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# Optimizing Solvent Extraction for Potent Antioxidant and Antidiabetic Activities: A Study on *Ampelocissus martini* Root

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**Abstract**

The selection of extraction solvents is a critical factor influencing the composition and concentration of phytochemicals in plant extracts, thereby leading to variations in their biological activities. In this study, *Ampelocissus martini* Planch., an edible plant traditionally used for its medicinal properties, was investigated for its phytochemical profile and biological activities. We assessed the impact of different solvents used in fractionating the aqueous methanolic root extract of the plant on its phytochemical content, as well as its antioxidant, antidiabetic, and antibacterial properties. Among the 3 fractions—ethyl acetate, butanol, and water-soluble—the butanol fraction exhibited significantly higher levels of total phenolics and proanthocyanidins, while the ethyl acetate fraction contained the highest levels of total flavonoids and saponins. The water-soluble fraction showed lower concentrations of these compounds compared to the other 2 fractions. The butanol fraction demonstrated superior antioxidant activity, outperforming other fractions and several standard antioxidants, as evidenced by DPPH, ABTS, FRAP, and CUPRAC assays. Additionally, the butanol fraction showed greater antidiabetic activity, indicated by a lower $IC_{50}$ value, although it was still less effective than acarbose, a synthetic antidiabetic drug. Correlation analysis revealed a significant association between total proanthocyanidin content and the observed levels of antioxidant and antidiabetic activities across the fractions. All fractions also exhibited antibacterial activity against Gram-positive bacteria. Based on these findings, butanol emerges as the optimal solvent for extracting specific phytochemicals with potent antioxidant, antidiabetic, and antibacterial activities from the aqueous methanolic extract of *A. martini* root. The butanol fraction holds promise as a natural source of antioxidants for managing diabetes and its associated complications.

**Keywords:** α-amylase inhibitory, *Ampelocissus martini*, Antidiabetic, Antimicrobial, Antioxidant, Fractionation, Solvent extraction.

## Introduction

Free radicals are highly reactive molecules characterized by the presence of an unpaired electron in their outer shell. These molecules are generated both through normal metabolic processes within the body and as a result of exposure to environmental pollutants. An overproduction of free radicals, coupled with a reduction in antioxidant defense mechanisms, induces oxidative stress, which is implicated in the pathogenesis of numerous diseases, including diabetes [1].

Diabetes, one of the most prevalent chronic conditions globally, is marked by elevated blood glucose levels (hyperglycemia). The disease primarily arises from either insufficient insulin production (type 1 diabetes) or impaired insulin action (type 2 diabetes) [2]. The complications associated with diabetes include cardiovascular diseases, nephropathy (kidney disease), neuropathy (nerve damage), and retinopathy (eye disease). Additionally, individuals with diabetes are more vulnerable to microbial infections, which can trigger inflammation. This inflammation plays a critical role in the excessive generation of free radicals, further exacerbating the progression of diabetic complications [3,4].

A key therapeutic strategy for managing type 2 diabetes involves the inhibition of carbohydrate-digesting enzymes (α-amylase and α-glucosidase) using synthetic inhibitors such as acarbose, voglibose, and miglitol. However, these inhibitors are often associated with adverse side effects, including diarrhea, flatulence, and abdominal pain. Consequently, there is a pressing need for natural and safer enzyme inhibitors with potent antioxidant properties to treat diabetes and its complications [5].

Plants are potential sources of natural bioactive compounds that may offer safer alternatives for the treatment of type 2 diabetes and its complications. Numerous studies have identified plant-derived phytochemicals, such as phenolic compounds [6], flavonoids [7], proanthocyanidins [8], and saponins [9], as possessing both antioxidant and antidiabetic activities. However, the chemical diversity of phytochemicals can influence the types and concentrations of these compounds in different solvent extracts, leading to variability in their biological activities [10].

Wild grape (*Ampelocissus martini* Planch), which is widely distributed in Southeast Asia, including Thailand, has been traditionally used as a herbal remedy for various ailments. Phytochemicals, along with antioxidant and antimicrobial activities, have been documented in the plant's fruits [11], vines and rhizomes [12], and roots [13]. Notably, an aqueous methanolic extract from the root of this plant has demonstrated significant antidiabetic activity [13]. In this study, we investigated the phytochemical content, and *in vitro* antidiabetic, antioxidant, and antimicrobial activities of fractions obtained through various solvent extractions of the plant's aqueous methanolic root extract. Additionally, we examined the correlation between the phytochemical composition and the observed biological activities.

## Materials and methods

### Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and were sourced from QRec (Auckland, New Zealand), Sigma-Aldrich (Missouri, USA), Ajax Finechem (Auckland, New Zealand), Carlo Erba (Milan, Italy), and Acros Organics (New Jersey, USA).

### Plant sample collection and preparation

*Ampelocissus martini* specimens were collected from the wild in Roi-Et Province, Thailand, in January 2023. A voucher specimen (Siripipatthana 1) has been deposited at the Khon Kaen University (KKU) Herbarium, Thailand [14]. The fresh roots of the plant were thoroughly washed, shade-dried, and then ground into a fine powder using an electronic grinder. The powder was stored in a sealed glass container at 25 °C in darkness to preserve its integrity.

