

From Phenolics to Function: Anti-Diabetic Potentials of Hawthorn, Pomegranate, and Jujube Vinegars

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Abstract: This study aimed to compare the phenolic profile and antidiabetic activities of extracts and vinegars of pomegranate, hawthorn, and jujube. Extracts and vinegars of plants were evaluated by LC–MS/MS for their individual phenolic profiles. Antidiabetic potential was assessed in vitro by measuring α -amylase and α -glucosidase inhibition with acarbose as a positive control. Results showed unique phenolic profiles: pomegranate extracts and vinegar (especially pomegranate vinegar 2) were enriched in ellagic acid (68.76 mg/g) derivatives; hawthorn 70% ethanolic extract in quinic acid (89.355 mg/g) and hawthorn vinegar 1 in quinic acid (35.899 mg/g) and flavonol glycosides; and jujube ethanolic extract in quinic acid (77.803 mg/g), jujube vinegar 1 in quinic acid (101.568 mg/g) and protocatechuic derivatives. Vinegar fermentation enhanced phenolic recovery, resulting in higher levels of bioactive compounds compared to extracts, and it improved the recovery of phenolics, as the amounts of bioactive compounds were higher in vinegars than in extracts. Both pomegranate and hawthorn vinegar 2 exerted the highest α -glucosidase inhibitions (110.97 ± 3.01 and 101.08 ± 1.71). Jujube vinegars (especially jujube vinegar 1) showed complementary features with greater enzyme inhibitions (97.96 ± 1.27) along with previously reported insulin-sensitivity properties. This is the first study to provide a comparative evaluation of phenolic composition and antidiabetic potential of pomegranate, hawthorn, and jujube vinegars.

Keywords: Pomegranate; hawthorn; jujube; vinegars; phenolics; enzyme inhibition. © 2025 ACG Publications. All rights reserved.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder resulting from the combined effects of impaired insulin secretion and insulin resistance, leading to chronic hyperglycaemia and dysregulation of blood glucose levels. Postprandial hyperglycaemia is a target of prevention and treatment efforts, and the inhibition of carbohydrate-hydrolysing enzymes such as α -amylase and α -glucosidase is one of the most effective nutritional strategies for glycaemic management [1]. Although synthetic inhibitors, such as acarbose, exist and are used in clinical settings, their gastrointestinal side effects have led to increased interest in safer plant-derived food supplements containing phenolic compounds [2].

Pomegranate (*Punica granatum* L.) is a polyphenol-containing fruit that is commonly eaten in the form of juice, extract, or vinegar. Its ellagitannins, anthocyanins, and phenolic acids have been demonstrated to selectively inhibit α -glucosidase activity and improve insulin sensitivity, thereby reducing postprandial glucose fluctuations [3, 4]. Jujube (*Ziziphus jujuba* Mill.), commonly known as Chinese jujube or red date, contains flavonoids (quercetin, rutin), triterpenes, and polysaccharides as its active compounds and has been shown to exert both enzyme inhibition and PI3K/Akt-mediated insulin-sensitizing activities. An increasing body of evidence from preclinical and clinical studies supports its antidiabetic potential [5]. Hawthorn (*Crataegus* spp.), used in traditional medicine for its cardiovascular health benefits, also contains flavonoid glycosides (hyperoside, vitexin) and proanthocyanidins. These compounds have been documented to inhibit α -amylase and α -glucosidase, lower starch digestibility, and regulate metabolism [6,7].

In addition to fresh fruits and extracts, fermentation procedures can improve bioactivity. Fruit-based vinegars maintain polyphenols and produce low-molecular-weight, easily bioavailable phenolic metabolites and organic acids during acetic acid fermentation that further enhance antioxidant and anti-diabetic effects [8,9]. Recent investigations have demonstrated the ability of pomegranate and tropical fruit vinegars to potentiate enzyme inhibition and modulate glucose homeostasis; however, systematic comparisons of their effects remain limited.

Although individual phenolic profiles of pomegranate, hawthorn, and jujube have been reported, no comparative assessment of their vinegars and extracts using a standardized LC–MS/MS platform coupled with enzyme inhibition profiling has been presented before. Given this background, this study was conducted to offer a systematic characterization of phenolic compounds and anti-diabetic effects of hawthorn, jujube, and pomegranate vinegars and their extracts. To our knowledge, no previous study has integrated a validated LC–MS/MS quantification of phenolics with enzyme inhibition assays across pomegranate, hawthorn, and jujube vinegars and extracts. This comparative approach provides new insight into how acetic fermentation modulates phenolic composition and bioactivity among different fruit matrices. This approach offers new perspectives on these fruits as functional foods in the control of diabetes.

2. Materials and Methods

2.1. Chemicals

Pyrocatechol, quercetin, quinic acid, fumaric acid, aconitic acid, gallic acid, epigallocatechin, protocatechuic acid, catechin, gentisic acid, chlorogenic acid, protocatechuic aldehyde, tannic acid, epigallocatechin gallate, cynarin, 4-hydroxybenzoic acid, epicatechin, vanillic acid, caffeic acid, syringic acid, vanillin, syringic aldehyde, daidzin, epicatechin gallate, piceid, p-coumaric acid, ferulic acid-D3-IS, ferulic acid, sinapic acid, coumarin, salicylic acid, cyranoside, miquelianin, rutin-D3-IS, rutin, isoquercitrin, hesperidin, o-coumaric acid, genistin, rosmarinic acid, ellagic acid, cosmosiin, quercitrin, astragalin, nicotiflorin, fisetin, daidzein, quercetin-D3-IS, naringenin, hesperetin, luteolin, genistein, kaempferol, apigenin, amentoflavone, chrysin, and acacetin, all these standards were purchased from certified suppliers (Merck, Türkiye) and used for the qualitative and quantitative analysis of phenolic compounds in the samples. Analytical grade reagents, enzymes, solvents, and substrates were also purchased from Merck.

2.2. Sample Preparation and Extraction

Fresh fruits of pomegranate (*Punica granatum* L.), jujube (*Ziziphus jujuba* Mill.), and hawthorn (*Crataegus* spp.) were purchased from a local market in Istanbul, Türkiye. Since the samples were obtained from commercial markets, taxonomic authentication was based on morphological comparison with authenticated specimens, and Dr. Çağlayan Ünsal Gürer, a pharmacognosyist at İstanbul University, identified the fruits. Approximately 500 g of each fruit was shade-dried, ground into powder, and subjected to sequential maceration. Samples were extracted three times with either ethanol (EtOH) or ethanol: water (70: 30, v/v) (1000 mL) at room temperature for 24 h. After extraction, the filtrates were pooled, and the solvents were evaporated under reduced pressure. The crude extracts were then freeze-dried at -50°C to obtain dry powders. Extraction yields were calculated as follows: pomegranate EtOH extract 28.88% and 70% EtOH extract 18.33%; jujube EtOH extract 14% and 70% EtOH extract 49%; hawthorn EtOH extract 3.3% and 70% EtOH extract 1.5% (w/w). All dried extracts were stored at -18°C until further analysis.

