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Quantification of Phenolic Compounds in the Extract of Wine Production Residues of Wild Grape Fruits and their Antibacterial Activity

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The objectives of this work are to quantify the phenolic compounds in the extract of wine production residues using high performance liquid chromatography (HPLC) and its antibacterial activity. The results indicated that gallic acid and quercetin are the highest contents for phenolic acid and flavonoid, respectively. The extract showed efficiency against 12 bacterial strains which have more potency on *Salmonella typhi* (DMST 16122), *Staphylococcus aureus* (ATCC 25293) and *Bacillus cereus* (ATCC 11778). The concentration for inhibition (MIC) and bactericidal (MBC) of the extract on selected bacterial was in range of 500-250 µg/mL. This suggested that the wild grape residues from wine production composed of different phenolic substances, which could be used for inhibition of many bacteria.

Keywords: Phenolic compounds, Wine production residue, Wild grape, Antibacterial activity.

INTRODUCTION

Since ancient times, plants derived substances were considered as important materials for folk medicines [1-3]. Until now, various kinds of natural substances and their biological activity have been reported [4-6]. However, the new source of the natural products is still a subject of research worldwide. Phenolic compounds are the main plant-derived secondary metabolites which exhibited different activities including antibacterial, antiviral, anticarcinogenic, anti-inflammatory and antioxidant [7-9]. Among activities, antibacterial is one purpose to test [10]. The antibacterial activity of the natural substances for health supplement and food preservation gradually increased attention [11-13]. These factors have influenced the widespread studies of new source for possible antibacterial properties, while the isolation and determination of various phytochemicals [14-16].

In process of product like wine or juice, fruit byproducts are as waste and no further generally use special proposes. However, some reports have been mentioned that fruit by products are enrich of nutraceutical compounds, especially polyphenols [17-19]. Grape wine is an alcoholic beverage, which popularly consumed worldwide. The grape byproducts from wine production have been studied for their active compounds as well as biological activities [20-23]. The fruit byproducts composed high content of phenolic compounds which exhibited health supplementation [24].

A native plant called "wild grape (*Ampelocissus martinii* Planch.)", dispersed along area of the northeastern, Thailand is known as herb and applied as folk medicine with long history. Present work found that the wild grape fruit composed different phytochemicals and antioxidant and antibacterial activities [25,26]. This has attracted considerable work interest to investigate the phytochemicals and antibacterial activity of wild grape fruits residues, which derived from wine production. Therefore, the aim of this work is to extract the wine residues of wild grape fruits derived from wine production using ethanol as solvent. The extracts were then analyzed for their phytochemicals by high performance liquid chromatography (HPLC) technique and antibacterial activity by agar well diffusion method. Results will be used as a supplement to this plant nutraceutical and biological databases.

EXPERIMENTAL

The red fruits of wild grape (mature stage) were collected from the local community forest, Suvannaphumi, Roi-Et, Thailand.

The fruits were washed with distilled water before preparation of wine. The wild grape fruits residues were separated, dried in an oven at 80 ºC. The dried residues were grinned to powder and stored at 4 ºC until extraction.

Crude extraction: The powder of wild grape fruit residues was macerated by ethanol in the ratio of 1g:10 mL for 24 h. The extract solution was separated *via* filter paper, evaporated to obtain the crude extract. The crude was then mixed with ethanol into solution before use.

Phenolic compounds quantification: The individual phenolic compounds were analyzed by RP-HPLC analysis followed by previous report [27]. The tent external standards were used for comparison and identification.

