Phytochemical and Biological Activities of the Wild Grape Fruit Extracts Using Different Solvents

Boonsod Yardpiroon[1], Saengdee Aphidech[2] and Srihanam Prasong[1]*

[1]*Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand*

[2]*Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand*

ABSTRACT

Aims: To investigate the phytochemical, antioxidant and antibacterial activities of the solvent extracts of wild grape fruits in different colors.

Study design: The solvent extracts of wild grape (*Ampelocissus martinii* Planch.) fruits were prepared by using soxhlet extractor, before investigation of phytochemical and biological activities.

Place and Duration of Study: Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand, between August 2012 and May 2013.

Methodology: All extracts were investigated for their total phenolic (TPC) and flavonoid contents (TFC) by Folin-Ciocalteu and colorimetric aluminum chloride assays, respectively as well as antioxidant activity using 2,2´-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. They also tested for their antibacterial activity against infective bacteria using agar well diffusion method.

Results: Methanolic extracts showed the highest of TPC comparison to other. The methanolic extract from green wild grape has the highest of TPC, followed by ethanolic extract. The ethanolic extracts of red and black wild grape fruits found the highest of TFC whereas the green wild grape using methanol showed the highest TFC. All methanolic extracts showed the lowest of IC_{50} values when compared to other solvents in the same color. Among them, the methanolic extract from green wild grape has the lowest of IC_{50} values which considered to be the highest powerful of antioxidant activity. The obtained results were directly trend with the FRAB values. However, the ethanolic extract showed antioxidant activity similar as the methanolic extract. The methanolic extract from green wild grape showed good antibacterial activity. All ethanolic extracts showed widely and similarly inhibition of selected bacteria, but no activity in all water extracts. The MIC and MBC of all extracts were arranged of 500-250 µg/mL.

Conclusion: Methanol and ethanol should be used as good solvent extraction of wild grape fruits to obtain high TPC and TFC and good biological activities.

Keywords: wild grape, solvent, phenolic, flavonoid, biological activities

1. INTRODUCTION

In last decade, the study of free radical and they affect on human health has been increased since this free radical involved directly on living system damage, especially degenerative

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

diseases [1,2]. Various kinds of diseases were occurred by the condition called “oxidative stress”. This stress was caused from existence of free radical [3]. It is well known that oxidative stress can be treated by antioxidant substances [4]. Several studies have shown that plants phytochemical could be used as therapeutically benefit for treatment of diseases [5,6]. Generally, plants produce various secondary metabolites including phenols, flavonoids, quinines, tannins, alkaloids, saponins and sterols [7]. Those of metabolites are being used as pharmaceutical drugs [8-10]. Recently, natural phytochemical have been interested to explore and apply to instead of synthetic drugs [11]. The phytochemical of dietary and non-dietary are reported to modulate different kinds of degenerative and chronic diseases [12-14]. In the past, plants have been used as herbal materials for treatment infection diseases [15]. The plant-based drugs have been shown as few side effects, cheap and easy availability [16]. Plants are known as a large source of natural phytochemical which contained of biological activities [17-19]. Natural antimicrobial components in plants have been proved to inhibit the growth of bacteria [16,20]. This activity has going to be new hot spots for pharmacological studies in the following years [21]. In recent, several kinds of plant containing pharmacological substances have been studied and characterized, especially medicinal herbs [22-25]. To investigate of plant phytochemical, an important step is extraction process. This step is related to the content activity as well as chemical structure of substances [26]. Solvent extraction has been used for preparation plants extract [27]. Previously, various solvent such as hexane, methanol, isopropanol and ethyl acetate have been applied for extraction of phytochemical [28].

Wild grape *(Ampelocissus martinii* Planch.) is generally found in Thailand. It is a traditional herb ingredient and has been used for a long history. The stem and fruits of wild grape are similar to cultivated grape as well as color and stage of fruit development. Therefore, the phytochemical and their activities of the wild grape fruits may similar to the phytochemical found in the grape. Until now, information about some activities of wild grape phytochemical is not available.

2. MATERIAL AND METHODS

2.1 Plant Material

Fresh fruits of wild grape (*Ampelocissus martinii* Planch.) were collected from Suwannaphumi district, Roi-Et province, in August 2012. The plant material was identified by taxonomy professor, Department of Biology, Faculty of Science, KhonKaen University, Thailand. The fruits were washed twice with water and grouped followed they colors (green, red and black). All of fruits were kept at 4 °C and then used in urgent.

