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Antioxidant and antibacterial potentials of some Thai native plant extracts

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Abstract

Eight native Thai plant rarely studied were extracted by using distilled water and 95% ethanol then were investigated for their total phenolics, free radical scavenging, metal chelating and five antibacterial capacities. Five bacteria consisted of one gram-negative (*Esherichia coli*) and four gram-positive (*Stapphylococcus aureus*, *Stapphylococcus epidermidis*, *Bacillus cereus* and *Bacillus subtilis*). Significant difference was observed among solvent extractions used and the plant species. The aqueous extracts gave higher antioxidant abilities but lower antibacterial capacities than the ethanol ones. Principle component analysis (PCA) revealed that the free radical scavenging was correlated to the total phenolics. Among the eight species, *S. gratum* extracts showed the strongest free radical scavenging ability, while, *C. harmandiana* showed the strongest chelating capacity. The *G. cowa* extracts were exception in antibacterial capacities because their activities against all the test bacteria. Some native Thai plant rarely studied could be used as alternative natural sources for antibacterial and antioxidant substances.

Introduction

Nowadays consumers widely concern about hazards of synthetic antibacterial and antioxidant agents, and their preferences in using natural products for good quality with eco-friendliness continue to grow (Gupta *et al.*, 2008). On the other hand, various plant extracts have been used as flavoring, coloring, and preservative agents for thousand years throughout the world (Brandi *et al*., 2006). As tropical regions provide tremendous resource of plants with wide range of biodiversity, various plants have been known to be beneficial; however many species are still not well investigated for their valuable bioactive phytochemicals. The largest class of such beneficial phytochemicals is phenolic compounds which possess physiological or functional properties such as anti-inflammatory, antimicrobial and antioxidant (Segura *et al*., 1998; Kazłowska *et al*., 2010; Maddox *et al.*, 2010). Among those properties, antioxidation and antimicrobial are of our interest. Normally, human body is equipped with mechanisms to eliminate the free radicals and reactive oxygen species which are produced from oxygen consumption in aerobic metabolisms (Barros *et al*., 2007). In addition, external factors such as radiation, air pollutant and tobacco smoke may also generate free radicals. When these reactive radicals are excessive or the antioxidants are insufficient, damage of cellular components such as lipids, proteins and nucleic acids can be enhanced and eventually lead to cell death, tissue damage, aging, and lesions such as cancer, diabetes and cardiovascular disease metabolisms (Rojas *et al*, 2003; Barros *et al.*, 2007). Antioxidant activities of phenolics can act as radical scavengers and metal chelators (Deetae *et al*., 2012; Fawole *et al*., 2012). Together with phenolics, various phytochemicals such as alkaloids, terpenes and coumarins exhibit antibacterial properties (Arruda *et al.*, 2011). Such properties of plant extracts are also applied in preservation of raw and processed food as well as for alternative treatments. Interestingly, these compounds also possesses antimicrobial activities as observed in preventing rancidity, off-flavor, changes of color and texture, and spoilage of fish, fruits and vegetables products (Ponce *et al.*, 2008; Maqsood *et al.*, 2013).

Nowadays, a rich diversity of plants exists in many regions of Thailand. Some indigenous plants are infrequent found, however their potential bioactivities cannot be neglected. Therefore, the plant species

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namely *Clausena harmandiana*, *Clausena excavate*, *Garcinia cowa*, *Dolichandrone serrulata*, *Syzygium gratum*, *Limnophila aromatic*, *Boesenbergia pulcherrima* and *Ocimum gratissimum* were investigated. Their leaves are edible and commonly used as local vegetables in Thailand; however a few studies related to their antioxidation and antibacterial activities have been conducted (Daduang *et al*., 2011; Maneerat *et al.*, 2012). In this work, total phenolic contents, antioxidant properties and antibacterial abilities against some foodborne pathogens of the 8 native plant extracts were evaluated.

