TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FROM 13 THAI TRADITIONAL PLANTS.

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ABSTRACT

Water and ethanol (EtOH) crude extracts from 13 Thai traditional plants were screened for their total phenolic content, antioxidant and antibacterial properties. The highest total phenolic levels and antioxidant activity of water crude extracts were detected in *Syzygium cumini* (L.) Skeels at 358.250 $\pm$ 0.014 mgGAE/g_{dw} and 332.425 $\pm$ 0.21 mM Trolox , respectively. *Piper betle* Linn. EtOH crude extracts were found the highest total phenolic contents 474.083 $\pm$ 0.005 mgGAE/g_{dw} and *Anacardirm occidentale* Linn. 411.916 $\pm$ 0.05 mM Trolox as the highest antioxidant activity in EtOH extracts. Almost plants in this study demonstrated the correlation between phenolic content and antioxidant capacity but some were no correlation between them. The antimicrobial activity was found in 7 species of water crude extracts and 2 species of EtOH crude extracts. *Syzygium cumini* (L.) Skeels water crude extract showed the highest inhibition zone to *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) at the concentration of 100 mg/ml. The highest inhibition zone of EtOH crude extracts was found in *Piper betle* Linn. (100 mg/ml) to *S. aureus*, MRSA, *Escherichai coli* and *Pseudomonas aeruginosa*. Minimal inhibitory concentration (MIC) and Minimal bacteriocidal concentration (MBC) of *Syzygium cumini* (L.) Skeels water crude extracts was found at 6.25 and 12.5 mg/ml to *S. aureus* and MRSA, respectively. *Piper betle* Linn. EtOH crude extracts was found at 12.5 mg/ml for MIC and MBC to *S. aureus* and MRSA.

KEY WORDS : Antimicrobial activity, Antioxidant activity, Phenolic content, Thai traditional plants.

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ปริมาณฟีนอลิกทั้งหมด สารต้านอนุมูลอิสระและฤทธิ์ต้านจุลชีพของสารสกัดสมุนไพรไทย 13 ชนิด

บทคัดย่อ

จากการศึกษาปริมาณฟีนอลิกทั้งหมด สารต้านอนุมูลอิสระ และฤทธิ์ต้านจุลชีพของสารสกัดสมุนไพรไทย 13 ชนิดด้วยน้ำและเอทานอล พบว่าสารสกัดยอดหว้าที่สกัดด้วยน้ำ มีปริมาณฟีนอลิกทั้งหมดและปริมาณสารต้านอนุมูลอิสระสูงสุด เท่ากับ 358.250 $\pm$ 0.014 mgGAE/g_{dw} และ 332.425 $\pm$ 0.21 mM Trolox ตามลำดับ ในขณะที่สารสกัดใบพลูที่สกัดด้วยเอทานอล มีปริมาณฟีนอลิกทั้งหมดสูงสุดเท่ากับ 474.083 $\pm$ 0.005 mgGAE/g_{dw} และยอดยาร่วง (มะม่วงหิมพานต์) ที่สกัดด้วยเอทานอล มีปริมาณสารต้านอนุมูลอิสระสูงสุด เท่ากับ 411.916 $\pm$ 0.05 mM Trolox นอกจากนี้ยังพบว่าในสารสกัดส่วนใหญ่จะมีปริมาณฟีนอลิกและสารต้านอนุมูลอิสระสัมพันธ์กัน สารสกัดที่สกัดด้วยน้ำ 7 ชนิด และสารสกัดที่สกัดด้วยเอทานอล 2 ชนิดแสดงฤทธิ์ต้านจุลชีพ โดยสารสกัดยอดหว้าที่สกัดด้วยน้ำสามารถยับยั้งการเจริญของเชื้อ *Staphylococcus aureus* และ Methicillin-resistant *Staphylococcus aureus* (MRSA) ได้ดีที่สุดที่ระดับความเข้มข้น 100 mg/ml สารสกัดในพลูที่สกัดด้วยเอทานอล (100 mg/ml) สามารถยับยั้งการเจริญของเชื้อ *S. aureus*, MRSA, *Escherichai coli* และ *Pseudomonas aeruginosa* ได้ เมื่อนำมาศึกษาหาระดับความเข้มข้นต่ำสุดของสารสกัดสมุนไพรที่สามารถยับยั้งและทำลายจุลชีพ (Minimal inhibitory concentration : MIC และ Minimal bacteriocidal concentration : MBC) พบว่าสารสกัดยอดหว้าที่สกัดด้วยน้ำมีค่า MIC เท่ากับ 6.25 mg/ml และมีค่า MBC เท่ากับ 12.5 mg/ml ในการยับยั้งและทำลายเชื้อ *S. aureus* และ MRSA ตามลำดับ สารสกัดใบพลูที่สกัดด้วยเอทานอล มีค่า MIC และ MBC เท่ากับ 12.5 mg/ml ในการยับยั้งและทำลายเชื้อ *S. aureus* และ MRSA

