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Phytochemical Content and Antioxidant Activity in *Ampelocissus martini* Planch. Root Extracts

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ABSTRACT

Extracts of wild grape (*Ampelocissus martini* Planch.) roots were fractionated by silica gel chromatography using different solvent systems and were then assayed for their phytochemical contents and antioxidant activity. High levels of total phenolic and flavonoid content were found in both crude extract and chromatographic fractions. The ethyl acetate: methanol (75:25 %v/v) fraction had the highest level of total phenolics and flavonoids as well as antioxidant activity assessed all methods, except ABTS assay. This indicated that eluting solvents directly affected phytochemical profile and activity. Moreover, the phenolic and flanonoid substances showed highly positive correlation coefficient (*r*) to antioxidant activity. This suggested that the wild grape root is a natural source containing high phytochemicals with antioxidant activity which might be used as active ingredients supporting good health.

Keywords: Wild grape root, Phytochemical, Antioxidant activity, Silica gel column chromatography.

INTRODUCTION

Free radicals occur naturally during oxygen metabolism, especially as reactive oxygen species (ROS)[1,2]. They confer both advantages and disadvantages depending on their concentration in the living organism. At low or moderate concentrations, they are involved in the immune system, cellular signaling pathways and mitogenic responses However, at high concentration, free radicals lead to damage of nucleic acids, lipids and proteins and are involved in many diseases including diabetes mellitus, various cancers and cardiovascular diseases[2].

An antioxidant is a molecule which can stabilize or deactivate free radicals before they destroy cells[1]. Antioxidants can be produced by the body or obtained from the diet. Endogenous antioxidants (enzymatic and non-enzymatic) found in the body are important for maintenance of optimal cellular functions, but exogenous antioxidants from the diet or from dietary supplements may be needed to protect cells of the body from free radical attack under conditions which can cause oxidative stress[3].

Plants are well known as a source of phytochemicals including vitamin C, β-carotene

and polyphenols or phenolic compounds[1]. Phenolic compounds are plant secondary metabolites, and universally occur in many plant parts including fruits, vegetables, nuts, seed, leaves, roots, and barks[4]. These compounds can be grouped into phenolic acids, flavonoids, proanthocyanidins, stilbenes, and lignans[5].

Grape is a popularly consumed fruit. Different parts of the grape plant contain phenolic compounds which show antioxidant, anti-cancer, anti-inflammatory, antibacterial, and antihypertensive activities[5-11]. A similar plant known as "wild grape (*Ampelocissus martini* Planch.)" has similar characters to table grape including those of the stem, leaf and fruit. This plant has been recognized as a Thai medicinal plant and applied in folk medicine. Many studies relating to antioxidants and phytochemicals in grape have been reported. Almost all of these studies concern extracts of seeds, leaves[12-13], juice[14-15], pomace[10], skin and pulp[5,16], stem[8-9,17-18] and fruits[19]. However, there are only a few studies of grape root extract[20-21].

In this study, crude extract and fractions obtained from silica gel chromatography of the wild grape root were assessed for phytochemical content and antioxidant activity by spectrophotometric assay. The correlation coefficient (*r*) between phytochemical content and antioxidant activity was also determined.

MATERIALS AND METHODS

Material and reagents

Wild grape roots were gathered from forest in Roi-Et province, Thailand in June 2018. All chemicals and reagents were analytical grade.

Preparation of wild grape root powder

Wild grape roots were first washed with tap water to remove dirt, and then dried at room temperature. The dried roots were chopped into small pieces, ground to powder, and kept in sealed plastic bag until use.

Extraction of crude extract

Crude extract was prepared following the previously published method[9] with slight modification. Briefly, 15 g of dried root powder were mixed with 300 mL of methanol : hydrochloric acid (99 : 1 v/v) under stirring for 2 h at room temperature. The extracted solution was separated by filtration using a filter paper (Whatman No.1), and kept at 4 °C. The residual solid was re-extracted using 200 mL of the same solvent for 6 hours. The extracted solutions were pooled, evaporated to a slurry using a rotary vacuum evaporator at 45-50 °C to remove solvent. The slurry was dissolved in 50 mL mixture of methanol:water (1:1), then extracted with 3 x 50 mL of petroleum ether for lipid removal. The remaining solution was concentrated under vacuum, mixed with 100 mL of 3.5% (w/v) sodium chloride in water, extracted repetitively with ethyl acetate (6 x 50 mL). The organic layers were pooled, and dried under vacuum. The remaining solid was weighed and dissolved in methanol to obtain 2 mg/mL, and kept at-20 °C until use.

Fractionation of compounds

Silica gel column chromatography was used to fractionate the crude extract using the previously published method[13]. The solvent mixtures used in the elution steps were ethyl acetate:methanol (100:0, 75:25), and methanol:water:acetic acid (87:10:3).

