

## Free Radical Scavenging Activity and Reducing Capacity of Five Southern Thai Indigenous Vegetable Extracts

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### Abstract

The phenolic compounds of five southern Thai indigenous vegetables including Mon-pu (*Glochidion wallichianum* Muell Arg), Cha-plu (*Piper sarmentosum* Roxb.), white popinac (*Leucaena leucocephala* de Wit.), djenkol tree (*Archidendrom jiringa* I. C. Nielsen.) and stink bean (*Parkia speciosa* Hassk.) were extracted using different solvents (50 % acetone, 80 % methanol and distilled water) at a ratio of sample to extracting medium of 1:25 (w/v). The extracts were analyzed for total phenolic content using the Folin-Ciocalteu procedure, free radical scavenging capacity by using 2',2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods, and reducing capacity by using a ferric reducing antioxidant power (FRAP) assay. The acetone extracts of all plants possessed the highest total phenolic content followed by the methanolic and distilled water extracts, respectively. The highest total phenolic content was found in the acetone extracts of Mon-pu and white popinac ( $p < 0.05$ ). The acetone extracts of all plants showed higher free radical scavenging capacity and reducing capacity than those of their methanolic and water extracts counterparts, respectively ( $p < 0.05$ ). Among all plants tested, the extracts of Mon-pu extracted with all extracting media exhibited the highest free radical scavenging and reducing abilities ( $p < 0.05$ ). The present study suggested that the acetone extract of Mon-pu was a potential source of natural antioxidants.

**Keywords:** Free radical scavenging activity, reducing capacity, local vegetables, extracts, phenolic compounds

### Introduction

Lipid oxidation in fat based food products not only brings about food deterioration but also produces free radicals or active oxygen species such as peroxy, hydroxyl and alkoxy free radicals, which are purportedly associated with aging, carcinogenesis, mutagenesis, atherosclerosis, diabetes and arthritis [1-3]. The harmful effects of free radicals in living systems could be attenuated by endogenous and dietary antioxidants. Antioxidants may directly scavenge the free radicals, form chelating complexes with transition metals, act as reducing agents, induce the production of antioxidant enzymes, and/or suppress the generation of oxidative enzymes, such as cyclooxygenase, in biological systems [4]. Recently, natural antioxidants are in high demand because of their potential in health promotion and disease prevention, and their improved safety and

consumer acceptability. Plants are rich sources of natural antioxidants, such as phenolic substances. Several studies have showed that increased dietary intake of natural phenolic antioxidants correlates with decreased coronary heart disease [5-6].

Phenolic compounds are secondary metabolites of the plant kingdom. There are a diverse group of compounds that are composed of an aromatic benzene ring substituted with hydroxyl groups, including their functional derivatives. Many polyphenolic compounds are known to be free radical scavengers or antioxidants that have beneficial health effects [7-8]. Many researches indicated that antioxidant activities of plant phenolics were stronger than that of synthetic antioxidants such as BHA and BHT [9-10]. It is generally accepted, however, that natural antioxidants are more potent, efficient and safer than synthetic antioxidants [11]. For

example,  $\alpha$ -tocopherol is the most active form of vitamin E and natural 2*R*,4'*R*,8'*R*- $\alpha$ -tocopherol is more potent than synthetic racemic  $\alpha$ -tocopherol primarily because the  $\alpha$ -tocopherol transfer protein selectively recognises natural  $\alpha$ -tocopherol. As such, natural antioxidants are more favorably accepted than synthetic antioxidants [11]. The antioxidant activities are related to the structure of phenolic compounds [12-13]. Generally, antioxidant activity depends on the number and positions of hydroxyl groups and other substitutes, and glycosylation of the phenolic molecule [12-13]. The presence of certain hydroxyl groups on the phenolic nucleus enhances antioxidant activity. Substitution patterns in the phenol ring with hydroxyl groups at the ortho and para position and an ethyl/butyl group at the para position also affect antioxidant activity of phenolic compounds [12-13]. In addition, substitution of the phenol ring with bulky groups, such as 2,6-di-tertiary-butyl-4-methoxyphenol, at the ortho position can favor antioxidant activities [12-13]. Thus, antioxidant activities of plant containing phenolic substances involve chemical structure and are correlated with phenolic components in plants.