2.2 Preparation of Extracts

The wild grape fruits were dried using an oven at 40 °C for 3 days to obtain the final moisture less than 5% of dried fruits. All of wild grape groups were grinded and blended with distilled water, methanol and ethanol using a blender with high speed. The mixture was then homogenized in high speed blender for 5 min and filtered with vacuum under an ice bath. The filtrates were evaporated using a rotary evaporation at 45 °C until the weight of evaporated filtrate were less than 10% of the original weight. All of extractions were performed in triplicate and were stored at -40 °C until use.

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

2.3 Chemicals

The DPPH (2,2-diphenyl-1-Picrylhydrazyl) was purchased from Sigma-Aldrich (Singapore). Aluminium chloride ($AlCl_3$) was purchased from Merck (England). Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) 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 ($FeSO_4 \cdot 7H_2O$) and gallic acid were purchased from Univar. Butylated hydroxyanisole (BHA, $C_{16}H_{16}O_2$) and Butylated hydroxytoluene (BHT, $C_{15}H_{24}O$) were purchased from Fluka. All other chemicals and reagents of analytical grade were used.

2.4 Evaluation of Total Phenolic Content

The amount of total phenolic content (TPC) in the extract of wild grape fruits was determined using the Folin-Ciocalteu reagent according to the method of Bonoli et al. [29] 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 in 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 content. 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).

2.5 Evaluation of Total Flavonoid Content

The total flavonoid content (TFC) of the extract was evaluated according to the modified method of Yang et al. [30]. The two hundred and fifty microliters of the extract was mixed with 1.25 mL of deionized water, 75 µL of 5% sodium nitrite ($NaNO_2$) solution and allowed to stand for 5 min at room temperature. One hundred and fifty microliters of 10% aluminium chloride ($AlCl_3$) was added to the mixture solution and left to react for 6 min at room temperature. Five hundred microliters of 1M sodium hydroxide (NaOH) and 775 µL 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).

2.6 Free-Radical Scavenging Activity

Free radical scavenging activity of the extract was determined by using a stable 2,2´-diphenyl-1-picrylhydrazyl (DPPH) following a modified method of Chan et al. [31]. A total of 1.0 mL of the 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 as calculated using the following equation;

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

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.

2.7 Ferric Reducing Antioxidant Power (FRAP) Assay

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

The reducing power of the extract was detected using a ferric reducing antioxidant power (FRAP) assay described by Benzie and Strain [32] with some modifications. Briefly, the fresh solution of FRAP reagent contained 2.5 mL of 10 mL 2,4,6-Tri (2- pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl with 2.5 mL of mM $FeCl_3$ 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 °C for 4 min. The absorbance of the mixture solution was measured at 593 nm using UV-Vis spectrophotometer. The ethanolic solution of know Fe (II) concentration in the range of 50-500 μM ($FeSO_4$) was used as calibration curve. The ferric reducing ability of the crude extracts was expressed as mM of $FeSO_4$ equivalent concentration (EC) per 100 gram of fresh weight (FW). BHT and quercitin was used as positive controls. The experiment was performed in triplicates.

2.8 Bacteria Culture

The different 17 types of infection bacterial were chosen substrate as for determination of antibacterial activity of the extract of wild grape (*Ampelocissus martinii* Planch.). All of 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.

2.9 Antibacterial Activity of Extracts

The inhibition activity on bacteria of extracts was tested using Agar well diffusion method. The 1 mL of cultured bacteria at equal turbidity of McFarland No.0.5 was swab and placed into the surface of Mueller-Hinton Agar. The agar media was punctured into 3 holes per each 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.

2.10 Statistical Analysis

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

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Flavonoid Contents

The wild grape fruits showed high quantity of phytochemical as indication by total phenolic (TPC) and flavonoid (TFC) contents. The TPC and TFC of the solvent extracts using distilled water methanol and ethanol were summarized in Table 1. The methanolic extract found the highest of TPC, especially from green wild grape (12.558 ± 0.345 mg GAE/gFW), then red and black wild grape fruits. The ethanolic extract showed TPC content similar trend with the methanolic extract. The water extract showed the lowest of TPC compared to other extracts. The total flavonoid content (TFC) was the highest in the methanolic extract of green wild grape (21.349 ± 0.69 mg CE/gFW) which was equal content to ethanolic extract (20.901 ± 0.24 mg CE/gFW). However, the extracts from the red color of

