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Antioxidant Activities and Phenolic Contents of Extracts from *Salvinia molesta* and *Eichornia crassipes*

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Abstract: The antioxidant activities and total phenolic contents of acetone/methanol extracts from *Salvinia molesta* and *Eichornia crassipes* were investigated. The antioxidant activities was evaluated by using scavenging of 2-2-Diphenyl-1-Picrylhydrazyl radical (DPPH˙). The Total Phenolic Content (TPC) was evaluated according to the Folin-Ciocaltue assay and gallic acid was used as standard. The Flavonoids Contents (TFC) were evaluated by using the aluminum chloride method. As the result, extract from *S. molesta* exhibited the highest antioxidant activity with IC_{50} value of 27.75±0.15 μg mL^{-1} followed by extract from leaf of *E. crassipes* (IC_{50} value of 145.33±0.89 μg mL^{-1}) and petiole of *E. crassipes* (IC_{50} value of 179.18±1.54 μg mL^{-1}), repectively. The TPC and TFC of extracts were in the range of 39.58±0.38-69.97±0.38 mg GAE g^{-1} dw and 12.67±0.02-23.16±0.08 mg QE g^{-1} dw, respectively. A High-Performance Liquid Chromatography (HPLC) method with photodiode array detection at 280 nm was used for identification and quantification. Nariginin was the major phenolic compounds (65.56-68.71 mg g^{-1} of crude extract) found in the extracts followed by myricetin (1.34-17.05 mg g^{-1} of crude extract) from *S. molesta* and *E. crassipes*.

Key words: Antioxidant activity, phenolic content, flavonoid content, *Salvinia molesta*, *Eichornia crassipes*

INTRODUCTION

The menace of aquatic weeds is reaching alarming problems in many parts of the world, but it is particularly severe in tropical countries, where abundant sunlight and favorable water temperature, increasing numbers of dams, barrage and irrigation channels foster aquatic growth (Kalita *et al*., 2007). Aquatic weed populations often reach nuisance proportions and interfere with beneficial uses of natural waters to such an extent that eradication measures have to be employed. A few of them are consumed by local people but many remain unutilized and go to waste. *Salvinia molesta* and *Eichornia crassipes* are free floating aquatic weed originating from tropical areas, with various growth habits under different environmental conditions. The phytochemical investigation on the *S. molesta* showed that it consists of 96% of amino (Lahdesaki, 1986) and *S. molesta* showed high radical scavenging in ethyl acetate extract (88.42%). Recently, two glycosides, 6′-o-(3,4-dihydroxy benzoyl)-β-D-glucopyranosyl ester and 4-o-β-D-glucopyranoside-3-hydroxy methyl benzoate, along with five known compounds as methyl benzoate, hypogallic acid, caffeic acid, paeoniflorin and pikuroside were found in *S. molesta* (Choudhary *et al*., 2008). The *E. crassipes* is supposed to contain high amounts of cysteine-based proteins like metallothioneins. It is well known that cysteine is also the main component of glutathione and displays antioxidative activity against $O_2^{\cdot -}$ and $OH^{\cdot}$ free radicals (Thornaleyand and Vasack, 1985).

The antioxidants are now known to play an important role in protection against disorders caused by oxidant damage (Chanwitheesuk *et al*., 2005). Natural antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seed) (Chanwitheesuk *et al*., 2005). Typical compounds that exhibit antioxidant activity include vitamins, carotenoids and phenolic compounds. It reported that phenolic compounds in plant possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free radical (Kahkonen *et al*., 1999).

The objectives of this investigation were to determine the antioxidant activity, TPC, TFC and to identify phenolic compounds of *S. molesta* and *E. crassipes* in the Northeastern of Thailand. Results from this preliminary

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study will provide a better understanding of the antioxidant properties of unutilized aquatic weeds and allow the identification of weeds with high antioxidant activity for future investigation and development into animals feed.

MATERIALS AND METHODS

Reagents: Acetone, methanol and hexane purchased from Merck (Darmstadt, Germany). Chemical used for determination of antioxidant activities and total phenolic contents including 2, 2-Diphenyl-2-Picrylhydrazyl Hydrate (DPPH), gallic acid, Folin-Ciocalteu's reagent and sodium carbonate (Na_2CO_3) were obtained from Fluka chemical and trolox was obtained from Sigma-Aldrich. Chemical employed for determination of phenolic compounds standards including naringenin and rutin were purchased from Sigma-Aldrich and catechin, epicatechin, gallic acid, kaempferol, myricetin and quercetin were obtained from Fluka chemical.

