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Antioxidant Capacities of *Pueraria mirifica, Stevia rebaudiana* Bertoni, *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees. and *Cassia alata* Linn. for the Development of Dietary Supplement

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ABSTRACT

This work reported on the antioxidant capacities of five different medicinal plants generally found throughout the country. The medicinal plants including *Pueraria mirifica, Stevia rebaudiana* Bertoni, *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees. and *Cassia alata* Linn. were subjected to extraction using a variety of solvents including ethanol, methanol, acetone, acetic acid, and distilled water. The method was based on inhibition in absorption of ABTS [2,2/-azino-bis (3-ethylbenthiazoline-6-sulfonic acid)] technique and the antioxidant capacity was recorded as TEAC (Trolox equivalent antioxidant capacity). The result showed that ethanol was the best solvent for extraction of all five medicinal plants to give the highest antioxidant capacities, followed by acetone, methanol, distilled water, and acetic acid, respectively. The highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni., followed by *Cassia alata* Linn., *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees., and *Pueraria mirifica*, respectively. Moreover, the interaction effect between solvents and medicinal plants on antioxidant capacity showed that the highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni extracted with acetone (2.96) and methanol (2.85), followed by *Stevia rebaudiana* Bertoni extracted with ethanol (2.64), and *Cassia alata* Linn. extracted with ethanol (2.57), while, the lowest antioxidant capacity was found in *Andrographis paniculata* (Burm.f.) Nees. extracted with acetone (0.04).

Key words: antioxidant capacity, *Pueraria mirifica, Stevia rebaudiana* Bertoni, *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees. *Cassia alata* Linn.

INTRODUCTION

Free radicals are also found in the environment, including exposure to ionizing radiation (from industry, sun exposure, cosmic rays, and medical X-rays), ozone and nitrous oxide (primarily from automobile exhaust), heavy metals (such as mercury, cadmium, and lead), cigarette smoke (both active and passive), alcohol, unsaturated fat, and other chemicals and compounds from food, water, and air. The body produces several antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, that neutralize many types

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of free radicals. Supplements of these enzymes are available for oral administration. However, their absorption is probably minimal at best. Supplementing with the "building blocks", the body requires to make SOD, catalase, and glutathione peroxidase which may be more effective. These building block nutrients include the minerals manganese, zinc, and copper for SOD and selenium for glutathione peroxidase. In addition to enzymes, many vitamins and minerals act as antioxidants in their own right, such as vitamin C, vitamin E, beta-carotene, lutein, lycopene, vitamin B2, coenzyme Q10, and cysteine (an amino acid). Herbs, such as bilberry, turmeric (curcumin), grape seed or pine bark extracts, and ginkgo can also provide powerful antioxidant protection for the body. Consuming a wide variety of antioxidant enzymes, vitamins, minerals, and herbs may be the best way to provide the body with the most complete protection against free radical damage (Halliwell *et al*., 1992).

Pueraria mirifica known in Thai as "Kwao krua" or "Kwao krua kao" or "White kwao krua" is a plant found in Thailand, Myanmar, and Southeast Asia and it belongs to the Leguminosae family. *Pueraria mirifica* has substantial health benefits. Its extracts are made by concentrating active ingredients from the rhizomes. The estrogenic substance in *Pueraria mirifica* is then isolated and tested on animals. Currently, there have been hundreds of researches on application to humans and animals. In humans, *Pueraria mirifica* is used as tonics, supplementary foods, and ingredients for cosmetics (Wang and Kurzer, 1997).

Stevia rebaudiana Bertoni is a small herb. The plant has been distributed to Southeast Asia including Thailand (as "Ya-wan"). More than 750 tons of stevia leaves per year are used as crude extract for consumption. The sweetening compound was isolated from stevia leaves by Rebaudi and Resenae (Suttajit *et al.*, 1993) and was named as "stevioside". Stevioside has high sweetening potency, 250-300 times that of sucrose, but little caloric value. Stevia and stevioside have been applied as a sugar substitute and used by those with obesity, diabetes mellitus, and heart disease. Stevioside can also inhibit the growth of certain bacteria (Suttajit *et al.*, 1993).

Curcuma longa Linn. known in Thai as "Kha-min" or turmeric, which belongs to the family of Zingiberaceae, is well known for all kinds of curry diets and has been widely used for food additive and condiments in most regions of southern Asia. Turmeric is species contain traditionally used mainly as spices and pigment but it is also an important medicinal plant whose fresh rhizome and dried powder are popular remedies for beautification. The rhizome juice is used as anthelmintic as well as in asthma, gonorrhea and urinary disease. The essential oil of the plant is used as antacid, carminative, stomachic and tonic (Rahman *et al.*, 2004).

