



## Natural Phytochemicals and Biological Activities of Wild Grape (*Ampelocissus martinii* Planch.) Root Extract

P. SIRIPATTHANA<sup>1,\*</sup>, P. SRIHANAM<sup>2</sup> and A. SANGDEE<sup>3</sup>

<sup>1</sup>Protein and Enzyme Technology Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand

<sup>2</sup>Creative and Innovation Chemistry Research Unit and Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand

<sup>3</sup>Microbiology and Applied Microbiology Research Unit, Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand

\*Corresponding author: Tel./Fax: +66 43 754246; E-mail: patthraporn.s@msu.ac.th; nanasara1@gmail.com

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A hydromethanolic root extract of *Ampelocissus martinii* Planch. (*A. martinii*) was analyzed by standard methods for its phytochemical content, antioxidant activity,  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitions and antibacterial activities. The root extract exhibited the highest content of saponins, followed by phenols, proanthocyanidin and flavonoids, respectively. It showed high antioxidant activity in FRAP and CUPRAC assays. The root extract and standard Trolox had similar antioxidant activities in the DPPH and ABTS assay. It also showed much higher  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity compared to standard acarbose. Moreover, the root extract inhibited all tested Gram-positive bacteria with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 6.25 mg/mL. These results indicate that *A. martinii* root can be pharmaceutically used as active ingredients to prevent bacterial infection and radical-related diseases especially diabetes.

**Keywords:** Phytochemicals, Enzyme inhibition, Antioxidant, *Ampelocissus martinii*.

### INTRODUCTION

Free radicals can be generated by metabolism in the human body and by exposure to X-rays and environmental pollution [1]. An imbalance between free radicals and antioxidants results in oxidative stress, which causes various degenerative diseases such as diabetes, cardiovascular disease, cancer, hypertension, neurological disorder and chronic obstructive pulmonary diseases [1-5].

To remove or prevent the formation of free radicals, many researchers have been interested in natural substances containing biological activities with pharmacological effects [6]. Natural substances from plants have been noted and tested for their pharmacological activities such as antioxidant, antibacterial action or protection against the onset of degenerative diseases caused by free radicals [5,7-13]. Phytochemicals such as flavonoids [14], tannin [15], alkaloids [16] and sterols [17,18]

as well as saponins [19] have shown effects on degenerative diseases. Moreover, the derived plant substances have been proved for their safety and minimum side effects compared to synthetic drugs [20]. Therefore, medicinal plants have been popularly used worldwide, from past to present to cure many disease symptoms [21].

Wild grape (*Ampelocissus martinii* Planch.) is a herb commonly found in Thailand. Fruits have been used as food while the leaves, roots and bark have been used as traditional herb ingredients to provide relief of symptoms. Morphology of the wild grape is similar to cultivated grape [22,23]. Some reports have indicated that wild grape extracts have high levels of phytochemicals and biological activities such as antibacterial and antioxidant activities [22,24-26]. However, there is limited information on the biological activity of wild grape root extract. Therefore, this study attempted to investigate phytochemical compounds and some biological effects including antioxidant

properties,  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition and antibacterial activities of *A. martinii* root extract to obtain more information about its pharmaceutical characteristics.

## EXPERIMENTAL

All chemicals and reagents used were of analytical grade.  $\alpha$ -amylase,  $\alpha$ -glucosidase and potato starch were purchased from Sigma-Aldrich (Missouri, USA). *p*-Nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) and acarbose were obtained from Acros Organic (New Jersey, USA).

**Plant material and root extraction:** Wild grape roots were collected from Roi-Et Province, in northeastern Thailand in January 2020. The roots were washed, chopped into small pieces and then dried in shade. The dried materials were ground into a fine powder using an electronic grinder and stored in an air-tight container at room temperature. Root extract was prepared following the method of Park & Jhon [27], with some modifications. In brief, 10 g of dried root powder was extracted overnight with 1,000 mL of 70% methanol at room temperature using a magnetic stirrer. Supernatant was obtained by centrifugation. The pellet was re-extracted twice using the same procedure. All the supernatants were pooled and evaporated to dryness in a rotary vacuum evaporator at 40-45 °C to obtain root extract.

