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Study of antioxidant activities, total phenolic content and total flavonoid content of the extracts of *Monochoria vaginalis* and *Cissus repens* Lamk. using different solvents

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Abstract

The present research was aimed to study of antioxidant activities, total phenolic content and total flavonoid content of leaves and fruits of *Monochoria vaginalis* and leaves and stem of *Cissus repens* Lamk. using ethyl acetate, acetone and ethanol as the solvents. The results showed that the ethanol extracts from *M. vaginalis* leaves (LME) had the highest total phenolic content (TPC) and total flavonoid content (TFC) at 336.30±16.17 mgGAE/g and 15.41±0.95 mgQE/g, respectively, while the ethyl acetate extracts from *C. repens* leaves (LCEA) had the lowest TPC and TFC at 58.27±13.88 mgGAE/g and 5.39±0.24 mgQE/g respectively. The analysis of antioxidant activity using DPPH assay found that the percentage of inhibition varies with the concentration of the extract. The lowest IC_{50} value, which has the best antioxidant activity, was found in the LME (169.93±13.42 ppm) which had moderate levels of activity compared with the standard solution of vitamin C (IC_{50} = 41.64±0.29 ppm) and standard solution Trolox (IC_{50} = 22.66±0.64 ppm). The analysis of ABTS scavenging gave consistent results with DPPH assay. LME showed the best antioxidant activity with IC_{50} equal to 159.76±16.05 ppm.

Keywords: Antioxidant activity, total phenolic content, total flavonoid content, *Monochoria vaginalis*, *Cissus repens* Lamk.

INTRODUCTION

Free radicals are atoms or molecules that have a single electron in the outermost orbital. It is an unstable substance and is sensitive to the reaction by losing or giving electrons to other substances so that the electrons are paired. Free radicals are biologically important, such as superoxide anion (O_2-•), hydroxyl (OH•), peroxyl (ROO•). There are two sources of free radicals, which are internal and external factors. Reactions that cause free radicals in the body such as the activity of xanthine oxidase (XOD) [1]. In the body there is a transition metal, such as iron, copper, which when reacting with hydrogen peroxide will produce hydroxyl radicals (OH•) [2]. It is a strong oxidant and highly reactive, therefore more harmful to molecules than other types of radicals. External factors that are free radicals such as drugs, especially cancer drugs such as adriamycin, mitomycin C, bleomycin. When eaten, free radicals develop and lipid oxidation, a process in which lipids are oxidized by free radicals [3].

Antioxidants are substances that are responsible for preventing or inhibiting oxidation. Here, including substances that can inhibit and control free radicals from stimulating the oxidation reaction, thus inhibiting free radicals from destroying cell composition. Antioxidants are both natural substances such as amino acid, ascorbic acid, carotenoids, flavonoids, tannins, tocopherols, etc. Phenolic compounds are substances found in plants. The amount of total phenolic compounds in food and beverages derived from vegetables and fruits will vary according to the type of plant, method of planting, degree of ripeness, processing and storage. The use of heat in the processing process reduces the total amount of phenolic compounds. The phenolic compound is a group of substances with antioxidant properties. It is combined aromatic ring compounds and have at least one hydroxyl group. Including derivatives of total phenolic compounds which are replaced by functional groups such as flavonoid, lignin, cinnamic acid and coenzyme Q. There are many types of combined phenolic compounds in nature, and have different chemical formula formulas from simple structural groups such as phenolic acids, to structural polymers such as lignin, the largest group is flavonoid compounds. Phenolic compounds found in plants are often included in sugar molecules in the form of glycosides. The most common type of sugar in the phenolic compound molecule is glucose and may be combined between the combined phenolic compounds. Some phenolic compounds are combined with other compound substances such as organic acids, alkaloids, proteins and terpenoids [4-6].

Monochoria vaginalis, in Thai, is called a “Phak Kiad” is a plant in the family Pontederiaceae. It is one of the weeds that are a problem in rice fields in Asia [7]. It has a short trunk, underground with roots deep in the soil. The leaves are a single leaf from the base of the stem alternately, round, succulent, hollow inside. The base of the petiole is a compressed sheath covering tightly. The leaves resemble a heart and the petals are separated. The outer petals are light green and often bloom during the rainy season. The fruit is like a capsule and contains a lot of brown seeds. In the literature review found that the ethanolic extract of leaves showed radical scavenging activity in 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonic acid) disodium salt (ABTS), superoxide and hydrogen peroxide assays. The hot water extract of leaves showed the inhibition of free radical in metal ion chelating assay [8].