Andrographis paniculata (Burm.f.) Nees. known in Thai as "Pha-ta-lai-jone" is an important medicinal plant in India, China, Thailand, and Scandinavia. The aerial parts of the plant (leaves and stems) are normally used for extraction of the active phytochemicals. Extracts of the plant and their constituents have been reported to exhibit a wide spectrum of biological activities of therapeutic importance including antibacterial, antiviral ,anti-inflammatory, antimalarial, immunostimulant, hepatoprotective, antithrombotic, anticancer, hypoglycemic, and hypotensive properties (Bhan *et al*., 2006).

Cassia alata Linn. known in Thai as "Chum-het-thet" is recommended for primary health care in Thailand to treat ringworm, constipation, and skin disease and it belongs to the legume family. Many reports have shown that some species of *Cassia* contain antimicrobial substances, particularly *Cassia alata* Linn. (Panichayukaranant and Intaraksa, 2003 ; Phongpaichit *et al*., 2004).

The objective of this study was to

evaluate the total antioxidant capacity of the medicinal plants including *Pueraria mirifica, Stevia rebaudiana* Bertoni*, Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) *Nees*., and *Cassia alata* Linn. which were subjected to extraction using a variety of solvents including ethanol, methanol, acetone, acetic acid, and distilled water. For this purpose, it was to find out the highest antioxidant capacities in the solvent and the medicinal plant.

MATERIALS AND METHODS

Plant materials and extraction

Pueraria mirifica, *Curcuma longa* Linn. and *Stevia rebaudiana* Bertoni were collected from Payao Province*, Andrographis paniculata* (Burm.f.) Nees. was collected from Chiang Mai Province and *Cassia alata* Linn. was collected from Phitsanulok Province, Thailand, during May to November, 2005. These medicinal plants were cleaned and cut into small pieces before being dried at room temperature and extracted with five solvents : 99% ethanol, 99% methanol, 99% acetone, 99% acetic acid, and distilled water. Each extraction step was completed in 24 hours. The extracts were filtered and concentrated in a rotavapor apparatus at approximately 40 °C. Finally, concentrated extracts were dried using freeze-vacuum dryer (FTS System, Flexi-Dry^{TM}) and stored at -40 °C for measurement of total antioxidant activity.

Chemical reagents

The chemical reagent ABTS or [2,2/-azino-bis (3-ethylbenthiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and Myoglobin were purchased from Fluka. All other chemicals used were of analytical grade. Metmyoglobin was purified after adding the stock myoglobin solution (100 µM) in 5 mM isotonic phosphate buffer saline (PBS buffer), pH 7.4 to an equal volume of freshly prepared 740 µM potassium ferricyanide. The solution was dialysed against PBS buffer, pH 7.4 twice at 4°C for 24 hours, and the metmyoglobin was collected and calculated for the concentration as described by Miller and Rice-Evans (1996). All compounds were dissolved in PBS buffer, except Trolox or samples dissolved in 95% ethanol.

Determination of total antioxidant capacity

The method was based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\bullet+}$, a blue-green chromophore with characteristic absorption at 734 nm, compared with that of Trolox, water-soluble vitamin E analog. The addition of antioxidants to the preformed radical cation reduced it to ABTS, determining a decolorization. Indeed, this was the basic feature of the methods developed for measuring the antioxidant capacity. The standard antioxidative curve of Trolox (concentration 0-2.5 mM) was obtained at the concentration of 108 µM H_2O_2 and 100 µM metmyoglobin at 20 minutes. The percentage inhibition of Trolox was calculated of the blank at an absorbance 734 nm by UV-vis spectrophotometer (Shimadzu, UV-1201V) and then was plotted as a function Trolox concentration. The total antioxidant capacity of several plant extracts were examined and compared with a standard antioxidant, Trolox. The Trolox equivalent antioxidant capacity was defined as the antioxidant capacity of 1 mg crude extracted to µmol of Trolox and represented as TEAC value. All determinations were carried out in triplicate. (Rice-Evans *et al*., 1994 ; Miller and Rice-Evans,1996 ; Poungbangpho *et al*., 2000a,b).