Bacterial culture: Twelve bacterial strains were chosen in this work. Nine reference bacterial strains including *Salmonella typhi* (DMST 5784), *S. typhi* (DMST 16122), *S. paratyphi* (ATCC 14028), *S. typhimurium* (ATCC 14028), *Shigella flexneri* (DMST 17569), *S. flexneri* (DMST 4423), *Staphylococcus aureus* (ATCC 25293), *Escherichia coli* 0157:H7 (DMST 12733) and *Bacillus cereus* (ATCC 11778), and 3 strains of clinical isolated including *S. typhi* (gr. D), *S. dysenteriae* and *Pseudomonas aeruginosa* were cultured in broth medium at 37 ºC for 2 days. The cultured bacteria were adjusted by adding 0.85% normal saline to give 1.5×10^{8} cells/mL bacterial density.

Antibacterial activity: Agar well diffusion method was applied for antibacterial testing. The prepared bacterial cultured was swab and placed into the surface of agar, pouring by extract within the holes of agar in plates. The diameters of inhibition zone (DIZ) were measured after incubated the culture plates at 37 ºC for 24 h.

Broth dilution assay: This assay used for testing the minimal inhibitor concentration (MIC) and minimal bactericidal concentration (MBC) of the extract. The concentration of the extract (mg/mL) were prepared. This solution was then added into 5 mL of Mueller-Hinton Broth. Selected bacterial solution (5 mL) was poured into the medium broth and then incubated at 37 ºC for 24 h. Antibiotics and the diluted solvent were used as control. The MIC and MBC were recorded as the lowest concentration, which could be inhibited and killed bacteria after incubation for 24 h, respectively.

RESULTS AND DISCUSSION

Individual phenolic compounds: Table-1 showed the presence of phenolic substances in the extract. The most phenolic acids were gallic acid (3.26 mg/g) and caffeic acid (0.30 mg/g) while flavonoids were quercetin (1.92 mg/g) and resveratrol (0.65 mg/g), respectively. The other substances found low contents and with similar content between phenolic acid and flavonoid. Both phenolic acids and flavonoids were produced for different function such as colours, favours and defense mechanism [28,29]. In this work, monoflavonoids; catechin (0.10 mg/g) and epicatechin (0.19 mg/g) were found in low contents comparing to other plants [30]. Moreover, a flavonol myricetin (1.92 mg/g) and flavonol glycoside; rutin (0.15 mg/g) generally found in low content [31] as like as obtaining result in this work. Resveratrol found in the second most content for flavonoid which in agreement with the previous works as it often found in fruit peels [32,33]. In general, several factors including the experimental conditions applied [20,24], management condition [29], the interaction with other environmental factors as well as sun (UV) light [34].

TABLE-1
PHENOLIC COMPOUNDS IN THE WILD GRAPE RESIDUE EXTRACT

Substances	Contents (mg/g DW)	Substances	Contents (mg/g DW)
Gallic acid	3.26 ± 0.00	Catechin	0.10 ± 0.00
Caffeic acid	0.30 ± 0.00	Epicatechin	0.19 ± 0.00
p-Coumaric acid	0.10 ± 0.00	Quercetin	1.92 ± 0.00
Ferulic acid	0.17 ± 0.00	Rutin	0.15 ± 0.00
Resveratrol	0.65 ± 0.00	Myricetin	0.12 ± 0.00

Antibacterial activity: The secondary metabolites in plant were produced for different function including against microbial pathogens [10-16]. Fig. 1 showed the effective activity of the extract against 12 bacterial strains. The extract exhibited antibacterial activity with variable DIZ (mm) (Table-2). It could be observed that the residues of mature wild grape fruits had effective antibacterial in moderate to high efficiency. The highest effectiveness found in *S. typhi* (DMST 16122) (20 mm), *S. aureus* (ATCC 25293) (20 mm) and *B. cereus* (ATCC 11778) (20 mm). The high efficacy was observed in *E. coli* 0157: H7 (DMST 12733), *S. typhimurium* (ATCC 14028) and *S. dysenteriae* with diameter of 14 mm. However, the extract also showed high antibacterial on *S. flexneri* (DMST 4423) and *S. typhi* (DMST 5784) with 13 mm inhibition zone. Other strains have moderate activity with the diameter of inhibition zone in ranged 10-11 including *S. typhi* gr. D, *P. aeruginosa*, *S. flexneri* (DMST 17569) and *S. paratyphi* (ATCC 14028). The active substances in plant could be inhibited bacterial growth by several actions such as destroying cytoplasmic membrane, interrupting membrane permeability or enhancing some components leakage as well as changes in energy metabolism [35,36].