Plant materials: *S. molesta* and *E. crassipes* were collected from natural sources in Srisaket province located in the Northeastern of Thailand during January, 2009. The leaves and petiole of *E. crassipes* were manually separated. The weeds were cleaned by distilled water several times or until it clean. After that, all of them were cut into small pieces before being dried in a hot air-oven at 60°C for 24 h. The dried weeds were ground into fine powder and stored in sealed plastic bags at 4°C, until ready for extraction.

Powder samples were extracted by soaking in hexane for 2 days in a glass container at room temperature followed by filtration with Whatman No. 1 paper. The residues were re-extracted with the same solvent for 2 times.

The extracts were evaporated under vacuum at 50°C using a rotary evaporator. After extraction with hexane, the residues was transferred for extraction with acetone/methanol (1:1), AcMeOH, in the same manner as hexane extract. The crude extracts were stored in dark vial and kept in 4°C, until further use on antioxidant activity, total phenolic contents, total flavonoid contents and HPLC analysis. Each evaporated thick and viscous extract (0.02 g±0.001 mg) was diluted with 25 mL methanol.

Extraction yield: The extraction yield was analyzed by Prasad with some modification. Briefly, the filtrate was evaporated to dryness by using a rotary evaporator (Buchi Model R-200, USA) under vacuum at 60°C. The yield of dried or power based on a percentage of dry weight basis was then calculates from Eq. 1 as:

$$\text{Yield (\%)} = \frac{W_1}{W_2} \times 100 \quad (1)$$

Where:

W_1 = The weight of extract after evaporation of acetone/methanol

W_2 = The dry weight of sample

DPPH radical scavenging assay: The free radical scavenging activity of the extract was measured using the DPPH assay followed the method by Liu *et al.* (2008). Briefly, 1 mL of 0.2 mM DPPH radical solution in ethanol mixed with 1 mL of extract sample solution at different concentration. Then, the samples were left to stand in a dark room at room temperature for 20 min. The 1 mL of ethanol absolute mixed with 1 mL of DPPH was used as blank. The absorbance was measured at 515 nm using an UV-visible spectrophotometer for triplicate measurements. All samples were analyzed in triplicate. Trolox equivalent was used as standard reference. The percentage of remaining DPPH against the sample concentration was plotted obtain the amount of antioxidant (μg) necessary to decrease free radicals by 50%. A smaller IC_{50} value corresponds to a higher antioxidant activity.

Determination of total phenolic contents (Folin-Ciocaltue method): Total Phenolic Contents (TPC) of extracts were assessed using the Folin-Coicalteau assay with some modification. The 0.5 mL of extract solutions were mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate.

The mixtures were agitated with a vortex mixer and allowed to stand at room temperature for 30 min in the dark room. The absorbance of extracts and prepared blank were measured at 765 nm by using UV-vis spectrophotometer. The TPC in all weed extracts were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram dry weight of weeds.

Determination of total flavonoid contents (aluminum chloride method): Total Flavonoid Contents (TFC) were measured using a modified colorimetric method (Liu *et al.*, 2008). The 0.25 mL extract solutions were added to the test tube containing 1.25 mL of distilled water. Then, 0.075 mL of 5% sodium nitrite was added to the mixture and maintained for 5 min and added the 0.15 mL of 10% aluminum chloride. After 6 min, 0.5 mL of 1 M sodium hydroxide was finally added and diluted the mixture with 0.275 mL of distilled water. The absorbance was measured

at 510 nm in comparison to a standard curve prepared by quercetin solution. The flavonoid contents were expressed as mg Quercetin Equivalent (QE) g^{-1} dry weight of weeds.

RP-HPLC separation of extracts: The HPLC analysis were performed with a Shimadzu HPLC system equipped with LC-10 Advp liquid chromatography, SPD-m10Avp diode array detector, SIL-10Advp auto-injection, SCL-10Advp system controller and CTO-10Avp column oven. Chromatographic separation was achieved on a C_{18} guard column and C_{18} reversed-phase packing column (4.5 mm × 25 cm, 5 μm) were used this study. The mobile phase consisted of solvent 3% acetic acid in water and solvent methanol; starting from 100% A for 5 min followed by linear increased of solvent B to 60% within 40 min and final increased to 100% A. Washing and recondition the column was done in 10 min. The detection wavelength was 254 nm, with flow rate 1 mL min^{-1} and injection volume 20 μL. All the prepared solutions were filtered though 0.45 μm membranes and the mobile phase were degassed before injection onto HPLC.