Statistical analysis

Three replicates values of antioxidant capacity of each sample were used for statistical analysis. Correlation analysis of antioxidant activity and solvents were carried out by using the statistical package for social science (SPSS)

program. Data were subjected to analysis of variance and means were compared by Duncan Multiple Range Test (DMRT), which differences at $p \leq 0.05$ considered to be significant.

RESULTS

The antioxidant capacity of Trolox at concentrations of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mM at 20 minutes were calculated as the percent inhibition of the absorbance and the standard curve was plotted (Figure 1). The result showed the directness in proportion of Trolox concentration to absorbance inhibition. The standard curve was therefore reproducible to use.

The antioxidant capacities of five different medicinal plants ; *Pueraria mirifica, Stevia rebaudiana* Bertoni*, Curcuma longa* Linn.*, Andrographis paniculata* (Burm.f.) Nees. and *Cassia alata* Linn. were studied. The method was based on inhibition in absorption of ABTS technique and the antioxidant capacity was recorded as TEAC (Trolox equivalent antioxidant capacity). The results showed that all five solvents had no effect on potentiating or attenuating the antioxidant capacity of the five medicinal plants. Ethanol was found to have the best solvent for extraction all of the medicinal plants which gave the highest antioxidant capacities, followed by acetone, methanol, distilled water and acetic acid, respectively (Table 1).

The antioxidant capacities of *Pueraria mirifica, Stevia rebaudiana* Bertoni*, Curcuma longa* Linn.*, Andrographis paniculata* (Burm.f.) Nees. and *Cassia alata* Linn. are shown in Table 2. The highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni, followed by *Cassia alata* Linn., and *Curcuma longa* Linn. *Andrographis paniculata* (Burm.f.) Nees. and *Pueraria mirific* had the lowest antioxidant capacity.

Moreover, the study of the interaction effect of solvents and medicinal plants on antioxidant capacity showed that the highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni extracted with acetone and methanol, followed by *Stevia rebaudiana* Bertoni extracted with ethanol, and *Cassia alata* Linn. extracted with ethanol, respectively. The lowest antioxidant capacity was found in *Andrographis paniculata* (Burm.f.) Nees. extracted with acetone (Table 3).

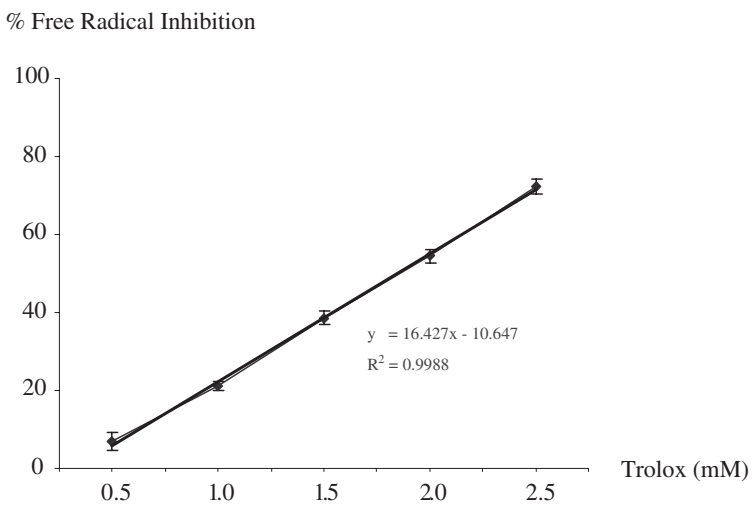

Figure 1 The standard curve of Trolox in reaction mixture of 500 µM ABTS, 100 µM metmyoglobin, 5 mM PBS buffer, and 108 µM H_2O_2.

Table 1 Trolox equivalent antioxidant capacity (TEAC) of extracts from five solvents.

Solvents	TEAC ± SD (μmol of Trolox /mg of crude extraction)
Ethanol	1.35±1.16
Methanol	1.23±1.23
Acetone	1.25±1.18
Acetic acid	0.76±0.70
Distilled water	1.08±0.57

Superscript letters a b and c in the row indicate significant differences at $p<0.05$.

Table 2 Trolox equivalent antioxidant capacity (TEAC) of extracts from medicinal plants.

Solvents	TEAC ± SD (μmol of Trolox /mg of crude extraction)
Pueraria mirific	0.26 ± 0.09^{c}
Stevia rebaudiana Bertoni	2.41 ± 0.58^{a}
Curcuma longa Linn.	1.17 ± 0.61^{b}
Andrographis paniculata (Burm.f.) Nees.	0.27 ± 0.29^{c}
Cassia alata Linn.	1.28 ± 0.84^{b}

Superscript letters a b and c in the row indicate significant differences at $p<0.05$.