**Determination of phytochemical contents:** Total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC) and total saponin content (TSC) were determined using the methods of Farhadi *et al.* [9], Pekal and Pyszynska [28], Li *et al.* [29] and Hiai *et al.* [30], respectively.

**Determination of antioxidant activity:** The antioxidant activity of the root extract was tested using 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical (DPPH<sup>\*</sup>) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS<sup>+</sup>) in the DPPH assay [9] and ABTS assay [31], respectively. The antioxidant activity was expressed as the half maximal inhibitory concentration (IC<sub>50</sub>) value, which is the concentration required to cause 50% inhibition. The metal reducing antioxidant power was also evaluated by ferric reducing antioxidant power (FRAP) [32] and cupric reducing antioxidant capacity (CUPRAC) [33]. The results were reported as Fe<sup>2+</sup> equivalents ( $\mu\text{mol Fe}^{2+}/\text{g DW}$ ) and Trolox equivalents (mg TE/g DW) for FRAP and CUPRAC, respectively.

**$\alpha$ -Amylase inhibition assay:** The  $\alpha$ -amylase inhibition assay proposed by Wickramaratne *et al.* [10] with slight modification was performed. A volume of 0.2 mL of 2 Units/mL of  $\alpha$ -amylase was mixed with 0.2 mL of the extract at varying concentrations, then incubated for 5 min at 37 °C. A volume of 0.2 mL of 1% (w/v) soluble potato starch was added and incubated for 3 min at 37 °C. Thereafter, 0.2 mL of 3,5-dinitrosalicylic acid (DNSA) reagent was added to the mixture to terminate the reaction and boiled for 10 min at 92 °C. The mixture was cooled to room temperature, then 1.5 mL of distilled water was added and the absorbance at 540 nm was measured using a spectrophotometer. For the control, the assay was conducted in an identical fashion but 0.2 mL of the buffer was used instead of root extract. Acarbose was used as a positive control. Percent inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where  $A_c$  = absorbance of the control;  $A_s$  = absorbance of the root extract or acarbose.

A graph was constructed by plotting % inhibition against sample concentration. Half maximal inhibitory concentration (IC<sub>50</sub>) value which is the concentration required to cause 50% inhibition was obtained from the graph.

**$\alpha$ -Glucosidase inhibition assay:**  $\alpha$ -Glucosidase inhibition was measured using the modified method of Elya *et al.* [34]. Sample at varying concentrations, 0.75 Unit/mL of  $\alpha$ -glucosidase and 5 mM of *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) were prepared in 0.1 M potassium phosphate buffer, pH 6.8. Sample (0.25 mL) was mixed with  $\alpha$ -glucosidase solution (0.125 mL), incubated for 15 min at 37 °C and added to the PNPG solution (0.125 mL). After incubation for 25 min at 37 °C, 1 mL of 0.2 M Na<sub>2</sub>CO<sub>3</sub> was added to the mixture. The reaction mixture was diluted with 1 mL of distilled water. *p*-Nitrophenol released from PNPG was measured at 400 nm on a spectrophotometer. Acarbose was used as positive control.  $\alpha$ -glucosidase inhibition (%) and IC<sub>50</sub> were calculated.

**Determination of antibacterial activity:** The method of Sangdee *et al.* [35] was used for determination of the antibacterial activity. Six reference strains of pathogenic bacteria were used *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Salmonella typhi* DMST 22842, methicillin susceptible *Staphylococcus aureus* MSSA 2933, methicillin resistant *S. aureus* MRSA 20651 and *S. aureus* MRSA 4738. The root extract was screened for antibacterial activity against these bacteria using an agar well diffusion method. The inhibition zones in each plate were measured and compared with the reference standard antibiotic tetracycline at 0.25 mg/mL. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined using the broth microdilution method.

**Statistical analysis:** The data were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) version 25 (SPSS Inc., Chicago, USA). Value are expressed as mean of triplicate determination  $\pm$  standard deviation (mean  $\pm$  SD).