คำสำคัญ : ฤทธิ์ต้านจุลชีพ, สารต้านอนุมูลอิสระ, ปริมาณฟีนอลิก, พืชสมุนไพรไทย

1. Introduction

Phenolic compounds are secondary metabolites which synthesize in plants. They were posed biological properties such as: antioxidant, antiapoptosis, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Han *et al*., 2007). Several studies have indicated that the antioxidant activities of some fruits, vegetables and herbs were highly correlated with their total phenolic contents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, T., 1994). Antioxidants compounds can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu, Y.S. *et al*., 1998; Emmons, C. L. *et al*., 1999).

Plants, which are rich in phenolic components, are of interest as sources of natural antioxidant. The biosynthesis of phenolic compounds and related substances is derived from some proteins, including tyrosine and tryptophan, in the shikimic acid pathway. In addition, the phenolic usually occur in bound form such as flavonoid glycosides and phenolic acid derivatives, which are synthesized from sugar. More than 3,000 plant species including Thai traditional plants have been presented on phenolic compounds and their antioxidant properties, antimutagenic properties (108 species) (Trakoontivakorn *et al*., 2001; Nakahara *et al*., 2002), and activity related to inflammation (four Thai plants) (Laupattarakasam *et al*., 2003), which were related to their antioxidant properties. Phenolics which occur in plant respiration and other phenolics also arise from this pathway and subsequent reaction such as cinnamic, p-coumaric, caffeic, furulic, chlorogenic, protocatechuic and gallic acids. These are derived from phenylalanine and tyrosine, which are amino acids. In addition, there are several processes to translocate carbohydrate from leave to various sink organs during photosynthesis (Maisuthisakul *et al*, 2008). The best method for determination of total phenolic content is Folin-Ciocalteu method which was published on the screening of natural antioxidants (Spigno *et al*., 2007).

Staphylococcus aureus, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are human pathogenic bacteria and can resistance to multiple drugs. This trended to search for new antimicrobial substances from various sourced as novel antimicrobial chemotherapeutic agents and the biologically active compounds derived from plants were considered to use. The aim of this study was evaluated on the total phenolic content, antioxidant properties and *in vitro* antibacterial activity of 13 Thai traditional plants to against gram positive and gram negative bacteria. These data should be useful for screening plants as potential sources and safe natural antioxidants and antibacterial compounds.

2. Materials and Methods

2.1 Plants used

Thirteen Thai traditional plants in Table 1 were collected from different natural habitats at Nakhonsrithammarat, Thailand. The fresh sample materials were rinsed with distilled water.

2.1.1 Water crude extracts

The sample materials were chopped and homogenized into distilled water in a blender for 1 min. After that the suspension was filtrated by whatman No.1 and then lyophilized by freeze dryer at – 20 ^{o}C for 20 h. The powder was stored at 4 ^{o}C until used.