Table 3 Trolox equivalent antioxidant capacity (TEAC) of the interaction effect of solvents and medicinal plants.

Solvents	TEAC ± SD (μmol of Trolox /mg of crude extraction)				
	P. mirifica	*S. rebaudiana* Bertoni	*C. longa* L.	*A. paniculata (Burm.f.)* Nees.	*C. alata* L.
Ethanol	0.17 ± 0.4^{fg}	2.64 ± 1.10^{ab}	0.62 ± 0.06^{efg}	0.77 ± 0.13^{efg}	2.57 ± 1.08^{ab}
Methanol	0.17 ± 0.3^{fg}	2.85 ± 0.92^{a}	2.17 ± 0.40^{abc}	0.14 ± 0.14^{fg}	0.80 ± 0.16^{defg}
Acetone	0.26 ± 0.06^{fg}	2.96 ± 0.90^{a}	1.30 ± 0.74^{cdef}	0.04 ± 0.07^{g}	1.69 ± 0.53^{bcde}
Acetic acid	0.33 ± 0.14^{fg}	1.93 ± 1.14^{abcd}	0.82 ± 0.9^{defg}	0.20 ± 0.02^{fg}	0.51 ± 0.10^{fg}
Distilled water	0.38 ± 0.22^{fg}	1.67 ± 1.61^{bcde}	0.93 ± 0.08^{defg}	0.21 ± 0.03^{fg}	0.84 ± 0.18^{defg}

Superscript letters a b c d e f and g in the same column and row indicate significant differences at $p<0.05$.

DISCUSSION

The results from this study indicated that all five solvents had no effect on potentiating or attenuating the antioxidant capacity of all five medicinal plants. Ethanol was the best solvent for extraction of all five medicinal plants which gave the highest antioxidant capacities, followed by acetone, methanol, distilled water, and acetic acid, respectively. Similar observation was made by Phongpaichit *et al.* (2004) who found antifungal activity from leaf extract of *Cassia alata* L., *Cassia fistula* L., and *Cassia tora* L. Panichayupakaranant and Kaewsuwan (2004) reported that using DPPH radical scavenging assay to investigate the antioxidant activity of crude methanol extracts from the leaves, flowers and pods of *Cassia alata* L. found the leaf extract to exhibit stronger antioxidant activity than flowers and pods. The highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni, followed by *Cassia alata* Linn., and *Curcuma longa* Linn.

Andrographis paniculata (Burm.f.) Nees. and *Pueraria mirific* had the lowest antioxidant capacity. Moreover, the interaction effect of solvents and medicinal plants on antioxidant capacity showed that the highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni extracted with acetone and methanol, followed by *Stevia rebaudiana* Bertoni extracted with ethanol and *Cassia alata* Linn. extracted with ethanol, while, the lowest antioxidant capacity was found in *Andrographis paniculata* (Burm.f.) Nees. extracted with acetone. This finding agreed with the previous report of Akowuah *et al.* (2006) on the free radical scavenging activity of methanol extracts than water extracts of *Andrographis paniculata*. Xi *et al.* (1998) reported that the antioxidant activity was more effectively inhibited by the hot water extract from *Stevia rebaudiana* Bertoni than DL-α-Toc or green tea extract. Then, *Stevia rebaudiana* Bertoni should be developed for dietary supplement and our study suggested that further compounds that exhibit antioxidant activities should be characterized in the future work.

CONCLUSION

The medicinal plants were subjected to extraction with five solvents including ethanol, methanol, acetone, acetic acid, and distilled water. The highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni, followed by *Cassia alata* Linn., *Curcuma longa* Linn., *Andrographis paniculata* (Burm.f.) Nees., and *Pueraria mirifica*, respectively. Ethanol was the best solvent for extraction all of the medicinal plants which gave the highest antioxidant capacities, followed by acetone, methanol, distilled water, and acetic acid, respectively. The highest antioxidant capacity was found in *Stevia rebaudiana* Bertoni extracted with acetone and methanol, followed by *Stevia rebaudiana* Bertoni extracted with ethanol, and *Cassia alata* Linn. extracted with ethanol, and the lowest antioxidant capacity was found in *Andrographis paniculata* (Burm.f.) Nees. extracted with acetone, respectively.

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