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Full Length Research Paper

Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts

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Total phenolic contents, antibacterial and antioxidant activities of aqueous and ethanolic extracts from Thai medicinal plants were investigated in this study. The antibacterial activity was carried out using agar disc diffusion and broth dilution methods against *Escherichia coli* O157:H7, *Propionibacterium acnes, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and methicillin resistant *S. aureus*. The aqueous and ethanol extracts of *Senna alata* showed the most effective activity against Gram positive bacteria, with inhibition zone ranging from 10.3 to 23.0 mm. The highest activity was observed from *S. alata* extracts against *P. acnes*, with minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of 1.9 and 3.9 mg/ml, respectively. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'– azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The strongest antioxidant activities of aqueous extract of *S. alata* were 22.11 ± 0.324 mg gallic/g extract and 214.99 ± 17.279 mg trolox/g extract when determined by DPPH and ABTS assay, respectively. Moreover, the highest total phenolic content of 70.90 ± 1.048 mg gallic/g extract was measured from the aqueous extract of *S. alata*. Therefore, the biological activities of these plants observed in this study will be useful to develop the plant extracts for primary treatment of diseases as new therapeutic agents.

Key words: Medicinal plants, antibacterial activity, pathogenic bacteria, antioxidant activity, total phenolic content.

INTRODUCTION

Several bacteria are commonly found on the human skin such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Propionibacterium acnes*. In some circumstance, they are major causes of human skin diseases such as carbuncles, cellulitis, furuncles impetigo and possibility progress to severe diseases (Oumeish et al., 2001). Many species of bacteria develop resistance to available antibacterial agents, creating serious problems in clinical treatment and this drives the search for new antimicrobial substances. Moreover, free radical and active oxygen species are associated with pathological conditions such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, cancerogenesis and the aging process (Martínez-Cayuela, 1995; Bagchi et al., 2000). Free radicals are highly unstable and strongly react with biomolecules, leading to the structural modification, abnormal function and cell or tissue damage.

Antioxidants are chemicals that can delay or inhibit oxidation by terminating the initiation and propagation chain reaction, and have potential for therapeutic uses and prevention of diseases. Phenolic compounds are powerful antioxidants that can protect the human body from free radicals by acting as hydrogen donors, reducing agents and radical scavengers. They cannot be produced by the human body and thus must be taken through diets, especially vegetables and fruits. Medicinal plants are also sources of antioxidants and antimicrobial substances, which have been used to treat human diseases based on ethnopharmacological and traditional used. Moreover, modern pharmaceutical and several drugs were initially isolated or derived from medicinal plants (Farnsworth and

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Bunyapraphatsara, 1992). In Thailand, several medicinal plants have been used as antioxidant substances (Kubola and Siriamornpun, 2011). Numerous studies have also demonstrated antimicrobial agents against pathogenic bacteria (Chomnawang et al., 2005, 2009; Vuddhakul et al., 2007).

This study aims at investigating the total phenolic contents, antibacterial and antioxidant activities of aqueous and ethanolic extracts of some Thai medicinal plants: *Thunbergia laurifolia*, *Andrographis paniculata*, *Vernonia cinerea, Senna alata*, *Zingiber cassumunar*, *Tinospora crispa*, *Derris scandens*, *Rhinacanthus nasutus*, *Momordica charantia* and *Pluchea indica*.

MATERIALS AND METHODS

Extracts of medicinal plants

Medicinal plants used in this study were purchased from Lampang Herb Conservation, Thailand. Plant materials were dried in ventilated oven at 60°C for 72 h, after which the dried plants were grounded and extracted with two solvents including distilled water and 95% ethanol with the proportion of 1:10 (w/v). In brief, 250 g of each plant were extracted with distilled water at 45°C for 3 h or macerated with 95% ethanol for 72 h with frequent agitation at room temperature (Houghton and Raman, 1998). The plant extracts were filtered through Whatman No.1 filter paper. The solvent was removed from filtrate of plant extracts by evaporation at 45°C under reduced pressure 50 mbar in a rotary evaporator (Buchi™) and the plant extract was dissolved in dimethylsulphoxide (DMSO) to give a concentration of 500 mg/ml.