RESULTS AND DISCUSSION

The extraction yield of *S. molesta* and *E. crassipes* from AcMeOH were shown in Table 1. The extraction yield of the samples varied from 14.2-18.5% with a decreasing order: *S. molesta*> leaf of *E. crassipes*> petiole of *E. crassipes*. It was found that hexane extract obtained in very small amounts. The mixture of acetone/methanol was more effective for extracting phenolic compounds in *S. molesta* and *E. crassipes* than those of hexane, which could be due to the solubility of phenolic compounds and flavonoids. One of the factors that may influence the extraction is polarity of solvents. Therefore, only AcMeOH extracts of *S. molesta* and *E. crassipes* were selected for further studies.

The TPC in weeds were in the range of 39.58±0.38-69.97±0.38 mg GAE g^{-1} dw. (Table 2). Petiole of *E. crassipes* presented the lowest amount of TPC (39.58±0.38 mg GAE g^{-1} dw) while the highest was observed in *S. molesta* with a value of 69.97±0.38 mg GAE g^{-1} dw (Table 2). A relationship was found between TPC and IC_{50} value that the high content of TPC related to good antioxidant capacities. It can be seen that the extracts studied in this research had potential to contain antioxidant substances. Phenolic compounds have been proved to be responsible for the antioxidant activity of plants (Liu *et al*., 2008). However, high TPC values did not linear correspond to a high antioxidant activity. Because Folin-Ciocalteu reagent is not specific to just polyphenols but to any other substances that could be oxidized by the reagent (Wong *et al*., 2006). TFC of the weed extracts was also determined. Regarding the content of total flavonoids (mg QE g^{-1} dw), TFC varied from 12.67±0.02-23.16±0.08. The *S. molesta* was highest amounts of TFC (23.16±0.08 mg QE g^{-1} dw), while the lowest amount (12.67±0.02 mg QE g^{-1} dw) was observed in petiole of *E. crassipes*. In this study, TFC were increased with increasing with TPC and TFC/TPC ratios were in the range of 24.17-33.11. It also found that all types and part of weeds contained a lower TFC than TPC, since the compounds besides flavonoids are phenolic substances in plants (Pietta, 2000).

The DPPH assay is often used to evaluate the ability of antioxidant to scavenge free radicals. However, DPPH scavenging activity is best to presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution (Karagozler *et al*., 2008). A smaller IC_{50} value corresponds to a higher antioxidant activity of plant extracts (Maisuthisakul *et al*., 2008). In the DPPH test, the IC_{50} values of *S. molesta* and *E. crassipes* extracts were shown in Table 2. The *S. molesta* sample showed higher radical scavenging activity (IC_{50} 27.75±0.15 μg mL^{-1}) than *E. crassipes*. Leaf and the extract of *E. crassipes* (IC_{50} value of 145.33±0.89 μg mL^{-1}) possessed the higher activity than petiole extract of *E. crassipes* (179.18±1.54 μg mL^{-1}). Data obtained revealed that *S. molesta* exhibited the higher activity than that of *E. crassipes*. The extracts from *S. molesta* and *E. crassipes* were analyzed by HPLC.

Table 1: Yield of AcMeOH extracts of *S. molesta* and *E. crassipes*

Weeds	Plant part	Yield (%)	Moisture (%)
S. molesta	Leaf	18.5	96.78
E. crassipes	Leaf	16.9	91.52
	Petiole	14.2	95.05

Table 2: Contents of IC_{50} and total phenolic and flavonoid contents values of aquatic weed extracts

Weeds	Plant part	IC_{50} (μg mL^{-1})	TPC (mg GAE g^{-1} dw)	TFC (mg GAE g^{-1} dw)	TFC/ TPC ratio
S. molesta	Leaf	27.75±0.15	69.97±0.38	23.16±0.08	33.11
E. crassipes	Lleaf	145.33±0.89	62.49±0.18	15.10±0.02	24.17
	Petiole	179.18±1.54	39.58±0.38	12.67±0.02	32.01