2.1.2 Ethanol crude extracts

The fresh sample materials were air dried and ground to fine powder. Samples were soaked in 95% ethanol at room temperature for 1 week, filtrated by and evaporated by vacuum rotary evaporator at 50 ^{o}C. The samples were kept at 4 ^{o}C until used.

2.2 Preparation of crude extracts

The crude extracts were dissolved with the same solvent (Distilled water or EtOH), centrifuged at 6,000 rpm for 30 min and then filtrated with sterilized pyrogen free membrane No. 0.22 μm. To evaluate the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values two fold dilutions method was used (Zaidi *et al*., 2009). Final concentrations of each crude extract were 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.20 mg/ml in the mixture.

Table 1 13 Species of Thai traditional plants

Scientific name	Family	Traditional name	Part of use
Anacardirm occidentale Linn.	*Anacardiaceae*	Ya-ruang	Leaves
Clausena cambodiana Guill.	*Rutaceae*	Sa-mui	Leaves
Ficus racemosa L.	*Moraceae*	Ma-duar	Fruits
Glochidion wallichianum Muell.	*Euphorbiaceae*	Mun-pu	Leaves
Litsea petiolata.	*Lauraceae*	Tum-mung	Leaves
Ocimum basilicum Linn.	*Labiatae*	Ho-rapa	Leaves
Ocimum canum Sims.	*Lamiaceae*	Mang-luk	Leaves
Piper betle Linn.	*Piperaceae*	Plu	Leaves
Piper sarmentosum Roxb.	*Piperaceae*	Cha-plu	Leaves
Pseuderanthemum palatiferum (Nees) Radlk.	*Acanthaceae*	Phraya-vanon	Leaves
Spondias pinnata Kurz.	*Anacardiaceae*	Ma-kok	Leaves
Syzygium cumini (L.) Skeels.	*Myrtaceae*	Wa	Leaves
Syzygium gratum (Wight) S.N. Mitra var. gratum	*Myrtaceae*	Sa-med-shun	Leaves

2.3 Bacterial strains used

Total four bacterial strains [*Staphylococcus aureus* (SA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA)] were used in this study and they were supplied by Microbiology Laboratory of Science and Technology Faculty of NSTRU University, Nakhonsrithammarat, Thailand. Bacterial were maintained on Nutrient agar (NA) (Merck, Germany).

2.4 Determination of total phenolic content and antioxidant activity

The amount of total phenolics in water/EtOH crude extract of 13 Thai traditional plants were determined with the Folin-Ciocalteu reagent using the method of Lister and Wilson (2001) as modified by this study. To 20 µl of each sample (three replicates), 100 µl of 2 N Folin-Ciocalteu's reagent were added and incubated at room temperature for 5 min. 300 µl of Na_2CO_3 (25% w/v) were mixed and incubated at 45 ^{o}C for 30 min. The absorbance of all samples was measured at 765 nm using UV–visible spectrophotometer. Results were expressed as milligram of gallic acid equivalent per gram of dry weight (mgGAE/g_{dw}). The antioxidant capacity of water/EtOH crude extracts was determined by antioxidant assay kit (Sigma).

2.5 Antimicrobial activity

2.5.1 Disc diffusion assay

The extracts were carried out by disc diffusion assay (Jorgensen *et al*., 1999) with minor modifications. Briefly, the suspension was containing 10^8 CFU/ml (McFarland No. 0.5) of bacteria in trypticase soy broth (TSB) (Merck). Cotton swab were impregnated into each bacterial test strains and were swab on mueller hinton agar (MHA) (Merck) medium. Steriled 6 mm diameter filter paper discs were impregnated with 50 µl of the sterilize test material and placed onto MHA. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Vancomycin (320 µg/ml, 50 µl/disc) used as positive reference standards to determine the sensitivity of gram positive bacterial species tested and gentamycin (40 µg/ml, 50 µl/disc) was used as positive reference to gram negative bacteria. The inoculated plates were incubated at 37 ^{o}C for 24 h. The antibacterial activity was measured as the diameter (mm.) of clear zone of growth inhibition and each test was run in triplicate.