Bacterial strains

The tested bacterial strains, *Escherichia coli* O157:H7 DMST12743 and *Propionibacterium acnes* DMST14916 were obtained from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Thailand, while *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes*, methicillin resistant *S. aureus* (MRSA) and *Pseudomonas aeruginosa* were obtained from the Microbiology Section, Department of Medical Technology, Faculty of Associated Medical Science, Chiang Mai University, Chiang Mai, Thailand.

Investigation of antibacterial activity

Agar disc diffusion assay

The tested bacteria were cultured in Mueller-Hinton Broth (MHB, Merck®) at 37°C for 18 to 24 h. Turbidity of the bacterial culture was adjusted to approximately 1.0×10^8 CFU/ml. Subsequently, the culture of bacteria was swabbed on Mueller-Hinton Agar (MHA, Merck®). Then, a sterile paper disc (Macherey-Nagel) with 6 mm diameter was soaked in 500 mg/ml of each crude plant extracts and the discs were placed on the agar compared with solvent control, DMSO. These plates were incubated at 37°C for 24 h under aerobic condition. *P. acnes* was cultured in Brain Heart Infusion Broth (BHI, Merck®) at 37°C for 72 h and adjusted to approximately 1.0×10^8 CFU/ml. The extracts were also tested against *P. acnes* with previously mentioned procedure and the plates were incubated at 37°C for 72 h under anaerobic condition. The diameters of the inhibition zone around the discs were measured to access antibacterial activity. All experiments were performed in triplicates and the mean of inhibition zone was calculated.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The minimum inhibitory concentrations (MIC) were measured by broth dilution method. Two-fold serial dilutions of crude plant extracts were prepared in 0.5 ml MHB or BHI before inoculating with 0.5 ml of bacterial culture. The test tubes were incubated at 37°C for 72 h under anaerobic condition for *P. acnes* and incubated at 37°C for 24 h for other bacterial strains. MIC was recorded as the lowest concentration of crude extracts in which bacterial growth was inhibited. For MBC evaluation, the tubes with no growth were streak-plated on MHA or BHI agar and incubated under the mentioned condition for different bacterial strains. The MBC was recorded as the lowest concentration showing no visible growth of bacterial strains.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging

The DPPH radical scavenging assay was conducted according to the modified method of Brand-Williams et al. (1995) and Hoong Ho et al. (2010). The extracts were dissolved with methanol to prepare various concentrations, then 0.5 ml of each concentration was incubated with 1.5 ml of 0.1 mM methanolic solution of DPPH in the dark at room temperature for 20 min. Next, the absorbances were determined at 517 nm. Methanol was used as a blank solution, and DPPH without extract was used as a control. The percentage of free radical inhibition by the extract was calculated by the following equation:

$$\% \text{ Inhibition} = [(A0 - A1)/A0] \times 100$$

where A0 is the absorbance of control and A1 is the absorbance of the tested sample after treatment with extracts. Antioxidant activity of the extracts was expressed as gallic acid equivalent antioxidant capacity (GAE), which was determined from standard curve of gallic acid ($y = 9{,}187.9707x + 3.8635$, $R^2 = 0.9861$).

2,2'– azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical anion scavenging assay

The ABTS radical scavenging assay was performed according to the method of Re et al. (1999) with some modification. Briefly, the ABTS radical was generated by oxidation of ABTS with potassium persulfate ($K_2S_2O_8$). ABTS (7 mM) 10 ml was mixed with potassium persulfate (2.45 mM) 176 µl and kept in dark condition at room temperature for 12 to 16 h before use. ABTS working solution was diluted with 95% ethanol to obtain absorbance of 0.700 ± 0.020 at 734 nm. Then, 20 µL of crude extract was mixed with ABTS working solution and left in room temperature for 6 min. The absorbance was measured at 734 nm using 95% ethanol for standard blank. The percentage of free radical inhibition by the extract was calculated by the following equation:

$$\% \text{ Inhibition} = [(A0 - A1)/A0] \times 100$$

A0 is the absorbance of control and A1 is the absorbance of the tested sample after treatment with extracts. Antioxidant activity of extracts was expressed as trolox equivalent antioxidant capacity (TEAC), which was calculated from standard curved of trolox ($y = 193.9742x + 1.6930$, $R^2 = 0.9998$).