### Extraction and fractionation

The extraction method was adapted from Siripipatthana *et al.* [13] and Park and John [15] with several modifications. Root powder (10 g) was extracted with 300 mL of 70 % aqueous methanol at room temperature using a magnetic stirrer for 3 h. The mixture was then centrifuged (Rotanta 42R; Andres Hettich GmbH & Co. KG, Germany) to separate the supernatant from the pellet. The pellet was re-extracted twice using the same procedure. All supernatants were combined, filtered through Whatman No. 1 filter paper, and the methanol was removed by evaporation using a rotary vacuum evaporator (Hei-VAP G3; Heidolph Instruments GmbH & Co. KG, Germany) at 45 °C. The remaining aqueous solution was then extracted with hexane to remove lipophilic compounds, followed by sequential fractionation using solvents of increasing polarity (ethyl acetate and butanol). This process yielded 3 fractions: the ethyl acetate fraction, the butanol fraction, and the water-soluble fraction. The ethyl acetate and butanol fractions were concentrated using a

rotary vacuum evaporator at 45 °C, while the water-soluble fraction was dried using a combination of rotary vacuum evaporation and freeze-drying.

**Determination of phytochemical content**

Total phenolic content (TPC) and total proanthocyanidin content (TPAC) were quantified following the methods described by Farhadi *et al.* [16] and Li *et al.* [17], respectively. Total flavonoid content (TFC) and total saponin content (TSC) were assessed using the methods of Pekal and Pyrzynska [18] and Hiai *et al.* [19], respectively. The TPC was expressed as milligrams of Gallic Acid Equivalent per gram of Dry Weight (mg GAE/g DW), the TPAC as milligrams of Catechin Equivalent per gram of Dry Weight (mg CE/g DW), the TFC as milligrams of Quercetin Equivalent per gram of Dry Weight (mg QE/g DW), and the TSC as milligrams of Aescin Equivalent per gram of Dry Weight (mg AE/g DW).

**Determination of antioxidant activity**

The scavenging abilities of the samples against DPPH radicals ($DPPH^{\bullet}$) and ABTS radical cations ($ABTS^{\bullet+}$) were evaluated using the methods purposed by Farhadi *et al.* [16] and Re *et al.* [20], respectively. The samples' capacities to reduce metal ions ($Cu^{2+}$ and $Fe^{3+}$) were determined using the Cupric Reducing Antioxidant Capacity (CUPRAC) assay [21] and the Ferric Reducing Antioxidant Power (FRAP) assay [22], respectively. DPPH and ABTS activities were reported as the half-maximal inhibitory concentration ($IC_{50}$), indicating the concentration required to achieve 50 % inhibition. CUPRAC values were expressed as milligrams of Trolox Equivalent per gram of Dry Weight (mg TE/g DW), while FRAP values were reported as micromoles of Ferrous ion ($Fe^{2+}$) Equivalent per gram of Dry Weight (μmol $Fe^{2+}$/g DW).

**Determination of antidiabetic activity**

The inhibition of α-amylase activity was assessed using the method described by Wickramaratne *et al.* [23] to evaluate the antidiabetic potential of the samples. The antidiabetic activity was quantified as the $IC_{50}$, which represents the concentration of the sample required to achieve 50 % inhibition of enzyme activity.

**Determination of antibacterial activity**

Antibacterial activity of the samples was evaluated using the method described by Sangdee *et al.* [24]. The pathogens tested included Gram-negative bacteria (*Salmonella typhi* DMST 22842 and *Escherichia coli* ATCC 25922) and Gram-positive bacteria (*Staphylococcus aureus* MSSA 2933, *Staphylococcus aureus* MRSA 20651, *Staphylococcus aureus* MRSA 4738, and *Bacillus cereus* ATCC 11778). The agar well diffusion method was employed to screen the antibacterial activity of the samples against these bacterial strains. Additionally, the microdilution method was utilized to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the samples.

**Statistical analyses**

Experiments were conducted in triplicate, and results were expressed as the mean ± standard deviation (mean ± SD). Statistical analyses were performed using SPSS software version 25.0 (IBM, Armonk, NY, USA). Data were analyzed using analysis of variance (ANOVA), and significant differences between means were determined by Duncan's new multiple range test, with a significance level set at $p < 0.05$. Additionally, Pearson's correlation coefficients ($r$) were calculated to assess correlation.

