

Research Article

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PHYTOCHEMICAL AND BIOLOGICAL ACTIVITIES IN FRESH JUICE EXTRACTS OF WILD GRAPE (AMPELOCISSUS MARTINI PLANCH.) FRUITS

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ABSTRACT

The fresh juice extracts of wild grape (*Ampelocissus martini* Planch.) fruits in different colors; green, red and black were used for investigation of their phytochemicals and antioxidant activity. This fruit is much similar when compared to the cultivated grape, an important source of phytochemicals, and never been reported about their biological activities. The fresh juice extracts were evaluated for total phenolic content (TPC), and total flavonoid content (TFC) by using Folin-Ciocalteu assay and colorimetric aluminum chloride assay, respectively. The antioxidant activities were carried out using DPPH and FRAP assays. In addition, the antibacterial activity of the extracts was also performed by agar well diffusion method. The fresh juice extract of wild grape fruits showed high contents of TPC and TFC, especially the juice extract from black color (11.37 \pm 0.25 mg GAE/g FW and 19.41 \pm 0.30 mM FeSO₄/g FW for TPC and TFC, respectively). However, the fresh juice extracts from green color exhibited the highest antioxidant activities. In contrast, the antibacterial activity of the juice extract of green color. In conclusion, this work confirmed the strong antioxidant and antibacterial activities of wild grape fruits extracts of green color. In conclusion, this work confirmed the strong antioxidant and antibacterial activities of wild grape fruits extracts. The result suggested that this fruit might be used as a powerful source of phytochemicals and biological activities.

Keywords: Ampelocissus martini Planch., Antioxidant, Antibacterial, Phytochemical

INTRODUCTION

In recent years, studies on phytochemical extracted from plants have been gradually increased.¹⁻⁴ Since many phytochemicals were believed to protect the body from free radicals⁵ which are released when cells or the body are in oxidative stress and result in damage of living systems. Sometime, this damage is related with diseases and disorders such as cancer, multiple types of cardiovascular diseases, immune diseases, and aging.6 It is well known that the product of oxidation stress can be treated by substances called antioxidant. Antioxidants are substances that delay or prevent oxidative stress even present at low quantity.⁷ Plants have been reported as an important source of many substances that could be used in prevention of human diseases. From the past, there are strong evidences that medicinal plants are being used for treatment of diseases and revitalizing body system worldwide, especially in ancient civilizations including Thailand. Until now, medicinal plants are an important part of traditional medicine.8 Phytochemicals such as phenolics and flavonoids are classified as secondary metabolites.9 They usually comprised of various biological activities¹⁰ and are being used as pharmaceutical drugs.¹¹⁻¹⁴ Over past decades, synthetic substances were increasingly denied; therefore, plant metabolites have been explored and applied for medical application.15

Wild grape (*Ampelocissus martini* Planch.) is a native tropical plant found generally in the north and northeast of Thailand. The fruit of wild grape and stage of fruit development is very similar to cultivated grape. Therefore, the wild grape fruit may be contained of some

secondary metabolites as well as their biologicals. As far as our literature is concerned, the information on phytochemical and biological activities of wild grape fruit is rarely available. Thai people (especially in the northeast region) have been consuming it as food and used as medicine for a long history. Therefore, the aim of this work was to screen some phytochemicals and evaluate the biological activities of the wild grape fruit extracts.

MATERIALS AND METHODS Plant Material

Fresh fruits of wild grape (*Ampelocissus martini* Planch.) were collected from Kheeleg village, Tambon Huayhinlad, Suwannaphumi district, Roi-Et province, Thailand in August 2012. The fruits were washed twice with water and grouped (green, red and black colors). All fruits were kept at 4 °C and used as required.

Preparation of Fresh Juice

The wild grape fruits of each group were weighed and extracted by hand fingers forcing to obtain the fresh juice at room temperature. The yield of the extract was about 30% (w/v) in terms of starting material. The fresh juice extract was filtrated using Whatman No. 1 paper filter, and then stored in refrigerator at 4 °C (less than 35 h) for further studies of phytochemicals and biological activities.

Chemicals

The DPPH (2,2-diphenyl-1-Picrylhydrazyl) was purchased from Sigma-Aldrich (Singapore). Aluminium

chloride (AlCl₃) was purchased from Merck (England). Ferric chloride hexahydrate (FeCl₃·6H₂O) and Folin-Ciocalteu's reagent were purchased from Carlo Erba Reagents. 2,4,6-Tri (2-pyridyl)-s-triazine ($C_{18}H_{12}N_6$) was purchased from Acros organics. (±)-catechin hydrate ($C_{15}H_{14}O_6$), ferrous sulphate heptahydrate (FeSO₄·7H₂O) and L-ascorbic acid ($C_6H_8O_6$) were purchased from Univar. All other chemicals and reagents were of analytical grade.