Total phenolic contents

The total phenolic compound contents were determined by Folin-Ciocalteau method (Chandler and Dodds, 1983). Briefly, plant extract was dissolved with methanol to obtained a concentration of 1 mg/ml. Afterward, 250 µl of the extract was mixed with 1.25 ml of water and then 250 µl of 95% ethanol and 125 µl of 50% Folin-Ciocalteau were added and mixed thoroughly. The mixture was incubated for 5 min and subsequently, 250 µl of 5% Na_2CO_3 was added and incubated for 1 h. The absorbance was measured at 725 nm. The standard curve was prepared using 10 - 100 µg/ml solutions of gallic acid and the concentration of phenolic compounds of extract was calculated from standard curve ($y = 8.3373x-0.0616$, $R^2 = 0.9945$) and expressed as gallic acid equivalent antioxidant capacity (GAE).

Statistical analysis

All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA using the SPSS software (version 17.0 for windows). Significant differences between means were determined by Duncan's new multiple-range test. A significant difference was considered at the level of $P < 0.05$. Spearman's rho correlation analysis was used to determine the correlation between two antioxidant methods, and between antioxidant activity and the total phenolic content.

RESULTS AND DISCUSSION

Antibacterial activity

Reconstituted plant extracts at concentration at 500 mg/ml were used throughout the study as these were the highest concentration that plant extract could be dissolved in DMSO. The results indicated the potential of medicinal plant extracts against seven pathogenic bacteria, as shown by agar disc diffusion susceptibility test, MIC and MBC values. The diameters of inhibition zones obtained are presented in Table 1. Aqueous and ethanolic extracts of *S. alata* demonstrated effective broad-spectrum antibacterial activity against all the Gram positive bacteria with diameters of inhibition zones ranging from 10.3 to 23.0 mm. In addition, the ethanolic extracts of *D. scandens* exhibited promising results against all the Gram positive bacteria; *S. aureus*, *S. epidermidis*, *S. pyogenes*, *P. acnes* and MRSA, with diameters of inhibition zones ranging from 10.0 to 14.0 mm. Moreover, the aqueous extracts of *D. scandens*, *P. indica*, *R. nasutus*, *T. laurifolia*, *V. cinerea* and the ethanolic extracts of *A. paniculata, M. charantia*, *R. nasutus*, *T. crispa* and *T. laurifolia* showed moderate activity on Gram positive bacteria. Additionally, the aqueous extracts of *M. charantia, T. crispa*, *Z. cassumunar* and ethanolic extract of *P. indica*, *V. cinerea* and *Z. cassumunar* showed very low activity against tested bacteria. However, all the tested Gram positive and Gram negative bacteria were not inhibited by aqueous extract of *A. paniculata*. Moreover, all plant the extracts could not inhibit growth of Gram negative bacteria; *E. coli* O157: H7 and *P. aeruginosa*. The sensitivity between Gram positive and Gram negative bacteria after treatment with the plant extracts could be ascribed in morphological difference. The cell wall of Gram negative bacteria had the complex structure more than Gram positive bacteria. It contained an outer phospholipid membrane carrying the structural lipopolysaccharide components, which made the cell wall of Gram negative bacteria impermeable to antimicrobial substances (Nostro et al., 2000; Tadeg et al., 2005). Thus, the Gram negative bacterial cells might not be destroyed by the plant extracts.