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

wild grape fruit were also have high TFC content with 17.404 ± 0.41 mg CE/gFW (methanolic extract), 19.902 ± 0.48 mg CE/gFW (ethanolic extract). The ethanolic extract from black color of wild grape fruit has TFC of 15.628 ± 0.31 mg CE/gFW, which was the highest in the same color. The TFC of the water extracts were the lowest and have the same content in all colors. The phytochemicals found to gradually study and interested since they are more effective activity on human health [14]. The cultivated grape has been reported as a rich source of phytochemicals [33-35]. The cultivated green grape (cultivar Chardonnay) showed the TPC and TFC of 2.011 ± 0.05 and 1.664 ± 0.20 mg/mL, respectively, while the red grape (cultivar Concord) showed the TPC and TFC of 3.340 ± 0.13 and 1.682 ± 0.06 mg/mL, respectively [36]. The phytochemical composition in wild grape has been rarely available information so far. The results from this work reveal that the fruit of wild grape showed high content of TPC and TFC as like as grape. The contents of phytochemical found to be related directly with biological activities, especially antioxidant activity [37]. The contents of phytochemical were affected from both the solvent used and the color of wild grape fruits. It is well known those cultivars, maturity, colors, part of fruits as well as the types and quantity of phytochemicals [13]. In addition, both genetic and agronomic or environmental factors act main roles on the phytochemical composition and nutritional quality of the crops [33]. In this recent work, the TPC and TFC were higher in the green and red colors than black color of wild grape fruit. This may affect from the chlorophylls [38] and anthocyanins [39] composed in the green and red colors, respectively. Interestingly, anthocyanin is also found in black color, but it showed the lowest contents of both TPC and TFC. This might be affected from the method of extraction as well as the solvent used.

3.2 Antioxidant Activity

The antioxidant activity of the extracts was shown in Table 2. The IC_{50} was calculated from DPPH assay and expressed as the concentration of antioxidant exists in the extract which was able to decrease 50% amount of the DPPH. With IC_{50} value, the water extract has not show antioxidant activity in all of fruit extracts (ND). This obtained results showed difference profile comparison to FRAB since the FRAB value of water extracts showed ferric reducing power of 207.290 ± 7.75, 140.370 ± 5.38 and 138.790 ± 7.73 mM $FeSO_4$/gFW for green, red and black colors of wild grape fruits, respectively. Considering from IC_{50} value, both methanolic and ethanolic extracts have similar power of antioxidant activity. The methanolic extract (IC_{50} = 0.186 ± 0.004 µg/mL) of green wild grape has higher powerful than ethanolic extract (IC_{50} = 0.413 ± 0.017 µg/mL). The extract from red color of wild grape fruit has also revealed the antioxidant activity similar trend to the green since the methanolic extract (IC_{50} = 0.397 ± 0.017 µg/mL) indicated higher efficacy on DPPH radical than ethanolic extract (1.433 ± 0.064 µg/mL). Moreover, the methanolic extract of black wild grape fruit showed antioxidant activity by DPPH assay, but in the lowest capacity (52.265 ± 7.884 µg/mL). The methanolic and ethanolic extracts from green and red colors of wild grape fruits showed higher reducing ability than that of water extract. Furthermore, the methanolic extract showed the higher activity than ethanolic extract. The FRAB values of the methanolic extract from green wild grape (560.610 ± 9.370 mM $FeSO_4$/100gFW) showed the highest value as well as ethanolic extract (361.750 ± 6.507 mM $FeSO_4$/100gFW). With previous reports, polyphenols and flavonoids are used for prevention of various degenerative diseases [7,40]. It is well known that phenolics act as terminators of free radical from oxidation reaction, while flavonoids are responsible for the radical scavenging effects [5]. Generally, the extract with high total phenolic contents had higher antioxidant activity [33,38]. The methanolic extracts of green color of wild grape fruit showed the lowest value of IC_{50} and the highest of FRAP value. This means the antioxidant activity in the methanolic extract of green wild grape have the most potential of antioxidant activity. In the same time, ethanolic of green wild grape showed slightly lower antioxidant activity than the methanolic extract. Moreover, both methanolic and ethanolic extracts of red wild grape showed directly

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

trend following the results of phytochemical investigation. On the other hand, the IC_{50} of the water extracts did not be detect, but found little of FRAP values showed little activity. The water extract of black wild grape has the higher FRAP value even the extracts from green and red colors of wild grape fruit showed lower of FRAP value than alcoholic extracts. The result may suggest that the active compound in the water extract of black wild grape may not be phenolic or flavonoid. Many previous works have been reported that the bioactive substances found in plants or microorganisms are also composed of other biological activities such as antimicrobial activities [41], inhibition of plasma platelet aggregation and cyclooxygenase activity, histamine release suppression, anti-inflammatory and antiallergenic effects [42].