2.3. Vinegar Samples

Three commercially available pomegranate vinegars produced in accordance with the Turkish Food Codex and ISO quality standards were purchased from a local market in Istanbul, Türkiye. A vinegar from a widely marketed brand was selected as the reference sample for comparative purposes. Brand names are not disclosed to prevent potential legal issues. Values were expressed as mg/g of dry extract equivalent obtained from the vinegar sample.

2.4. Stock Solutions

Crude extract stock solutions (4000 µg/mL) were prepared, and working concentrations were obtained by serial dilution as described by Akdeniz *et al.* [10].

2.5. LC-MS/MS Analysis

Analysis of phenolic profiles in extractions and vinegars from the plants was performed using a validated LC-MS/MS method [10, 11]. Chromatographic separation was achieved with a Shimadzu Nexera UHPLC system (Shimadzu Corporation, Japan) coupled to an LC-MS/MS-8040 Shimadzu triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, operating in both positive and negative ion mode. The UHPLC system comprised an autosampler, column oven, binary pump, and degasser.

Chromatographic separation was performed on the Agilent Poroshell 120 EC-C18 analytical column (150 × 2.1 mm, 2.7 µm) at 40 °C with the mobile phase of elution buffer A (5 mM ammonium formate and 0.1% formic acid in water) and elution buffer B (5 mM ammonium formate and 0.1% formic acid in methanol). The gradient elution was performed with mobile phase B, increasing from 20% to 100% (0–25 min), holding at 100% B (25–35 min), and then returning to 20% B (re-equilibration) from 35 to 45 min. The flow rate was 0.5 mL/min, and the injection volume was 5 µL.

Fifty-six phenolics (53 phenolic compounds and 3 internal standards) were quantified in MRM mode. Product ions for each analyte were generated using predetermined precursor-to-product ion transitions and optimized collision energy to achieve selectivity and sensitivity. The operating conditions of MS were as follows: nitrogen drying gas flow, 15 L/min; nebulizing gas flow, 3 L/min; maintained at DL temperature, 250°C; heat block temperature, 400°C; interface temperature, 350°C; and the data acquisition and processing were performed by LabSolutions software (Shimadzu).

2.6. Enzyme Inhibition Assays

2.6.1. Antidiabetic Activity

Inhibition of α -amylase and α -glucosidase was evaluated by a modified spectrophotometric method as described by Lazarova *et al.* (2015) [12].

2.6.2. α -Amylase Inhibition Assay

Inhibition of the extracts and vinegars on α -amylase was evaluated by a modified spectrophotometric method. In brief, 1 mL of the sample solution, prepared in phosphate buffer (pH: 6.9), was mixed with 1 mL of porcine pancreatic α -amylase (1 U/mL). 1% starch solution (dissolved in the same buffer) was used as the substrate and added to the tube after a 10 min pre-incubation at 37°C.

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1 ml of dinitro salicylic acid (DNS) reagent was added to terminate the reaction after incubation at 37°C for 15 min. The tubes were placed in a boiling water bath for 5 min, cooled, diluted with distilled water, and the absorbance was read at 540 nm. The positive control was acarbose, and the inhibition percentage was measured relative to enzyme activity in the absence of the inhibitor.

2.6.3. α -Glucosidase Inhibition Assay

The inhibition of α -glucosidase was measured by the *p*-nitrophenyl- α -D-glucopyranoside (pNPG) as substrate. 50 μ L of sample solution was mixed with 100 μ L of α -glucosidase enzyme (0.5 U/mL in phosphate buffer pH: 6.8) before pre-incubating the mixture at 37°C for 10 min. The reaction was initiated by adding 50 μ L of 5 mM pNPG and incubating for 20 min at 37°C, then stopped by adding 2 mL of 0.1 M Na₂CO₃, and the absorbance of the released *p*-nitrophenol was measured at 405 nm. Acarbose was used as the reference inhibitor. Percent inhibition (%) was calculated as compared to the control absorbance. For these assays, the percentage inhibition values were calculated accordingly to Equation (1).

$$\text{Inhibition (\%)} = 100 - \left(\frac{OD_{\text{test well}}}{OD_{\text{control}}} \right) * 100 \quad (1)$$

2.7. Statistical Analysis

All experiments were conducted in triplicate, and data are presented as mean $\pm$ standard deviation. Independent variables were subjected to a one-way analysis of variance (ANOVA) to identify statistically significant differences at $p \leq 0.05$. When a significant effect was observed, a Tukey test was used to conduct post hoc comparisons of the means. Statistical computations and principal component analysis (PCA) were performed using JASP 0.19.1.0. Data visualization was executed with SR Plot [13].

3. Results and Discussion

3.1. LC-MS/MS Analysis for Phenolic Content

As shown in Figure 1 and Table S1, the phenolic composition of pomegranate, jujube, and hawthorn extracts varied notably with the solvent type. Pomegranate ethanolic extract contained 12 compounds (9.75 mg/g, Figure 2. (B)) while the 70% ethanolic extract showed fewer compounds but a slightly higher total content (10.44 mg/g, Figure 2. (C)). The 70% ethanolic extract had the richest profile, with 14 compounds (95.89 mg/g, Figure 2 (E)), compared to only 6 compounds in the ethanolic extract (0.81 mg/g, Figure 2 (D)). In jujube, the ethanolic extract contained 14 compounds (83.17 mg/g; Figure 2 (F)), whereas the 70% ethanolic extract contained only 4 compounds (0.75 mg/g; Figure 2 (G)).