## RESULTS AND DISCUSSION

**Phytochemical contents:** Plants are well known as sources of natural products that have various biological and pharmaceutical activities. Therefore, they are the main ingredient for use on human health in traditional medicine [36]. The plants are composed of several types of phytochemicals, especially saponins and phenolic compounds [37]. In this work, the hydro-methanolic extract of wild grape root was investigated for its phytochemical contents. The phytochemical contents of the root extract were, in decreasing order, TSC (1040.54  $\pm$  2.62 mg AE/g DW), TPC (469.95  $\pm$  0.33 mg GAE/g DW), TPAC (243.15  $\pm$  3.03 mg CE/g DW) and TFC (16.39  $\pm$  0.07 mg QE/g DW). This correlates with the observations of Abifarín *et al.* [13] that TPC was present at higher content than TFC in methanolic root extract of *Cucumis africanus* and by Ngo *et al.* [11] that phytochemical levels were in the following ranked

order: TSC > TPC > TPAC for *Salacia chinensis* root. The TPC value in the present study was similar to reported results [25] and in the value ranges found in root extracts of *Urtica dioica* [38]. The TPAC content of the present study was almost 5.5X, 6X, 9X higher than the value found in root, stem and leaf, respectively of *S. chinensis* which is a plant used for treatment of various diseases including diabetes, skin diseases and inflammation. Moreover, present saponin value was almost 1.3X higher than that of root extract from *Salacia chinensis* [11]. The variation in types and contents of phyto-chemicals can be affected by different extraction solvents, plant parts, geographical regions from which the plant is sourced, seasons and maturity stages, polarity of phytochemicals, conditions used for study as well as analytical procedures [39-42].

**Antioxidant activity:** Several methods have been proposed to measure the antioxidant activity of substances based on a single-electron transfer (SET) reaction mechanism to reduce certain compounds, including metals and radicals. These methods include DPPH, ABTS, FRAP and CUPRAC assays [43]. The DPPH assay is based on the reducing ability of antioxidants towards DPPH<sup>\*</sup>, which results in a decrease of the absorbance value at 515 nm of a purple-coloured solution. The IC<sub>50</sub> value is the concentration of the substance required to cause 50% inhibition and a lower IC<sub>50</sub> reflects higher antioxidant activity [44]. As shown in Table-1, for the DPPH assay, the IC<sub>50</sub> value of the root extract (10.22 ± 0.02 mg/L) was lower than that of the standard Trolox (13.78 ± 0.04 mg/L). This means that the wild grape root extract showed more potent inhibition of DPPH radicals compared to Trolox. Its IC<sub>50</sub> value was also lower than the values found in roots of *Ferula gummosa* [45], root of *Zizyphus lotus* [46], bark of *Cordia dichotoma* [47], stem bark of *Anogeissus leiocarpus* [44] and different maturity stages of wild grape fruit [39]. Using the ABTS assay of the present study, the IC<sub>50</sub> of the root extract and Trolox had similar values at 5.57 ± 0.04 mg/L and 5.33 ± 0.01 mg/L, respectively. The Trolox activity result conformed to the findings of Wongnarat & Srihanam [39]. Compared to the reports of Wongnarat & Srihanam [39] and Thonpho *et al.* [40], the ABTS IC<sub>50</sub> value of the root extract was very much lower than the values for the fruits and pulp, but similar to that of seeds of wild grape.

For reducing antioxidant power, FRAP and CUPRAC assays were chosen because both methods are simple and widely used [9,32]. For the FRAP assay, the antioxidant activity of the extract is reflected through the reductive ability of ferric tripyridyltriazine (Fe<sup>3+</sup>-TPTZ) complex (colourless complex) to form the ferrous tripyridyltriazine (Fe<sup>2+</sup>-TPTZ) complex (blue coloured complex), which has an absorbance maximum at 593 nm [48]. In case of CUPRAC, an ability of the extract to