The aqueous and ethanolic extracts that possessed anti-bacterial effect were determined for their MIC and MBC values by broth dilution method. The MIC and MBC values were summarized in Table 2. The extract of *S. alata* exhibited the highest antibacterial activity against all the Gram positive bacteria with MIC ranging from 1.9 to 62.5 and MBC ranging from 3.9 to 125 mg/ml, followed by *D. scandens* with MIC from 3.9 to 125 and MBC from 7.8 to 250 mg/ml, *T. laurifolia* with MIC from 7.8 to 125 and MBC from 31.3 to 250 mg/ml, *R. nasutus* with MIC from 62.5 to 125 and MBC from 125 to 250 mg/ml and *A. paniculata* showed MIC from 125 to 250 and MBC from 125 to 250 mg/ml, respectively. Interestingly, most of crude plant extracts could inhibit MRSA and the aqueous extract of *D. scandens* showed higher inhibition than other extracts. Therefore, the result indicated that these plant extracts had a potential to be developed an antibacterial agents for MRSA strains. Moreover, Owoyale et al. (2005) reported that the alcoholic extract of *S. alata* showed high activity against fungi; *Mucor* sp., *Rhizopus* sp., *Aspergillus* sp., and yeast; *Candida albicans* and *Saccharomyces*. Antibacterial activity of *S. alata* was also observed on both Gram positive and Gram negative bacteria; *E. coli*, *Bacillus subtilis*, *Salmonella typhi*, *P. aeruginosa*, *S. aureus* with MIC ranging from 70-860 µg/ml. In addition, Idu et al. (2006) reported the activity of methanolic, chloroform, petroleum ether and aqueous extracts of *S. alata* leaves against *B. subtilis*, *C. albicans*, *E. coli, Proteus vulgaris*, *P. aeruginosa* and *S. aureus* with MIC ranging from 7.8 to 250 µg /ml. The result showed that the differences of antibacterial activity of *S. alata* might be attributed to the different procedures of testing antibacterial activity, difference in geographical environments, cultivar types, seasonality and plant aging of *S. alata*.

Antioxidant activity

The antioxidant activity is influenced by many factors that cannot be assessed by a single method; hence at least two test models have been recommended for the evaluation of antioxidant activity (Schlesier et al., 2002). In this study, the antioxidant activity was determined by DPPH and ABTS radical scavenging methods. The DPPH assay is widely used to determine the free radical scavenging ability in plant extract. DPPH, a free radical

Table 1. Effect of plant extracts on growth of pathogenic bacteria by agar disc diffusion method.