LC-MS/MS results indicated that pomegranate extracts were rich in ellagic acid (7.608 mg/g), with higher levels in the 70% ethanol extract (9.690mg/g) compared to the ethanol extract. Although punicalagin was not quantified, ellagic acid derivatives quantified in our study are known hydrolysis products of punicalagin, thereby indirectly reflecting ellagitannin content. Hawthorn 70% ethanolic extract exhibited a markedly different profile, dominated by quinic acid (89.355 mg/g), isoquercitrin (2.915mg/g), and quercetin (1.335mg/g). In contrast, jujube ethanol extract contained high levels of quinic acid (77.803mg/g) and moderate levels of protocatechuic acid (1.823mg/g), isoquercitrin (2.406mg/g), and quercitrin (0.581mg/g). In general, phenolic recovery was greater with 70% ethanol, highlighting the role of solvent polarity in extracting polar phenolics, such as ellagitannins and flavonoid glycosides.

The phenolic profiles (Figure 1, Figure 2(A), Figure 3(A), Figure 4(A), Figure 5(A), and Table S1) highlight the unique phenolic signatures of each fruit. In pomegranate, predominance of ellagitannins, particularly ellagic acid, supports previous studies showing that punicalagin and related derivatives are the principal phenolics in both peel and juice [14, 15]. These compounds are well known for their antioxidant effects and their role in modulating carbohydrate metabolism. More recently, Shahkoomahally et al. [16] confirmed that punicalagin A and B remain the most abundant phenolics across diverse pomegranate cultivars, underlining their importance as biomarkers.

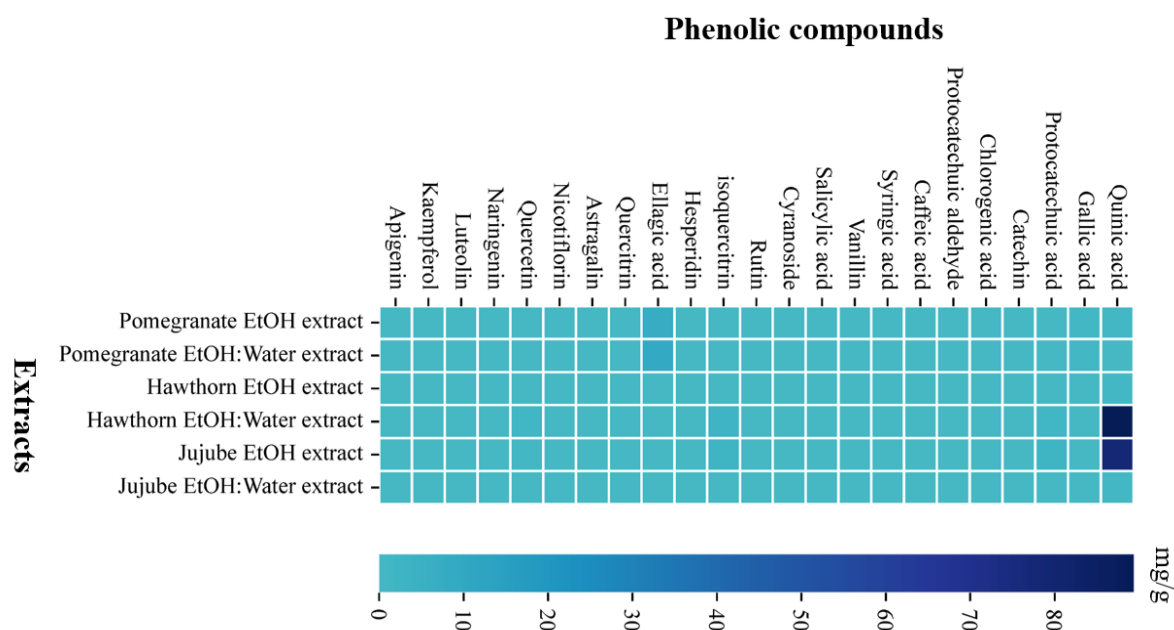


Figure 1. Matrix heatmap designation of phenolic compounds found in pomegranate EtOH extract, pomegranate EtOH: water extract, hawthorn EtOH extract, hawthorn EtOH: water extract, jujube EtOH extract, and jujube EtOH: water extract. Values in mg/g (w/w) of extracts. EtOH means ethanolic extract, and EtOH: water shows 70% ethanolic extract.

Hawthorn extracts, in contrast, contained high levels of quinic acid and quercetin derivatives, which is consistent with earlier findings that *Crataegus* spp. are rich in flavanol glycosides (e.g., hyperoside, isoquercitrin) and procyanidins [17, 18]. These compounds are associated not only with cardioprotective effects but also with inhibitory activity against carbohydrate-hydrolyzing enzymes, indicating their relevance to both cardiovascular and metabolic health. The relatively high concentration of quinic acid found here further supports its contribution to hawthorn's bioactivity.

Jujube extracts displayed a simpler but distinctive profile, dominated by quinic acid, protocatechuic acid, isoquercitrin, and quercitrin. This is in agreement with previous reports describing chlorogenic acid, rutin, catechin, and protocatechuic acid as the characteristic phenolics of jujube fruits [19, 20]. These constituents have been linked to antioxidant activity as well as anti-diabetic and anti-inflammatory effects, suggesting multiple pathways through which jujube may exert health benefits.

The phenolic composition of pomegranate, hawthorn, and jujube vinegars exhibited distinct differences (Figure 6. (A), Table S2). Pomegranate vinegars (Figure 3. (B), (C), (D)) were characterized by high levels of ellagic acid (62.64–68.76 mg/g). A substantial amount of epicatechin (68.37mg/g) in pomegranate vinegar 1 and gallic acid (up to 169.90 mg/g) in pomegranate vinegar 2 were observed. Hawthorn vinegars (Figure 4. (B), (C), (D)) contained relatively lower ellagic acid but notable levels of quinic acid (8.39–35.89 mg/g) and flavonoid glycosides such as isoquercitrin (0.16–0.91mg/g) and rutin (0.07–0.76mg/g). Jujube vinegars (Figure 5. (B), (C), (D)) were dominated by quinic acid (6.59–101.57mg/g), together with epicatechin (40.62mg/g in jujube vinegar 3) and moderate amounts of protocatechuic acid (up to 7.99 mg/g). Jujube vinegar 1 also contained higher levels of flavonoids such as quercitrin (3.36mg/g) compared to the other jujube vinegars. These findings confirm that fermentation not only preserves but also enhances the release of specific phenolic compounds depending on the fruit substrate.