TABLE 2
EFFECT OF WILD GRAPE RESIDUE EXTRACT ON BACTERIAL INHIBITION

Bacterial strains	Diameter of inhibition zone (mm)
S. typhi (DMST 5784)	13
S. typhi (DMST 16122)	20
S. paratyphi (ATCC 14028)	11
S. typhimurium (ATCC 14028)	14
S. flexneri (DMST 17569)	11
S. flexneri (DMST 4423)	13
S. aureus (ATCC 25293)	20
B. cereus (ATCC 11778)	20
E. coli 0157: H7 (DMST 12733)	14
S. typhi (gr. D)	10
S. dysenteriae	14
P. aeruginosa	11

The different strains of bacteria; *S. typhi* (DMST 16122), *S. flexneri* (DMST 4423), *S. aureus* (ATCC 25293), *B. cereus* (ATCC 11778), *E. coli* 0157:H7 (DMST 12733), *S. typhi* gr. D, *S. dysenteriae* and *P. aeruginosa* were selected for finding MIC and MBC values according they were sensitive affected

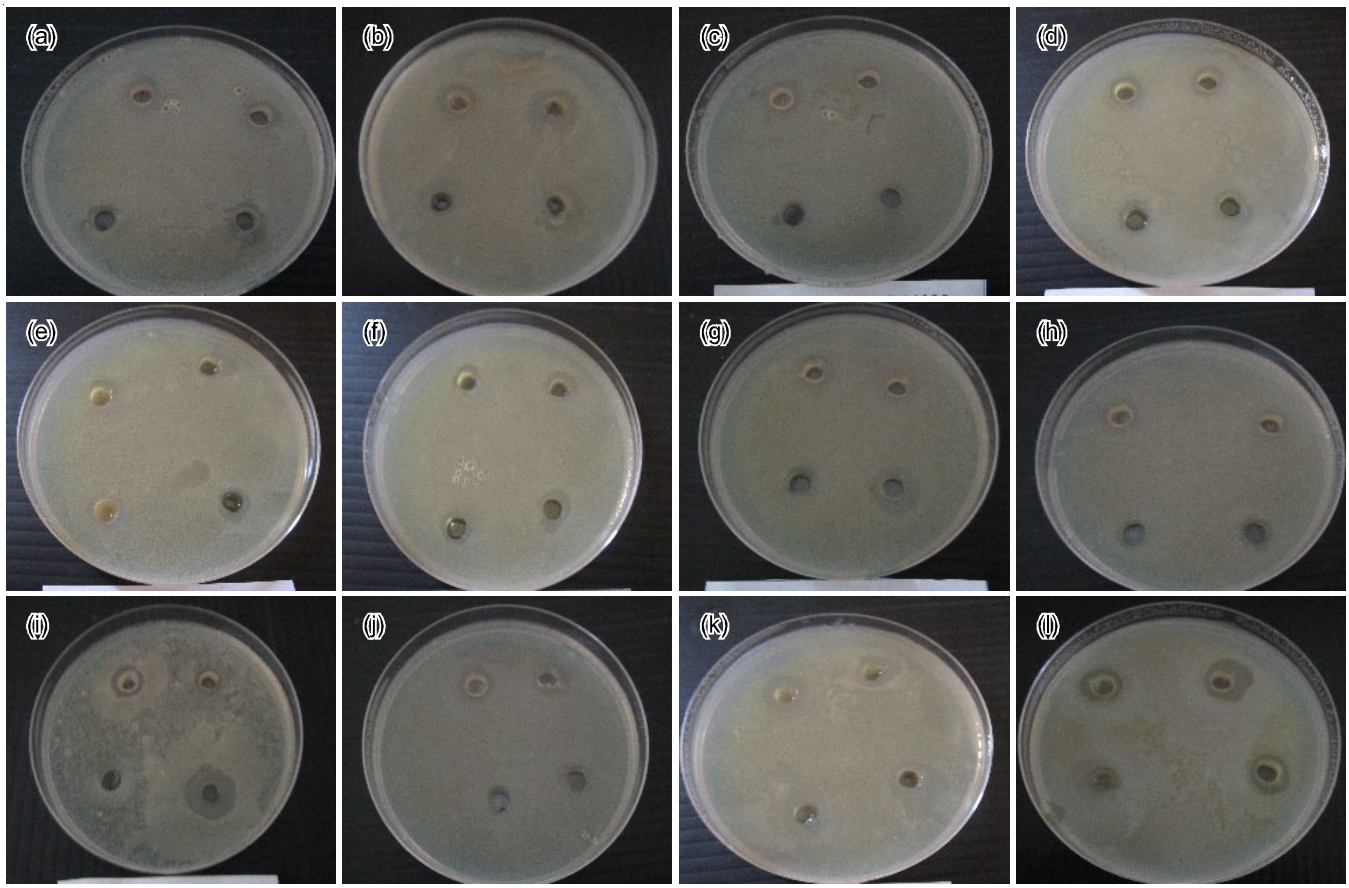

Fig. 1. Effect of wild grape residues extract against different bacterial strains. *S. typhi* (DMST 5784) (a), *S. typhi* (DMST 16122) (b), *S. paratyphi* (ATCC 14028) (c), *S. typhimurium* (ATCC 14028) (d), *S. flexneri* (DMST 17569) (e), *S. flexneri* (DMST 4423) (f), *S. aureus* (ATCC 25293) (g), *E. coli* 0157: H7 (DMST 12733) (h), *B. cereus* (ATCC 11778) (i), *S. typhi* (gr. D) (j), *S. dysenteriae* (k), *P. aeruginosa* (l)

by the extract. The results indicated that the MIC and MBC values were found to be 250-500 µg/mL as shown in Table-3. This suggested that the residues of wild grape fruits had efficiency of antibacterial activity according the different types of phenolic compounds.

TABLE-3
MIC AND MBC VALUES OF THE