Plant species	Parts used	Extract (500 mg/ml)	Zone of Inhibition (mm)						
			Bacterial strains[a]						
			E. coli	***P. aeruginosa***	***S. aureus***	**MRSA**	***S. epidermidis.***	***S. pyogenes***	***P. acnes***
Andrographis paniculata	Whole plant	Aqueous	0	0	0	0	0	0	0
		Ethanol	0	0	9.0 ± 1.0^{abcd}	9.0 ± 1.0^{abcd}	12.3 ± 1.5^{abcd}	13.0 ± 1.7^{abcd}	0
Derris scandens	Leaves	Aqueous	0	0	20.7 ± 0.6^{bcd}	21.3 ± 1.2^{bcd}	18.7 ± 1.5^{bcd}	0	0
		Ethanol	0	0	10.7 ± 0.6^{bcd}	14.0± 1.7^{bcd}	10.0 ± 0.0^{bcd}	10.7 ± 0.6^{bcd}	12.7 ± 1.2^{bcd}
Momordica charantia	Leaves	Aqueous	0	0	0	0	10.7 ±0.6^{a}	0	0
		Ethanol	0	0	10.0 ± 0.6^{abcd}	0	0	15.3± 0.6^{abcd}	17.3 ± 0.6^{abcd}
Pluchea indica	Leaves	Aqueous	0	0	9.0±1.0^{ab}	13.7 ± 0.6^{ab}	0	0	0
		Ethanol	0	0	7.3± 0.6^{a}	0	0	0	0
Rhinacanthus nasutus	Leaves	Aqueous	0	0	11.7 ± 0.6^{abc}	15.3 ± 0.6^{abc}	0	0	0
		Ethanol	0	0	8.0 ± 0.0^{abc}	8.7 ± 1.2^{abc}	0	9.3 ± 0.6^{abc}	0
Senna alata	Leaves	Aqueous	0	0	14.7 ± 0.6^{d}	14.7 ± 0.6 ^{d}	11.7 ± 0.6 ^{d}	15.0 ± 0.0 ^{d}	23.0 ± 1.0 ^{d}
		Ethanol	0	0	11.3 ± 0.6^{cd}	17.7 ± 1.2^{cd}	15.0 ± 0.0^{cd}	10.3 ± 0.6^{cd}	14.7 ± 0.6^{cd}
Thunbergia laurifolia	Leaves	Aqueous	0	0	0	7.3 ± 0.6^{abc}	0	18.3 ± 1.5^{abc}	0
		Ethanol	0	0	10.0 ± 0^{abcd}	7.7 ± 0.6^{abcd}	8.0 ± 1.7^{abcd}	19.7 ± 1.5^{abcd}	0
Tinospora crispa	Stem	Aqueous	0	0	0	0	0	11.7 ± 0.6^{a}	0
		Ethanol	0	0	0	11.7 ± 1.5^{abc}	6.7 ± 0.6^{abc}	10.0 ± 1.0^{abc}	0
Vernonia cinerea	Whole plant	Aqueous	0	0	13.0 ± 0.0^{abc}	15.0 ± 0.0^{abc}	0	0	0
		Ethanol	0	0	0	0	0	0	9.5 ± 0.6^{a}
Zingiber cassumunar	Rhizome	Aqueous	0	0	0	0	0	8.3 ± 0.6 ^{a}	0
		Ethanol	0	0	0	0	0	11.3± 1.5 ^{a}	0

Data were given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between plant species and bacterial strains was done using post hoc Duncan's test. Values with different alphabets within each column were significantly different (*P<0.05*).

Table 2. MIC and MBC values of crude plant extracts against pathogenic bacteria using broth dilution method.

Plant species	Extract	MIC and MBC (mg/ml)													
		Bacterial strains[a]													
		E. coli		*P. aeruginosa*		*S. aureus*		MRSA		*S. epidermidis*		*S. pyogenes*		*P. acnes*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
A. paniculata	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	250	250	125	250	125	250	125	125	-	-
D. scandens	Aqueous	-	-	-	-	3.9	7.8	3.9	7.8	31.3	31.3	-	-	-	-
	Ethanol	-	-	-	-	62.5	125	62.5	125	125	250	62.5	125	125	125
M. charantia	Aqueous	-	-	-	-	-	-	-	-	125	250	-	-	-	-
	Ethanol	-	-	-	-	62.5	125	-	-	-	-	15.6	31.3	15.6	31.3
P. indica	Aqueous	-	-	-	-	125	250	125	250	-	-	-	-	-	-
	Ethanol	-	-	-	-	125	250	-	-	-	-	-	-	-	-
R. nasutus	Aqueous	-	-	-	-	62.5	125	62.5	125	-	-	-	-	-	-
	Ethanol	-	-	-	-	62.5	125	125	250	-	-	62.5	125	-	-
S. alata	Aqueous	-	-	-	-	62.5	62.5	31.3	31.3	62.5	125	15.6	31.3	1.9	3.9
	Ethanol	-	-	-	-	62.5	125	7.8	15.6	15.6	15.6	62.5	125	31.3	62.5
T. laurifolia	Aqueous	-	-	-	-	-	-	125	250	-	-	31.3	31.3	-	-
	Ethanol	-	-	-	-	125	250	125	250	125	250	7.8	15.6	-	-
T. crispa	Aqueous	-	-	-	-	-	-	-	-	-	-	31.3	62.5	-	-
	Ethanol	-	-	-	-	-	-	125	250	125	250	62.5	125	-	-
V. cinerea	Aqueous	-	-	-	-	125	125	62.5	125	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	31.3	125
Z. cassumunar	Aqueous	-	-	-	-	-	-	-	-	-	-	125	125	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	62.5	125	-	-