Determination of total phenolic content

Total phenolic content (TPC) of the extracts was analyzed following a previously reported method[12]. Gallic acid was used as standard and TPC was reported as mg gallic acid equivalent/g dry weight (mg GAE/g DW).

Determination of total flavonoid content

The total flavonoid content (TFC) was measured following the previously published method[22] except that quercetin was used as standard. The TFC was expressed as mg quercetin equivalent/g dry weight (mg QE/g DW).

Determination of total proanthocyanidin content

The total proanthocyanidin content (TPAC) was determined based on a previous report[23] except that ethanol was used as solvent for preparation of vanillin. Catechin was used as standard and TPAC was reported as mg catechin equivalent/g dry weight (mg CE/g DW).

DPPH assay

The ability of extracts to scavenge DPPH radicals was evaluated following a previous report[12] except that the absorbance measurement was performed at 517 nm. The scavenging activity was

expressed as IC_{50} value indicating the concentration required to cause 50% inhibition.

ABTS assay

The ability of extracts to scavenge ABTS was evaluated following a previous report[24] except that sample was prepared at various concentrations using methanol instead of ethanol. The scavenging activity was reported as IC_{50} value.

FRAP assay

The ferric reducing antioxidant power of the extracts was determined following previous report[23]. A standard curve was constructed from different concentrations of $FeSO_4$ solution and absorbance values at 593 nm. The FRAP value was reported as μmol Fe^{2+}/g dry weight (μmol Fe^{2+}/g DW).

CUPRAC assay

The cupric reducing antioxidant capacity of the extracts was assayed following a previously published method[25]. Trolox was used as standard and the CUPRAC value was reported as mg Trolox equivalent/g dry weight (mg TE/g DW).

Statistical analysis

The mean ± standard deviation (SD) and Duncan's new multiple range test were used to evaluate the significant differences with $p<0.05$. Pearson's correlation coefficient (r) was used to indicate data correlation.

RESULTS AND DISCUSSION

Crude extract and fractionation by silica gel column chromatography

Based on peak absorbance (A_{280}), fractions eluting from the silica gel column were pooled into three major fractions designed as F1, F2 and F3 that were eluted with ethyl acetate, ethyl acetate: methanol (75:25) and methanol:water:acetic acid (87:10:3), respectively.

Phytochemical contents (TPC, TFC, TPAC)

Table 1 shows the phytochemical contents of crude extract and fractions. TPC was highest in the fraction eluted by ethyl acetate: methanol (75:25) (F2), while fraction F3, eluted by methanol:water:acetic acid (87:10:3) had the lowest content. The crude extract and the fraction eluted by ethyl acetate (F1) had moderate content of the tested phytochemicals, and was not significantly different ($p<0.05$) compared to crude extract. The trend in variation of TFC in the different fractions was similar to that for TPC, which had the highest and lowest contents in F2 and F3, respectively. The TFC of F2 was almost 15X higher than that of F3. Crude extract had the highest TPAC while F3 showed the lowest content.

Table 1: TPC (GAE/g DW), TFC (mg QE/g DW) and TPAC (mg CE/ g DW) of crude and different fractions from wild grape root (mean ± SD, n = 3)

Samples	TPC	TFC	TPAC
Crude	468.90 ± 2.71^{b}	873.14 ± 2.40^{c}	40.91 ± 0.40^{a}
F1	471.23 ± 1.56 ^{b}	974.15 ± 8.33^{b}	15.68 ± 0.04^{c}
F2	552.42 ± 3.18^{a}	1235.95 ± 4.13^{a}	24.84 ± 0.15^{b}
F3	29.21 ± 0.17^{c}	83.33 ± 0.42^{d}	0.46 ± 0.03^{d}

$^{a-d}$Superscripts in each column indicates significant difference ($p<0.05$).

The results indicated that polarity of solvent influenced the phytochemical content of chromatographic fractions of wild grape root extract. The solvent mixture of ethyl acetate: methanol (75:25) was the best mobile phase to elute fractions with high TPC and TFC from silica gel chromatography. This result was in agreement with previous work about effect of solvent elution on fractions of wild grape leaves[13].

DPPH, ABTS, FRAP and CUPRAC assays

Plant phenolic compounds are important secondary metabolites. They have a variety of mechanisms for antioxidant activity[26]. The scavenging activity of the extracts for DPPH and ABTS radicals and an ability to reduce Fe and Cu ions are shown in Table 2.

The reduction of DPPH$^{\bullet}$ resulting from the ability of the test samples to trap radicals caused the loss of absorbance at 517 nm[26-27]. A low IC_{50} value reflected high radical scavenging activity[27]. The results showed that F2 had the highest scavenging activity followed by crude > F1 > F3, respectively. It was interesting to find that F2 has almost 21X more potency as a scavenger against DPPH$^{\bullet}$ than did F3. For the ABTS assay, all samples possessed different levels of radical-scavenging activity which were in the following ranked order: F1 > F2 > crude > F3.