Evaluation of Total Phenolic Content

The amount of total phenolic content (TPC) in the crude extract of wild grape fruits was determined using the Folin-Ciocalteu reagent according to the method of Bonoli et al.¹⁶ by using gallic acid as a standard. For the modified procedure, fifty microliters of crude extract was mixed with 3 mL of 10% Folin-Ciocalteu reagent (diluted 10 fold with distilled water). The mixture solution was stand at room temperature for 15 min. After that 1.5 mL of 10% (w/v) sodium carbonate solution was added to the mixture and then left at room temperature for 15 min. The absorbance of all samples was measured at 750 nm using an UV-Vis spectrophotometer (UV-1610, Shimadzu). The experiment was carried out in triplicate and averages of values were calculated. The TPC was analyzed against gallic acid calibration curve standard and expressed as milligrams of gallic acid equivalents (mg GAE) per grams of fresh weight (g of FW).

Evaluation of Total Flavonoid Content

The total flavonoid content (TFC) of crude extract was evaluated according to the modified method of Yang et al.¹⁷ Two hundred and fifty microliters of crude extract was mixed with 1.25 mL of deionized water, 75 μ L of 5% sodium nitrite (NaNO₂) solution and allowed to stand for 5 min at room temperature. One hundred and fifty microliters of 10% aluminium chloride (AlCl₃) were added to the mixture solution and left to react for 6 min at room temperature. Five hundred microliters of 11M sodium hydroxide (NaOH) and 775 μ L of distilled water were added to the mixture. The absorbance of all samples was immediately measured at 510 nm. TPC was calculated using the standard curve of (±)-catechin, and expressed as milligrams of catechin equivalents (mg CE) per gram of fresh weight (g of FW).

Free-Radical Scavenging Activity

Free radical scavenging activity of crude extract was determined by using a stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) following a modified method of Chan et al.¹⁸ A total of 1.0 mL of crude extract was added to 2.0 mL of 0.1 mM DPPH solution. The mixture solution was incubated at room temperature in a dark room for 30 min. Absorbance of all samples was measured at 517 nm using an UV-Vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following equation;

Radical scavenging activity (%) = $[A_{control} - A_{sample}] / A_{control} \times 100$

Where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the crude extract. BHA dissolved in methanol was also analyzed as control. DPPH radical scavenging activity was expressed as IC_{50} value, which represented the amount of antioxidant in the crude extract necessary to reduce the initial DPPH concentration by 50%. The experiment was performed in triplicates.

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing power of the crude extract was detected using a ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain¹⁹ with some modifications. Briefly, the fresh solution of FRAP reagent contained 2.5 mL of 10 mL 2,4,6-Tri (2- pyridyl)-striazine (TPTZ) solution in 40 mM HCl with 2.5 mL of mM FeCl₃ and 25 mL of 0.3M acetate buffer pH 3.6 was freshly prepared. The 20 µL of crude extract was mixed with 180 µL of FRAP reagent and allowed to stand at 37 ⁰C for 4 min. The absorbance of the mixture solution was measured at 593 nm using UV-Vis spectrophotometer. The ethanolic solution of known Fe (II) concentration in the range of 50-500 µM (FeSO₄) was used as calibration curve. The ferric reducing ability of the crude extracts was expressed as mM of FeSO₄ equivalent concentration (EC) per 100 gram of fresh weight (FW). BHT and quercitin was used as positive controls. The experiment was performed in triplicates.

Antibacterial Activity

The different 17 types of pathogenic bacteria was chosen as substrate for determination of antibacterial activity of the juice extract of wild grape (*Ampelocissus martinii* Planch.). All bacteria including *S. typhi* (DMST 5784), *S. flexneri* (DMST 17569), *E. cloacae*, *S. aureus* (ATCC 25293), *S. typhi* (gr. D), *S. paratyphi* (ATCC 14028), *S. typhi* (DMST 16122), *S. flexneri* (DMST 4423), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), Enterobacter sp, *B. cereus* (ATCC 11778), *E. coli* (0157:H7 DMST 12733), *Ps. aeruginosa*, *S. aureus* (MRSA DMST 20625), *K. pneumonia* and *S. dysenteriae* were cultured in Mueller-Hinton broth at 37°C for 48 h. The cultured bacteria were diluted with 0.84% normal saline by adjusting turbidity of bacterial suspension as equal to McFarland No. 0.5 for obtaining bacterial density of about 1.5×10^8 cell/mL.