compound, is stable in room temperature and produces a violet solution in organic solvents with maximum absorbance at 517 nm. Antioxidant compound scavenges free radical by hydrogen donation and reduction of DPPH$^{\bullet}$ (violet color) to DPPH-H (yellowish color) (Jadhav et al., 1996; Yamaguchi et al., 1998). In this study, the scavenging effects of plant extract on DPPH radical were shown in Table 3. The results

Table 3. Antioxidant activities and total phenolic contents of medicinal plant extracts.

Plant species	DPPH (mg gallic/g extracts)		ABTS (mg trolox/g extracts)		Phenolic content (mg gallic/g extracts)	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
A. paniculata	0.33 ± 0.004^{a}	0.85 ± 0.014^{d}	25.00 ± 0.103^{a}	8.32 ± 0.067^{b}	7.99 ± 0.302^{e}	10.67 ± 0.240^{c}
D. scandens	10.87 ± 0.152^{j}	7.14 ± 0.100^{f}	46.34 ± 0.172^{de}	58.53 ± 0.488^{ef}	48.12 ± 1.201^{h}	46.56 ± 1.816^{h}
M. charantia	1.89 ± 0.138^{d}	2.04 ± 0.014^{d}	8.99 ± 0.051^{a}	4.10 ± 0.048^{a}	11.63 ± 0.36^{a}	22.02 ± 1.390^{d}
P. indica	1.77 ± 0.010^{d}	9.30 ± 0.255^{hi}	23.38 ± 0.814^{b}	63.91 ± 0.480^{f}	13.83 ± 0.360^{b}	27.5 ± 0.660e
R. nasutus	9.09 ± 0.026^{h}	16.53 ± 0.074^{l}	204.66 ± 3.567^{i}	41.83 ± 0.795^{cd}	22.38 ± 0.485^{d}	33.13 ± 1.445^{f}
S. alata	22.11 ± 0 .324^{m}	9.09 ± 0.011^{h}	214.99 ± 17.279^{i}	31.29 ± 0.519^{bc}	70.9 ± 1.048^{j}	50.8 ± 0.421^{i}
T. laurifolia	6.71 ± 0.121^{e}	9.64 ± 0.108^{i}	30.83 ± 0.149^{bc}	33.87 ± 0.176^{bcd}	22.18 ± 1.269^{d}	33.13 ± 1.113^{f}
T. crispa	1.31 ± 0.012^{c}	7.71 ± 0.011^{g}	157.54 ± 28.491^{h}	56.81 ± 1.098^{ef}	11.19 ± 1.088^{a}	43.18 ± 0.421^{g}
V. cinerea	7.48 ± 0.104^{fg}	16.48 ± 0.138^{l}	32.99 ± 0.349^{bcd}	34.50 ± 0.308^{bcd}	21.94 ± 1.066^{d}	29.06 ± 0.591^{e}
Z. cassumunar	0.76 ± 0.025^{b}	11.24 ± 0.354^{k}	151.61 ± 5.047^{h}	90.05 ± 0.814^{g}	11.35 ± 0.138^{a}	43.17 ± 0.668^{g}

IC_{50} values of gallic acid and trolox were 0.005 and 0.249 mg/ml, respectively. Data in table were given as mean ± standard deviation (SD) of triplicate experiments. Statistical comparison between groups applied using Post hoc Duncan test. Values with different alphabets within each test were significantly different (*P<0.05*).

indicated that the greatest activity was found in aqueous extract of *S. alata* with 22.11 mg gallic/g extract, followed by the ethanolic extracts of R. nasutus (16.53 mg gallic /g extract) and *V. cinerea* (16.48 mg gallic /g extract), while the aqueous extract of *A. paniculata* had the lowest DPPH radical scavenging activity with concentration 0.33 mg gallic /g extract.

