OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM LEAVES AND STEMS OF THAI WATER SPINACH (*Ipomoea aquatic* var. *aquatica*)

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Abstract

In order to investigate the optimal microwave-assisted extraction condition of bioactive compounds from fresh leaf and stem of Thai water spinach (TW). Bioactive compounds were extracted with ethanol and water by microwave at 450, 600 and 850 watt for 60 seconds on ratio of 1 to 5 (sample: solvent). Phytochemical screening, total flavonoid content (TFC) and DPPH-radical scavenging activity of the extracts were determined. The highest yield was found in ethanolic extract of leaf at 600 watt (1.58%). From phytochemical screening test, all samples showed positive for alkaloids, carbohydrates, terpenes, phenols, tannins and flavonoids. The ethanolic extracts showed higher TFC than those of water extracts in all experiment conditions. The ethanolic stem extracts showed higher DPPH radical scavenging activity than those of aqueous extracts. While the aqueous leaf extracts were higher scavenging activity than those of ethanolic leaf extracts. The significantly highest flavonoid contents were found in ethanolic leaf extracted at 850 watt of power (349.69 mg QE/g dw), whereas the highest antioxidant activity was occurred in ethanolic stem extract at highest power (54.49 mg TEAC/g dw) ($p<0.05$, ANOVA). In conclusion, the optimal condition for extracting active compounds from TW by using microwave energy was the ethanol extracted at 850 watt for 60 seconds. This finding suggested that this plant can be used as the alternative source of active ingredient in food and cosmetics industry.

Keywords: DPPH, flavonoids, *Ipomoea aquatica*, red-stem Thai water spinach

Introduction

In Thailand, there are two kinds of *Ipomoea aquatica* that are wild varieties red-stem Thai water spinach (TW; *I. aquatic* var. *aquatica)* and green-stem Chinese water spinach (CW; *I. aquatica* var. *reptan*). The green CW has widely been studied that is one of the richest sources of bioactive compounds (e.g. carotenoids, polyphenols, flavonols) and their bioactivities (e.g. antioxidant and antiproliferative properties) (Huang et al. 2005; Prasad et al. 2005). However, the red-stem TW has little been investigated. Red TW is a traditional medicinal plant that is cultivated throughout Thailand as common food and low value. In Thai traditional wisdom, the stem and leaf of TW are used for anti-hair loss therapy. Although, the anti-hair loss property of TW has not been scientifically investigated, this plant is one of the interested sources for anti-hair loss actives. Recently, the microwave-assisted extraction (MAE) is interestedly used for extraction bioactive compounds from plant material. MAE had many advantages (shorter time, less solvent and higher extraction rate) compared with other method (Song et al. 2011). Therefore, the objective of this study was to investigate the optimization extraction of bioactive compounds from red-stem TW using MAE method as preliminary observation for further our anti-hair loss study.

Methodology

Plant preparation

The red-stem TW was purchased from market in Chiang Rai province. The plant was washed, cleaned, separated part of leaf and stem and cut into small piece (~0.5x0.5 cm). Samples were kept in polyethylene bag and stored in refrigerator at 4 ^{o}C.

Microwave-assisted extraction of bioactive compounds

The 40 g of each leaf and stem of TW were soaked with 200 mL of either DI water or 95% ethanol at the ratio of sample: solvent (1:5 w/v). Bioactive compounds were extracted with household microwave (Samsung M183GM) by various powers (450, 600 and 850 watt) for 60 seconds (with turn on 20 seconds and turn off 20 seconds until total turn on 60 seconds). The mixtures were filtered through a Whatman filter paper No.1. The filtrates were freeze dried and then stored at 4 ^{o}C.

Phytochemical screening test

The phytochemical compounds (e.g. alkaloids, carbohydrates, terpenes, phenol, tannins and flavonoids) were investigated. All extracts were dissolved with DI water at ratio 0.01g: 2 mL (sample: solvent) and then filtered by using Whatman filter paper No.1. Alkaloids in extracts were detected by Wagner’s test, whereas the carbohydrates were determined by using Bendict’s test. Vanillin-Sulphuric acid test was used to detect the terpene in extract. Phenol, tannins and flavonoids of extracts were determined by using Ferric chloride test, Gelatin test and alkaline reagent test, respectively.

Total flavonoid content (TFC)

The 250 μL of samples were mixed with 50 μL of aluminium chloride, 50 μL of potassium acetate, 750 μL of 95% ethanol and 1400 μL of DI water. The mixture was incubated at room temperature for 30 minutes and then measured the absorption at 415 nm by using spectrophotometer. Quercetin (QE) was used as standard.

DPPH radical scavenging activity

Sample solution (1.5 mL) was added with 1.5 mL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 95% ethanol. The mixture was mixed vigorously and allowed to stand for 30 min in dark at room temperature: The absorbance of resulting solution was measured at 517 nm. The activity was expressed as μmol trolox eauivalent (TE)/ mg protein.

Statistical analysis

The obtained data were statistically analyzed using SPSS program for window (SPSS version 11.5, SPSS Inc, Chicago, IL, USA) and the differences will be considered significant when $p<0.05$.