## Results and discussion

**Effect of different solvents used in fractionation on phytochemical contents**

Numerous studies indicate that aqueous mixtures of organic solvents are highly effective for phytochemical extraction [10]. Consequently, a 70 % aqueous methanol solution with magnetic stirring was employed as the primary solvent to obtain the crude extract. Methanol was removed *via* evaporation, leaving an aqueous residue. Lipids were then extracted from this aqueous residue using hexane. Due to the varying chemical structures of phytochemicals and their associated polarity [9,25], multiple solvents with increasing polarities—ethyl acetate and butanol—were used sequentially to fractionate the phytochemicals into 3 distinct fractions: Ethyl acetate, butanol, and water-soluble fractions. **Figure 1** presents the phytochemical contents for these 3 fractions. TPC, expressed as mg GAE/g DW, and TPAC, expressed as mg CE/g DW,

exhibited a similar trend, increasing significantly in the order: water-soluble fraction (246.41 ± 1.15 and 119.19 ± 1.13) < ethyl acetate fraction (481.76 ± 1.47 and 136.23 ± 2.61) < butanol fraction (495.51 ± 2.98 and 236.46 ± 2.82). TFC, reported as mg QE/g DW, and TSC, reported as mg AE/g DW, also showed similar trends, increasing in the order: Water-soluble fraction (8.02 ± 0.00 and 642.76 ± 2.79) < butanol fraction (18.35 ± 0.04 and 1098.13 ± 5.92) < ethyl acetate fraction (20.72 ± 0.04 and 1274.86 ± 9.84). The observed variations in phytochemical contents across the fractions can be attributed to the polarity of the solvents used and the diverse structures of the phytochemical compounds [25]. The impact of extraction solvents on phytochemical content has been corroborated by several studies [10,12,26].

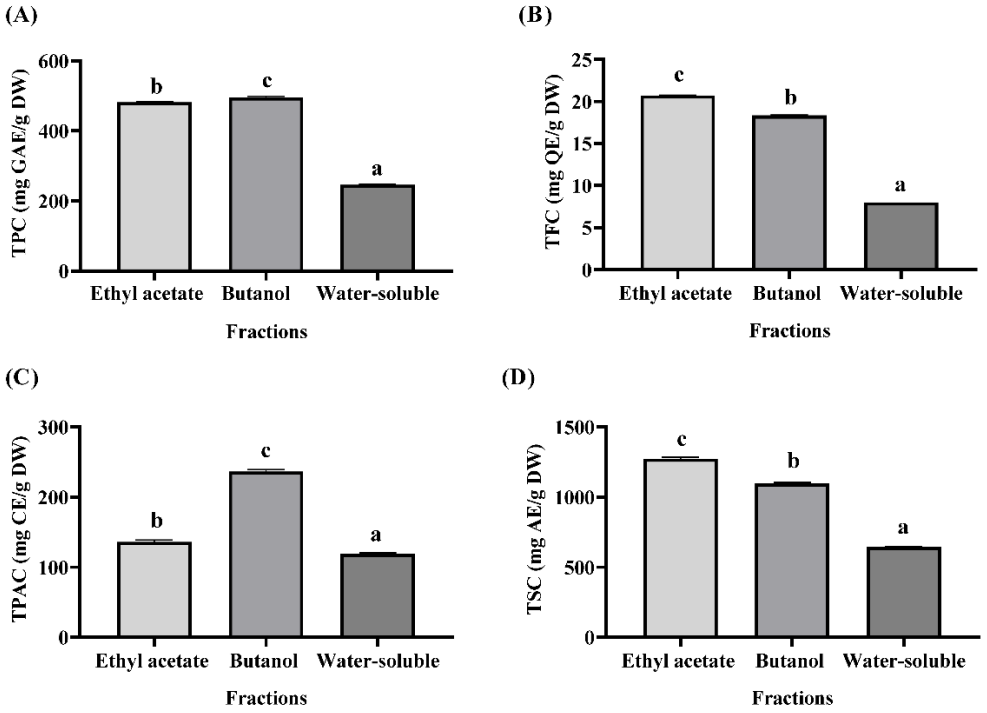


**Figure 1** Phytochemical content in fractions (A) total phenolic content (TPC), (B) total flavonoid content (TFC), (C) total proanthocyanidin content (TPAC) and (D) total saponin content (TSC). Different lowercase letters above the bars indicate significant differences ($p < 0.05$).

**Effect of different solvents used in fractionation on antioxidant activity**

Numerous studies indicate that different aqueous mixtures of organic solvents not only influence phytochemical content but also affect antioxidant activity [10,27]. In this study, we employed several well-established *in vitro* assays—namely DPPH, ABTS, FRAP, and CUPRAC—to evaluate antioxidant activity across all 3 fractions. These assays are favored for their simplicity and widespread use [10,25,28].

The DPPH assay is based on electron transfer principles. DPPH$^{\bullet}$, a stable free radical with a purple color, absorbs light at 517 nm [28]. Antioxidants in the sample reduce DPPH$^{\bullet}$ to a non-radical form, leading to a fading of the purple color. The extent of this color change correlates with the antioxidant activity of the sample [10,25,28]. The $IC_{50}$ value, representing the concentration required to achieve 50 % inhibition of DPPH$^{\bullet}$, was calculated from a plot of inhibition percentage versus extract concentration. A lower $IC_{50}$ value indicates greater free radical scavenging activity [29]. Similarly, the ABTS assay measures the reduction of the blue-green ABTS$^{\bullet+}$ radical cation, which absorbs light at 734 nm [10]. The addition of antioxidants to the ABTS$^{\bullet+}$ solution results in a reduction of the radical and a decrease in color intensity and absorbance. The $IC_{50}$ value for ABTS was determined from graphical analysis.