Moreover, the ABTS radical scavenging activity is another method widely used to evaluate antioxidant activity. The ABTS radical cation can be generated by strong oxidizing agents such as potassium permanganate ($KMnO_4$) or potassium persulfate ($K_2S_2O_8$) and these molecules can be detected at absorbance of 734 nm. $ABTS^{\bullet+}$ radical is more sensitive than DPPH radical, although the mechanisms of scavenging are alike. Antioxidant in plant extracts could reduce blue-green color ($ABTS^{\bullet+}$) to colorless neutral form by electron transferring (Miller and Rice-Evans, 1997). The result of ABTS radical scavenging (Table 3) showed that the aqueous extracts of *S. alata* had the significant highest activity (P<0.05) with 214.99 mg trolox/g extract, followed by the aqueous extract of *R. nasutus* (204.66 mg trolox/g extract), *T. crispa* (157.54 mg trolox/g extract) and *Z. cassumunar* (151.61 mg trolox/g extract). The ethanolic extracts of the plants, however, showed low ABTS radical scavenging activity. Therefore, the different compositions of extract were obtained from the different extraction solvent reflecting the different antioxidant activity (Pinelo et al., 2004). There was also significantly different antioxidant activity between aqueous and ethanolic extracts. For spearman correlation analysis, the result showed a low association between two antioxidant methods (R=0.224). The reason might be that the mechanisms to generate free radicals between DPPH and ABTS methods were different (Figure 1). The DPPH radical scavenging assay indicated the ability of the extract to transfer electrons or hydrogen atoms, while the ABTS radical scavenging activity indicated the hydrogen donating and the chain-breaking capacity of the extract to free radical (Perez-Jimenez and Saura-Calixto, 2005). Moreover, there were differences of solubility of each antioxidant component. In general, DPPH could evaluate hydrophilic compound, while ABTS can be used to investigate both hydrophilic and lipophilic compounds (Arnao et al., 2001).

Total phenolic contents

Phenolic compounds are major plant secondary metabolite, which have several biological functions including antioxidant and antibacterial activities. The total phenolic contents of the tested medicinal plant extracts were determined using the Folin–Ciocalteau colorimetric method by manipulation of the regression equation of gallic acid calibration curve ($y = 8.3373x - 0.0616$, $R^2 = 0.9945$). From Table 3, the aqueous extract of *S. alata* had the highest total phenolic contents (70.90 mg gallic/g extract). Moreover, the ethanolic extract of *S. alata*, *Z. cassumunar*, *T. crispa* and *R. nasutus* had also shown the high total phenolic contents by 50.80, 43.17, 43.18 and 33.13 mg gallic/g extract, respectively. In general, the antioxidant activity of medicinal plants is associated with total phenolic content (Chew et al., 2009; Liu et al., 2009). For spearman correlation analysis, it was found that there was a high correlation between the antioxidant capacities obtained from DPPH method and total phenolic content (R=0.720, P<0.001), whereas there was a low correlation between ABTS model and total phenolic compound (R=0.396) as shown in Figure 1. Furthermore, the mechanism of Folin- Ciocalteu method could be disturbed by other components from plant extracts