EXTRACT ON THE SELECTED BACTERIAL

Selected bacterial strains	MIC (µg/mL)	MBC (µg/mL)
S. typhi (DMST 16122)	250	250
S. flexneri (DMST 4423)	500	500
S. aureus (ATCC 25293)	250	250
B. cereus (ATCC 11778)	250	250
E. coli 0157: H7 (DMST 12733)	500	500
S. typhi (gr. D)	500	500
S. dysenteriae	500	500
P. aeruginosa	500	500

Conclusion

The phenolic compounds in the extract of wild grape fruits from wine production using HPLC. Comparing to 10 external standards, the highest phenolic acid and flavonoid were gallic acid and quercetin, respectively. Other substances found gradually decreased and varied contents. Agar diffusion method indicated the antibacterial activity of the extract against twelve bacterial stains both Gram-negative and Gram-positive. Three bacterial strains were inhibited by the extract with high potency including *Salmonella typhi* (DMST 16122), *Staphylococcus aureus* (ATCC 25293), and *Bacillus cereus* (ATCC 11778) with the MIC and MBC values of 500-250 µg/mL. The wild grape residues from wine production composed various types of phenolic compounds, which were associated on the antibacterial activity. Therefore, the extract of wild grape residues would be used as a pharmaceutical source to prevent infective bacteria. The finding results are led to support for further research of this extract on bacteriostatic or bactericidal profiles and tumor cytotoxicity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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