Materials and Methods

Chemicals

Folin–Ciocalteu's phenol reagent, gallic acid monohydrate and absolute ethanol were supplied from Sigma-Aldrich (Steinheim, Germany). Ferrozine (3-(2-pyridyl)-5, 6-diphenyl-1,2,4-triazine), 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) were obtained from Fluka (Buchs, Switzerland). Glycerol and tryptone powder were obtained from Bio basic Inc. Agar and yeast extract was obtained from HIMEDIA (Mumbi, India). Ampicilin sodium salt was United States Pharmacopeia grade.

Plant materials

C. harmandiana, *C. excavata*, *B. pulcherrima*, *S. gratum* and *L. aromatic* were collected from Mahidol university, Kanchanaburi campus. *D. serrulata*, *G. cowa* and *O. gratissimum*, were purchased from local markets at Pathum Thani province. The plant leaves were washed with distilled water and dried at 45°C for 2 days. The dried leaves of the plants were finely homogenized using a blender (Philips, Japan) and kept in plastic bags.

Extractions

Solvent extraction was performed according to Ceyhan *et al.* (2012) with a few modifications. Sample 25 g (dried basis) was mixed with 250 ml of sterile distilled water or 95% ethanol, continuously stirred at 37°C for 14 h, and then filtered over sterile cheesecloth. After that, the crude extract was concentrated using a rotary evaporator (BUCHI Rotavapor R-205) at 45°C under vacuum. The concentration of dried extract was adjusted to 1000 mg/ml with the extracting solvent and stored at -20°C prior to further analysis.

Determination of total phenolic contents

Total phenolic content of the plant extract was determined using the Folin–Ciocalteu assay (Deetae *et al*., 2012). Briefly, 300 µl of each plant extract was reacted with 1.5 ml of Folin–Ciocalteu's reagent and 1.2 ml of sodium carbonate (7.5% w/v). Then, the reaction mixture was left in the dark for 30 mins and measured at 765 nm using a spectrophotometer (Thermo Spectronic model 4001/4, USA). The concentration of total phenolic compounds in the plant extract was expressed as mg of gallic acid equivalent per g sample.

Antioxidant activities

ABTS assay

The ABTS free radical-scavenging activity of each plant extract was determined according to Deetae *et al.* (2012). The ABTS solution was diluted with distilled water to obtain the absorbance of 0.700 ± 0.050 at 734 nm. After that, the prepared ABTS solution (3.9 ml) was reacted with the crude extract (20 µl) in the dark at room temperature for 6 min. The absorbance for the sample at 734 nm was recorded. The antioxidant capacity was calculated using an equation as reported by Deetae *et al.* (2012). Extract concentration providing the 50% effective concentration (EC_{50}) was calculated from the graph plotted in percentage against each extract concentration. The EC_{50} values were adjusted to antiradical activity (AAR) determined as $1/EC_{50}$ due to this factor increases follow their antioxidant activity.

FIC assay

The Ferrous Ion-Chelating (FIC) ability of each plant extract was determined according to Deetae *et al*. (2012). One millilitre of the crude extracts was reacted with 0.1 mM $FeSO_4$ (1 ml) and 0.25 mM ferrozine (1 ml). After the prepared mixture was kept at room temperature in the dark for 10 min, absorbance at 562 nm was measured. The percentage of metal chelating ability was calculated using an equation as reported (Deetae *et al*., 2012) and the EC_{50} and $1/EC_{50}$ was calculated as described above.