Cissus repens Lamk. is known in the Thai language as “Tao Kan", a plant belonging to the family Vitaceae. It likes to wrap around a large tree. The leaves are about 3 petiolule and slightly hairy. When fresh, succulent, the leaves are ovate, 1–4 cm wide, 1.5-6 cm long. The flowers look like a bouquet of large, flat and tight clusters. The fruit is round, 0.5–1 cm in size. If squeezed, the water will turn purple red. *C. repens* roots showed analgesic and anti-

inflammatory activities, used for snake bites, rheumatic pain and carbuncles in folk medicine. In addition, the stems were also used for the treatment of nephritis, long-term coughing and diarrhea [9].

Monochoria vaginalis and *Cissus repens* Lamk are another native plant that can be found in many locals. There are reports of uses for cooking, medicinal benefits and biological activity research results. The aim of this research was to study of antioxidant activities, total phenolic content and total flavonoid content of the extracts using different solvents and to be a scientific database to increase the value of Thai folk plants to have more value can be developed and applied in the cosmetic and dietary supplement industries.

METERIALS AND METHODS

Plants sampling

Collecting plant specimens consisting of the leaves and the stem of *M. vaginalis*, the leaves and the fruits of *C. repens* Lamk. from Lan Khoi Subdistrict, Pa Payom District, Phatthalung, Thailand, each 1 kilograms, then all samples were baked in a hot air oven at a temperature of 80 ^{o}C for 8 hours until the specimens were completely dry and then ground into a fine powder.

Extraction of plant samples

Sample powder was weighed 25 grams, added 150 ml of 95% ethanol, then extract with Soxhlet extractor for 4 hours. After that, the solvent that has been evaporated by Rotary evaporator until the crude extract was obtained. The obtained extracts were weighed and stored in an opaque container at room temperature until the next step was to be tested. The extraction was performed in the same manner using acetone and ethyl acetate solvents, repeated 3 times for each solvent.

Determination of total phenolic content

Quantitative analysis of total phenolic content by Folin-Ciocalteu colorimetric uses gallic acid as a standard substance (concentration in the range of 0.01-0.05 mg/ml). The method was based on the 96-well microplate adapted from Ahmad's method [10]. Dissolve each extract with methanol to a concentration of 1 mg/ml. Pipette 12.5 µl sample solution, add 12.5 µl of 10% v/v Folin-Ciocalteu solution, put in 96-well plate, set aside for 10 minutes. Then add 125 µl of 7.5% w/v solution Na_2CO_3, leave at room temperature for 30 minutes. Take the solution to measure the absorbance using a Microplate reader with a wavelength of 765 nm. Calculate the amount of phenolic content in extracts compared with the standardized gallic acid chart in milligrams equivalent of gallic acid per gram of extract (mgGAE/g extract) from the formula;

$$TPC = \frac{GAE \times V \times D}{W}$$

Where TPC is the total phenolic content, GAE is the equivalent value of gallic acid obtained from substitution in a linear equation (mg/ml), V is the sample volume (ml), D is the dilution factor and W is the sample weight (g)

Determination of total flavonoid content

Analysis of the amount of flavonoid content was slightly modified from Chatattikun and Choabchalard's method [11]. Preparing by 0.05 g of each plant crude extract in a 10 ml volumetric flask, adjust the volume with methanol, get a crude extract with a concentration of 5 mg/ml. After that, 100 µL of the prepared sample was pipetted in 96-well plate and add 100 µL of 10% w/v $AlCl_3$. Set aside for 10 minutes, then add 1 M CH_3COOK with a volume of 100 µL and set aside for 30 minutes. The absorbance is measured at 435 nm wavelength with a Microplate reader using quercetin as a standard substance. Total flavonoid content is expressed in micrograms equivalent of quercetin per gram of extract (µgQE/g extract) from the formula;

$$TFC = \frac{QE \times V \times D}{W}$$

Where TFC is the total flavonoid content, QE is the equivalent value of quercetin obtained from substitution in a linear equation (mg/ml), V is the sample volume (ml), D is the dilution factor and W is the sample weight (g)

Testing the antioxidant activity by DPPH method

Antioxidant activity by DPPH method was slightly modified from Al-Saeedi's methodby preparing sample solutions at various concentrations using methanol as a solvent [12]. (concentration in the range 25-1000 ppm). Pipette 100 µl of sample solution pipette into 96-well plate, add 100 µl of methanol and 100 µl of 0.3 mM DPPH solution and incubated in a dark, room temperature for 30 minutes. The absorbance was measured using a Microplate reader at a wavelength of 517 nm by using methanol as blank and vitamin C and Trolox as standard substance. Calculate the percentage of free radicals scavenging by using the absorption equation at various concentrations.

