactive compounds and extensive phytochemical can break on structures and cell membranes of bacteria are possible antibacterial activity (Ghasemzadeh *et al.*, 2016). It has been reported

that the phenolic compounds such as anthraquinones can antibacterial activity (Tamokou *et al.,* 2009). A similar study Krishna *et al.* (2011) reported the compound from *T. grandis* leaves extract as anthraquinones and activity against *S. aureus* and *K. pneumoniae*.

The anti-tyrosinase activity fromleaves extract of *T. grandis* showed that the fallen leaves extract highest percentage of inhibitory tyrosinase activity. The research was found the compounds such as gallic acid, rutin, quercitin, ellagic acid and sitosterol of the methanolic leaves extract of *T. grandis* (Nayeem and MD, 2011). According, the previous study the phenolic compounds as gallic acid effect inhibition tyrosinase enzyme (Alam *et al.,* 2012). However, this compound increased amount of stress on teak and different soil-weather have effects on the total phenolic content and antioxidant activity (Kusnadi *et al.,* 2016).

T. grandis leaves extract indicated to be a valuable bioactive source, and to be applied for the health and medical purpose. These natural compounds may also prove to have free radicals, antibacterial and anti-tyrosinase.

It concluded that the methanol extract from leaves of *T. grandis* showed that the fresh and fallen leaves from Phrae of plus tree at Thongphaphum silviculture research station inhibited *S. aureus, S. epidermidis* and *P. acnes*. Fresh and fallen leaves from Chaingmai, Sukhothai of plus tree from Thonhphaphum and Pitsanulok silviculture research station inhibition tyrosinase enzyme greater than fifty percentage. Further investigations are needed to identify the bioactive compounds of fractions for developed in the future.

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