Antibacterial activity

Antibacterial activity was determined using an agar disc diffusion method (Thummajitsakul *et al*., 2012). Five bacterial strains, i.e. *Esherichia coli* (TISTR780), *Stapphylococcus aureus* (TISTR1466), *Stapphylococcus epidermidis* (TISTR518), *Bacillus cereus* (TISTR687) and *Bacillus subtilis* (TISTR008) obtained from Thailand Institute of Scientific and Technological Research were used as indicator strains. Single colony of each bacterial strains was cultured in 25 ml Plate Count Broth (PCB) at 37°C, shaking for 18 h. After that, cell density was adjusted to the range

of 0.1-0.5 OD at 600 nm using a spectrophotometer (Thermo Spectronic model 4001/4, USA) and spread on Plate Counting Agar (PCA). Each Paper disc (6 mm in diameter) was immersed in varied amounts of plant extract and placed on the surface of the inoculated plate which was incubated for 18 h at 37°C. The inhibition zone around the paper disc was calculated by subtraction of diameter of a clear zone with diameter of the paper disc.

Statistical analysis

Values were means ± standard deviation. Analysis of variance (ANOVA) and Pearson correlation analysis was performed using the method of Soper, 2013 ($p < 0.05$). Principle component analysis (PCA) was performed using Multibase 2013 implemented on Excel.

Results and Discussion

Total phenolic contents

Among the 8 tested plants, the aqueous extract of *S. gratum* showed the highest total phenolic contents (38.87 ± 8.60 mg gallic acid/g extract), followed by *C. excavate* > *C. harmandiana* > *D. serrulata* > *O. Gratissimum* > *B. pulcherrima* > *L. aromatic* > *G. cowa*, respectively. Likewise, the *S. gratum* plant exhibited also the highest value of total phenolics in ethanol solvent (31.73 ± 0.42 mg gallic acid/g extract), followed by *D. serrulata* > *C. excavate* > *G. cowa* > *O. Gratissimum* > *C. harmandiana* > *L. aromatic* > *B. pulcherrima*, respectively (Table 1). The results of statistical analysis showed that types of plant and extracting solvents significantly influenced on the total phenolic contents. The aqueous extracts of all tested plants showed significantly higher total phenolic contents than the ethanol extract. Generally, the phenolic compounds are more soluble in high polar solvent (Grigonisa *et al.*, 2005; Naczk *et al.*, 2006). However, non-phenolic components can be co-extracted in the crude extract if water content is too high in the extracting solvent. Extractable phenolic compounds mostly found in the extracts are phenolic acids, phenylpropanoids, flavanoids and quinones, and non-soluble phenolic compounds are tannin and lignins (Rice-Evans *et al.*, 1997). It has been reported that the amounts of phenolics can be affected by geographic locations, environment, season, part used, processing and storage (Perez-Jimenez and Saura-Calixto, 2005; Prior *et al.*, 2005).

Antioxidant activities

ABTS free radicals produced from oxidation reaction with potassium persulfate were used to measure the capacity of a primary antioxidant species

Table 1. Total phenolic contents, free radical scavenging activity and metal-chelating activity of plant extracts. Values are means ± standard deviation, n=4.

Plant species	FIC ($1/EC_{50}$)		ABTS ($1/EC_{50}$)		Total Phenolic contents (mg gallic acid eq./g extract)	
	aqueous	95% Ethanol	aqueous	95% Ethanol	aqueous	95% Ethanol
C. harmandiana	42.19×10^{-2}*	2.33×10^{-2}	24.94×10^{-2}*	3.10×10^{-2}	30.93±1.52	16.94±0.31
C. excavata	15.80×10^{-2}*	2.33×10^{-2}	23.58×10^{-2}*	7.61×10^{-2}	31.05±4.38	26.83±0.14
G. cowa	2.16×10^{-2} *	1.79×10^{-2}	6.73×10^{-2}*	9.18×10^{-2}	7.55±1.16	20.84±0.46
D. serrulata	14.68×10^{-2}*	2.29×10^{-2}	19.69×10^{-2}*	12.72×10^{-2}	24.03±2.71	27.13±0.33
S. gratum	22.57×10^{-2}*	1.91×10^{-2}	126.58×10^{-2}*	18.73×10^{-2}	38.87±8.60	31.73±0.42
L. aromatic	8.87×10^{-2} *	2.09×10^{-2}	7.66×10^{-2}*	2.85×10^{-2}	8.24±1.35	15.70±0.31
B. pulcherrima	19.57×10^{-2} *	2.16×10^{-2}	9.81×10^{-2}*	2.66×10^{-2}	10.35±1.13	9.42±0.23
O. Gratissimum	37.04×10^{-2}*	2.07×10^{-2}	8.21×10^{-2}*	12.21×10^{-2}	13.33±0.70	18.54±0.14