Testing the antioxidant activity by ABTS method

ABTS scavenging activity was analyzed by investigating their ability to scavenge the ABTS·+ using the method as described previously in the literature [13-14]. The 7 mM ABTS·+ was mixed with 2.45 mM potassium persulfate, and this mixture was stored in the dark at room temperature for 12-16 h to create stable, darkblue–green radical solution. The solution was then diluted with 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.70 ± 0.02 at 734 nm to form the test reagent. After the addition of 0.5 ml of plant extract to 1.5 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing with UV–Vis spectrophotometer. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. The scavenging activity of ABTS radicals was calculated using the following equation:

$$\% \text{ Free radical scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where % free radical scavenging is the percentage of inhibition of free radicals of the substance, $A_{control}$ is the absorbance value of the control substance and A_{sample} is the absorbance value of extracts, vitamin C or Trolox.

When the percentage of inhibition of free radicals of various concentrations was plotted, the graph gave the inhibitory concentration at 50 percent (IC_{50}).

RESULTS

Each 1 kilogram of each plant sample was dried in a hot air oven until the specimen was completely dry. The percentage of moisture of each plant is shown in Table 1. When the 25-gram dried plant samples were extracted by 3

solvents, ethyl acetate, acetone and ethanol (repeated extraction 3 times), the percentage of extraction is shown in Table 2.

Table 1 The percentage of moisture from each type of sample.

Plant sample	Plant weight before baking (g)	Plant weight after baking (g)	Percentage of moisture (%)
Leaves of *M. vaginalis*	1000	125	87.5
	1000	314	68.6
Stem of *M. vaginalis*	1000	115	88.5
	1000	223	77.7
Leaves of *C. repens* Lamk.			
Fruits of *C. repens* Lamk.			

Table 2 % Yield of extracts from plant samples with each type of solvent.

Plant sample	Solvent	Abbreviation	%Yield
Leaves of *M. vaginalis*	Ethyl acetate	LMEA	8.36±0.08
	Acetone	LMA	7.27±0.15
	Ethanol	LME	8.08±0.12
Stem of *M. vaginalis*	Ethyl acetate	SMEA	16.28±1.79
	Acetone	SMA	12.60±0.51
	Ethanol	SME	13.82±0.34
Leaves of *C. repens* Lamk.	Ethyl acetate	LCEA	7.96±0.20
	Acetone	LCA	6.20±0.18
	Ethanol	LCE	7.23±0.14
Fruits of *C. repens* Lamk.	Ethyl acetate	FCEA	7.92±0.28
	Acetone	FCA	6.37±0.09
	Ethanol	FCE	7.25±0.43

Phenolic acid is an important phenolic compound that occurs widely in the plant kingdom, especially in fruits and vegetables [15]. The total phenolic content (TPC) was estimated using the Folin-Ciocalteu (FC) reagent method. TPC concentrations ranged from 58.27 to 336.30 mgGAE/g. The results of TPC from each extract are shown in Table 3. Calibration curve from gallic acid showed maximum absorbance at 765 nm wavelength (equation $y = 18.06x + 0.1208$, $R^2 = 0.9939$). Total flavonoid content (TFC) was determined by aluminium chloride colorimetric assay. TFC concentrations ranged from 5.39 to 15.41 mgQE/g. The result of TFC of each crude extract is given in Table 3. Equation of calibration curve of quercetin standard was $y = 3.8233x + 0.0729$, $R^2 = 0.9986$.

Table 3 Total phenolic and total flavonoid content of each extract.

Crude extract	Total phenolic content (mgGAE/g)	Total flavonoid content (mgQE/g)
LMEA	227.48±2.49^{c}	12.48±1.01^{bc}
LMA	253.32±7.98^{b}	14.46±1.03^{ab}
LME	336.30±16.17^{a}	15.41±0.95^{a}
SMEA	261.59±6.58^{b}	12.76±1.38^{b}
SMA	184.66±5.13^{de}	10.26±0.36^{cd}
SME	163.40±4.52^{ef}	9.25±0.99^{d}
LCEA	58.27±13.88^{h}	5.39±0.24^{e}
LCA	131.35±3.27^{g}	9.05±1.15^{d}
LCE	201.79±3.44^{d}	9.82±0.26^{d}
FCEA	147.01±6.87^{fg}	8.83±0.41^{d}
FCA	73.18±3.46^{i}	8.07±0.29^{d}
FCE	98.43±8.20^{h}	9.32±0.47^{d}

$^{a-i}$ significant ($p < 0.05$) differences within a column.

DPPH and ABTS scavenging ability assay were used to evaluate the antioxidant activity of each extract. The capacities of extract in scavenging DPPH and ABTS radical are shown in Table 4.

Table 4 The half maximal inhibitory concentration of each extract.