An enormous variety of plants have been studied as new sources of phenolic substances but there are only a few reports about phenolic content and antioxidant activities from edible indigenous vegetables grown in southern Thailand. Local edible plants especially some vegetables served with Thai fermented rice noodles could be used as a potential source of new natural antioxidants. Therefore, the aims of this study were to evaluate

the total phenolic content, free radical scavenging activity and reducing capacity of indigenous southern Thai plant extracts.

## Materials and methods

### Chemicals

2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), Ferric chloride, potassium persulfate, Folin-Ciocalteu reagent and sodium acetate were purchased from Sigma/Aldrich (St. Louise, Mo., U.S.A.). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Fluka (Buchs, Switzerland).

### Sample Preparation

Five fresh indigenous vegetables (Table 1) were obtained from Thasala market, Nakhon Si Thammarat, Thailand approximately 12 hours after being harvested. All plants (5-kg each), were dried by using a hot air oven at 60 °C for 72 h until the moisture content reached 3 - 7 % to prevent or minimize the degradation and/or oxidation of active compounds present in the samples. Dried samples were ground to a fine powder using a grinder (Panasonic MK-G20MR, Japan) and passed through a 25 mesh sieve. The ground samples were vacuum-packed and stored at -40 °C until required for phenolic extraction.

**Table 1** Indigenous plants for the experiment.

Scientific name	Common name	Plant part
<i>Piper sarmentosum</i> Roxb.	Cha-plu	Leaf
<i>Glochidion wallichianum</i> Muell Arg.	Mon-pu	Leaf
<i>Leucaena leucocephala</i> de Wit.	White popinac	Leaf
<i>Archidendron jiringa</i> I. C. Nielsen.	Djenkol tree	Fruit
<i>Parkia speciosa</i> Hassk.	Stink bean	Fruit

### Different Solvent Extraction

Samples of dried powder (2 g each) were extracted with 50 ml of distilled water, 80 % methanol, or 50 % acetone in a shaking water bath at 50 °C for 15 h. Different concentrations of extracting media were used to represent differences in polarity. Each extract was filtered through Whatman no. 4 filter paper (Whatman International Limited, Kent, UK). The filtrate was subjected to analysis for total phenolic content, radical scavenging activities and reducing capacity.

### Total Phenolic Content (TPC)

TPC was estimated using the Folin-Ciocalteu colorimetric method according to the method of Yu *et al* [14]. Samples (100 µl) were mixed thoroughly with 2 ml of 2 % (w/v) Na<sub>2</sub>CO<sub>3</sub>. After 2 min, 100 µl of Folin-Ciocalteu reagent (prepared by mixing Folin-Ciocalteu and methanol in a ratio of 1:1, v/v) was added to the mixture. The resulting mixture was allowed to stand at ambient temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as milligrams of gallic equivalents (GAE) per gram of dry weight (mg/g DW) of vegetable material.

### DPPH<sup>•</sup> - Scavenging Activity

The DPPH free radical scavenging activity was measured according to Agbor *et al* [15]. The DPPH radical (DPPH<sup>•</sup>) solution (60 µM) was prepared in 80 % ethanol. DPPH<sup>•</sup> solution (3.9 ml; 0.68 ± 0.005 at 515 nm) was added to 100 µl of the extract. The reaction was carried out at room temperature in the dark for 2 h. After incubation, the absorbance was recorded at 515 nm. Trolox (0 - 1 mM) was used as a standard. The result was expressed as mmol equivalents per gram dry weight (mmol/g DW) of vegetable material.