demonstrated that aqueous extracts showed significantly higher properties than ethanol extracts ($P < 0.05$)

to donate hydrogen atoms (Naczk and Shahidi, 2006). The $1/EC_{50}$ values indicating antiradical activity (AAR) were in the range of 2.66×10^{-2} to 126.58×10^{-2}. The types of plant and extraction solvents dominated on antioxidant capacities. The aqueous extract of *S. gratum* had the strongest free radical scavenging ability with the $1/EC_{50}$ of 126.58×10^{-2}, followed by *C. harmandiana* > *C. excavate* > *D. serrulata* > *B. pulcherrima* > *O. Gratissimum* > *L. aromatic* > *G. cowa*. Likewise, the ethanol extract of *S. gratum* showed the strongest antioxidant ability (18.73×10^{-2}), followed by *D. serrulata* > *O. Gratissimum* > *G. cowa* > *C. excavate* > *C. harmandiana* > *L. aromatic* > *B. pulcherrima* (Table 1). The results were consistent with the previous report that the free radical scavenging capacities were found in extracts of *L. aromatic* and *G. cowa* (Daduang *et al*., 2011). The ability of chelate off metal ions was determined by the FIC assay. This involves blocking of a Fenton reaction which generates harmful hydroxyl radicals leading to occurrence of many diseases (Aruoma *et al.*, 1987). In our results, the aqueous extracts of all tested plants showed also significant higher chelating abilities than ethanol extracts with the $1/EC_{50}$ values in range 1.91×10^{-2} to 42.19×10^{-2}. The aqueous extract of *C. harmandiana* showed the strongest chelating ability with $1/EC^{50}$ of 42.19×10^{-2}, followed by *O. Gratissimum* > *S. gratum* > *B. pulcherrima* > *C. excavate* > *D. serrulata* > *L. aromatic* > *G. cowa.* For the ethanol extract, *C. harmandiana* and *C. excavate* had also the strongest chelating ability (2.33×10^{-2}), followed by *D. serrulata* > *B. pulcherrima* > *L. aromatic* > *O. Gratissimum* > *S. gratum* > *G. cowa* (Table 1).

Antibacterial activities

The results of statistical analysis showed that types of plant and extracting solvents significantly impacted on antibacterial activities. The aqueous extract of *G. cowa* demonstrated the highest antibacterial activity effective against all tested bacteria with mean zones of inhibition ranging from 6.17±1.72 to 11.33±1.37 mm, followed by *D. serrulata* and *S. gratum*. For the ethanol extracts, *G. cowa* also had the highest antibacterial activity (6.67±0.52 to 9.17±0.98), followed by *O.*

Table 2. Antibacterial activities of eight plant extracts against five pathogens. Values are means ±standard deviation, n = 3