2.5.2 Broth dilution assay

For initial screening, crude extracts that showed 100% inhibition (no visual growth of bacterial colonies compared to control) were further evaluated for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values with each sample. 1 ml of crude extracts was added to 1 ml of bacterial reference were inoculated in TSB (200 fold dilution compare with McFarland No. 0.5). The test tubes were incubated at 37 ^{o}C for 24 h. Vancomycin and gentamycin were used as positive references. After incubation, the first tubes that visually and no grow of bacteria reference were determined as MIC values. Then, the clear tubes were selected and 0.1 ml were spread on trypticase soy agar (TSA) (Merck) and incubated at 37 ^{o}C for 24 h. The MBC was defined as the test sample at which there were less than 5 colonies of bacterial at the latter cultivation.

3. Results

3.1 Total phenolic content and antioxidant activity

The highest total phenolic content in 13 species of plants was found in water and EtOH crude extract of *Syzygium cumini* (L.) Skeels. and *Piper betle* Linn. at 358.250 $\pm$ 0.014 and 474.083 $\pm$ 0.005 $mgGAE/g_{dw}$ and the highest antioxidant in water and EtOH crude extract are *Syzygium cumini* (L.) Skeels. and *Anacardirm occidentale* Linn. at 332.425 $\pm$ 0.21 and 411.916 $\pm$ 0.05 mM Trolox. The data of total phenolic content and antioxidant activity were demonstrated in Table 2.

3.2 Antimicrobial activity

The antimicrobial activity of 13 Thai traditional plants were tested by disc diffusion assay at different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.20 mg/ml) and 7 species of water crude extract showed the inhibitory activities against the tested bacteria. *Syzygium cumini* (L.) Skeels. showed the highest diameter of inhibition zone at 12.48 mm (Conc. 100 mg/ml of crude extract) to *Staphylococcus aureus* and has shown inhibition range of 6.92 to 12.48 mm to *Staphylococcus aureus*. The antimicrobial activities of *Syzygium cumini* (L.) Skeels. were found to against Methicillin-resistant *Staphylococcus aureus* in the ranged from 8.46 – 13.85 mm which more sensitive than *Staphylococcus aureus*. *Anacardirm occidentale* Linn. and *Piper betle* Linn. were 2 species from EtOH crude extract of 13 plant species which has shown the highest activity *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The EtOH crude extract of *Piper betle* Linn. was inhibited all indicator strains and has shown the highest inhibition zone on *Staphylococcus aureus* at the concentrations of 12.5–100 mg/ml, the diameter to *Staphylococcus aureus* ranged from 9.00 to 22.06 mm The inhibition of *Piper betle* Linn. EtOH crude extract has shown the sensitivity to Methicillin-resistant *Staphylococcus aureus* at the concentration of 12.5–100 mg/ml and 50–100 mg/ml to *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activity of water and EtOH crude extract of 13 species Thai traditional plants are presented in Table 3.

3.3 Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) determination

The active crude extracts from disc diffusion assay were studied on MIC and MBC. The MIC and MBC of active crude extract on different tested strains are given in Table 4. The results indicated that *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* were most sensitive to *Syzygium cumini* (L.) Skeels. of water crude extract at the MIC of 6.25 mg/ml and 12.5 mg/ml of MBC to both strains. The EtOH crude extract of *Piper betle* Linn. has shown the most sensitivity to *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at MIC of 12.5, 12.5, 50 and 50 mg/ml and MBC tested on *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* are 12.5 mg/ml and 50 mg/ml to *Escherichia coli* and *Pseudomonas aeruginosa*.

4. Discussion

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providing health benefit effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insect, and herbivores (Vaya *et al*., 1997). However, the correlation between phenolic compounds and antioxidant activities are not clear.