### ABTS<sup>•+</sup> - Scavenging Activity

The free radical scavenging activity was also determined by the ABTS<sup>•+</sup> method [16-17]. ABTS<sup>•+</sup> was prepared by oxidizing 7 mM ABTS in 80 % ethanol with 2.45 mM potassium persulfate and incubated at ambient temperature in the dark for 16 h. The ABTS<sup>•+</sup> solution was then diluted with 80% ethanol to obtain an absorbance of 0.700±0.005 at 734 nm. The ABTS<sup>•+</sup> solution (3.9 ml; absorbance of 0.700 ± 0.005) was added

to 100 µl of the sample and mixed thoroughly. The reaction mixture was allowed to stand at ambient temperature in the dark for 6 min and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using Trolox standard in the concentration ranging from 0 - 1 mM. The result was determined in terms of Trolox equivalent antioxidant capacity (TEAC) and expressed as mmol equivalents per gram dry weight (mmol/g DW) of vegetable material.

### Reducing Capacity

Ferric reducing antioxidant power (FRAP) assay was used to determine the reducing capacity of extracts. FRAP assay was performed according to Agbor *et al* [15] with some modifications. The FRAP reagent was prepared by adding 1 vol of 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate and 16 ml glacial acetic acid), 1 vol of 10 mM TPTZ prepared in 1 M HCl and 1 vol of 20 mM FeCl<sub>3</sub>. This reagent (3 ml) was mixed with 100 µl of the sample similar to those used for the ABTS and DPPH assays. The mixture was shaken and incubated at ambient temperature for 8 min and the absorbance was read at 593 nm. A standard curve was made with Trolox (0 - 1 mM) and the result was expressed as mmol Trolox equivalents (TE) per gram dry weight (mmol/g DW) of material.

### Statistical Analysis

Data were reported as mean ± SD for triplicate determinations. Analysis of variance (ANOVA) and least significant difference tests (SPSS for window, Version Rel. 10.0.5., 1999, SPSS Inc., Chicago, Ill., U.S.A.) were conducted to identify differences among means. Duncan's multiple range test (DMRT) was used to determine the correlation between means. Statistical significance was declared at  $p < 0.05$ .

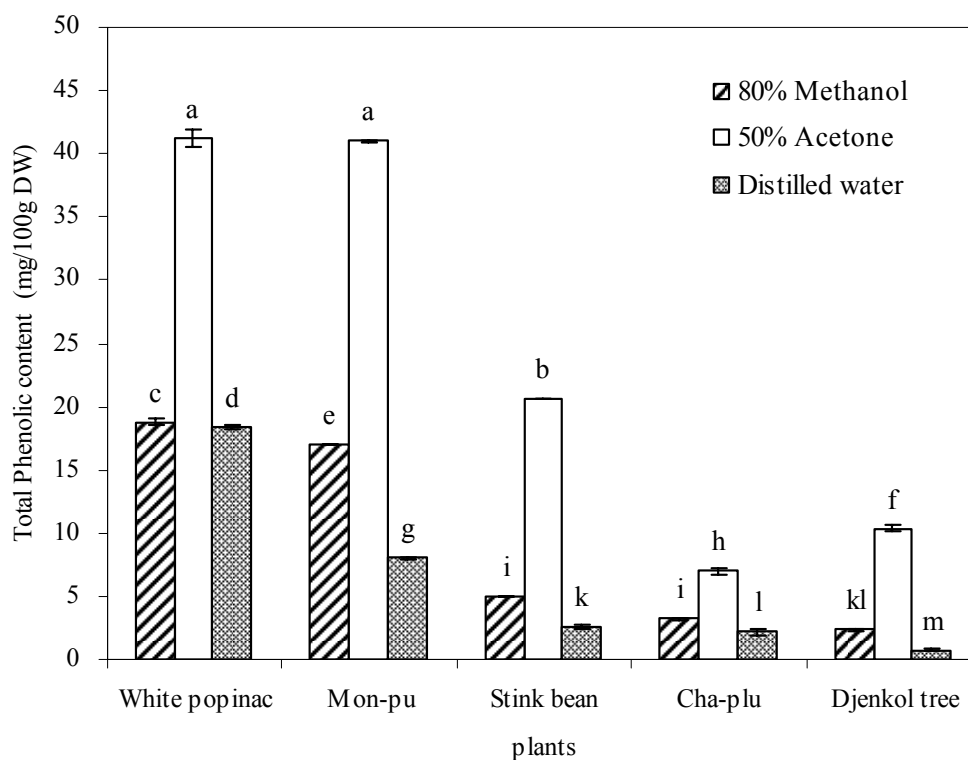
## Results and discussion

### Total Phenolic Content of Different Solvent Extracts

Phenolic compounds, the principal antioxidant constituents of natural plant products, are composed of phenolic acid and flavonoids [18]. These compounds are potent radical terminators by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step [18]. The high potential of polyphenols to scavenge free