reduce the Cu<sup>2+</sup>-neocuproine complex to Cu<sup>+</sup>-neocuproine is measured. The high ability to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> or Cu<sup>2+</sup> to Cu<sup>+</sup> results in high calculated values of FRAP or CUPRAC. As shown in Table-1, the FRAP value of root extract was 550.45 ± 4.02 μmol Fe<sup>2+</sup>/g DW. This FRAP value was higher than that of extracts from many vegetables studied by Tiveron *et al.* [49] and extracts of root, stem, leaf and fruit from medicinal plants species [50], although it was much less than that of seed extract from wild grape [40]. For the CUPRAC assay, the root extract exhibited a high value (1328.28 ± 5.61 mg TE/g DW), which was higher than the values of extracts from wild grape fruits [39], seeds and pulps at different maturity stages [40]. These results indicated that the wild grape root extract has high antioxidant potential in both free radical scavenging (DPPH and ABTS assays) and reducing power (FRAP and CUPRAC assays). Therefore, the wild grape root could be considered as a powerful source of natural antioxidants.

#### Inhibition of α-amylase and α-glucosidase enzymes:

Inhibitory potential of the root extract on the enzymes α-glucosidase and α-amylase was chosen for evaluation because the root extract might help to protect against diabetes. α-Amylase hydrolyzes complex polysaccharides to oligosaccharides, which are then hydrolyzed by intestinal α-glucosidase to liberate glucose, which then enters the bloodstream. Inhibition of these enzymes can reduce postprandial blood glucose level [51]. There are many synthetic drugs used as inhibitors such as acarbose, voglibose and miglitol; however, they cause undesirable side effects including abdominal pain, diarrhea and flatulence [5,51].

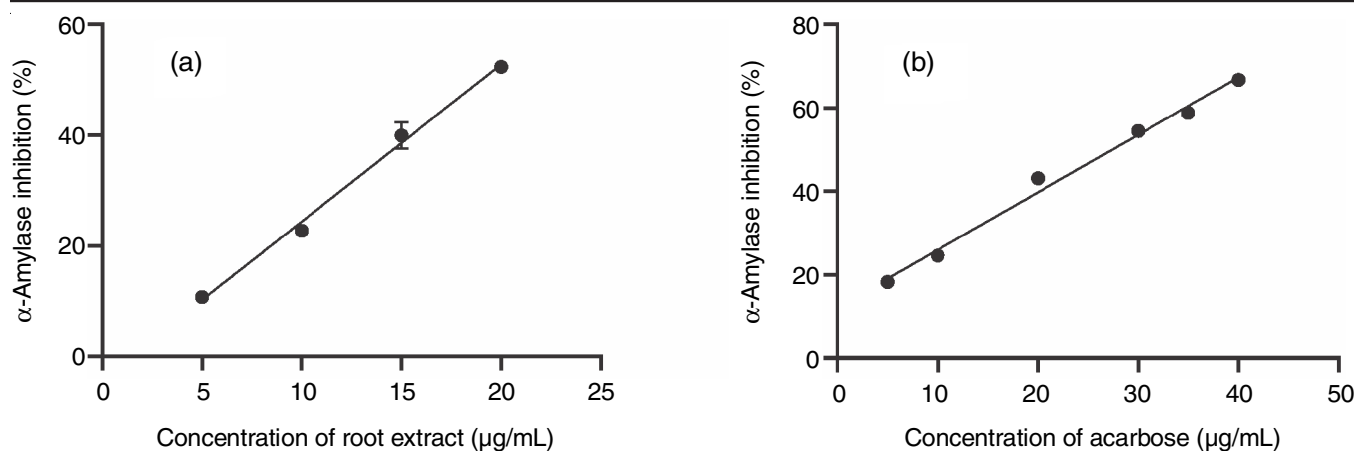
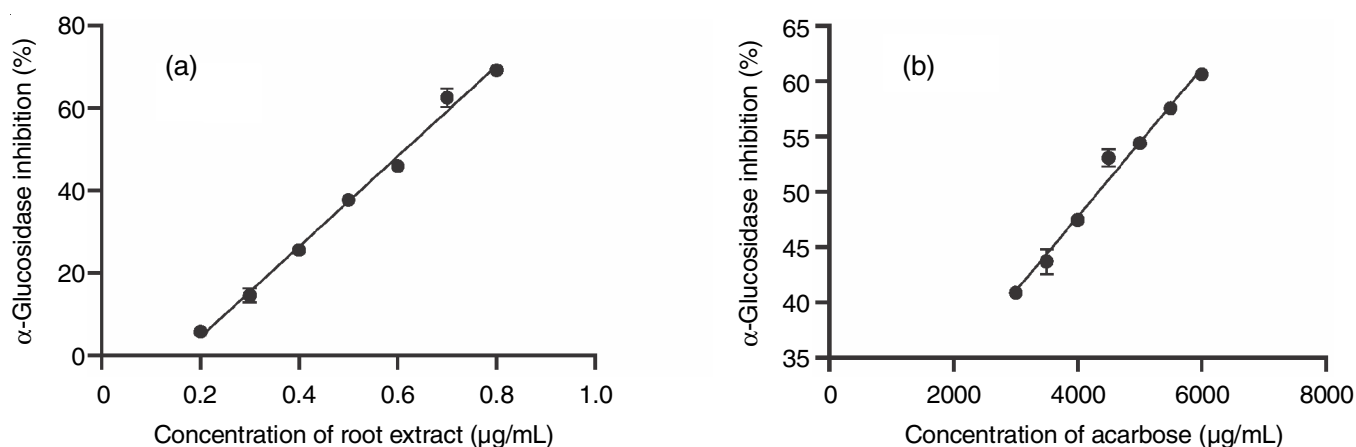
The enzyme inhibition activity of wild grape root extract is shown in Figs. 1 and 2. The root extract exhibited dual inhibiting potential against both enzymes and inhibitory activity increases in a dose dependent manner as the extract concentration increases. The root extract and acarbose showed α-amylase inhibition (IC<sub>50</sub> = 9.03 ± 0.27 and 27.42 ± 0.03 μg/mL, respectively) and α-glucosidase inhibition (IC<sub>50</sub> = 0.62 ± 0.01 and 4337.62 ± 30.80 μg/mL, respectively). These results indicated that the wild grape root extract had higher potential inhibition for both enzymes compared to acarbose, which was used as a standard inhibitor. The results were in agreement with previous reports about extracts of grape seed [52] and skin [53], *Rumex crispus* root [54] as well as *Moringa oleifera* root [55] that showed higher α-glucosidase inhibition activity than did acarbose. There are several reports showed that root extract of many plants exhibited more α-glucosidase inhibition than α-amylase inhibition [8,54,55].