The $IC_{50}$ values for both DPPH and ABTS assays are summarized in **Table 1**. All 3 fractions (ethyl

acetate, butanol, and water-soluble) and standards (Trolox, BHT, and ascorbic acid) demonstrated dose-dependent inhibition of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. In the DPPH assay, all 3 fractions exhibited higher inhibition (i.e., lower $IC_{50}$ values) compared to BHT ($IC_{50}$ = 50.81 ± 0.09 mg/L). Notably, the butanol fraction displayed the strongest antioxidant effect ($IC_{50}$ = 13.21 ± 0.05 mg/L) compared to Trolox ($IC_{50}$ = 13.77 ± 0.02 mg/L), although it was less effective than ascorbic acid ($IC_{50}$ = 7.04 ± 0.01 mg/L). For the ABTS assay, the butanol fraction showed the highest antioxidant activity with the lowest $IC_{50}$ value of 4.12 ± 0.01 mg/L. Additionally, the ethyl acetate fraction exhibited greater $ABTS^{\bullet+}$ inhibition compared to BHT and Trolox.

**Table 1** Free radical scavenging effect of the fractions and standards (Trolox, BHT, and ascorbic acid).

| Sample | DPPH assay $IC_{50}$ (mg/L) | ABTS assay $IC_{50}$ (mg/L) |
|---|---|---|
| Ethyl acetate fraction | 19.39 ± 0.04$^{d}$ | 4.97 ± 0.02$^{c}$ |
| Butanol fraction | 13.21 ± 0.05$^{b}$ | 4.12 ± 0.01$^{a}$ |
| Water-soluble fraction | 22.71 ± 0.05$^{e}$ | 10.34 ± 0.03$^{f}$ |
| Trolox | 13.77 ± 0.02$^{c}$ | 5.34 ± 0.01$^{d}$ |
| BHT | 50.81 ± 0.09$^{f}$ | 5.91 ± 0.02$^{e}$ |
| Ascorbic acid | 7.04 ± 0.01$^{a}$ | 4.28 ± 0.01$^{b}$ |

BHT= Butylated hydroxytoluene; Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

Antioxidant activity was also assessed by measuring the reduction of metal ions such as $Cu^{2+}$ and $Fe^{3+}$. In the FRAP assay, the reduction of the $Fe^{3+}$-TPTZ complex to the $Fe^{2+}$-TPTZ complex results in a color change from colorless to blue, with absorbance at 593 nm [28]. The CUPRAC assay involves the reduction of the $Cu^{2+}$-neocuproine ($Cu^{2+}$-Nc) complex to $Cu^{+}$-Nc, resulting in a color change from light blue to yellow-orange, with absorbance at 450 nm [28]. Higher absorbance values indicate greater antioxidant capacity.

As detailed in **Table 2**, antioxidant activities in the FRAP assay were lower than that of ascorbic acid (1145.05 ± 3.12 μmol $Fe^{2+}$/g DW), although the butanol fraction exhibited significantly higher activity than the other fractions and BHT (287.16 ± 1.17 μmol $Fe^{2+}$/g DW). In the CUPRAC assay, all fractions showed greater antioxidant activity compared to BHT (719.02 ± 1.55 mg TE/g DW), with the butanol fraction displaying the highest antioxidant activity (1520.66 ± 11.42 mg TE/g DW). Overall, butanol was identified as the optimal solvent for extracting high antioxidant activity from the aqueous methanolic root extract of *Ampelocissus martini*.

**Table 2** Reducing power of the fraction and standards (BHT and ascorbic acid).

| Sample | FRAP assay (μmol $Fe^{2+}$/g DW) | CUPRAC assay (mg TE/g DW) |
|---|---|---|
| Ethyl acetate fraction | 262.58 ± 3.08$^{a}$ | 926.71 ± 5.86$^{c}$ |
| Butanol fraction | 710.50 ± 1.53$^{d}$ | 1520.66 ± 11.42$^{e}$ |
| Water-soluble fraction | 419.48 ± 1.84$^{c}$ | 859.34 ± 0.00$^{b}$ |
| BHT | 287.16 ± 1.17$^{b}$ | 719.02 ± 1.55$^{a}$ |
| Ascorbic acid | 1145.05 ± 3.12$^{e}$ | 1452.25 ± 1.17$^{d}$ |

BHT= Butylated hydroxytoluene; Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

**Effect of different solvents used in fractionation on antidiabetic activity of extracts**

α-amylase and α-glucosidase are 2 pivotal enzymes associated with type 2 diabetes. In the human small intestine, α-amylase catalyzes the hydrolysis of starch into oligosaccharides, which are subsequently broken down by α-glucosidase. This enzymatic activity results in the release of glucose molecules into the bloodstream, leading to a significant rise in postprandial blood glucose levels. While synthetic inhibitors such as acarbose and miglitol have been utilized to mitigate starch degradation and control postprandial glucose levels in type 2 diabetes, their use is often accompanied by adverse side effects. Consequently, there is growing interest in exploring phytochemicals as potential safer alternatives for antidiabetic therapy [30].

