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Research Article
Screening on Phytochemicals and Comparative Antioxidant Activity of Wild Grape Wine Production Pomaces Extracts

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Abstract

Background and Objective: Wild grape (*Ampelocissus martini* Planch.) fruits are composed of different contents of phytochemicals which posed various biological activities. This work aimed to extract the wild grape pomace from wine production and to screen the phytochemicals and comparative antioxidant activity of the obtained extracts. **Materials and Methods**: Different growth stages of wild grapefruits were separated by colour before fermentation to wine for 15 days. The wild grape pomaces were separated, filtered and dried before ethanolic extraction. The UV-Vis spectrophotometry was used for phytochemical contents determination as well as the antioxidant activity. The correlation between the active compounds and antioxidant activity was also performed by correlation analysis. **Results:** The highest phytochemical contents were found in the immature pomace extract with high contents of total Condensed-Tannins (CDT), Proanthocyanidins (TPAC), Phenolics (TPC) and Saponins (TSC) contents. With DPPH and ABTS radicals scavenging assays, all extracts have low values of IC_{50}, which revealed the highest antioxidant capacity. All extracts showed low reducing power potential by FRAP assay, but have high reducing power on CUPRAC assay. The phytochemicals showed a different correlation to antioxidant activity. **Conclusion:** The wild grape pomace from wine production is a good source of bioactive phytochemicals. This finding results might be used as guide information for further study, especially their pharmaceutical activities.

Key words: Antioxidant activity, extract, phytochemical, pomace, screening, wine, wild grape

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently, the oxidative stress, causing by free radicals, in humans is an interesting issue and increasing study. This stress led to the onset of many types of degenerative diseases caused by free radicals such as cancer, heart coronary disorder disease and brain disorder disease, Parkinson and Alzheimer[1-3]. Previous works revealed that the degenerative diseases caused by free radicals could be protected by a kind of substance called "antioxidant". These substances are divided generally into 2 groups, natural and synthetic substances. Among them, the natural antioxidant is properly proposed according to its safety. The main natural antioxidants are derived from plants, especially vegetables, fruit and herb. The plant-derived antioxidants are called "phytochemicals"[4,5].

Thailand is one of the most biodiversity-rich countries which is composed of various medicinal herbs and fruits, especially in the north and northeast of Thailand. These herbs are composed of many bioactive phytochemicals, which are beneficial effects for health and reduce the harmful of many diseases[6,7]. Moreover, many parts of these Thai fruits or herbs have been reported for their active compounds and activities[8-10].

The grape has been reported as a kind of fruit that composed many types of phytochemicals and all showed various biological activities[3,11,12]. All parts of the grape are reported as a good source of phytochemicals[1,12-14]. Moreover, the grape products such as wine grape are the main product which is popularly consumed worldwide[11,15]. The by-product of the wine process is grape pomace. This pomace has been studied about phytochemicals and their biological activity. The wine grape pomace also has been found many types of phytochemicals with biological activity including antioxidant[16].

Besides the cultivated grape, a herb from northeastern, Thailand namely "wild grape (*Ampelocissus martini* Planch.)" has gained attractive study. This was according to its taxonomy was similar to the commercial grape. We have been reported that the wild grape composed many types of phytochemicals and various activities[17-19].

However, studies on wild grape pomace derived from wine production have never been reported, especially in Thailand. Therefore, this study aimed to work on the wine of wild grapefruit was performed. After that, the wild grape pomaces were separated and extracted with methanol. The obtained crude extract was then screened for phytochemicals and antioxidant activity.

MATERIALS AND METHODS

Study area: All parts of the experiments were done at the Department of Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham, Thailand for 6 months from October, 1, 2020-March, 30, 2021.

Materials: The different growth stages (immature, mature and ripe) of wild grape (*Ampelocissus martini* Planch.) fruits were defined by colours from the author hometown at Roi-Et Province, Thailand. Each fruit stage of the wild grape was used for wine production without yeast. After 2 weeks, the wild grape pomaces were separated, dried and ground to powder.

Methods

Crude extracts and phytochemical screening: The extraction of crude was followed by previous reported[20]. The obtained extracts were then screened for Total Phenolic Content (TPC) by reaction with folin-ciocalteu reagent and using gallic acid as standard (mg GAE/g DW) following the previously described method[21]. The total flavonoid content (TFC) was determined by reaction aluminium chloride ($AlCl_3$) and using catechin as standard (mg CE/g DW) following the previously described method[8]. The total saponin content (TSC) was determined by mixing with the vanillin-ethanol solution following the previously described method[22] and using aescin as standard (mg AES/g DW). Total condensed-tannins content (CDT) was investigated using vanillin-methanol and HCl system following the previously described methods[23], comparing to catechin as standard (mg CE/g DW). The total proanthocyanidin content (TPAC) was also analyzed by vanillin-methanol and HCl system with the previously described methods[24], comparing to catechin as standard (mg CE/g DW).