**Antibacterial activity:** The wild grape root extract was screened for antibacterial activity. As shown in Table-2, the

TABLE-1  
ANTIOXIDANT ACTIVITY OF WILD GRAPE ROOT EXTRACT

Samples	DPPH assay IC <sub>50</sub> <sup>a</sup> (mg/L)	ABTS assay IC <sub>50</sub> (mg/L)	FRAP assay (μmol Fe <sup>2+</sup> /g DW)	CUPRAC assay (mg TE/g DW)
Root extract	10.22 ± 0.02	5.57 ± 0.04	550.45 ± 4.02	1328.28 ± 5.61
Trolox	13.78 ± 0.05	5.33 ± 0.01	ND	ND

<sup>a</sup>The concentration of plant extract required to cause 50% inhibition. The results are expressed as mean ± SD of triplicate measurements. ND: not determined, DW: dry weight, DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP: ferric reducing antioxidant power, CUPRAC: cupric reducing antioxidant capacity.

Fig. 1.  $\alpha$ -Amylase inhibitory activity of (a) wild grape root extract and (b) acarbose standardFig. 2.  $\alpha$ -Glucosidase inhibitory activity of (a) wild grape root extract and (b) acarbose standard

root extracts exhibited antibacterial activity against 4 strains of Gram-positive bacteria (*S. aureus* MSSA 2933, *S. aureus* MRSA 20651, *S. aureus* MRSA 4738 and *B. cereus* ATCC 11778), whereas no antibacterial activity was shown against Gram-negative bacteria (*S. typhi* DMST 22842 and *E. coli* ATCC 25922). The inhibition zone of the extracts ranged from 20-23 mm. However, the bacteria were less sensitive to the root extract than they were to tetracycline. The MIC and MBC of wild grape root extract were evaluated using a microdilution assay. The results showed that the MIC and MBC values of the root extract were both 6.25 mg/mL (Table-3). The difference in inhibition among Gram-positive and Gram-negative bacteria may reflect differences in bacterial cell surface structures between the two groups of bacteria. Gram-negative bacteria contain lipophilic ends of the lipoteichoic acids of the cell