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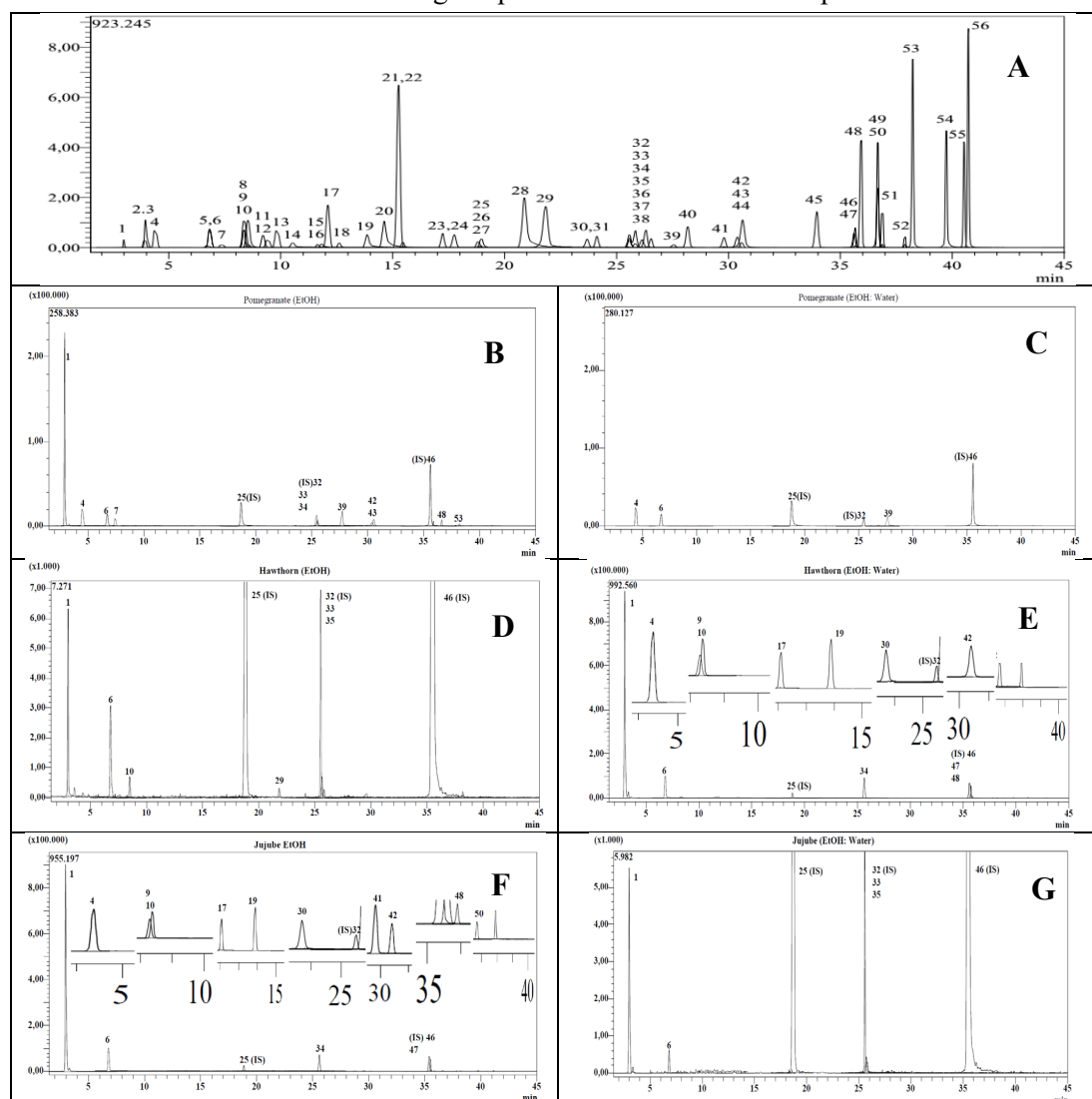


Figure 2. LC-MS/MS chromatograms of the reported vinegars

A: TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanillic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: *p*-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: *o*-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B:** LC-MS/MS chromatogram of the Pomegranate (EtOH), **C:** LC-MS/MS chromatogram of the Pomegranate (EtOH: Water), **D:** LC-MS/MS chromatogram of the Hawthorn (EtOH), **E:** LC-MS/MS chromatogram of the Hawthorn (EtOH: Water), **F:** LC-MS/MS chromatogram of the Jujube (EtOH: Water), **G:** LC-MS/MS chromatogram of the Jujube (EtOH: Water).