radicals may be because of their many phenolic hydroxyl groups [19]. In the present study, the total phenolic content of the extract was affected by the extracting solvent used. From the results, it was found that the 50 % acetone extracts of all plants exhibited the highest total phenolic content when compared to those of 80 % methanol and distilled water extracts (**Figure 1**). In all cases, the lowest amount of phenolic compounds was found in the distilled water extract, ranging from 0.75 (djenkol tree) to 18.35 (white popinac) mg/100g DW. Therefore, 50 % acetone was found to be the most efficient solvent to extract antioxidants containing phenolic constituents from all five plants. This result was in agreement with Su *et al* [4] who reported that acetone was an effective solvent for extraction of phenolics in black peppercorn, nutmeg, rosehip, cinnamon and

oregano leaf. It was postulated that the polarity of 50 % acetone was almost similar to that of plant phenolic components. Therefore, the phenolic compounds were effectively extracted. Among all plant samples, Mon-pu and white popinac extracts in 80 % methanol had the highest phenolic content whereas the lowest value was found in the distilled water extract of the djenkol tree. When comparing the effectiveness of 80 % methanol with distilled water on the extraction of phenolic compounds from Mon-pu and white popinac, it was noted that 80 % methanol was a more appropriate extracting medium rather than distilled water. The results suggest that the polarity of phenolic constituents found in these plants might be the same as that of 80 % methanol.

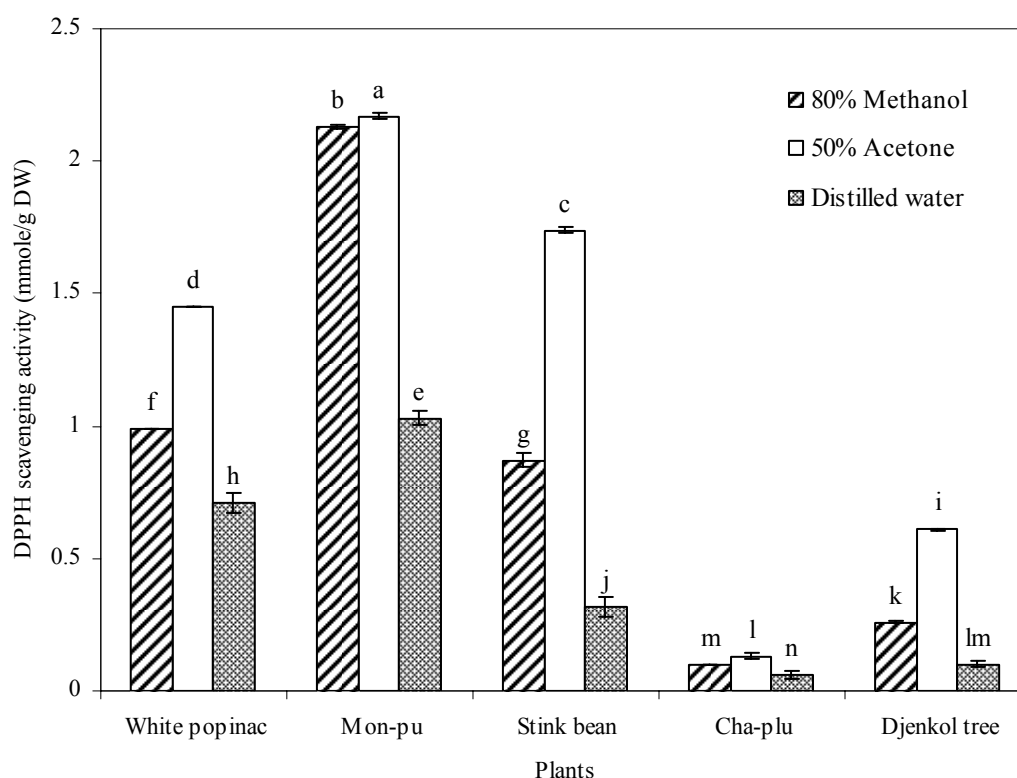


**Figure 1** Total phenolic content of five Thai indigenous plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ( $p < 0.05$ ).