Plant species	Extract (1 g/ml)	Inhibition zone (mm) Bacterial strains				
		E. coli	*B. subtilis*	*B. cereus*	*S. aureus*	*S. epidermis*
C. harmandiana	aqueous	0	0	0	0	0
	95% Ethanol	4.50±1.04*	6.00±0.63*	8.17±1.17*	7.33±0.82*	8.67±0.52*
C. excavata	aqueous	0	0	0	0	0
	95% Ethanol	5.50±1.05*	5.33±0.52*	6.83±0.41*	8.83±0.75*	8.17±0.40*
G. cowa	aqueous	6.17±1.72	7.83±1.47	8.67±1.51	6.17±0.98*	11.33±1.37*
	95% Ethanol	6.67±0.52	6.83±0.75	8.33±0.82	9.17±0.98*	8.33±0.82*
D. serrulata	aqueous	0	0	0	0	0
	95% Ethanol	0	6.67±2.34*	8.83±0.41*	0.67±0.26*	8.33±1.63*
S. gratum	aqueous	0	3.00±1.55	4.00±0.00	3.67±0.52	3.83±0.41
	95% Ethanol	0	3.83±0.41	4.00±1.09	4.00±0.63	5.00±1.67
L. aromatic	aqueous	0	0	0	0	0
	95% Ethanol	2.50±1.22*	2.83±0.41*	3.67±0.52*	3.67±0.52*	4.50±0.55*
B. pulcherrima	aqueous	0	0	0	0	0
	95% Ethanol	1.50±1.76*	5.83±0.75*	3.17±1.47*	3.33±1.63*	6.17±3.49*
O. Gratissimum	aqueous	0	0	0	0	0
	95% Ethanol	6.33±0.82*	6.66±1.03*	7.16±0.57*	9.33±0.81*	9.33±0.81*

* demonstrated that ethanol extracts showed significantly higher activities than aqueous extracts ($P < 0.05$)

Gratissimum > *C. excavata* > *D. serrulata* > *C. harmandiana* > *B. pulcherrima* > *S. gratum* and *L. aromatic*, respectively (Table 2). However, the ethanol extracts of *D. serrulata* and *S. gratum* showed limited effect against the gram-negative bacteria *E. coli.* Our results consist with previously reported (Siridechakorn *et al*., 2012), who found new dihydrobenzopyran and xanthone derivatives from *G. cowa* stem exhibited the antibacterial activities against *E. coli*, *S. typhimurium*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA). Similarly, the new carbazole alkaloids from *C. harmandiana* twigs had the antibacterial activity against *E. coli*, *S. typhimurium*, *S. aureus* and methicillin-resistant *S. aureus* (MRSA) (Maneerat *et al*., 2012). It has been reported that phenolics can form complexes with proteins and bacterial membranes, or involve membrane disruption to inactivate microbial growth (Prior *et al*., 2005). However, the gram-negative bacteria were more resistant to the plant extracts than the gram-positive bacteria because the cell wall of gram-negative bacteria had morphology more complex than those of gram-positive bacteria. It might be explained by the effect of lipopolysaccharides in their outer membrane (Spigno *et al.*, 2007).

Statistical analysis

Pearsons'correlation coefficient between each mean variable was determined. The correlation between the content of total phenolics and the 1/EC_{50} values of ABTS assay was strongly significant positive ($r = 0.641$, $p < 0.05$), indicating that higher phenolic components affected to increase free-radical scavenging abilities of each plant extract. The correlation between the 1/EC_{50} values of FIC assay and the total phenolic contents was not significant ($r = 0.228$, $p > 0.05$), indicating that some phenolic compounds in each plant extract might not show binding activities to metal ions. The correlation of antibacterial activities with the total phenolic contents and the EC_{50} of ABTS assay were not significant ($r = -0.085$, -0.110: $p > 0.05$, respectively). The antibacterial activities and the 1/EC_{50} values of FIC assay showed a significant negative correlation ($r = -0.691$, $p < 0.05$). It may be explained that some phenolic compounds or antioxidants in the extracts were not function as antibacterial agents, and other non-phenolics in plant extracts can act as antibacterial substances such as antibacterial peptides from edible plant leaves (Song *et al.*, 2012), and organic acids which can disrupt the bacteria cell wall of some pH-sensitive pathogens such as *E. coli* (Raybaudi-Massilia *et al*., 2009). Therefore, the antibacterial abilities could not refer solely to total phenolic contents or antioxidants, even though, the FIC assay showed a significant negative correlation.