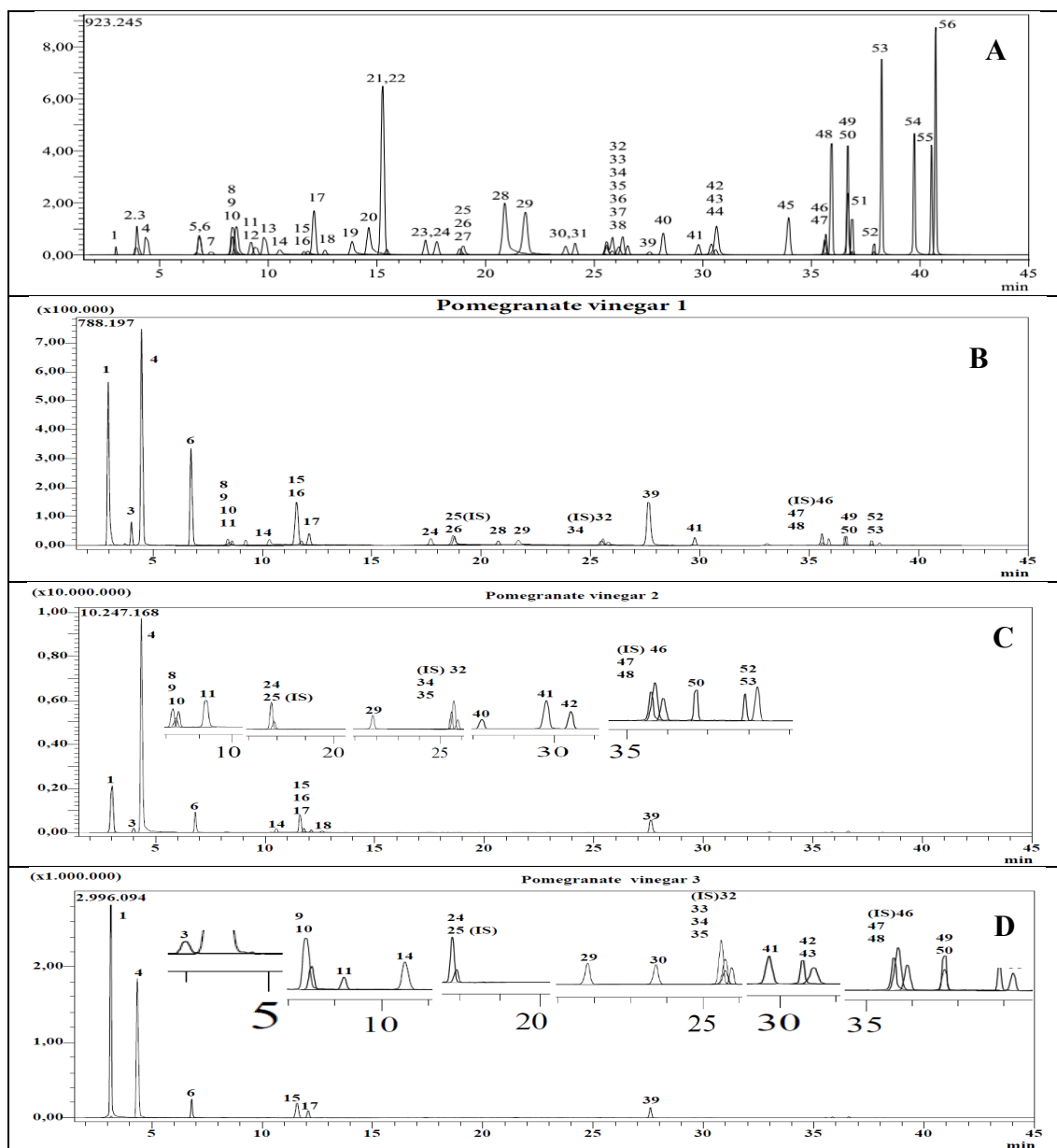


Figure 3. LC-MS/MS chromatograms of reported vinegars

A: TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanillic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: *p*-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: *o*-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B:** LC-MS/MS chromatogram of the Pomegranate vinegar 1, **C:** LC-MS/MS chromatogram of the Pomegranate vinegar 2, **D:** LC-MS/MS chromatogram of the Pomegranate vinegar 3.

Functional fruit vinegars: phenolics and anti-diabetic potential

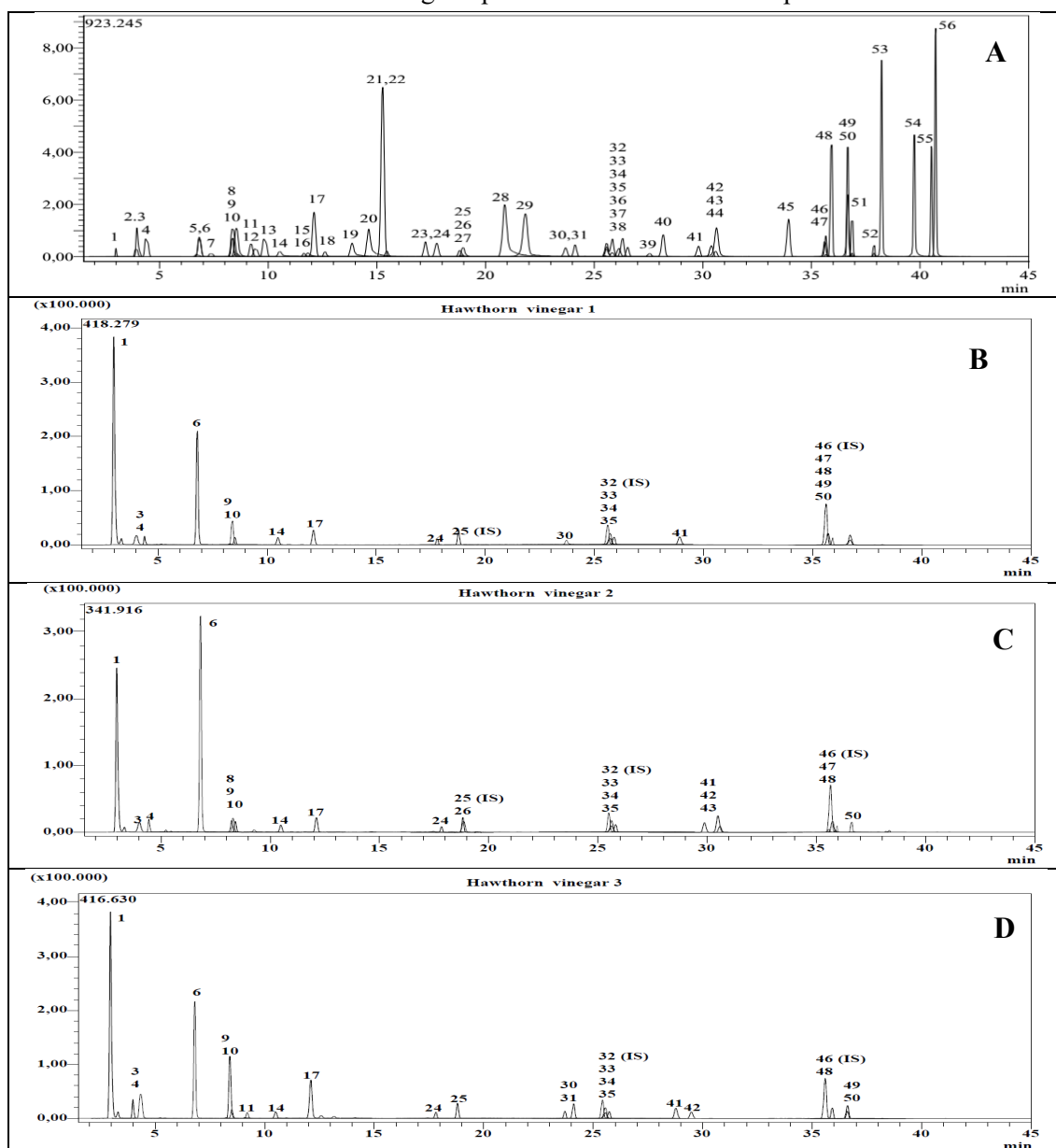


Figure 4. LC-MS/MS chromatograms of reported vinegars

A: TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanillic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: p-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: o-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B:** LC-MS/MS chromatogram of the Hawthorn vinegar 1, **C:** LC-MS/MS chromatogram of the Hawthorn vinegar 2, **D:** LC-MS/MS chromatogram of the Hawthorn vinegar 3.