In this study, we evaluated the antidiabetic activity of 3 fractions from *Ampelocissus martini* root, along with acarbose, by assessing their inhibition of α-amylase activity. All tested samples, including acarbose, demonstrated dose-dependent inhibition of α-amylase, as illustrated in **Figure 2**. $IC_{50}$ values, which represent the concentration required to achieve 50 % enzyme inhibition, were calculated from the linear regression equations derived from the graph. Lower $IC_{50}$ values indicate more effective inhibition of α-amylase. The $IC_{50}$ values ranged from 27.70 ± 0.40 to 116.13 ± 0.37 µg/mL, with the butanol fraction showing significantly greater enzyme inhibition compared to the other fractions, though it was less effective than acarbose (**Table 3**).

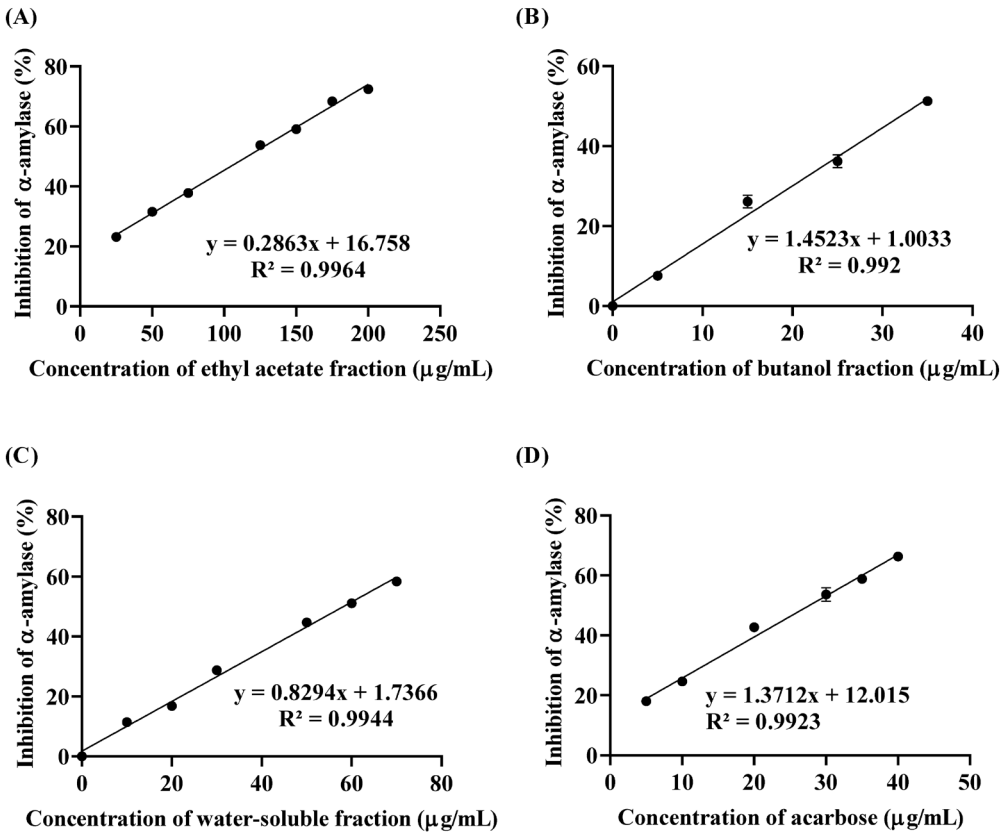


**Figure 2** Graph plot of α-amylase inhibition (%) against various concentrations of (A) ethyl acetate fraction, (B) butanol fraction, (C) water-soluble fraction, and (D) acarbose.

**Table 3** $IC_{50}$ values of the fractions and acarbose for α-amylase inhibition.

| Sample | α-amylase $IC_{50}$ (μg/mL) |
|---|---|
| Ethyl acetate fraction | $116.13 \pm 0.37^{d}$ |
| Butanol fraction | $33.75 \pm 0.73^{b}$ |
| Water-soluble fraction | $58.19 \pm 0.38^{c}$ |
| Acarbose | $27.70 \pm 0.40^{a}$ |

Different lowercase letters in the same column indicate significant differences ($p < 0.05$).

The inhibition of α-amylase by phenolic compounds, particularly proanthocyanidins, is primarily attributed to hydrophobic interactions and hydrogen bonding between these compounds and the enzyme. These interactions likely alter the enzyme's active site, reducing its ability to catalyze starch breakdown [31]. On the other hand, saponins appear to inhibit α-amylase through competitive inhibition, where they compete with the enzyme's natural substrate for binding at the active site, effectively blocking its activity [9].

**Correlation between phytochemical contents and biological activities**

The potential correlations between phytochemical contents (total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC), and total saponin content (TSC)), antioxidant activity (measured by DPPH, ABTS, FRAP, and CUPRAC assays), and α-amylase inhibitory activity across the 3 fractions were evaluated using Pearson's correlation analysis. The results, detailed in **Table 4**, are expressed as Pearson's correlation coefficients ($r$). TPC demonstrated a significant correlation with antioxidant activities measured by the DPPH ($r = -0.796$) and ABTS ($r = -0.997$) assays. Furthermore, ABTS assay results showed a significant correlation with both TFC ($r = -0.954$) and TSC ($r = -0.921$). Notably, TPAC was the only phytochemical that significantly correlated with antioxidant activities across all 4 assays. The inhibition of α-amylase activity exhibited a significant correlation solely with antioxidant activity assessed by the FRAP assay ($r = -0.920$).