Crude extract	DPPH Scavenging IC_{50} (ppm)	ABTS Scavenging IC_{50} (ppm)
LMEA	298.90±14.64^{de}	322.72±6.47^{d}
LMA	211.88±6.24^{c}	236.43±11.32^{c}
LME	169.93±13.42^{b}	159.76±16.05^{b}
SMEA	359.82±19.08^{f}	316.90±7.62^{d}
SMA	425.95±13.40^{g}	384.53±10.88^{e}
SME	625.25±19.21^{h}	633.37±19.92^{f}
LCEA	>1000^{j}	>1000^{h}
LCA	318.28±14.83^{e}	352.35±14.40^{de}
LCE	278.12±12.95^{d}	213.70±7.39^{c}
FCEA	784.34±16.41^{i}	898.76±16.14^{g}
FCA	>1000^{j}	>1000^{h}
FCE	>1000^{j}	939.02±35.14^{g}
Ascorbic acid	41.64±0.29^{a}	80.02±1.40^{a}
Trolox	22.66±0.64^{a}	55.90±0.32^{a}

$^{a-j}$ significant ($p < 0.05$) differences within a column.

DISCUSSION

Different parts of the plant may have different phytochemical compounds, which may have different pharmacological effects of each parts [16]. The polarity of the solvent is important to the total amount of phenolic [17]. TPC in leaves and stem of *M. vaginalis* were evaluated in the present study. The highest amount of phenolic compounds was present in LME (336.30±16.17 mgGAE/g) and the lowest was in SME (163.40±4.52 mgGAE/g). The leaves and fruits of *C. repens* were found to have less total phenolic content than *M. vaginalis*. LCE showed more total phenolic content (201.79±3.44 mgGAE/g) than LCA and LCEA, while FCEA showed more total phenolic content (147.01±6.87 mgGAE/g) than FCE and FCA. Phenolic compounds are better soluble in polar solvents because they contain hydroxyl groups, in accordance with Wang and Weller [18]. Comparing the works of literature, report a TPC in range of 72.66±0.46 to 292.65±0.42 mgGAE/g of eight selected wild vegetables from Nepal in methanol extract, a TPC of 348.36±38.53 mgGAE/g in methanol extract of the leaves of *M. ferrea* L. and a TPC of 3.6±0.089 mgGAE/g in the methanol extract of *P. oleracea* [19-21]. Flavonoids belong to the phenolic compound class. They are ingested by humans and they seem to display important antiinflammatory, anti-allergic and anti-cancer activities [22]. Likewise, *M. vaginalis*

showed more total flavonoid content than *C. repens*. LME was found to high flavonoid content of 15.41±0.95 mgQE/g follow by LMA, SMEA and LMEA with 14.46±1.03, 12.76±1.38 and 12.48±1.01 mgQE/g, respectively.

DPPH molecules are free radicals by delocalization of electrons in molecules. The single electron moves, resulting in a dark purple solution. If the DPPH solution is mixed with a sample capable of providing the DPPH hydrogen atom, the color of solution is converted into colorless. Trolox and ascorbic acid are compounds with the highest antioxidant capacity, which we can use to assess the antioxidant strength of the sample. From this research, all plant extracts have the ability to trap free radicals with increasing concentration. It is found that LME has the best antioxidant activity with an IC_{50} value of 169.93 ± 13.42 ppm which gives a result that is consistent with the total phenolic and flavonoid content. LME, LCE, LMEA and LCA exhibit moderate antioxidant activity with an IC_{50} value of 211.88±6.24, 278.12±12.95, 298.90±14.64 and 318.28±14.83 ppm, respectively. LCEA, FCA, FCE are extracts that do not show antioxidant activity. All data were compared with the IC_{50} value of standard ascorbic acid (41.64±0.29 ppm) and trolox (22.66±0.64 ppm). The ability to inhibit free radicals is consistent with the total phenolic and total flavonoid content. Many literary works have reported the important role of phenolic compounds in eliminating free radicals as well as the high extraction capability of rich phenolic plant samples. Correlation analyses demonstrated that TPC and TFC exhibited high correlation with bamboo antioxidant activity [23]. The water-acetone mixture extract was presented TPC, DPPH and ferric reduction ability (FRAP) values of 58.44 mg GAE/g, 250.20 µmol TE/g and 720.15 µmol TE/g, respectively [24].

CONCLUSIONS

This study reveals that the leaves of *M. vaginalis* in ethanol extracts showed the highest antioxidant activity, total phenolic and total flavonoids content. The leaf extract shows better antioxidant activity than other parts. The ethanol extract of leaves of *M. vaginalis* and *C. repens* showed moderate antioxidant activities. Further phytochemical studies can be done for the isolation of compounds from the ethanol extracts of leaves from *M. vaginalis*. It constitutes a natural source of potent antioxidants that may prevent many diseases and could be potentially used in cosmetics or supplement food.

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