Antioxidant activity screening: The antioxidant activity of the extracts was investigated by two mechanisms, one is $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals scavenging activity was determined according to a previously published method[21,24]. The 50% Inhibition Concentration (IC_{50}) value on free radicals was calculated to express the activity of the extract. The last mechanism by Ferric Reducing Antioxidant Power (FRAP)[25] and Cupric Reducing Antioxidant Capacity (CUPRAC)[26] were applied in this work.

Statistical analysis: All the assays were performed for triplicate to obtain Mean$\pm$Standard Deviation (SD). Duncan and Pearson's tests were used to find the significance with $p<0.05$ of the data and correlation analysis, respectively.

RESULTS

Screening content of phytochemicals: The yields of the wild grape pomace extracts showed that the mature fruit has the highest percentage (13.752±0.552% w/w), then ripe stage (9.612±0.422% w/w) and the lowest extraction yield was an immature stage (6.497±0.470% w/w). The results revealed that the % yield was a statistically significant difference ($p<0.05$). As shown in Table 1, the crude extract of immature had the highest of all phytochemical contents. In the immature extract, CDT was the predominant substance, followed by TPC, TSC, TPAC and TFC, respectively. The mature extract showed slightly higher TFC, TSC and CDT contents than the ripe extract but in lower TPC and TPAC contents. The order of dominant phytochemicals in the mature extract was similar to the immature extract, while the ripe extract showed switching between TSC and TPAC.

Screening of antioxidant activity: The antioxidant activity of the wine pomace extracts was shown in Table 2. Using DPPH and ABTS assays, all extracts showed low IC_{50} values that mean they have a high potential for free radical scavenging. Among them, the immature had the highest antioxidant activity and then ripe, mature extracts, respectively. Moreover, all extracts had more effect on the ABTS radicals scavenging activity than DPPH. The immature extract was found to have higher antioxidant activity than mature and ripe when analyzed by FRAP and CUPRAC. Furthermore, the reducing power on ferric ion (FRAP) exhibited lower than cupric ion (CUPRAC). In comparison with the growth stage of fruits, all extracts showed cupric-reducing power activity than ferric-reducing power which was about 170% in immature and 115% in both ripe and mature extracts.

Correlation analysis: The correlation analysis was conducted among the total phenolic, flavonoid, saponin, condensed tannin and proanthocyanidin contents and the antioxidant ability (Table 3). The positive correlation means more phenolic contents resulted in the extract having a high antioxidant activity (reducing power). The highest positive correlations ($r>0.900$) were obtained from TPC, TPAC and CDT and both FRAP and CUPRAC. The high positive correlations ($r>0.690$)

Table 1: Phytochemical contents (PC/g DW) the wine pomace extracts in different growth stages

Samples	TPC (mg GAE)	TFC (mg QE)	TSC (mg AES)	CDT (mg CE)	TPAC (mg CE)
Immature	105.444^c±0.347	22.323^c±1.400	77.143^c±3.780	334.222^b±2.673	120.800^c±1.622
Mature	46.500^a±0.882	20.000^b±0.606	51.048^b±1.574	237.333^a±1.333	31.067^a±2.095
Ripe	66.500^b±0.667	18.081^a±0.350	23.571^a±0.714	224.389^a±3.355	56.889^b±1.540

Results are expressed as Mean±SD of triplicate measurements. Mean with different letters in the same column represent significant differences at p<0.05,TFC: Total flavonoid content, TPC: Total phenolic content, TFC: Total flavonoid content, TSC: Total saponin content, CDT: Total condensed-tannin content, TPAC: Total proanthocyanidins content, GAE: Gallic acid equivalent, AES: Aescin equivalent, CE: Catechin equivalent

Table 2: Antioxidant activity of the wine pomace extracts in different growth stages

Samples	DPPH (IC_{50} mg mL^{-1})	ABTS (IC_{50} mg mL^{-1})	FRAP (μM Fe^{2+} g^{-1} DW)	CUPRAC (mg TE g^{-1} DW)
Immature	0.179^a±0.005	0.011^a±0.000	2.165^c±0.010	350.926^c±11.578
Mature	0.282^b±0.018	0.018^c±0.000	1.048^a±0.017	115.370^a±3.902
Ripe	0.189^a±0.005	0.016^b±0.001	1.349^b±0.043	220.741^b±3.699