### DPPH• Scavenging Activity of Different Solvent Extracts

DPPH• is a free radical compound and has been widely used to test the free radical scavenging activity of various samples. When a hydrogen atom or electron is transferred to the odd electron in DPPH•, the absorbance at 515 nm decreased proportionally to the increases of non radical forms of DPPH. Conventionally, high free radical scavenging ability is regarded as high antioxidant activity. Therefore, measurement of DPPH• scavenging activity has been used as one of the basic screening steps for searching new antioxidant compounds from natural resources [20]. The present investigation demonstrates that 50 % acetone extracts of all plants show the highest DPPH• scavenging activity whereas distilled water extracts exhibited the lowest ( $p < 0.05$ ) (Figure 2). The result suggests that 50 % acetone is an appropriate solvent for extracting DPPH radical scavenging agents from these plants. This is similar to the results of Su *et al* [4] who found that botanical extracts in 50 % acetone showed higher DPPH• scavenging activity than those in 80 % methanol. In the case of different plant samples, the extract of Mon-pu in 50 % acetone had the greatest DPPH• scavenging activity (2.17 mmol/g DW) followed by that in 80 % methanol (2.13 mmol/g DW), the extract of stink bean in 50 % acetone (1.74 mmol/g DW), the

extract of white popinac in 50 % acetone (1.45 mmol/g DW), the extract of Mon-pu in distilled water (1.03 mmol/g DW), the extract of white popinac in 80 % methanol (0.99 mmol/g DW), the extract of stink bean in 80 % methanol (0.87 mmol/g DW) and the extract of white popinac in distilled water (0.71 mmol/g DW), respectively ( $p < 0.05$ ) (Figure 2). The result indicates that DPPH• scavenging activity is positively correlated with phenolic content of the extract (Figure 1). A good relationship between DPPH• scavenging activity and phenolic content was previously reported by Surveswaran *et al* [21] in 133 Indian medicinal plants and by Maisuthisakul *et al* [22] in some Thai indigenous plants. The different DPPH radical scavenging activity of the extracts was probably due to the combined action of the present substances in variable concentrations and their high hydrogen atom donating abilities. Chen and Ho [23] reported that the potency of a molecule for scavenging the radical was due to the number of hydrogen atoms available for donating by the hydroxyl group. In addition, the additive or synergistic effects of phenolic compounds made the antioxidants activity of the crude extracts higher than that of the isolated compounds. On the other hand, the presence of glycoside in the phenolic extracts could also decrease the antioxidant activity (DPPH• scavenging ability) by affecting the donation of the hydrogen atom [24].



**Figure 2** DPPH<sup>•</sup> scavenging activity of five Thai indigenous plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ( $p < 0.05$ ).