4.3 Antibacterial activity

The water extract of dried wild grape fruits did not have the antibacterial activity against all of infective bacteria. As shown in Table 3, methanolic extract from green color of wild grape fruits was moderate effective against over 7 strains including *S. typhi* DMST 5784, *S. typhi* gr. D, *S. paratyphi* ATCC 14028, *S. typhi* DMST 16122, *B. cereus* ATCC 11778, *E.coli* 0157: H7 DMST 12733 and *Ps. aeruginosa* with diameter inhibition zone (DIZ) in range of 10-12 mm. The methanolic extract of red wild grape has the highest antibacterial activity for *S. typhi* DMST 16122 (16 mm). However, it showed narrow antibacterial activity against only *S. typhi* gr. D (DIZ=10 mm), *E. coli* 0157: H7 DMST 12733 (DIZ=9 mm) and *Ps. aeruginosa* (DIZ=12 mm). The extract of black wild grape showed similar antibacterial activity as like as the extract of red wild grape, since it can be inhibited only 4 strains of infective bacteria. The highest effective antibacterial activity of the extract from black color of wild grape fruit was found in *B. cereus* ATCC 11778 (DIZ=17 mm). In addition, it can be moderately inhibited of *S. typhi* DMST 16122, *E. coli* 0157: H7 DMST 1273 and *Ps. aeruginosa* with diameter inhibition zone in range of 10-11 mm. Generally, the ethanolic extract of wild grape fruits showed widely inhibited of bacteria comparison to the methanolic extract as shown in Table 4. All of wild grape fruit colors showed similar pattern against infective bacteria. However, those of extracts cannot have antibacterial activity against 6 strains include *E. cloacae*, *S. aureus* ATCC 25293, *S. typhi* gr. D, *S. paratyphi* ATCC 14028, *E. coli* ATCC 25922, *B. cereus* ATCC 11778 and *S. aureus* MRSA DMST 20625. The ethanolic extracts from all of wild grape fruit colors showed moderately antibacterial activity against *S. flexneri* DMST 4423, *S. typhimurium* ATCC 14028, *E. coli* 0157: H7 DMST 12733, *Ps. aeruginosa* and *S. dysenteriae* with diameter inhibition zone in range from 9-13 mm. The *S. typhi* DMST 5784 (DIZ=11 mm) and *S. typhi* DMST 16122 (DIZ=12 mm) were inhibited by the ethanolic extracts from green and red colors of wild grape fruit. Moreover, *S. flexneri* DMST 17569 was only inhibited by ethanolic extract of red wild grape (11 mm), while *S. paratyphi* ATCC 14028 was inhibited by the ethanolic extract of black wild grape (DIZ=10 mm), respectively. As shown in Table 5, the MIC and MBC values were found between 500-250 µg/mL of the methanolic extract. Beside the MBC and MIC, the methanolic extracts from red wild grape showed the highest power on *S. typhi* DMST 16122 whereas the extract from black wild grape showed the highest power on *B. cereus* ATCC 11778 with the values of 250 µg/mL. On the other hand, *S. typhi* DMST 5784**,** *S. flexneri* DMST 17569, *S. paratyphi* ATCC 14028, *S. flexneri* DMST 4423, *S. typhimurium* ATCC 14028, *E. coli* 0157: H7 DMST 12733, *Ps. aeruginosa* and *S. dysenteriae* were selected for MIC and MBC assays of ethanolic extract. The results indicated that both MBC and MIC values of tested bacteria were 500 µg/mL, except *Ps. aeruginosa* was 250 µg/mL. The methanolic and ethanolic extracts showed widely effective antibacterial activity. However, the profiles of each extracts were varied depending on solvent and fruit color. This result suggested that the antibacterial activity of the extracts was affected by different kinds of phytochemical composed in each stages of the fruit development. It is well known that the active compounds called