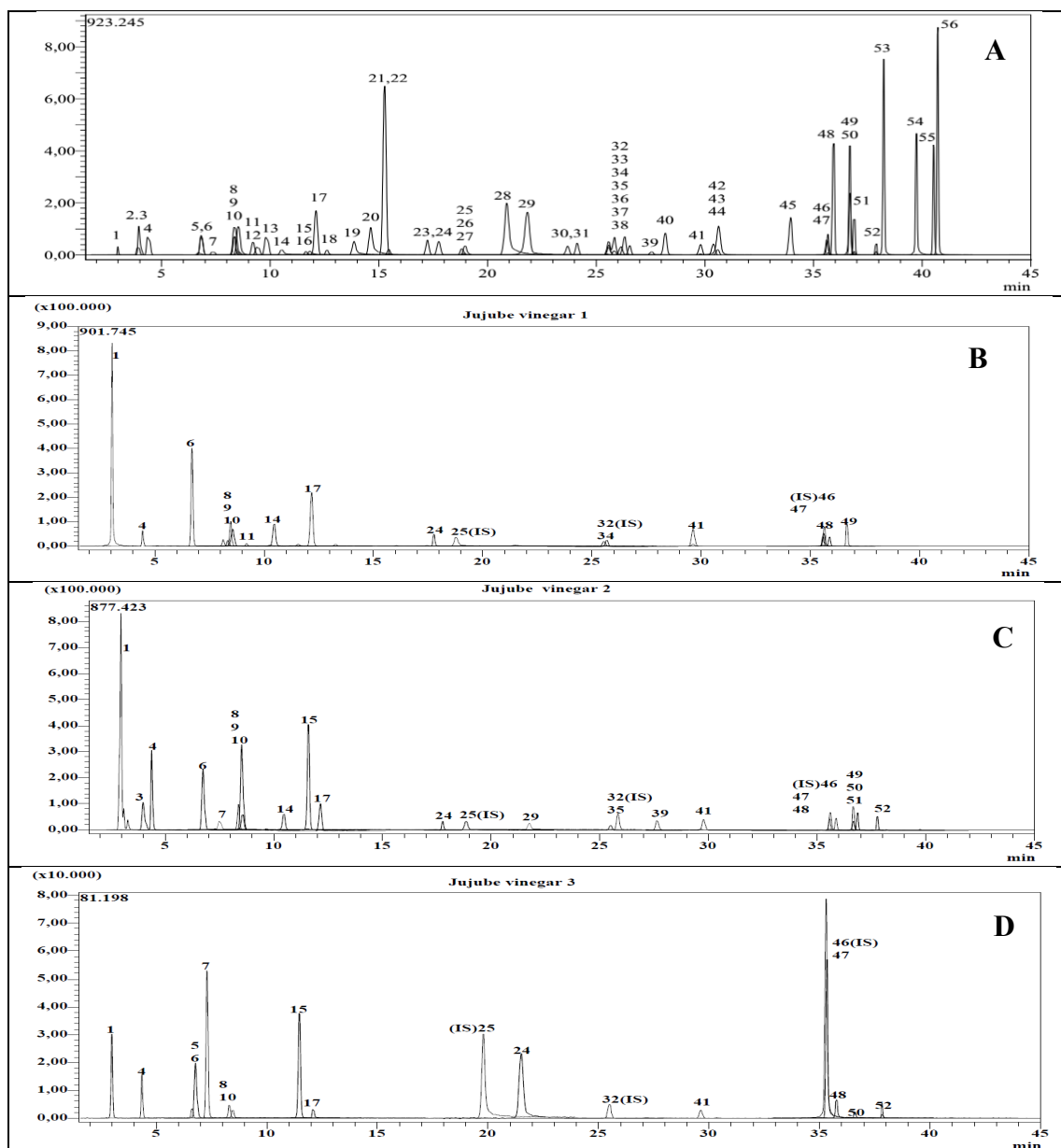


Figure 5. LC-MS/MS chromatograms of reported vinegars

A: TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: Quinic acid. 2: Fumaric acid. 3: Aconitic acid. 4: Gallic acid. 5: Epigallocatechin. 6: Protocatechuic acid. 7: Catechin. 8: Gentisic acid. 9: Chlorogenic acid. 10: Protocatechuic aldehyde. 11: Tannic acid. 12: Epigallocatechin gallate. 13: 1,5-dicaffeoylquinic acid. 14: 4-OH Benzoic acid. 15: Epicatechin. 16: Vanillic acid. 17: Caffeic acid. 18: Syringic acid. 19: Vanillin. 20: Syringic aldehyde. 21: Daidzin. 22: Epicatechin gallate. 23: Piceid. 24: p-Coumaric acid. 26: Ferulic acid. 27: Sinapic acid. 28: Coumarin. 29: Salicylic acid. 30: Cynaroside. 31: Miquelianin. 32: Rutin. 34: isoquercitrin. 35: Hesperidin. 36: o-Coumaric acid. 37: Genistin. 38: Rosmarinic acid. 39: Ellagic acid. 40: Cosmosiin. 41: Quercitrin. 42: Astragalin. 43: Nicotiflorin. 44: Fisetin. 45: Daidzein. 47: Quercetin. 48: Naringenin. 49: Hesperetin. 50: Luteolin. 51: Genistein. 52: Kaempferol. 53: Apigenin. 54: Amentoflavone. 55: Chrysin. 56: Acacetin; **B:** LC-MS/MS chromatogram of the Jujube vinegar 1, **C:** LC-MS/MS chromatogram of the Jujube vinegar 1, **D:** LC-MS/MS chromatogram of the Jujube vinegar 3.

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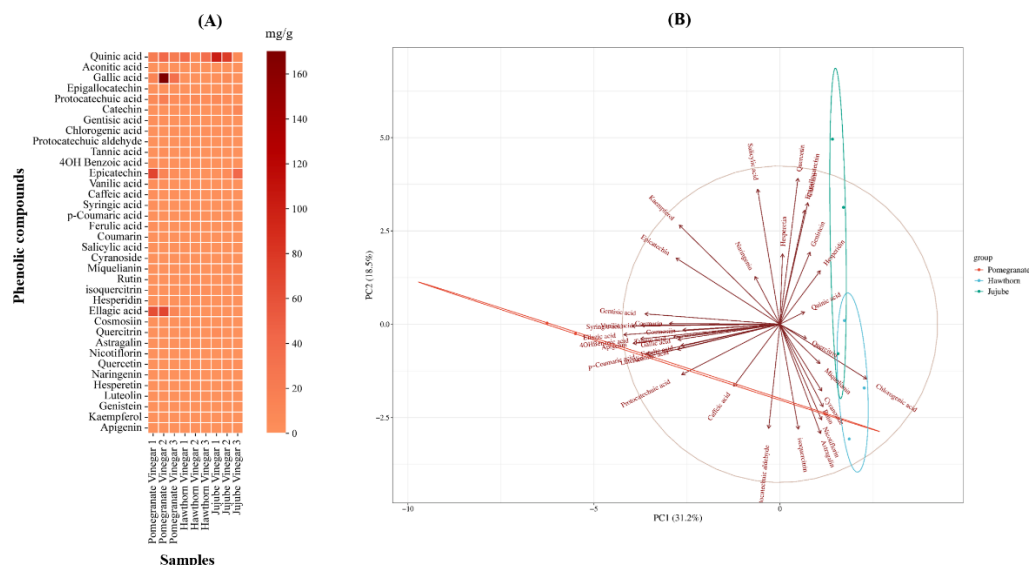


Figure 6. (A) Heatmap and (B) Biplot from PCA of phenolic compounds determined in pomegranate, hawthorn, and jujube vinegars.