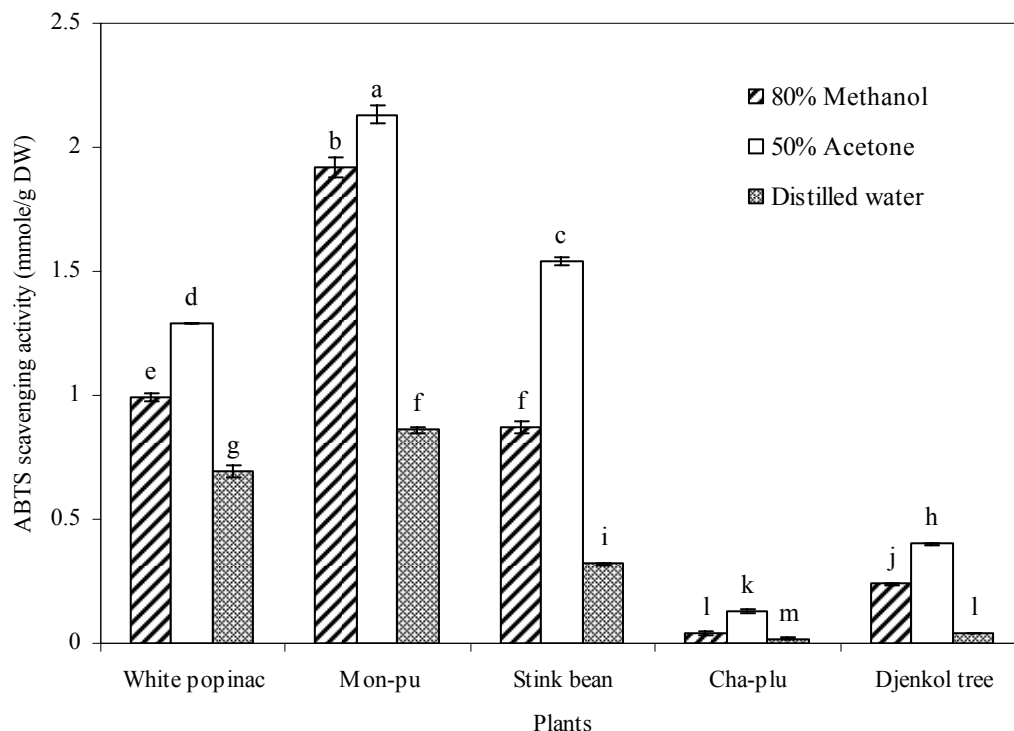
#### Scavenging Effect of Different Solvent Extracts from Various Plant Samples on ABTS<sup>•+</sup>

The reduction of the 2,2'-azinobis(3-ethylbenzothiazoline sulphonate) radical cation (ABTS<sup>•+</sup>) has been widely used to measure the antioxidant capacity of natural extracts [16-17]. The reductions of ABTS<sup>•+</sup> with free radical scavengers present in the tested sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm. The reaction time of the improved ABTS assay is only 6 min, much shorter than that of DPPH assay (120 min in the present study). Thus, ABTS assay can be conducted for systematically assessing total antioxidant capacity of the crude extract from plant materials on a large scale. However, it has been recommended that at least two methods should be used due to the differences between the test system investigated [25].

Different solvent extracts of five Thai indigenous plants in the south of Thailand were evaluated and compared for their ABTS<sup>•+</sup>-scavenging capacities, using spectrophotometry. The result obtained was similar to that of DPPH<sup>•</sup> scavenging activity. 50 % acetone extracts showed highest ABTS<sup>•+</sup>-scavenging capacities while distilled water extracts had the lowest ( $p < 0.05$ ) (**Figure 3**). It has been recognized that the extracting medium may significantly alter the antioxidant activity estimation. The result indicated that 50 % acetone was the best extraction solvent for all plant samples when compared to 80 % methanol and distilled water. This suggested that the polarity of major free radical scavenging active compounds present in all plants was close to that of 50 % acetone. This result was supported by Su *et al* [4]. From the result, the extract of Mon-pu in 50 % acetone possessed the highest ABTS<sup>•+</sup>-scavenging capacity whereas the Cha-plu extract in

distilled water showed the lowest ABTS<sup>•+</sup>-scavenging capacity ( $p < 0.05$ ) (**Figure 3**). This result was in accordance with the total phenolic content found in the extract (**Figure 1**). A good

relationship between radical scavenging activity and phenolic content was observed in Indian medicinal plants [21] and in some Thai indigenous plants [22].



**Figure 3** ABTS<sup>•+</sup> scavenging activity of five Thai indigenous plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ( $p < 0.05$ ).