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

phytochemicals were produced for plant against microbial pathogens which were considered to be potent source of novel compounds with having biological activities such as antioxidant and antimicrobial activities [7,43]. The development of drug from natural medicinal plants instead of commercial antimicrobial drugs has been focused in recent years [44]. The obtained results from this work indicated that alcoholic extracts of wild grape fruits are effective against the selected bacteria with slightly differed in types of bacteria and efficacies. This result might be caused from the characteristics of each bacterial cell wall [45]. With previous reports, many bioactive produced by plants have been found to protect plants against bacteria, fungi and pests [44,46]. Therefore, it is not surprise that the extracts of wild grape fruits have be composed of antibacterial activity. With MIC and MBC studies, the methanolic and ethanolic extracts revealed similar potential of antibacterial activity, but in different profiles. This result should be reflected by the phytochemicals composed in the extracts. The results of TPC and TFC contents found to relate directly on antioxidant and antibacterial activities of the extracts. However, the temperature of soxhlet extraction was different for each solvent used which could be affected the phytochemical and biological activities of the obtained extracts. Therefore, this point may an important to different content of TPC and TFC as well as antioxidant and antibacterial activities. In further study, other extraction method, solvents and active compounds such as steroids, alkaloids or tannins may be involved on the tested biological activities which should be further performed.

4. CONCLUSION

The phytochemicals and their biological activities; antioxidant and antibacterial activities of the wild grape fruit solvent extracts were reported in this work. The results can be concluded that methanolic extract from green color of wild grape fruit has the highest of total phenolic content (TPC), total flavonoid content (TFC), high potential of antioxidant activity. The water extract showed the lowest of total phenolic content (TPC), total flavonoid content (TFC) and has not composed of antioxidant and antibacterial activities. On the other hand, methanolic extracts from black color of wild grape fruit and ethanolic extract from red color of wild grape fruits showed widely antibacterial activity against infective bacteria with MIC and MBC arranged from 500-250 µg/mL. From all of results, the solvent extracts of wild grape (*Ampelocissus martinii* Planch.) fruits are rich in phytochemical contents which possessed high antioxidant and antimicrobial activities. Therefore the data found in this work might be used for further study of the wild grape extract on various applications such as health supplement and pharmaceutical benefits.

ACKNOWLEDGEMENTS

We would like to thank Dr. Gusavadee Sangdee and Miss Jiraporn Krasaetep for their kindly support both chemical reagent and technique. We also great fully thank Division of Research Facilitation and Dissemination, Department of Chemistry, Faculty of Science, Mahasarakham University and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand for financial support.

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Srihanam Prasong designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.
Saengdee Apidech and Jirum Jenjira managed the analyses of the study and collected all data. All authors read and approved the final manuscript.

REFERENCES

1. Chouchouli V, Kalogeropoulos N, Konteles SJ, Karvela E, Makris DP, Karathanos VT. Fortification of yoghurts with grape (*Vitis vinifera*) seed extracts. LWT-Food Sci Technol. 2013; 53: 522-529.
{http://dx.doi.org/10.1016/j.lwt.2013.03.008}

2. Dani C, Oliboni LS, Vanderlinde R, Bonatto D, Salvador M, Henriques JAP. Phenolic content and antioxidant activities of white and purple juices manufacture with organically-or conventionally-produced grapes. Food Chem Toxicol. 2007;45:2574-2580.
{http://www.ncbi.nlm.nih.gov/pubmed/17683842}

3. Poudel PR, Tamura H, Kataoka I, Mochioka R. Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. J Food Compos Anal. 2008; 21:622-625.
{http://dx.doi.org/10.1016/j.jfca.2008.07.003}

4. Javanmardi J, Stushnoff C, Locke E, Viranco JM. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. Food Chem. 2003;83:547-550.
{www.sciencedirect.com/science/article/pii/S0308814603001511}

5. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy. 2011;46:81-88.
{www.uctm.edu/journal/j2011-1/12_Maria_Atanasova.pdf}

6. Soylu EM, Soylu S, Kurt S. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*. Mycopathologia. 2006; 161:119-128.
{www.ncbi.nlm.nih.gov/pubmed/16463095}

7. Alghazeer R, El-Saltani H, Saleh N, Al-Najjar A, Hebail F. Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. Natural Science. 2012;4:324-335.
{www.scirp.org/Journal/PaperInformation.aspx?paperID=19119}
8. Liu YS, Zhang KQ. Antibacterial activity of selected *Cyathus* species. Mycopathologia. 2004;157:185-189.

{www.ncbi.nlm.nih.gov/pubmed/15119855}

9. Mares D, Romagnoli C, Tosi B, Andreotti E, Chillemi G, Poli F. Chicory extracts from *Cichorium intybus* L. as potential antifungals. Mycopathologia. 2005;160:85-92.

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

{www.ncbi.nlm.nih.gov/pubmed/16160773}

10. Yazaki K, Sugiyama A, Morita M, Shitan N. Secondary transport as a efficient membrane transport mechanism of plant secondary metabolites. *Phytochemistry Review*. 2008;7:513-524.
{link.springer.com/article/10.1007%2Fs11101-007-9079-8}

11. Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. *Journal of Food composition and Analysis*. 2006;19:513-537.