Results are expressed as Mean±SD of triplicate measurements. Mean with different letters in the same column represent significant differences at p<0.05, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis3-ethylbenzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power, CUPRAC: Cupric reducing antioxidant capacity, IC_{50}: Half minimum inhibitory concentration, mg: Milligram, mL: Milliliter, uM: Micro molar, g: Gram and DW: Dried weight

Table 3: Correlation (r) of phytochemical contents and antioxidant activity of the wine pomace extracts in different growth stages

Factors	TPC	TFC	TSC	TPAC	CDT	DPPH	ABTS	FRAP	CUPRAC
TPC	1	0.636	0.635	0.894**	0.997**	-0.799**	-0.991**	0.996**	0.991**
TFC		1	0.907**	0.889**	0.665	-0.948**	-0.676*	0.691*	0.553
TSC			1	0.676*	0.899**	-0.180	-0.660	0.691*	0.532
TPAC				1	0.916**	-0.761*	-0.992**	0.997**	0.982**
CDT					1	-0.480	-0.913**	0.925**	0.833**
DPPH						1	0.776*	-0.751*	-0.864**
ABTS							1	-0.992**	-0.980**
FRAP								1	0.997**
CUPRAC									1

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), TPC: Total phenolic content, TFC: Total flavonoid content, TSC: Total saponin content, TPAC: Total proanthocyanidins content, CDT: Total condensed-tannin content, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power, CUPRAC: Cupric reducing antioxidant capacity were obtained from TFC, TSC and FRAP while moderate positive correlations were obtained from TFC, TSC and CUPRAC assays. In contrast, negative correlations between the phenolic compounds and IC_{50} value (DPPH and ABTS assays) was observed. The low IC_{50} value corresponds to high antioxidant activity. The TPC exhibited significant negative correlations ($p<0.05$) with DPPH ($r = -0.799$) and to ABTS ($r = -0.991$). Considering TFC, it exhibited high significant negative correlations ($p<0.05$) with DPPH ($r = -0.948$), but was moderate correlation to ABTS ($r = -0.676$). The TPAC exhibited significant negative correlations ($p<0.05$) with ABST (-0.992), but it was high correlation to DPPH ($r = -0.761$). The CDT showed a significant negative correlation on only ABTS assay ($r = -0.913$).

DISCUSSION

Phytochemicals test indicated that the types and contents of phytochemicals from wine pomace dramatically varied by the stages of fruit development. This indicated that the growth stage of fruit is one factor in the phytochemical content[17]. In the immature fruit, the wild grape should be collected various chemical substances for fruit development and those substances were gradually degraded after the mature stage. At the last stage (ripe), the collected or synthesized substances should be applied during growth and remained in the low content. Moreover, the phytochemicals in the plant extract varied by factors such as derived parts, time harvest, instrument analysis, solvents, methods and procedures used and cultivation practices[24].

Interesting in plants' derived substances on human health benefits have been known for long history[27] as well as their activities[28]. Antioxidant activity is one of study and test. However, it is well known that no individual technique is sufficient for the evaluation of antioxidant activity[29]. Therefore, this work used 4 methods for the detection of antioxidant activity including scavenging free radicals (DPPH and ABTS assays) and metal-reducing power (FRAP and CUPRAC assays) mechanism. The results strongly revealed that the phytochemicals in wine pomace are chiefly responsible for their antioxidant property, but with a different mechanism. The conclusion is in agreement with the previous reports[25,30,31]. The difference in the antioxidant activity concerned with the contents and types of phytochemicals in each wine pomace extract which was agreed with the previous works[32]. The phenolic compounds which contain many hydroxyl, ortho-dihydroxyl groups and a double bond in the aromatic ring are good antioxidants and were involved in antioxidant activity[33-36]. This was also confirmed by the correlation test since almost all phytochemicals showed a high correlation to the antioxidant activity assays, especially TPC and TPAC.

In further works, other pharmaceutical activities like antibacterial and anti-enzymatic activities would be tested for wide grape wine pomace extracts to obtain more information.

CONCLUSION

The growth stages of wild grapefruit are directly related to the phytochemicals in the pomaces. The crude extract of the immature showed the highest total phytochemicals content and antioxidant activity. The CDT is the most abundant substance found in the wine pomace extracts. The phytochemical contents exhibited significant positive and negative correlations to their antioxidant activity depending on analysis purpose. The wild grape pomace from wine production might be used as the phytochemical source, promising antioxidant activity via free radicals scavenging and reducing power mechanisms. This would be interesting to focus more studies on other activities for application in health benefits.

SIGNIFICANCE STATEMENT

This work screened phytochemicals in the extract of wild grapefruit pomaces from wine production. The results would be helped the researcher to uncover the critical areas of native herbs from local diversity that are not able to explore by many researchers. Thus, a new theory on wine production residues of wild grapefruits may be arrived at.

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