These findings suggest that proanthocyanidins, a class of phenolic compounds, are pivotal in mediating both free radical scavenging and metal ion reduction activities. Additionally, fractions with strong $Fe^{3+}$ reducing power demonstrated notable α-amylase inhibitory activity. Therefore, the use of butanol as a solvent appears to be an effective method for isolating fractions rich in proanthocyanidins, antioxidants, and α-amylase inhibitors. Our results align with the findings of Li *et al.* [8], which identified proanthocyanidins as key contributors to antioxidant and α-amylase inhibitory activities.

**Antibacterial activity**

The rise in antibiotic use has been directly linked to the emergence of antibiotic-resistant bacteria, prompting the exploration of alternative antimicrobial agents derived from phytochemicals in plant extracts. These natural compounds are appealing due to their cost-effectiveness, safety, and accessibility relative to synthetic pharmaceuticals [32]. To assess the antibacterial potential of the 3 fractions and a synthetic drug (tetracycline), an agar-well diffusion assay was performed, with results presented in **Table 5**. At a concentration of 50 mg/mL, all 3 fractions demonstrated antibacterial activity against 4 strains of Gram-positive bacteria, producing inhibition zones ranging from 13 to 26 mm. None of the fractions exhibited activity against Gram-negative bacteria (*S. typhi* DMST 22842 and *E. coli* ATCC 25922). In comparison, all bacterial strains showed greater sensitivity to tetracycline than to the fractions. MIC and MBC values, determined by the microdilution method, are detailed in **Table 6**. Of the 3 Gram-positive strains tested (*S. aureus* MRSA20651, *S. aureus* MRSA4738, and *B. cereus* ATCC11778), the ethyl acetate fraction was found to be more effective, with an MIC value of 6.25 mg/mL, compared to 12.5 mg/mL for the other fractions.

These findings align with Bahri-Sahloul *et al.* [33], who reported that Gram-negative bacteria

generally exhibit higher resistance compared to Gram-positive bacteria. This resistance is likely due to the structural differences in cell membranes: Gram-negative bacteria possess an outer membrane that renders their surfaces highly hydrophilic, whereas the lipophilic components of the lipoteichoic acids in Gram-positive bacteria's membranes may facilitate the entry of hydrophobic compounds [33]. Phytochemicals such as saponins and phenolic compounds—including phenolic acids, flavonoids, stilbenes, and proanthocyanidins—are known to inhibit microbial growth and activity. Their antimicrobial effects are attributed to their diverse structural and chemical properties, which can lead to cell membrane permeabilization, destabilization, or inhibition of extracellular enzymes [34-36].

**Table 4** Pearson's correlation coefficient (*r*) of phytochemical content, antioxidant activity, and α-amylase inhibitory activity.

| Assay | TPC | TFC | TPAC | TSC | DPPH | ABTS | FRAP | CUPRAC | Amylase inhibition |
|---|---|---|---|---|---|---|---|---|---|
| **TPC** | 1 | 0.975** | 0.650 | 0.948** | −0.798** | −0.997** | 0.218 | 0.617 | 0.180 |
| **TFC** | | 1 | 0.463 | 0.995** | −0.643 | −0.954** | −0.005 | 0.426 | 0.396 |
| **TPAC** | | | 1 | 0.374 | −0.976** | −0.706* | 0.883** | 0.998** | −0.630 |
| **TSC** | | | | 1 | −0.565 | −0.921** | −0.103 | 0.335 | 0.484 |
| **DPPH** | | | | | 1 | 0.842** | −0.762* | −0.966** | 0.449 |
| **ABTS** | | | | | | 1 | −0.293 | −0.676* | −0.104 |
| **FRAP** | | | | | | | 1 | 0.902** | −0.920** |
| **CUPRAC** | | | | | | | | 1 | −0.662 |
| **Amylase inhibition** | | | | | | | | | 1 |

* = Correlation is significant at the 0.05 level; ** = Correlation is significant at the 0.01 level.

**Table 5** Zone of inhibition (mm) of the fractions and tetracycline against pathogenic bacteria.

| Microorganisms | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | **Ethyl acetate fraction (50 mg/mL)** | **Butanol fraction (50 mg/mL)** | **Water-soluble fraction (50 mg/mL)** | **Tetracycline (0.25 mg/mL)** |
| **Gram-positive bacteria** | | | | |
| *S. aureus* MSSA2933 | 23 | 19 | 15 | 37 |
| *S. aureus* MRSA20651 | 26 | 20 | 14 | 23 |
| *S. aureus* MRSA4738 | 23 | 19 | 13 | 33 |
| *B. cereus* ATCC11778 | 22 | 20 | 15 | 35 |
| **Gram-negative bacteria** | | | | |
| *S. typhi* DMST 22842 | - | - | - | 13 |
| *E. coli* ATCC 25922 | - | - | - | 12 |

(-) = No inhibition zone.

