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Research Article
Determination of Wild Grape Phenolic Compounds in the Extracts of Winemaking Byproducts and Their Antioxidant Competency

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Abstract

Background and Objective: Various medicinal herbs and fruits in Thailand composed of many bioactive phytochemicals, which are support health and reduce the harmful of many diseases. The main objectives of this study were to extract wild grape residues obtained from wine production and fractionate them by silica column chromatography and investigate the chemical substances and antioxidant competency. **Materials and Methods:** Methanolic crude extract of wild grape pomace was fractionated by silica gel chromatography using the mixture methanol/ethyl acetate as eluting solvents. The chemical substances including total phenolic, flavonoid, saponin and condensed-tannin were investigated by colorimetric spectrophotometer. The antioxidant activities with free radical scavenging (DPPH and ABTS) and reducing power antioxidants (FRAP and CUPRAC) were tested. Finally, High Performance Liquid Chromatography (HPLC) was applied for the analysis of the individual phenolic compounds. **Results:** The fractionated extracts had higher chemical substances than crude extract, except total phenolic. Among the substances, condensed-tannins showed the highest content in the fractionated extracts. The active substances showed higher ABTS free radical scavenging activity than DPPH and metal-reducing power antioxidant by CUPRAC than FRAP assays. The dominant phenolic substances in the fractionated extracts were gallic acid, resveratrol, quercetin, epicatechin and caffeic acid. **Conclusion:** The pomace of immature wild grape fruits from wine production contained various types of chemical substances and antioxidant competency. The obtained results provide more information on the wild grape fruits in terms of phytochemical source and their activity.

Key words: Fractionation, residues, substances, wild grape, wine

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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

Medicinal plants have been used for treatment and disease prevention worldwide for a long history since they are composed of various chemical substances and safe utilization[1-3]. The chemical substances in plants revealed various biological activities including antioxidant activity[4-6]. With these results, the plant-derived substances have been applied in health supplement products and cosmetics[7]. However, the biodiversity of plants in the world is known which were the goal for the study of a new source of phytochemicals and their biological activities[8-10].

Ampelocissus martinii Planch. or wild grape in a local name, has been interested in biological potency and its phytochemical composition. This was due to it showing all parts similar to the cultured grape which proved high phytochemicals and excellent biological properties[4,11-13]. The people in the northern and northeastern of Thailand generally used this plant for relied upon some symptoms as the herb. The deep information on its phytochemicals and biological activities have been studied[14,15]. However, the chemical substances in wine products of wild grape are rarely reported, especially in wine pomace. To expand more informative data on the chemical composition in wild grape pomace from wine production, the fractionation of crude extract was performed before the determination of its phytochemicals. To confirm the active ingredient potential, antioxidant activity was also tested and discussed in this study.

MATERIALS AND METHODS

Study area: The experiment was done at the Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand for 6 months from January 1 to June 30, 2021.

Materials: The fresh and green of wild grapefruits were collected by colours from August-October, 2020, at the forest in Roi-Et Province, Thailand. The fruits were washed with tap water followed by distilled water before storing at -4°C until further process.

Methods

Fractionation process: The wild grape pomaces were firstly separated from wine for crude extraction by ethanol using Soxhlet. The extract was concentrated by the evaporation technique. The ethanol was used for dissolving the crude extracts before loading them on a silica gel glass column. The different polarity of ethyl acetate/methanol mixture (100, 70:30, 50:50, 70:30 and 100) was prepared for substance elution. The collection volume of the eluting mixture was then measured at 280 nm for checking the purposed phenolic compounds and changed other eluting solvents.

Determination of phytochemicals: Different types of phytochemicals were determined following previous reports. Total Phenolic Content (TPC) using gallic acid as standard, Total Flavonoid Content (TFC) using catechin as standard, Total Saponin Content (TSC) using aescin as standard and total Condensed Tannins content (CDT) using catechin as standard were investigated[16-18].

Antioxidant competency: The antioxidant competency of the fractionated extracts was investigated by various assays of colorimetric spectrophotometer. $DPPH^{\bullet}$ radicals scavenging activity was determined according to a previously published method[19] and expressed by the 50% inhibition (IC_{50}) value. The $ABTS^{\bullet+}$ radicals scavenging activity was also determined using colorimetric analysis[16]. The metal-reducing power activity was determined by the ferric reducing antioxidant power (FRAP)[19] and cupric reducing antioxidant capacity (CUPRAC)[20]. The FRAP results were expressed as μM Fe^{2+} g^{-1} DW, while CUPRAC results were expressed as milligrams Trolox equivalent per gram dry weight (mg TE g^{-1} DW), respectively.