In pomegranate vinegars, the dominance of ellagic acid and punicalagin derivatives is consistent with earlier studies reporting that pomegranate vinegar retains high levels of hydrolysable tannins and anthocyanins, responsible for its potent antioxidant and antimicrobial properties [21, 22]. These compounds have been directly linked to their anti-diabetic potential through the inhibition of α -glucosidase and a reduction in postprandial glycemia [23].

Hawthorn vinegars were enriched in quinic acid and quercetin glycosides, aligning with reports that hawthorn-based fermented products contain significant amounts of gallic acid, chlorogenic acid, and flavonoids such as hyperoside and rutin [18, 24]. These bioactives are associated with cardiovascular protection and modulation of glucose metabolism, supporting the traditional use of hawthorn in the management of cardiometabolic disorders.

Jujube vinegars showed high concentrations of quinic acid, catechins, and protocatechuic acid, in agreement with recent studies that demonstrated traditionally fermented jujube vinegars to be rich in protocatechuic aldehyde, syringaldehyde, vanillic acid, and chlorogenic acid compared to commercial products [25, 26]. These phenolics are widely recognized for their antioxidant, anti-inflammatory, and anti-diabetic activities.

PCA of phenolic compounds detected in vinegars is shown in Figure 6. (B) and S1. It revealed that the first two principal components (PCs) explained cumulative variances of 31.2% and 18.5%, respectively. As shown in the biplot, the positioning of the vinegars (especially pomegranate vinegars) indicates a clear separation. Vinegars derived from jujube and hawthorn were clustered closer together. At the same time, those from pomegranate were separated, suggesting clear differences in phenolic compounds. Taken together, the results highlight distinct phytochemical fingerprints: pomegranate vinegar is rich in ellagitannins and gallic acid derivatives; hawthorn vinegar is characterized by quinic acid and flavonol glycosides; and quinic acid, catechins, and protocatechuic derivatives dominate jujube vinegar. This diversity suggests complementary health-promoting properties, supporting the positioning of fruit vinegars as functional beverages for managing oxidative stress, glucose homeostasis, and cardiovascular health.

3.2. Enzyme Inhibition Activity Results

The enzyme inhibition assays revealed a marked difference between pomegranate, jujube, and hawthorn vinegars and crude extracts (in ethanol and 70% ethanol) across all tested enzymes as shown in Figure 7 and Table S3.

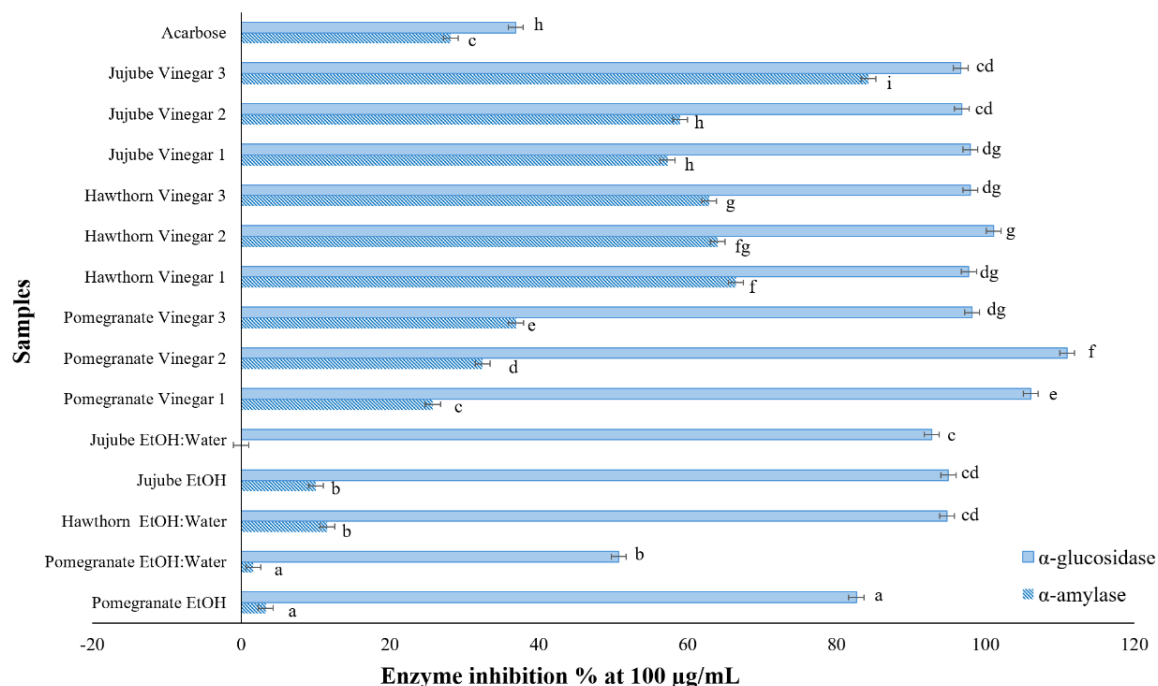


Figure 7. Anti-diabetic activities of extracts and vinegars of pomegranate, hawthorn, and jujube. EtOH means ethanolic extract, and EtOH: water shows 70% ethanolic extract. Samples with common lower-case letters are not significantly different ($p > 0.05$).