{www.sciencedirect.com/science/article/pii/S0889157505000232}

12. Renuka Devi R., Arumughan C. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. *Bioresource Technology*. 2007;98:3037–3043.

13. Lako J, Craige Trenery V, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S, Premier R. Phytochemical flavonols, cartenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. Food Chem. 2007;101:1727-1741.
{DOI: 10.1016/j.foodchem.2006.01.031}
14. Liu RH. Whole grain phytochemical and health. J Cereal Sci. 2011;46: 207-219.
{www.sciencedirect.com/science/article/pii/S0733521007001166}

15. Desai MN, Chaven NS. Antibacterial activity and phytochemical screening of *Cynometra iripa* kostel. Int J Pharm Biol Sci. 2010;1:1-4.
{www.ijpbs.net/issue-3/14.pdf}
16. Chatterjee SK, Bhattacharjee I, Chandra G. Isolation and identification of bioactive antibacterial components in leaf extracts of *Vangueria spinosa* (Rubiaceae). Asian Pac J Trop Med. 2011;4:35-40.
{www.ncbi.nlm.nih.gov/pubmed/21771412}
17. Lee S, San D, Ryu J, Lee YS, Jung SH, Kang J. Anti-oxidant activities of Acanthopanax senticosus stems and their lignin components. Arch Pharm Res. 2004; 27:106-110.
{www.ncbi.nlm.nih.gov/pubmed/14969348}
18. Tsao R, Deng Z. Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatog B. 2004;812:85-99.
{www.ncbi.nlm.nih.gov/pubmed/15556490}
19. Kang NS, Lee JH. Characterisation of phenolic phytochemicals and quality changes related to the harvest times from the leaves of Korean purple perilla (*Perilla frutescens*). Food Chem. 2011;124:556-562.
{www.sciencedirect.com/science/article/pii/S030881461000782X}
20. Chun SS, Vattem DA, Lin YT, Shetty K. Phenolic antioxidants from clonal (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. Process Biochem. 2005;40:809-816.
{www.sciencedirect.com/science/article/pii/S0032959204001189}
21. Ma H, He X, Yang Y, Li M, Hao D, Jia Z. The genus Epimedium: An ethanopharmacological and phytochemical review. J Ethnopharmacol. 2011;134:519-541.
{www.ncbi.nlm.nih.gov/pubmed/21215308}
22. Jang HD, Chang KS, Huang YS, Hsu CL, Lee SH, Su MS. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. Food Chem. 2007;103: 749-756.
{www.sciencedirect.com/science/article/pii/S030881460600731X}

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

428 23. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity
429 and *in vitro* inhibit potential against key enzymes relevant for hyperglycemia and
430 hypertension of commonly used medicinal plants, herbs and spices in Latin America.
431 Bioresource Technol. 2010;101:4676-4689.
432 {www.ncbi.nlm.nih.gov/pubmed/20185303 }
433 24. Rosso GL. Ins and outs of dietary phytochemicals in cancer chemoprevention. Biochem
434 Pharmacol. 2007;74:533-544.
435
436 {www.ncbi.nlm.nih.gov/pubmed/17382300}
437 25. Astelbauer F, Gruber M, Brem B, Greger H, Obwaller A, Wernsdorfer G, Congpuong K,
438 Wernsdorfer WH, Walochnik J. Activity of selected phytochemicals against *Plasmodium*
439 *falciparum*. Acta Tropical. 2012;123(2):96-100.
440 {www.ncbi.nlm.nih.gov/pubmed/22537982}
441
442 26. Xu C, Zhang Y, Wang J, Lu J. Extraction, distribution and characterization of phenolic
443 compounds and oil in grape seeds. Food Chem. 2010;122:688-694.
444 {www.sciencedirect.com/science/article/pii/S0308814610003110}
445
446 27. Cheng VJ, Bekhit AE-DA, MaConnell M, Mros S, Zhao J. Effect of extraction solvent,
447 waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts
448 from wine residue from cool climate. Food Chem. 2012;134:474-482.
449 {www.sciencedirect.com/science/article/pii/S0308814612002919}
450
451 28. Lai P, Li KY, Lu S, Chen HH. Phytochemical and antioxidant properties of solvent
452 extract from *Japonica* rice bran. Food Chem. 2009;177:538-544.
453 {www.sciencedirect.com/science/article/pii/S0308814609004932}
454
455 29. Bonoli M, Verardo V, Marconi E, Caboni MF. Antioxidant phenols in barley (*Hordeum*
456 *vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free
457 and bound phenolic compounds. J Agri Food Chem. 2009;5:5195-5200.
458 {www.ncbi.nlm.nih.gov/pubmed/15291496}
459
460 30. Yang J, Martinson TE, Liu RH. Phytochemical profiles and antioxidant activities of wine
461 grapes. Food Chem. 2009;116:332-339.
462 {www.sciencedirect.com/science/article/pii/S0308814609002088}
463
464 31. Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etlingera*
465 species (Zingiberaceae) in Peninsular Malaysia. Food Chem. 2007;104:1586-1593.
466 {www.sciencedirect.com/science/article/pii/S0308814607002257}
467
468 32. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of
469 “antioxidant power”: The FRAP assay. Anal Biochem. 1996;239:70-76.
470 {www.ncbi.nlm.nih.gov/pubmed/8660627 }
471
472 34. Chuang C-C, Bumrungpert A, Kennedy A, Overman A, West T, Dawson B. et al. Grape
473 powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin
474 resistance in primary cultures of human adipocytes. J Nutr Biochem. 2011;22: 89-94.
475 {www.ncbi.nlm.nih.gov/pubmed/20382011}
476
477 35. Anastasiadi M, Pratsinis H, Kletsas D, Skaltsounis A-L, Haroutounian SA. Bioactive non-
478 coloured polyphenols contents of grapes, wines and verification by products: Evaluation of
479 the antioxidant activities of their extracts. Food Res Int. 2010;43:805-813.
480