Table 2 Total phenolic content and antioxidant activity from 13 Thai traditional plants

Thai traditional plants	Phenolic content ($mgGAE/g_{dw}$)		Antioxidant (mM Trolox)	
	Water	EtOH	Water	EtOH
Anacardirm occidentale Linn.	99.917 ± 0.003	305.750 ± 0.008	63.177 ± 0.12	411.916 ± 0.05
Clausena cambodiana Guill.	130.750 ± 0.001	38.250	4.921 ± 0.30	9.274 ± 0.13
Ficus racemosa L.	317.417 ± 0.010	ND	209.630 ± 0.16	ND
Glochidion wallichianum Muell.	163.250 ± 0.003	225.750 ± 0.004	160.572 ± 0.10	341.055 ± 0.15
Litsea petiolata	183.250 ± 0005	44.917 ± 0.001	58.165 ± 0.05	6.912 ± 0.04
Ocimum basilicum Linn.	4.083 ± 0.001	8.250 ± 0.001	2.430 ± 0.06	ND
Ocimum canum Sims.	ND	20.750	ND	ND
Piper betle Linn.	25.750 ± 0.002	474.083 ± 0.005	8.759 ± 0.01	12.491 ± 0.025
Piper sarmentosum Roxb.	14.083 ± 0.006	35.750 ± 0.001	ND	ND
Pseuderanthemum palatiferum (Nees) Radlk.	30.750 ± 0.003	29.083 ± 0.001	7.926 ± 0.01	ND
Spondias pinnata Kurz.	33.250 ± 0.002	70.750 ± 0.005	13.968 ± 0.05	21.099 ± 0.19
Syzygium cumini (L.) Skeels.	358.250 ± 0.014	75.750 ± 0.001	332.425 ± 0.21	25.664 ± 0.06
Syzygium gratum (Wight) S.N. Mitra var. gratum.	111.583 ± 0.001	84.917 ± 0.001	38.375 ± 0.16	5.731 ± 0.16

*Values are mean ± S.D. of three replicates and ND are undetected of phenolic compound and antioxidant activity

Table 3 Antimicrobial activities of water and EtOH crude extracts from 13 Thai traditional plants by disdiffusion assay

Crude extracts	Bacterial indicator	Diameter of inhibition zone (mm) (50 µl/disc)					
		Concentration of crude extracts (mg/ml)					
		100	50	25	12.5	6.25	3.125
Negative control							
Water	SA	-					
	MRSA	-					
	EC	-					
	PA	-					
EtOH	SA	-					
	MRSA	-					
	EC	-					
	PA	-					
Positive Control							
Vancomycin (320 µg/ml.)	SA	14					
	MRSA	13.5					
Gentamycin (30 µg/ml.)	EC	21.5					
	PA	24.2					
Water crude extract							
Anacardirm occidentale Linn.	SA	8.3	7.35	6.26	-	-	-
	MRSA	8.78	7.94	6.88	-	-	-
	EC	7.42	-	-	-	-	-
	PA	8.76	7.25	-	-	-	-
Clausena cambodiana Guill.	SA	6.10	-	-	-	-	-
	MRSA	6.10	-	-	-	-	-
Ficus racemosa L.	SA	7.00	6.42	6.12	-	-	-
	MRSA	7.09	6.35	-	-	-	-
Glochidion wallichianum Muell.	MRSA	8.80	7.39	6.24	-	-	-
Litsea petiolata	SA	8.26	6.45	-	-	-	-
	MRSA	6.92	6.10	-	-	-	-
Spondias pinnata Kurz.	SA	7.39	-	-	-	-	-
	MRSA	7.75	-	-	-	-	-
	PA	6.10	-	-	-	-	-
Syzygium cumini (L.) Skeels.	SA	12.48	9.13	8.00	7.13	6.92	-
	MRSA	13.85	12.84	11.51	9.45	8.46	-
Ethanol crude extracts							
Anacardirm occidentale Linn.	SA	6.38	-	-	-	-	-
	MRSA	6.60	-	-	-	-	-
	EC	6.40	-	-	-	-	-
	PA	6.57	-	-	-	-	-
Piper betle Linn.	SA	22.06	16.52	14.20	9.00	-	-
	MRSA	16.53	16.47	15.50	10.06	-	-
	EC	11.00	8.00	-	-	-	-
	PA	9.22	7.25	-	-	-	-