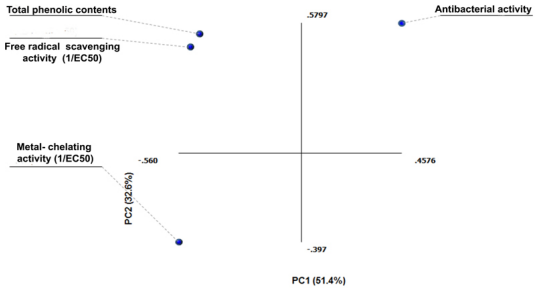

Figure 1. PCA loading plot of variables, i.e. total phenolic contents, free radical scavenging activity, metal-chelating activity and antibacterial activity

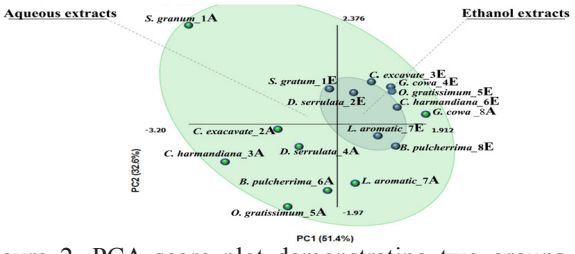

Figure 2. PCA score plot demonstrating two groups of variances, i.e. plant water extracts and plant ethanol extracts of 8 species (*C. harmandiana*, *C. excavate*, *G. cowa*, *D. serrulata*, *S. gratum*, *L. aromatic*, *B. pulcherrima* and *O. Gratissimum*). Last letter followed the species name of A or E denoted water extract and ethanol extract respectively. Numbers 1 to 8 illustrated the descending order of total phenolic contents found in aqueous and ethanol extracts.

PCA was performed to get insight of interrelation between variables and group of variances. PC_1 and PC_2 explained 51% and 33% of variances, respectively. PC1 loadings consisted of free radical scavenging activity, metal-chelating ability and antibacterial activity, while PC2 loading was only the total phenolic content. Factor loading plot showed that total phenolic content located separately from free radical scavenging, metal-chelating and antibacterial activities (Figure 1). On the other hand, score plot showed that the plant extracts were divided into two groups based on their extracting solvents, i.e. water and 95% ethanol (Figure 2). When both of the plots were superimposed, interrelation among extracting solvent, antibacterial activity, the total phenolic content, free radical scavenging activity and metal-

chelating ability was demonstrated. It was clear that most of ethanol extracts showed higher antibacterial activity and metal chelating activity. It has been reported that plant ethanol extracts mostly has higher antibacterial activity than the aqueous extracts (Rispail *et al*., 2005). On the other hand, free radical scavenging activity of ethanol extracts of *C. excavata*, *L. aromatic* and *B. pulcherrima* were outstandingly high. However, these functional properties of interest seem not directly related to the total phenolic contents. It has been reported that some edible vegetables (i.e. *L. aromatic* from shoot tip) with low phenolic contents had high free radical scavenging activity abilities in ethanol extracts (Daduang *et al.*, 2011). It may be affected by other antioxidants which are non-phenolic compounds such as pigments and ascorbic acids (Conklin *et al*., 2004).

Conclusions

Our studies demonstrated that eight native Thai plants rarely studied had both antioxidant and antibacterial properties. However, the capacities depended on solvent extraction used and plant species. The aqueous plant extracts gave higher in total phenolic contents, free radical scavenging and metal-chelating abilities but lower in antibacterial activity than the ethanol ones. Among the 8 species, *S. gratum* extracts were the exception for free radical scavenging activity. *C. harmandiana* extract gave the highest metal chelating property. *G. cowa* extracts were outstanding for antibacterial capacities since their activities against all tested bacteria including *Esherichia coli*, *Stapphylococcus aureus*, *Stapphylococcus epidermidis*, *Bacillus cereus* and *Bacillus subtilis*. Therefore, some native Thai plants rarely studied had antioxidant and antibacterial potentials. They may be used as alternative natural sources applicable to agriculture, cosmetic and food products.

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