Quantification of chemical substances: A reversed-phase HPLC-UV system and a diode array detector using column Inertsil ODS-3, C18 was used for determination of individual chemical substances[21]. The variable UV-diode array wavelength at 280, 320, 306 and 360 nm was applied for 10 external standards gallic acid, catechin, epicatechin, caffeic acid, p-coumaric acid, ferulic acid, resveratrol, quercetin, rutin and myricetin. The relative retention times, peak areas and UV spectra of phenolic compounds were identified against an external standard method.

Statistical analysis: All experiment was performed in triplicate and expressed by Mean±Standard Deviation (SD). The significant differences with $p<0.05$ were analyzed by Duncan's new multiple range test.

RESULTS

Phytochemical content: The chemical substances known as phytochemicals in many sub-fractions of wine pomace of wild grape (*A. martinii* Planch.) are presented in Table 1. The Total Phenolic Content (TPC) of all fractionated extracts was

Table 1: Chemical substances in the crude and fractionated extracts of winemaking byproducts

Samples	TPC (mg GAE g^{-1} DW)	TFC (mg QE g^{-1} DW)	TSC (mg AES g^{-1} DW)	CDT (mg CE g^{-1} DW)
Crude	105.44±0.35^{f}	22.32±1.40^{a}	77.14±3.78^{a}	334.22±2.67^{a}
SF-1	6.28±0.06^{a}	54.80±0.44^{e}	390.4±4.128^{c}	833.33±0.33^{c}
SF-2	19.22±0.50^{b}	45.81±0.09^{d}	314.2±4.299^{b}	488.89±3.89^{b}
SF-3	27.50±0.55^{c}	36.31±0.46^{c}	697.62±3.76^{d}	1184.44±5.40^{d}
SF-4	37.94±0.10^{e}	33.28±0.76^{b}	319.05±8.25^{b}	1851.11±3.85^{f}
SF-5	30.17±0.44^{d}	37.32±1.27^{c}	324.29±7.43^{b}	1562.22±3.88^{e}

Triplicate values of each measurement were expressed as Mean±SD, different letters in the same column showed values significant differences at $p<0.05$, ND: Non-detected, SF: Sub-factions, TPC: Total phenolic content, TFC: Total flavonoid content, TSC: Total saponin content and CDT: Total condensed-tannins content

Table 2: Antioxidant competency of the crude extract of winemaking byproducts compared to fractionate samples

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)
Crude	1.80±0.00^{a}	1.10±0.03^{a}	1.16±0.01^{b}	35.93±1.58^{a}
SF-1	2.3±0.018^{b}	4.68±0.02^{c}	1.32±0.31^{a}	39.59±1.24^{a}
SF-2	2.10±0.00^{b}	2.01±0.02^{b}	3.00±0.73^{c}	77.2±5.406^{b}
SF-3	1.80±0.00^{a}	1.6±0.025^{b}	5.24±0.34^{d}	151.48±4.40^{d}
SF-4	2.80±0.00^{c}	1.90±0.03^{b}	3.87±0.97^{c}	107.37±5.95^{c}
SF-5	3.58±0.01^{d}	2.08±0.01^{b}	2.26±0.31^{b}	62.78±5.56^{b}

Triplicate values of each measurement were expressed as Mean±SD, different letters in the same column showed values significant differences at $p<0.05$, ND: Non-detected, FRAP: Ferric reducing antioxidant power, CUPRAC: Cupric reducing antioxidant capacity, DPPH: 2,2-diphenyl-1-picrylhydrazyl and ABTS: 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic acid

determined by the Folin-Ciocalteu method, which was calculated according to the equation as gallic acid equivalent (mg GAE g^{-1} DW). The total phenolic contents of crude extract showed 105.44±0.35 mg GAE g^{-1} DW. The TPC of sub-fractions showed lower content than that of the crude extract and have between 6.28-37.94 mg GAE g^{-1} DW depending on the eluting solvents. Among them, sub-fraction 4 (SF-4) showed the highest TPC (37.94 mg GAE g^{-1} DW). The TFC was investigated by colorimetric aluminium chloride assays and calculated according to the equation as quercetin equivalents (QE mg g^{-1} DW). Results for the TFC of all sub-factions showed values between 36.31-54.80 mg QE g^{-1} DW, which were higher content than crude extract (22.32 mg QE g^{-1} DW). As among fractionated extracts, the SF-1 extract contained the highest contents of flavonoids (54.80 mg QE g^{-1} DW), compared to other extracts. The Total Saponin Contents (TSC) were investigated by colorimetric vanillin assays and calculated according to the equation as aescin equivalents (mg AES g^{-1} DW). The TSC of all sub-factions showed values between 314-698 mg AES g^{-1} DW which were higher content than crude extract (77.14 mg AES g^{-1} DW). The SF-3 extract contained the highest contents of saponins (698 mg AES g^{-1} DW), compared to other fractionated extracts. The total condensed-tannin contents (CDT) were investigated by colorimetric vanillin assays and calculated according to the equation as Catechin Equivalents (mg CE g^{-1} DW). The CDT of all sub-factions showed higher values than the crude (334.22 mg CE g^{-1} DW) between 488-1851 mg AES g^{-1} DW. The SF-4 extract contained the highest contents of condensed-tannins (698 mg AES g^{-1} DW), compared to other fractionated extracts.