Antibacterial Activity of Aqueous Extracts

The inhibition activity on bacteria of aqueous extract was tested using Agar well diffusion method. The 1 mL of cultured bacteria at equal turbidity of McFarland No.0.5 was swabed and placed into the surface of Mueller-Hinton Agar. The agar media was punctured into 3 holes per culture plates of 0.5 cm diameter. Twenty five micro-liters of the juice extracts were poured into 2 holes of agar and another hole was used as control (without the juice extract). The culture plates were incubated at 37 °C for 24 h. Finally, the diameters of inhibition zones (DIZ) were measured in millimeter (mm) and were recorded as the mean of triplicate experiments. Moreover, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the fresh juice extracts were carried out using agar two folds serial dilution assay.

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Table 1: DPPH radical scavenging and reducing capacity (FRAP)) of the fresh juice extract of wild grape
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Colors	DPPH radical scavenging	Ferric reducing antioxidant power (FRAP)
	$IC_{50} (\mu g/mL) \pm SD$	$(mM FeSO_4/100g FW) \pm SD$
Green	0.974 ± 0.882	304.740±0.555
Red	39.620±2.556	365.000±0.962
Black	1.380±0.071	612.120±1.923

Table 2: Antibacterial activity of the fresh juice extract of wild grape against pathogenic bacteria

Bacterial strains	Diameter of zone of inhibition (mm)		
	Green	Red	Black
S. typhi DMST 5784	-	14	-
S. flexneri DMST 17569	-	13	-
E. cloacae	-	11	-
S. aureus ATCC 25293	12	11	-
S. typhi gr. D	-	16	-
S. paratyphi ATCC 14028	-	13	-
S. typhi DMST 16122	-	13	20
S. flexneri DMST 4423	13	15	19
E. coli ATCC 25922	10	20	-
S.typhimurium ATCC 14028	-	-	-
Enterobacter sp.	13	15	-
B. cereus ATCC 11778	-	13	-
E.coli 0157: H7 DMST 12733	13	11	-
Ps. aenainosa	-	-	-
S. aureus MRSA DMST 20625	11	13	10
K. pneumoniae	-	-	-
S. dysenteriae	-	16	-

*(-) mean no activity

Table 3: MIC and MBC values of the fresh juice extract of wild grape against selected pathogenic bacteria

Bacterial strains	Juice colors	MIC (µg/mL)	MBC (µg/mL)
S. typhi gr. D	Red	500	500
S. typhi DMST 16122	Black	250	250
S. flexneri DMST 4423	Red	250	250
S. flexneri DMST 4423	Black	250	250
S. dysenteriae	Red	250	250
E. coli ATCC 25922	Red	500	500

Statistical Analysis

Data were expressed as means \pm standard deviations (SD) of triplicate experiments.

RESULTS

Total Phenolic and Flavonoid Contents

The fresh juice extracts of wild grape (Ampelocissus martini Planch.) fruits in different colors (green, red and phytochemical black) were analyzed for their compositions. The results indicated that wild grape fruit is rich in total phenolic (TPC) and flavonoid (TFC) contents. The highest TPC was found in black color (11.37±0.25 mg GAE/g FW), followed by green (3.63±0.03 mg GAE/g FW), and then red color $(2.51\pm0.12 \text{ mg GAE/g FW})$ with gallic acid as standard. The fresh juice extract of wild grape was good source of TFC which was indicated as mg cathecin equivalent per g of FW. The TFC of all extracts were higher than 10 mg CE/g FW. The highest TFC value was found in the juice of black color (19.41±0.30 mg CE/g FW), followed by green (16.83±0.04 mg CE/g FW), and red color (12.97±0.03 mg CE/g FW), respectively.

Antioxidant Activity

The antioxidant activity of the juice extract was analyzed using DPPH scavenging assay and ferric reducing antioxidant power (FRAP) assay. The DPPH assay results are shown in Table 1. The IC₅₀ was expressed as the concentration of antioxidant comprise in the extracts, able to decrease the DPPH amount by 50%. Almost extracts showed free radical scavenging capacity, especially green and black colors. Their capacities were indicated IC₅₀ of 0.97±0.88 µg/mL and 1.38±0.07 µg/mL, respectively while red color was 39.62±2.56 µg/mL. Moreover, the reducing ability (FRAP values) of the juice extracts of wild grape fruits was about 304.740±0.555, 365.000±0.962 and 612.120±1.923 mM FeSO₄/100g FW for green, red and black colors, respectively.