The FRAP assay has also been widely used for reducing metal ions of the antioxidants[26] which reduced Fe^{3+} to Fe^{2+} to form the blue color[28]. In this study, F2 showed the highest FRAP value while F3

showed the lowest FRAP value. The CUPRAC assay was used to measure the reducing power of Cu(II) to Cu(I) by antioxidants[26]. In the reaction mixture, the Cu(II)-neocuproin complex is reduced to the Cu(I)-neocuproin form which shows an absorption maximum at 450 nm[28]. The variation in CUPRAC activity followed similar trends to that found in FRAP activity. The effect of solvents for extraction and elution of crude extract on antioxidant activity was also reported[13,29].

Correlations analysis

The correlation coefficients (*r*) between phytochemical types and antioxidant activity from the various assay methods are presented in Table 3.

Table 2: Scavenging activity by DPPH and ABTS assays and reducing power by FRAP and CUPRAC assays of wild grape root crude and fraction extracts (mean ± SD, n = 3)

Samples	DPPH assay IC_{50} (mg polyphenols/L)	ABTS assay IC_{50} (mg polyphenols/L)	FRAP assay (μmol Fe^{2+}/g DW)	CUPRAC assay (mg TE/g DW)
Crude	21.61 ± 0.00^{c}	13.35 ± 0.02^{b}	413.02 ± 8.04^{c}	706.23 ± 1.87^{c}
F1	24.40 ± 0.17^{b}	7.53 ± 0.02^{d}	472.02 ± 2.86^{b}	957.58 ± 4.30^{b}
F2	14.30 ± 0.07^{d}	8.94 ± 0.03^{c}	548.05 ± 4.91^{a}	1134.76 ± 2.75^{a}
F3	303.21 ± 0.49^{a}	287.30 ± 0.25^{a}	31.76 ± 1.47^{d}	N.D.

$^{a-d}$Superscripts in each column indicates significant difference ($p < 0.05$)
N.D. indicates not detected

Table 3: Pearson's correlation coefficient (*r*) of wild grape root extracts

Trait	TPC	TFC	TPAC	DPPH	ABTS	FRAP	CUPRAC
TPC	1	0.987**	0.759**	-0.991**	-0.987**	0.993**	0.971**
TFC	-	1	0.671*	-0.959**	-0.954**	0.996**	0.992**
TPAC	-	-	1	-0.790**	-0.777**	0.680*	0.585*
DPPH	-	-	-	1	0.999**	-0.975**	-0.942**
ABTS	-	-	-	-	1	-0.973**	-0.941**
FRAP	-	-	-	-	-	1	0.992**
CUPRAC	-	-	-	-	-	-	1

*p<0.05, **p<0.01 indicate significant differences values

TPC showed highly positive correlation coefficient (p<0.01) with TFC and TPAC, and TFC showed moderate correlation coefficient with TPAC. High correlation coefficients between TPC and TFC have been reported by other researchers[13,29]. Highly positive correlation coefficients were also found between DPPH and ABTS, and between FRAP and CUPRAC. Moreover, FRAP and CUPRAC showed high correlation coefficient to TPC and TFC, but moderate correlation coefficient to TPAC. The results suggested that high content of phenolics and flavonoids resulted in high metal reducing power. IC_{50} values have inversed meaning compared with antioxidant activity[20]; therefore, the obtained correlation coefficients (*r*) values where be negative. From the correlation coefficients values, the phenolics and flavonoids had antioxidant mechanisms involving the scavenging of free radicals, but had lower capacity than FRAP assay. Therefore, the FRAP assay would be an appropriate technique for antioxidant activity determination in wild grape root. The result was in accord with the data of Feng and coworkers[30], who found that the FRAP technique could be a good technique to determine antioxidant activity in sugarcane extract since the technique had a higher correlation coefficient with phenolics and flavonoids, compared to the ABTS technique.

CONCLUSION

Silica gel chromatography could partially separate crude extract of wild grape root. The fraction eluted by ethyl acetate:methanol at 75:25 (v/v) (F2) showed highest values of TPC and TFC, while highest TPAC was found in the crude extract. Fraction F2 also demonstrated the highest scavenging of free radicals and reducing power antioxidant activity from all assays, except ABTS assay which showed the highest antioxidant activity in the ethyl acetate fraction (F1). High correlation coefficient between phytochemicals and antioxidant activity suggests that there are substances in the wild grape root that play a role in free radical inhibition through radical scavenging activity, and metal reducing ability are phenolics and flavonoids. The results obtained from this work suggest that wild grape root is a good exogenous antioxidant because it contains high concentrations of phytochemicals with high

antioxidant activity. Further study might involve quantification of phenolic components in each fraction and *In vivo* assay for biological activity to obtain more information of these phytochemicals.

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Conflict of interest

The authors declare no conflict of interest.

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