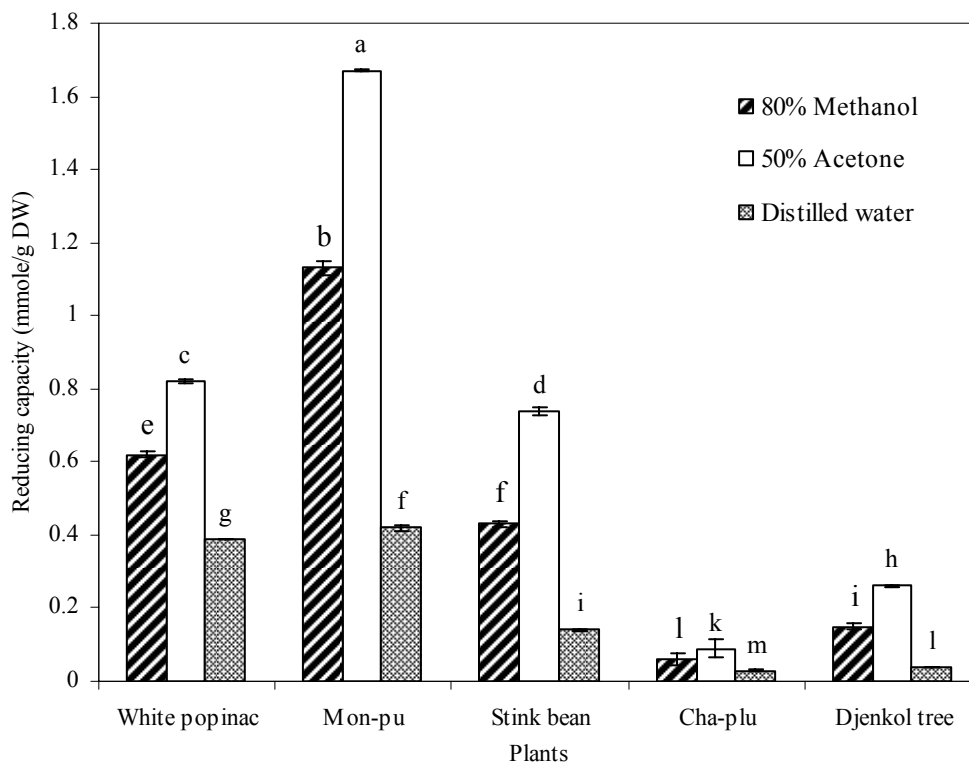
#### Reducing Power of Different Solvent Extracts from Five Indigenous Plants

Several reports indicated that the reducing power of bioactive compounds was associated with antioxidant activity [26-27]. Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power. The reducing power of 80 % methanol, 50 % acetone and distilled water extracts of different plant samples is shown in **Figure 4**. The reducing power of 50 % acetone extracts appeared to be higher in all plants than those of 80 % methanol and distilled water extracts, respectively ( $p < 0.05$ ). The result was well correlated with the amount of phenolic

content present in the respective extracts (**Figure 1**). Siddhuraju and Becker [28] reported that the reducing power of the water extract of the moringa leaf was found to be the lowest and it was correlated with the phenolic content in that extract. For different plant extracts, the extract of Mon-pu in 50 % acetone exhibited the highest reducing power (1.67 mmol/g DW) while the lowest value was found in the distilled water extract of Cha-plu (0.03 mmol/g DW) ( $p < 0.05$ ). Thus, phenolics present in the 50 % acetone extract of Mon-pu possibly acted as electron donors and could terminate the radical chain reactions by converting free radicals to more stable products. Reische *et al* [29] reported that primary antioxidants react with lipid and peroxy radicals and convert them to more

stable, non-radical products and less readily available to further promote auto-oxidation. The result showed that the different plant species resulted in the difference in reducing power. This

was probably due to the difference in substances with different concentrations and electron donating abilities found in the different plants.



**Figure 4** Reducing capacity of five Thai indigenous plant extracts. Bars represent the standard deviation from triplicate determinations. Different letters indicate significant differences ( $p < 0.05$ ).

### Conclusions

Fifty percent acetone was an appropriate solvent for phenolic constituents extraction in all five indigenous Thai vegetables. The 50 % acetone extract of Mon-pu exhibited the strongest DPPH<sup>•</sup> and ABTS<sup>•+</sup>-scavenging activities with the highest reducing capacity. Generally, the antioxidant activity of the extracts positively correlated with the content of total phenolics.

### Acknowledgements

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