Determination of antioxidant competency: The antioxidant competency was tested for different actions, free radical scavenging and metal ion reducing power. The results for antioxidant competency showed in Table 2. The fractionated extracts showed the difference of DPPH scavenging activity with IC_{50} of 1.80- 3.58 mg mL^{-1}. Besides the fractions, SF-3 has the lowest IC_{50} value and was similar to IC_{50} of the crude extract (1.80 mg mL^{-1}). With the $ABTS^{\bullet+}$ radical scavenging test, the results found that the fractionated extracts showed lower IC_{50} values (1.65-4.68 mg mL^{-1}) than that by DPPH method, except SF-1 extract (2.38 and 4.68 mg mL^{-1}). Comparison antioxidant competency of crude extracts tested between ABTS and DPPH methods, the $ABTS^{\bullet+}$ radical scavenging activity has lower IC_{50} values (1.10 mg mL^{-1}) than DPPH radical. By FRAP assay, the fractionated extracts have activities arranged between 1.32-5.24 μM, higher than crude extract (1.16 μM Fe^{2+} g^{-1} DW). The cupric-reducing power activity (CUPRAC assay) showed a similar trend with FRAP assay since the fractionated extracts (39.59-151.48 mg TE g^{-1} DW) have metal-reducing power activity higher than the crude extract (35.93 mg TE g^{-1} DW). Among the fractionated extracts, SF-3 extract has the highest reducing power activity in both FRAP and CUPRAC assays.

Chemical substances identification: The individual chemical substances in the fractionated extracts are presented in Table 3. The dominant phenolic compounds in the crude extract were gallic acid (1.43 mg g^{-1} DW), resveratrol (1.87 mg g^{-1} DW), epicatechin (1.07 mg g^{-1} DW) and quercetin (3.53 mg g^{-1} DW). The fractionated extracts found variable substance contents. The SF-1 showed a high content of gallic

Table 3: Individual chemical substances (mg g^{-1} DW) comparison between crude and fractionated extracts analyzed by RP-HPLC

	Samples					
Substances	Crude	SF-1	SF-2	SF-3	SF-4	SF-5
Gallic acid	1.43±0.00^{d}	1.04±0.02^{d}	1.52±0.00^{c}	0.90±0.00^{c}	0.93±0.00^{c}	1.05±0.00^{c}
Caffeic acid	0.28±0.00^{b}	0.41±0.00^{b}	0.51±0.00^{b}	0.32±0.00^{b}	0.30±0.00^{b}	0.30±0.00^{b}
P-coumaric acid	0.10±0.00^{a}	0.11±0.00^{a}	0.14±0.00^{a}	0.17±0.00^{a}	0.17±0.00^{a}	0.17±0.00^{a}
Ferulic acid	0.31±0.10^{b}	0.17±0.00^{a}	0.18±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}	0.16±0.00^{a}
Resveratrol	1.87±0.08^{e}	0.72±0.02^{c}	1.07±0.04^{a}	4.19±0.09^{e}	4.54±0.10^{e}	1.10±0.20^{c}
Catechin	0.10±0.00^{a}	0.13±0.00^{a}	0.40±0.15^{b}	0.10±0.01^{a}	0.26±0.02^{b}	ND
Epicatechin	1.07±0.03^{c}	0.10±0.01^{a}	0.14±0.01^{a}	0.19±0.01^{a}	0.11±0.01^{a}	ND
Quercetin	3.53±0.04^{f}	1.89±0.04^{e}	1.94±0.02^{d}	1.51±0.08^{d}	1.77±0.01^{d}	1.98±0.02^{d}
Rutin	0.13±0.01^{a}	0.12±0.00^{a}	0.15±0.00^{a}	0.12±0.00^{a}	0.15±0.00^{a}	0.10±0.01^{a}
Myricetin	0.12±0.00^{a}	0.11±0.00^{a}	0.10±0.00^{a}	0.12±0.01^{a}	0.12±0.02^{a}	0.17±0.00^{a}