- = no inhibition zone

Table 4 Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) from Thai traditional plants by broth dilution assay

Crude extracts	MIC (mg/ml)				MBC (mg/ml)			
	SA	MRSA	EC	PA	SA	MRSA	EC	PA
Water crude extracts								
Anacardirm occidentale Linn.	25	25	100	50	25	25	100	100
Clausena cambodiana Guill.	100	100	-	-	100	100	-	-
Ficus racemosa L.	25	50	-	-	50	50	-	-
Glochidion wallichianum Muell.	-	25	-	-	-	25	-	-
Litsea petiolata	50	50	-	-	100	100	-	-
Spondias pinnata Kurz.	100	100	-	100	100	100	-	100
Syzygium cumini (L.) Skeels.	6.25	6.25	-	-	12.5	12.5	-	-
Ethanol crude extracts								
Anacardirm occidentale Linn.	100	100	100	100	100	100	100	100
Piper betle Linn.	12.5	12.5	50	50	12.5	12.5	50	50

There were correlation between total phenolic content and antioxidant activity in some plants but some were not on this study. Antioxidant activity correlated well with phenolic and flavonoid content, whereas the total phenolic compounds correlated weakly with other components except flavonoid contents (Maisuthisakul *et al*, 2008). No correlation results in some plants is could possibly be due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity and the Folin-Ciocalteu method is not and absolute measurement of the amount of phenolic materials. The crude extracts possibly contain different type of phenolic compounds, which have different antioxidant capacities (Javanmardi *et al*., 2003 ; Sengul *et al*., 2009). Also some results suggest that phenolic compounds do not make a major contribution to the antioxidant activity of the extracts. There were no correlation between the antioxidant activity and total phenolic contents (Modoressiet, A. *et al*., 2009).

Syzygium cumini (L.) Skeels. water and EtOH crude extracts showed the highest total phenolic content, antioxidant property and against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*. In Brazil, the bark, fruits, seeds and leaves of *Syzygium cumini* (L.) Skeels. were used for the treatment of diabetes and administered in various pharmaceutical preparations (Braga *et al*., 2007). Seeds have shown hypoglycemic and antioxidant activities. Bark is also used for dysentery and diarrhea. Moreover, *Syzygium cumini* (L.) Skeels. has been shown to have sedative and anticonvalsant effects and a potent central nervous system depressant effect (Pepato *et al*., 2004)

The highest total phenolic content and antibacterial activity of EtOH crude extracts were found on *Piper betle* Linn. The leaves of the *Piper betle* Linn. are used in traditional medicine and possess antioxidant, antibacterial, antifungal, antidiabetic, radioprotective and antiallergic activity (Wirotesangthong *et al*., 2008).

To find out the potential and safe natural therapeutic agents, total phenolic content, antioxidants and antimicrobial activity were considered on screening method. However, the toxicity of plant extracts should be tested, to confirm their safety for use and should be purified to clarify the pharmacological properties for pharmaceutical application.

Conclusion

The experiments described above demonstrated that Thai traditional plants are rich in phenolic component but some result suggests that phenolic compounds do not make a major contribution to the antioxidant activity of the extracts. *Syzygium cumini* (L.) Skeels. and *Piper betle* Linn. possess of the compounds which high in phenolic content, antioxidant and antimicrobial activity which demonstrated that Thai traditional plants can be the good sources of natural product for side dishes, commercial and medicinal uses.

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