membrane, which promotes penetration by hydrophobic compounds, whereas the external membrane of Gram-negative bacteria causes their surface to be highly hydrophilic [7]. The phytochemical compounds in wild grape root extract may participate in the bacterial inhibitions since many reports have indicated that phytochemical constituents present in plants could inhibit various types of microorganisms [56]. Phenolic compounds play an important role in inhibiting bacterial growth by disrupting the bacterial cytoplasmic membrane, leading to a change in membrane permeability and finally causing leakage of constituents such as proteins, nucleic acids and inorganic ions [57]. Flavonoids could inhibit bacterial pathogens by many actions such as inhibiting DNA gyrase, cytoplasmic membrane function or energy metabolism [58]. Zhang *et al.* [59] suggested that the methanolic extract of

TABLE-2  
SCREENING OF ANTIBACTERIAL ACTIVITY OF WILD GRAPE ROOT EXTRACT AGAINST  
6 STRAINS OF GRAM-POSITIVE AND GRAM-NEGATIVE PATHOGENIC BACTERIA

Samples	Diameter of inhibition zone (mm)					
	Gram-positive bacteria				Gram-negative bacteria	
	<i>S. aureus</i> MSSA2933	<i>S. aureus</i> MRSA20651	<i>S. aureus</i> MRSA4738	<i>B. cereus</i> ATCC11778	<i>S. typhi</i> DMST22842	<i>E. coli</i> ATCC25922
Root extract (25 mg/mL)	20 × 20	22 × 22	20 × 20	20 × 20	–	–
Root extract (50 mg/mL)	23 × 23	23 × 23	22 × 22	23 × 23	–	–
Tetracycline (0.25 mg/mL)	37 × 37	23 × 23	33 × 33	35 × 35	13 × 13	12 × 12

TABLE-3  
MIC AND MBC VALUES OF WILD GRAPE ROOT EXTRACT  
AGAINST 6 STRAINS OF PATHOGENIC BACTERIA

Bacterial species	MIC (mg/mL)	MBC (mg/mL)
<i>S. aureus</i> MSSA2933	6.25	6.25
<i>S. aureus</i> MRSA20651	6.25	6.25
<i>S. aureus</i> MRSA4738	6.25	6.25
<i>B. cereus</i> ATCC11778	6.25	6.25
<i>S. typhi</i> DMST22842	ND	ND
<i>E. coli</i> ATCC25922	ND	ND

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration; ND: Not determined

*Crataegus pinnatifida* containing high levels of flavonoids and polyphenols could inhibit *S. aureus* because they destroyed cell wall and plasma membrane integrity, inhibited enzymes and increased reactive oxygen species (ROS) in the cell together with modification of expression of associated genes and inducing apoptosis of the bacteria cell. There was a report that epicatechin-(4 $\beta$ →6)-epicatechin-(2 $\beta$ →O→7,4 $\beta$ →8)-catechin (EEC), a proanthocyanidin trimer from peanut skin, could inhibit *B. cereus* by interruption of the cell membrane and wall and by modification of nutritional metabolism [60]. Saponins of green tea showed ability to damage the bacterial cell wall and membrane [61]. Saponins of root and top of *Medicago* species exhibited high activity against many Gram-positive bacteria (*B. cereus*, *B. subtilis*, *S. aureus* and *Enterococcus faecalis*), but no inhibition (MICs > 0.5 mg/mL) against Gram-negative bacteria [62].

## Conclusion

The wild grape root extract contained high levels of phytochemicals and antioxidant activity. It showed higher potential of  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition as compared to acarbose. Moreover, the root extract exhibited antibacterial activity against certain Gram-positive bacteria with low MIC and MBC values at 6.25 mg/mL. These results indicate that wild grape root is a potential natural source of bioactive compounds which might be pharmaceutically used to prevent bacterial infection and radical-related diseases especially diabetes.

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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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