Antibacterial Activity

Antibacterial activity of the juice extract of wild grape (*Ampelocissus martini* Planch.) fruits was assayed by agar well diffusion method against 17 bacterial strains (Table 2). The juice extract from green color was highly effective against *S. aureus* ATCC 25293, *S. flexneri* DMST 4423,

E. coli ATCC 25922, Enterobacter sp., E.coli 0157:H7 DMST 12733 and S. aureus MRSA DMST 20625 with inhibition zone ranging from 10-13 mm while it has not shown antibacterial activity for S. typhi DMST 578, S. flexneri DMST 17569, E. cloacae, S. typhi gr. D, S. paratyphi ATCC 14028, S. typhi DMST 16122, S.typhimurium ATCC 14028, B. cereus ATCC 11778, Ps. Aenainosa, K. pneumonia and S. dysenteriae. All the juice extracts were not effective against 3 strains of pathogenic bacteria; S.typhimurium ATCC 14028, Ps. aeruginosa and K. pneumoniae. The juice extracts of black color had the lowest antibacterial activity. It was found to have antibacterial activity only against S. typhi DMST 16122 (20 mm), S. flexneri DMST 4423 (19 mm) and S. aureus MRSA DMST 20625 (10 mm). The results indicated that the juice extract from red color of wild grape fruits showed the highest effective antibacterial activity against all tested bacterial strains, except S. typhimurium ATCC 14028, Ps. aeruginosa and K. pneumoniae. The more effective antibacterial activity against S. typhi gr. D. S. typhi DMST 16122, S. flexneri DMST 4423, E. coli ATCC 25922 and S. dysenteriae were chosen for MIC and MBC assay. As shown in Table 3, the MIC and MBC values were found in range of 500-250 µg/mL of selected juice extracts.

DISCUSSION

Many kinds of fruits, vegetables, spices and medicinal plants have been reported to be good sources of phytochemicals. These phytochemicals have been found to play protective roles against chronic degenerative diseases.^{20,21} The phytochemicals including polyphenols, carotenoids and vitamin were found to be important for study and interested since they were found more effective in activity on human health.²² In addition, these phytochemicals are also composed of biological activities such as antioxidant and antimicrobial activities. This work was attempted to screen some phytochemicals, especially phenolics and flavonoids in the fresh juice of wild grape (Ampelocissus martini Planch.), a local herbal medicinal plant of Thailand. Phenolics are secondary metabolite products in plant which are important on the growth of The quantitative investigation of the plants.²³ phytoconstituent of juice extracted from different colors of wild grape fruits are moderately existed with total phenolics content (TPC) and total flavonoids (TFC). The contents of phytochemicals were varied depending on the colors of fruit. The black color showed the highest value in both phenolics and flavonoids in comparison to other. With reference to previous reports, the activities as well as the phytochemical compositions in plants were affected by many factors such as cultivars, maturity, environmental factors, colors as well as the types and quantity of phytochemicals.^{21,24} Many reports about the relationship between phytochemicals and antioxidant activity were found.^{20,25,26} Polyphenol and flavonoid are used for prevention of various diseases caused from free radicals.927 The phenolics act as terminators of free radical from oxidation reaction, while flavonoids are responsible for the radical scavenging effects.⁸ Generally, the extract with high total phenolic contents had higher antioxidant activity.^{17,28,29} The results in this work

indicated that green and black colors of wild grape fruits composed of antioxidant activity in higher potential both DPPH and FRAP assays. This may affected from the chlorophyll components of green color³⁰ and the anthocyanin in the black color.²⁵ The function of reducing power of the phytochemicals presented in the juice was reflected by the antioxidant capacity. The antioxidant capacity increased with increasing concentration in all samples. Many studies have been shown that flavonoids and phynolics contribute significantly to the total antioxidant activity of many fruits including grape, vegetable and medicinal plants.³¹⁻³³ Wild grape is similar to cultivated grape [Genus Vitis] including stem and fruit. The cultivated grape has been reported as a rich source of phytochemicals and shown to have protective effects on many regenerative diseases.^{17,34} However, study on phytochemical composition in wild grape has been rarely available information. The phytochemicals and antioxidant activity of the wild grape juice found to be the same as with the extracts from grape. The wild grape juice possessed high degree of antibacterial activity, especially on Gram negative bacteria over 14 selected strains. This suggested that the antibacterial activity of the fresh juice may be due to the presence of different kinds of phytochemicals. It is well known that the secondary metabolites are called phytochemicals and were produced in plant against microbial pathogens. Previous reports suggested that medicinal plants are considered to be potent source of novel compounds with having biological activities such as antioxidant and antimicrobial activities.9,35 In terms of MIC and MBC values, the wild grape juices possess high antibacterial activity. This suggests that the potential is mostly reflected by the concentration of phytochemicals in the extracts. However, other active compounds such as steroids, alkaloids or tannins may be involved this activity. The evaluation and characterization as well as biological investigation of other compounds are in process.

CONCLUSION

The present work showed that the wild grape juice extracts are composed of high content of phytochemicals, especially phenolics and flavonoids. They are also having high potential of biological activities such as antioxidant and antibacterial activities. Since the wild grape (*Ampelocissus martini* Planch.) is a local herb, it may be exploited in preparation of crude drugs for human health care. It can also be considered for using as natural antioxidant and antibacterial drugs with more potent efficiency in biomedical or pharmaceutical applications.

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