**Table 6** MIC and MBC values of the fractions against pathogenic bacteria.

| Microorganisms | MIC (mg/mL) | | | MBC (mg/mL) | | |
|---|---|---|---|---|---|---|
| | Ethyl acetate fraction | Butanol fraction | Water-soluble fraction | Ethyl acetate fraction | Butanol fraction | Water-soluble fraction |
| **Gram-positive bacteria** | | | | | | |
| *S. aureus* MSSA2933 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| *S. aureus* MRSA20651 | 6.25 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| *S. aureus* MRSA4738 | 6.25 | 12.5 | 12.5 | 12.5 | 25.0 | 12.5 |
| *B. cereus* ATCC11778 | 6.25 | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 |
| **Gram-negative bacteria** | | | | | | |
| *S. typhi* DMST 22842 | NT | NT | NT | NT | NT | NT |
| *E. coli* ATCC 25922 | NT | NT | NT | NT | NT | NT |

NT = Not Tested.

## Conclusions

The results from fractionating the aqueous methanolic extract of wild grape root indicate that the choice of solvent significantly influences both the composition and quantity of phytochemicals in the resulting fractions. Notably, the butanol fraction, which exhibited the highest proanthocyanidin content, demonstrated superior antioxidant and antidiabetic activities compared to the other fractions. Correlation analysis further confirmed a significant and direct relationship between proanthocyanidin content and the observed biological activities. Additionally, all fractions exhibited antibacterial properties. Given the high proanthocyanidin content and its associated biological effects, this study suggests that butanol is an effective solvent for fractionating the aqueous methanolic extract of wild grape root, yielding enhanced concentrations of specific phytochemicals and improved antioxidant and antidiabetic activities. The butanol fraction, therefore, shows potential as a valuable source of natural antioxidants for managing diabetes and its associated complications. Future research should focus on identifying the specific compounds within the butanol fraction and evaluating its *in vivo* antidiabetic effects.

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## References

[1] S Shohag, S Akhter, S Islam, T Sarker, MK Sifat, MM Rahman, MR Islam and R Sharma. Perspectives on the molecular mediators of oxidative stress and antioxidant strategies in the context of neuroprotection and neurolongevity: An extensive review. *Oxidative Medicine and Cellular Longevity* 2022; **2022**, 7743705.

[2] A Rosengren and P Dikaiou. Cardiovascular outcomes in type 1 and type 2 diabetes. *Diabetologia* 2023; **66(3)**, 425-437.

[3] MA Darenskaya, LI Kolesnikova and SI Kolesnikova. Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bulletin of Experimental Biology and Medicine* 2021; **171(2)**, 136-149.

[4] MU Sohail, F Mashood, A Oberbach, S Chennakkandathil and F Schmidt. The role of pathogens in diabetes pathogenesis and the potential of immunoproteomics as a diagnostic and prognostic tool. *Frontiers in Microbiology* 2022; **13**, 1042362.

[5] EA Makinde, C Ovatlarnporn, AE Adekoya, OF Nwabor and OJ Olatunji. Antidiabetic, antioxidant and antimicrobial activity of the aerial part of *Tiliacora triandra*. *South African Journal of Botany* 2019; **125**, 337-343.

[6] D Cheng, P Wang, J Huang, B Yang, M Ma, P Yu, Z Zeng, D Gong and S Deng. Antioxidant, antidiabetic and identification of phenolic constituents from *Potentilla discolor* Bge. *European Food Research and Technology* 2020; **246**, 2007-2016.

[7] TF Rakotondrabe, M Fan and M Guo. Exploring potential antidiabetic and anti-inflammatory flavonoids from *Euphorbia humifusa* with an integrated strategy. *Frontiers in Pharmacology* 2022; **13**, 980945.

[8] X Li, J Liu, Q Change, Z Zhou, R Han and Z Liang. Antioxidant and antidiabetic activity of proanthocyanidins from *Fagopyrum dibotrys*. *Molecules* 2021; **26(9)**, 2417.

[9] MO Nafiu and AOT Ashafa. Antioxidant and inhibitory effects of saponin extracts from *Dianthus basuticus* Burtt Davy on key enzymes implicated in type 2 diabetes *in vitro*. *Pharmacognosy Magazine* 2017; **13(52)**, 576-582.

[10] T Venkatesan, YW Choi and YK Kim. Impact of different extraction solvents on phenolic content and antioxidant potential of *Pinus densiflora* bark extract. *BioMed Research International* 2019; **29**, 3520675.

[11] J Jirum, A Sangdee and P Srihanam. Phytochemical and biological activities in fresh juice extracts of wild grape (*Ampelocissus martini* Planch) Fruits. *International Journal of Research in Ayurveda and Pharmacy* 2013; **4(3)**, 337-341.

[12] L Vittaya, S Khongsai, J Ui-Eng, C Chalad and N Leesakul. Effect of total phenolic and flavonoid contents of *Ampelocissus martini* in radical scavenging and antibacterial activities. *Agriculture and Natural Resources* 2019; **53(2)**, 154-160.

[13] P Siripipatthana, P Srihanam and A Sangdee. Natural phytochemicals and biological activities of wild grape (*Ampelocissus martinii* Planch.) root extract. *Asian Journal of Chemistry* 2021; **33(3)**, 545-550.