Table 3: Mean concentrations of phenolic compounds of extracts of *S. molesta* and *E. crassipes* detected and quantified by HPLC

Phenolic compounds	Amount of phenolic compounds (mg g^{-1} crude extract)		
	S. molesta	Leaf of *E.crassipes*	Petiole of *E.crassipes*
Gallic acid	-	-	-
Catechin	19.82	-	-
Epicatechin	4.67	-	-
Vanillin	0.44	1.28	-
Rutin	16.33	-	1.24
Myricetin	17.05	1.34	1.97
Quercetin	0.32	-	-
Naringenin	66.77	68.71	65.56
Keapferol	-	0.91	0.16

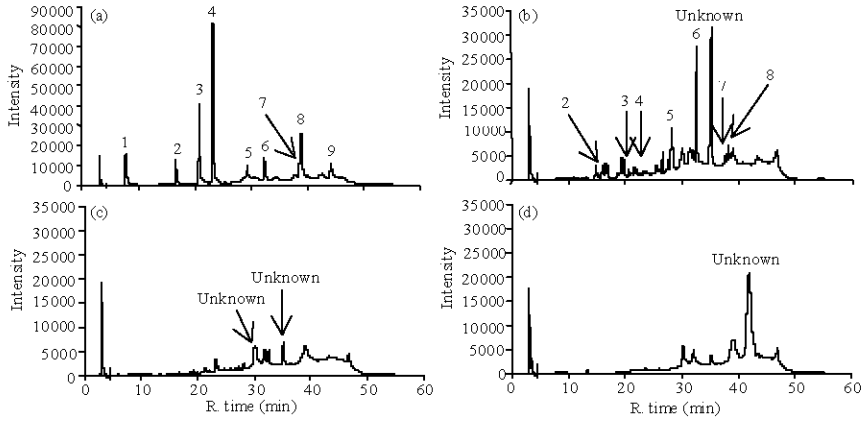

Fig. 1: Chromatograms of (a) stadards of 9 phenolic compounds; (b) in *S. molesta*; (c) leaf of *E. crassipes* and (d) petiole of *E. crassipes*: (1) gallic acid; (2) catechin; (3) epicatechin; (4) vanillin; (5) rutin; (6) myricetin; (7) quercetin; (8) naringenin and (9) kamepferols

The phenolic compounds were listed in Table 3, Figure 1a showed typical chromatograms of standard solutions, the compounds including gallic acid, rutin, vanillin, epicatechin, catechin, kaempferol, quercetin, myricetin and naringinin identified based on retention time. Figure 1b-d shows that the same numbers of peaks (corresponding to same retention time) were observed in the chromatograms of *S. molesta* and *E. crassipes*. The chromatograms of *S. molesta* (Fig. 1b) were different from that leaf of *E. crassipes* (Fig. 1c) and petiole of *E. crassipes* (Fig. 1d). The results shown that the major of phenolic compounds in *S. molesta* and *E. crassipes* were rutin, vanillin, epicatechin, catechin, kaempferol, quercetin, myricetin and naringinin. Naringinin and myricetin were the dominant phenolic compounds in *S. molesta* and *E. crassipes*, while the epicatechin, catechin and quercetin were not detect in leaf of *E. crassipes* and petiole of *E. crassipes* and kaempferol not detect in *S. molesta*. The separation and identification of individual phenolic compounds in weeds extracts were analyzed by RP-HPLC. The compounds identified can be divided into 2 groups, phenolic acid (gallic acid and caffeic acid) and flavonoids (rutin, vanillin, epicatechin, catechin, kaempferol, quercetin, myricetin and naringinin). According to the results, it is obviously that content of phenolic compounds of *S. molesta* and *E. crassipes* coming from different ages and growth under different environmental were totally different, this may be related of the nutritional from water and soil.

CONCLUSION

The results in this study demonstrated that there are differences in the contents of antioxidant activities and phenolic compounds of *S. molesta* and *E. crassipes*. The TPC and TFC were in relation to antioxidant activities of weed extracts. It was found that *S. molesta* had higher contents of total phenolic and antioxidant than *E. crassipes*.

The *S. molesta* and *E. crassipes* showed that naringinin was the main of phenolic compounds, while myricetin, vanillin, kaempferol and quercetin were also present in low concentrations. Thus, *S. molesta* can be utilized as a source of aquatic weeds antioxidant, with a potential use in feed animals.

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