Triplicate values of each measurement were expressed as Mean±SD, different letters in the same column showed values significant differences at $p<0.05$ and ND: Non-detected

acid (1.04 mg g^{-1} DW), quercetin (1.89 mg g^{-1} DW) and resveratrol (0.72 mg g^{-1} DW). The main chemical substances found in SF-2 and SF-3 and were similar the SF-1 including gallic acid (1.52 and 0.90 mg g^{-1} DW), quercetin (1.94 and 1.51 mg g^{-1} DW) and resveratrol (0.72 and 4.19 mg g^{-1} DW). Moreover, both fractions also found caffeic acid (0.51 and 0.32 mg g^{-1} DW).The SF-4 and SF-5 composed of chemical substances similar trend with different contents of gallic acid (0.93 and 1.05 mg g^{-1} DW), quercetin (1.77 and 1.98 mg g^{-1} DW) and resveratrol (4.54 and 1.10 mg g^{-1} DW). Other chemical substances but with low content were also identified. However, 2 types of flavonoids, catechin and epicatechin were not detected in the SF-5.

DISCUSSION

The plant is used as medicinal remedies according to it is rich in phytochemicals and possess various biological effects[5,22,23]. Many parts of the plant as well as their products including wine composed high chemical substances and biological activities[13,21,24]. The crude and fractionated extracts of winemaking by-products composed various types of phytochemicals and variable contents. Total phenol investigation indicated that the CDT found in this study has dramatically higher than that in the grape seed but flavan-3-ols (catechin and epicatechin) as well as flavonols (quercetin, myricetin) or natural pigment (rutin), were ranged in lower contents[25]. Using HPLC, almost tested phenolics in this study were in lower content than juice-byproducts (peach, guava and mango), except quercetin and caffeic acid[26]. Another study on grape, pomace and wine from 3 red varieties grown in Argentina found that all most phenolic profiles showed higher content than this study, except caffeic acid and gallic acid of grape and pomace were lower. Moreover, the extract of winemaking byproduct from wild grape composed of resveratrol in higher content than that in the 3 red varieties[27]. Results from Table 1 and 3 showed that the eluting solvent mixture between methanol and ethyl acetate related to the chemical structure of substances. This finding was in agreement with another study[4]. All phenolic compounds composed of benzene ring at least 1 unit in their structure. This resulted to act as a hydrophobic part of the substances. The phenolic compounds decreased their polarity when the benzene unit increased. Therefore, the eluting solvent is a key point for the extraction of the proposed phytochemicals[27]. However, phytochemical contents varied by different factors[12,28,29]. The phytochemical contents found to be concerning their antioxidant activity agree to other reports[5,26]. Two antioxidant mechanisms, scavenging and metal ion reducing power were applied in this study. The DPPH and ABTS methods were used to measure free radical scavenging activity by testing the oxidation reaction of the extracts. The antioxidant activity of all fractions as shown in Table 2 was variable antioxidant competency. This might be due to the different contents and types of phytochemicals in each fractions[5]. Among the tested results, the extracts showed specific action on ABTS in higher competency than that on DPPH. In addition, the fractionated extracts showed higher reducing antioxidant power (FRAP and CUPRAC) than that crude extract. The different activities vastly depend on phenolic substances both types and contents[30,31]. The substances composed of more aromatic rings in their structures such as flavonoids, saponins and condensed-tannins could have interacted well with metal ions via coordinate linkages[17]. Moreover, phenolic compounds which contain many hydroxyl groups like phenolic acids are known

as good antioxidants[32-35]. The finding results in this study surely suggested that all extracts are composed of many types of phenolic compounds.

In future study, *in vitro* assay for biological activity such as enzymatic inhibition effect, antibacterial activity or inhibition protein denaturation would be further performed for confirming the health benefit of these fractionated extracts.

CONCLUSION

The wine production pomaces extract both crude and fractionation samples composed of various chemical substances and exhibit antioxidant activity by free radical scavenging and reducing antioxidant power. Polyphenol compounds including flavonoids, saponins and condensed-tannins were the main chemical substances in the extract, especially condensed-tannins. All fractionated extracts expressed lower free radical scavenging activity than the crude but in higher reducing antioxidant power. The results from HPLC indicated that all extracts composed all tested substances with variable contents, except for SF-5. Among the individual substances, quercetin, resveratrol was the highest content of flavonoid, while the highest content of phenolic acid was gallic acid. This study indicated that by-product from wine production from wild grape pomace is a good source of active compounds. It would be interesting for more studies on its biological activities.

SIGNIFICANCE STATEMENT

This study discovers the chemical substances in wild grapefruit pomaces from wine production and their antioxidant competency. The obtained results can be beneficial for basic information about the source of active substances. This study will gain the benefits data for the researcher to uncover the critical areas of local help into the important source that other researchers were not able to explore. Thus, more data information on wild grapefruit pomaces activities may be arrived at.

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