[14] P Siripipatthana. Alpha-amylase inhibitory and antioxidant activities in aqueous acetone extract and its fractions from *Ampelocissus martini* root. *Indian Journal of Pharmaceutical Sciences* 2022; **84(4)**, 929-937.

[15] EJ Park and DY Jhon. The antioxidant, angiotensin converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. *LWT - Food Science and Technology* 2010; **43(4)**, 655-659.

[16] K Farhadi, F Esmaeilzadeh, M Hatami, M Forough and R Molaie. Determination of phenolic compounds content and antioxidant activity in skin, pulp, seed, cane and leaf of five native grape cultivars in West Azerbaijan province, Iran. *Food Chemistry* 2016; **199**, 847-855.

[17] Y Li, C Guo, J Yang, J Wei, J Xu and S Cheng. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry* 2006; **96(2)**, 254-260.

[18] A Pekal and K Pyrzynska. Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods* 2014; **7**, 1776-1782.

[19] S Hiai, H Oura and T Nakajima. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. *Planta Medica* 1976; **29(2)**, 116-122.

[20] R Re, N Pellegrini, A Proteggente, A Pannala, M Yang and C Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 1999; **26(9-10)**, 1231-1237.

[21] R Apak, K Güçlü, M Özyürek and SE Karademir. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *J. Agric. Food Chem.* 2004; **52(26)**, 7970-7981.

[22] LL Zhang and YM Lin. Tannins from *Canarium album* with potent antioxidant activity. *J. Zhejiang Univ. Sci. B* 2008; **9(5)**, 407-415.

[23] MN Wickramaratne, JC Punchihewa and DBM Wickramaratne. *In-vitro* alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonine*. *BMC Complementary and Alternative Medicine* 2016; **16**, 466.

[24] K Sangdee, W Nakbanpote and A Sangdee. Isolation of the entomopathogenic fungal strain Cod-MK1201 from a Cicada nymph and assessment of its antibacterial activities. *Int. J. Med. Mushrooms* 2015; **17(1)**, 51-63.

[25] R San Miguel-Chavez. *Phenolic antioxidant capacity: A review of the state of the art*. *In*: M Soto-Hernandez, M Palma-Tenango and MdR Garcia-Mateos (Eds.). Phenolic compounds-Biological activity. IntechOpen, Rijeka, 2017, p. 59-74.

[26] Z Zreen, A Hameed, S Kiran, T Farooq and MS Zaroog. A Comparative Study of *Diospyros malabarica* (Gaub) extracts in various polarity-dependent solvents for evaluation of phytoconstituents and biological activities. *BioMed Research International* 2022; **2022**, 746223.

[27] M Casagrande, J Zanela, A Wagner Júnior, C Busso, J Wouk, G Iurckevicz, PF Montanher, F Yamashita and CRM Malfatti. Influence of time, temperature and solvent on the extraction of bioactive compounds of *Baccharis dracunculifolia*: *In vitro* antioxidant activity, antimicrobial potential, and phenolic compound quantification. *Industrial Crops and Products* 2018; **125**, 207-219.

[28] IG Munteanu and C Apetrei. Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences* 2021; **22(7)**, 3380.

[29] G Zengin, YS Cakmak, GO Guler and A Aktumsek. *In vitro* antioxidant capacities and fatty acid compositions of three *Centaurea* species collected from Central Anatolia region of Turkey. *Food and Chemical Toxicology* 2010; **48(10)**, 2638-2641.

[30] G Oboh, AT Isaac, AJ Akinyemi and RA Ajani. Inhibition of key enzymes linked to type 2 Diabetes and sodium nitroprusside induced lipid peroxidation in rats' pancreas by phenolic extracts of avocado pear leaves and fruit. *International Journal of Biomedical Science* 2014; **10(3)**, 208-216.

[31] MA Asgar. Anti-diabetic potential of phenolic compounds: A review. *International Journal of Food Properties* 2013; **16(1)**, 91-103.

[32] B Khameneh, NAM Eskin, M Iranshahy and BSF Bazzaz. Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics* 2021; **10(9)**, 1044.

[33] R Bahri-Sahloul, RB Fredj, N Boughalleb, J Shriaa, S Saguem, JL Hilbert, F Trotin, S Ammar, S Bouzid and F Harzallah-Skhiri. Phenolic composition and antioxidant and antimicrobial activities of extracts obtained from *Crataegus azarolus* L. var.aronia (Willd.) Batt. ovaries calli. *Journal of Botany* 2014; **2014**, 623651.

[34] T Tamura, M Ozawa, N Tanaka, S Arai and K Mura. *Bacillus cereus* response to a proanthocyanidin trimer, a transcriptional and functional analysis. *Current Microbiology* 2016; **73(1)**, 115-123.

[35] MI Khan, A Ahhmed, JH Shin, JS Baek, MY Kim and JD Kim. Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study *in vitro* and *in vivo*. *Evidence-Based Complementary and Alternative Medicine* 2018; **26**, 3486106.

[36] M Takó, EB Kerekes, C Zambrano, A Kotogán, T Papp, J Krisch and C Vágvölgyi. Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants* 2020; **9(2)**, 165.