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

{doi:10.1016/j.foodres.2009.11.017}
36. Yang J, Martinson TE, Liu RH. Phytochemical profiles and antioxidant activities of wine grapes. Food Chem. 2009;116:332-339.
{doi:10.1016/j.foodchem.2009.02.021}
37. Wang W, Yagiz Y, Buran TJ, Nunes CDN, Gu L. Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. Food Res Int. 2011;44:2666-2673.
{www.sciencedirect.com/science/article/pii/S0963996911003383}

38. Irudayaraj V, Janaky M, Johnson M, Selvan N. Preliminary phytochemical and antimicrobial studies on a spike-moss *Selaginella inaequalifolia* (hook. & grev.) Spring. Asian Pacific J Trop Med. 2010;3:957-960.
{www.sciencedirect.com/science/article/pii/S1995764511600084}

39. Velioglu YS, Mazza G, Gao L, Oomah B. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998;46:4113-4117.
{pubs.acs.org/doi/abs/10.1021/jf9801973}

40. Deepa VS, Kumar PS, Latha S, Selvamani P, Srinivasan S. Antioxidant studies on the ethanolic extract of *Commiphora* spp. Afr J Biotechnol. 2009;8:1630-1636.
{www.ajol.info/index.php/ajb/article/download/60349/48587}

41. Skočibušić M, Bezić N, Dunkić V. Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata* Vis. Growing in Croatia. Food Chem. 2006;96:20-28.
http://dx.doi.org/10.1016/j.foodchem.2005.01.051

42. Cook NC, Samman S. Flavonoids; chemistry, metabolism, cardioprotective effects and dietary sources. J Nutr Biochem. 1996;7:66-76.
http://dx.doi.org/10.1016/S0955-2863(95)00168-9

43. Edziri H, Mastouri M, Cheraif I, Aouini M. Chemical composition and antibacterial, antifungal and antioxidant activities of the flower oil of *Retama raetam* (Forssk.) Webb from Tunisia. Nat Prod Res. 2010;24:789-796.
{www.ncbi.nlm.nih.gov/pubmed/20461625}

44. Patra JK, Dhal NK, Thatoi HN. *In vitro* bioactivity and phytochemical screening of *Suaeda maritime* (Dumort): A mangrove associate from Bhitarkanika, India. Asian Pacific Journal of Tropical Medicine. 2011; 4(9):727-734.
{doi: 10.1016/S1995-7645(11)60182-X.}

45. Scherrer R, Gerhardt P. Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. J Bacteriol. 1971;107:718-735.
{http://www.ncbi.nlm.nih.gov/pmc/articles/PMC246993/}

46. Aboaba OO, Smith SI, Olude FO. Antimicrobial effect of edible plant extract on *Escherichia coli* 0157: H7. Pak J Nutri. 2006;5:325-327.
{scialert.net/abstract/?doi=pjn.2006.325.327}

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of wild grape extracts in different colors (green, red and black) of wild grape fruits.