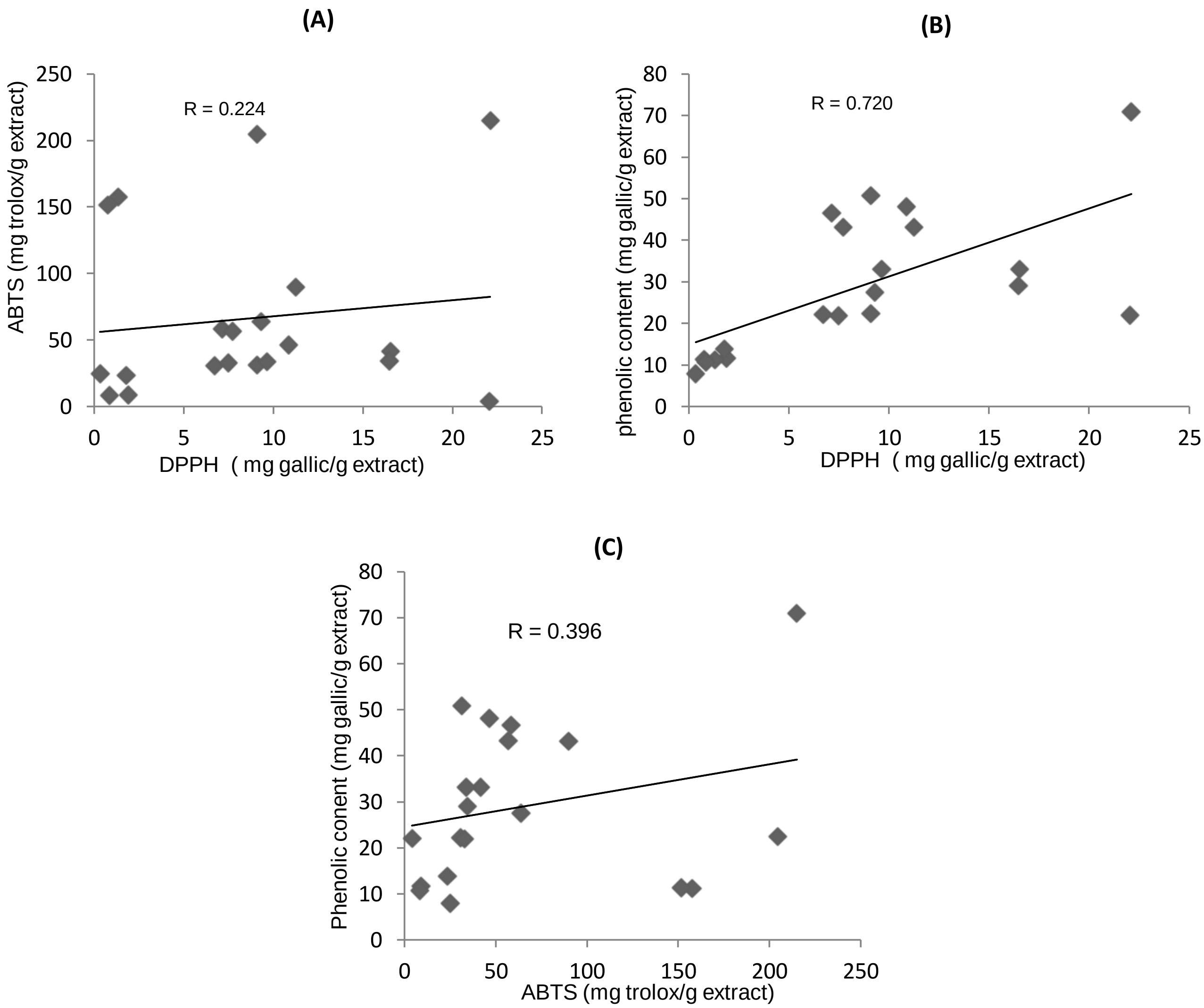

Figure 1. Spearman's correlation analysis between (A) DPPH and ABTS method (B) DPPH method and total phenolic content, and (C) ABTS method and total phenolic content.

including, proteins, peptides, polysaccharides, and pigments (Perez-Jimenez and Saura-Calixto, 2005; Prior et al., 2005).

Conclusion

The crude extracts of *S. alata* had strong and broad spectrum of antibacterial activities against Gram positive bacteria, including MRSA, *S. aureus*, *S. epidermidis*, *S. pyogenes* and *P. acnes*. Moreover, aqueous extract of *S. alata* showed strong antioxidant activity and high total phenolic content. Therefore, this extract could be used as accessible sources of natural antioxidants and health supplement products. In addition, the biological activities of the plants observed in this study will be useful for development of the potential plant extracts as new therapeutic agents for protecting and curing diseases.

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