Results and Discussion

The yields of ethanolic extracts from both stem and leaf of TW were higher than those of DI water extracts (Table 1), probably owing to extraction of both low- and high-molecular weight polar compounds (Prasad et al. 2005). The highest extractable yield was found in ethanolic extract of TW leaf at 600 watt (1.58%).

Table 1 The percentage of yield and phytochemical screening of stem and leaf of Thai water spinach (TW) extracts

Samples	Solvents	Microwave Power (watt)	% Yield	Phytochemical screening						
				Alk	Carb	Sap	Terp	Phenol	Tannin	Flavonoid
TW Stem	DI water	450	0.28	+	+	+	+	+	+	+
		600	0.38	+	+	+	+	+	+	+
		850	0.43	+	+	+	+	+	+	+
	95% Ethanol	450	0.50	+	+	-	+	+	+	+
		600	0.63	+	+	-	+	+	+	+
		850	0.73	+	+	-	+	+	+	+
TW Leaf	DI water	450	0.93	+	+	-	+	+	+	+
		600	1.03	+	+	-	+	+	+	+
		850	0.68	+	+	-	+	+	+	+
	95% Ethanol	450	1.33	+	+	-	+	+	+	+
		600	1.58	+	+	+	+	+	+	+
		850	1.13	+	+	-	+	+	+	+

Note: Positive (+) and negative (-) phytochemical compounds.

Phytochemical screening test, all extracts contained alkaloids, carbohydrates, terpenes, phenols, tannins, and flavonoids (Table 1). The total flavonoid contents were significantly different between each sample ($p<0.05$) that ranged from 58.62 to 349.69 mg QE/g dw. The TW ethanolic extracts were higher than water extracts at the same plant sample and extraction period (Table 2). Comparing between plant samples, the TW leaf extracts had the higher flavonoid contents than those of stem extracts at same extraction condition (Table 2). Both TW leaf and stem extracts tended to be higher flavonoid contents when increasing microwave power (600 and 850 watt) at the same solvents (Table 2). The highest of extractable flavonoid content was occurred from the leaf with 95% ethanol extracted at 850 watt of microwave power (349.69±9.98mg. QE/ g dw). Similar results were obtained in the green-stem water spinach (CW), where the ethanolic extracts had higher flavonoids than water extracts (Huang et al. 2005), and the CW leaf extracts showed the greater flavonoid contents than those of CW stem (Huang et al. 2005; James et al. 2009).

Table 2 The total flavonoid content and DPPH radical scavenging activity of stem and leaf of Thai water spinach (TW) extracts

Samples	Solvents	Microwave power (watt)	Total flavonoid content (mg. QE/g dw)	DPPH radical scavenging activity (mg TEAC/ g dw)
TW Stem	DI water	450	58.62±1.57^{i}	5.31±0.28^{f,g,h}
		600	194.16±2.92^{h}	4.19±0.50^{g,h}
		850	206.81±1.48^{g,h}	9.05±0.83^{e,f}
	95% Ethanol	450	194.84±7.55^{h}	45.02±1.06^{b}
		600	233.81±1.85^{g,h}	40.19±0.77^{c}
		850	305.42±6.41^{c}	54.49±0.75^{a}
TW Leaf	DI water	450	209.2±1.85^{g}	32.17±0.51^{d}
		600	245.26±4.87^{e}	31.84±0.58^{d}
		850	231.25±5.55^{f}	33.86±0.58^{d}
	95% Ethanol	450	322.00±3.87^{b}	2.02±3.34^{h}
		600	280.13±3.34^{d}	11.13±1.72^{h}
		850	349.69±9.98^{a}	2.14±0.53^{k}

Note: Mean±S.D. (n=3); Values within a column followed by different superscript differ statistically (ANOVA, $p<0.05$); QE= Quercetin equivalent; TEAC= Trolox equivalent.

The antioxidant activity was significantly different among samples ($p<0.05$) that ranged from 2.02 to 54.49 mg TEAC/ g dw. The DPPH scavenging activity of leaf samples with ethanol extracted was higher than those of leaf water extract. On the other hand, the ethanol extract of stem was lower than those of water extract (Table 2). The highest antioxidant activity was found from the stem sample with 95% ethanol extracted at 850 watt of microwave power (54.49±0.75 mg TEAC/ g dw). The results were similar to the previous studies that stem of the green CW (IC_{50}=33.18 μg/mL) had higher antioxidant activity than their leaf (IC_{50}= 672.38 μg/mL) (Huang et al. 2005; James et al. 2009). The results suggested that the flavonoid compounds from TW stem may directly exhibited the antioxidant activity but not in case of TW leaf that may contained other bioactive compounds with having antioxidant activity. The antioxidant activity may be directly correlated with the bioactive compounds (e.g. flavonoid or other) content of various extracts (Prasad et al. 2005).

Conclusion

The red-stem Thai water spinach (TW) have a significantly effect on antioxidant activity. The optimal extraction condition is 95% ethanol extracted at high watt of microwave power (850 watt). This plant can be used as the alternative source of antioxidant ingredient for the cosmetic and food industries.

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