Color	TPC (mg GAE/ gFW) ± SD			TFC (mg CE/gFW) ± SD		
	water	**methanol**	**ethanol**	**water**	**methanol**	**ethanol**
Green	0.697 ± 0.004	12.558 ± 0.345	7.148 ± 0.423	5.588 ± 0.013	21.349 ± 0.694	20.901 ± 0.236
Red	0.522 ± 0.135	6.445 ± 0.009	4.105 ± 0.038	5.245 ± 0.013	17.403 ± 0.412	19.902 ± 0.481
Black	0.324 ± 0.120	2.608 ± 0.122	1.150 ± 0.496	4.902 ± 0.000	9.243 ± 0.232	15.628 ± 0.305

Mean ± S.D. = mean values ± standard deviation of triplicate experiments.

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E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

Table 2. Antioxidant activity of wild grape extracts in different color (green, red and black) of wild grape fruits expressed by IC_{50} and FRAB values.

Colors	IC_{50} (µg/mL) ± SD			FRAP (mM FeSO4/100gFW) ± SD		
	water	methanol	ethanol	water	methanol	ethanol
Green	ND	0.186 ± 0.004	0.412 ± 0.017	207.290 ± 7.749	560.610 ± 9.370	361.750 ± 6.507
Red	ND	0.397 ± 0.017	1.432 ± 0.064	140.370 ± 5.381	239.010 ± 10.291	196.830 ± 6.476
Black	ND	52.264 ± 7.884	ND	138.790 ± 7.729	83.602 ± 4.789	68.379 ± 1.980

ND = no detection

* Tel.: +66 43 754246; fax: +66 43 754246.
E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

Table 3. Diameter of inhibition zone of methanolic extracts in different colors (green, red and black) of wild grape fruits.

Bacterial	Diameter of inhibition zone (mm)		
	Green	Red	Black
S. typhi DMST 5784	11	-	-
S. flexneri DMST 17569	-	-	-
E. cloacae	-	-	-
S. aureus ATCC 25293	-	-	-
S. typhi gr. D	11	10	-
S. paratyphi ATCC 14028	10	-	-
S. typhi DMST 16122	12	16	10
S. flexneri DMST 4423	-	-	-
E. coli ATCC 25922	-	-	-
S. typhimurium ATCC 14028	-	-	-
B. cereus ATCC 11778	12	-	17
E.coli 0157: H7 DMST 12733	10	9	11
Ps. aeruginosa	11	12	10
S. aureus MRSA DMST 20625	-	-	-
S. dysenteriae	-	-	-

(-) = no activity

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E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

Table 4. Diameter of inhibition zone of ethanolic extracts in different colors (green, red and black) of wild grape fruits.

Bacterial	Diameter of inhibition zone (mm)		
	Green	Red	Black
S. typhi DMST 5784	11	11	-
S. flexneri DMST 17569	-	11	-
E. cloace	-	-	-
S. aureus ATCC 25293	-	-	-
S. typhi gr. D	-	-	-
S. paratyphi ATCC 14028	-	-	10
S. typhi DMST 16122	12	12	-
S. flexneri DMST 4423	9	11	10
E. coli ATCC 25922	-	-	-
S. typhimurium ATCC 14028	9	11	10
B. cereus ATCC 11778	-	-	-
E. coli 0157: H7 DMST 12733	9	9	12
Ps. aeruginosa	13	14	10
S. aureus MRSA DMST 20625	-	-	-
S. dysenteriae	12	13	12

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E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.

Table 5 MBC and MIC values of solvent extracts on selected bacteria.

Bacterial (wild grape fruit color)	MBC (µg/mL)	MIC (µg/mL)
Methanolic extract		
S. typhi DMST 5784 (Green)	500	500
S. typhi gr. D. (Green)	500	500
S. typhi DMST 16122 (Red)	250	250
B. cereus ATCC 11778 (Black)	250	250
E. coli 0157: H7 DMST 12733 (Black)	500	500
Ps. aeruginosa (Red)	500	500
Ethanolic extract		
S. typhi DMST 5784 (Green)	500	500
S. flexneri DMST 17569 (Red)	500	500
S. paratyphi ATCC 14028 (Black)	500	500
S. flexneri DMST 4423 (Red)	500	500
S. typhimurium ATCC 14028 (Red)	500	500
E. coli 0157: H7 DMST 12733 (Black)	500	500
Ps. aeruginosa (Green/Red)	250	250
S. dysenteriae (Red)	500	500

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E-mail address: prasong.s@msu.ac.th;psrihanam@gmail.com.