All pomegranate vinegars demonstrated inhibitory activity against α -amylase and α -glucosidase, the key enzymes involved in carbohydrate digestion and postprandial glucose elevation. Notably, pomegranate vinegar 2 showed the most potent effect, suppressing α -glucosidase activity by $110.97 \pm 3.01\%$, which is almost three times higher than that of the standard antidiabetic drug acarbose ($36.89 \pm 0.35\%$) (Figure 7). This result suggests a promising role for pomegranate vinegar in glycemic regulation, in agreement with earlier studies that highlight the impact of pomegranate phenolics on glucose metabolism [27].

Among the extracts, both ethanol and 70% ethanol extracts exhibited minimal inhibition of α -amylase (3.34% and 1.67% , respectively) but retained potent α -glucosidase inhibition, with the ethanol extract reaching 82.65% . This selective action aligns with reports indicating that compounds such as ellagitannins and gallic acid derivatives preferentially target α -glucosidase while exerting weaker effects on α -amylase [28].

In contrast, vinegar samples displayed a broader spectrum of activity. Pomegranate vinegar 1 and 2 showed exceptional α -glucosidase inhibition (106.08% and 110.97% , respectively) alongside moderate α -amylase inhibition (25.76 – 36.93%). Pomegranate vinegar 3 followed a similar trend, with 98.20% inhibition of α -glucosidase and 36.93% inhibition of α -amylase. Such outcomes suggest that fermentation plays a key role in enhancing the antidiabetic potential, likely by generating low-molecular-weight phenolics, including gallic acid, ellagic acid, and catechin derivatives, which are recognized as inhibitors of carbohydrate-hydrolysing enzymes [28, 29]. Overall, the superior inhibitory capacity of pomegranate vinegars compared to extracts suggests that fermentation does more than conserve the phenolic fraction; it transforms it into more bioactive and bioavailable compounds. This observation is consistent with previous findings linking fermentation-driven increases in organic acids and depolymerized tannins to enhanced antidiabetic activity [28, 30].

Hawthorn vinegars exhibited strong α -glucosidase inhibition ($>90\%$) with potent α -amylase inhibition (Figure 7). This selective inhibition pattern aligns with earlier studies showing that hawthorn flavonoids, especially quercetin, hyperoside, and procyanidins, exert potent α -glucosidase inhibitory effects while only partially suppressing α -amylase activity [6, 7]. Such dual but selective inhibition is nutritionally advantageous, since excessive α -amylase inhibition can cause gastrointestinal discomfort, a limitation often reported for synthetic drugs such as acarbose [2].

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Recent *in vivo* data further confirm these enzyme-level findings. Seyidoglu et al. [31] demonstrated that hawthorn vinegar supplementation in rats reduced plasma glucose levels while simultaneously elevating GLP-1 levels, indicating a synergistic effect on both digestive enzyme inhibition and incretin hormone regulation. Similarly, ultrasound-processed hawthorn vinegars were found to enhance total phenolic content and antioxidant capacity, which may contribute to more vigorous anti-diabetic activity compared to traditional ones.

Jujube extracts exhibit potent inhibition of α -glucosidase and α -amylase (Figure 7), consistent with prior reports on phytochemicals. Jujube phenolics, particularly protocatechuic acid, gallic acid, and catechin derivatives, as well as triterpenes such as botulinic acid and lupeol, have been identified as contributors to glucose-lowering effects through both enzyme inhibition and intracellular signalling pathways [5]. These mechanistic insights are consistent with earlier reports that jujube fruit extracts improve glucose tolerance in diabetic models while simultaneously reducing oxidative stress markers [5, 17].

When the three fruits are considered together, apparent differences emerge in both phenolic composition and anti-diabetic activities. Pomegranate vinegars and extracts, dominated by ellagic acid derivatives, exhibited the most pronounced α -glucosidase inhibition, frequently surpassing that of acarbose, confirming the significant contribution of ellagitannins to glycemic control [3, 21]. Hawthorn, characterized by high levels of quinic acid and flavonol glycosides, also demonstrated robust α -glucosidase and α -amylase inhibition, consistent with studies showing that hawthorn phenolics modulate carbohydrate digestion and provide cardiometabolic benefits [6, 32]. Jujube, although exhibiting strong enzyme inhibition, contributes to complementary mechanisms through the presence of protocatechuic acid, rutin, and triterpenes, which enhance insulin sensitivity and regulate the PI3K/Akt and AMPK pathways, thereby improving systemic glucose utilization [5]. In conclusion, these findings suggest that pomegranate and hawthorn vinegars exhibit strong direct inhibitory effects on carbohydrate-hydrolysing enzymes, whereas jujube provides metabolic support through broader regulatory pathways. This complementary diversity underscores the potential to integrate all three fruits into functional food strategies to manage postprandial hyperglycaemia and type 2 diabetes mellitus.

4. Conclusions

This study provides a rare analytical–functional perspective by integrating a validated LC–MS/MS quantification of 53 phenolic compounds with comparative α -amylase and α -glucosidase inhibition assays. To our knowledge, it represents the first comprehensive comparison of the phenolic composition and *in vitro* antidiabetic potential of pomegranate (*Punica granatum* L.), hawthorn (*Crataegus* spp.), and jujube (*Ziziphus jujuba* Mill.) extracts and their corresponding vinegars. The LC–MS/MS analysis revealed distinct phytochemical fingerprints: ellagic acid derivatives were dominant in pomegranate, quinic acid and flavonol glycosides in hawthorn, and quinic acid, together with protocatechuic derivatives, in jujube. Fermentation markedly enhanced the release and bioaccessibility of these phenolics, resulting in stronger *in vitro* inhibition of α -amylase and α -glucosidase. All vinegar samples exhibited higher inhibitory capacity than their crude extracts and, in several cases, surpassed the reference inhibitor acarbose, underscoring the potential contribution of fermentation-derived phenolics to glycemic modulation.

A limitation of the present work is that punicalagin, the major ellagitannin in pomegranate, was not directly quantified because of analytical constraints, and the findings are confined to *in vitro* enzyme assays. Future studies should therefore include *in vivo* and clinical evaluations to confirm these effects under physiological conditions and to elucidate the metabolic transformations that occur during fermentation.

Overall, the results suggest that fruit vinegars may aid glycemic regulation *in vitro* by selectively inhibiting carbohydrate-hydrolyzing enzymes. However, these data provide mechanistic rather than therapeutic evidence. Further investigation into the bioavailability of fermentation-derived metabolites and their relationship with glucose metabolism will help clarify their functional roles. The complementary phenolic diversity of pomegranate, hawthorn, and jujube supports their promise as nutritionally valuable functional ingredients, although translational validation remains essential before any nutraceutical applications can be justified.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Supporting Information

Supporting information accompanies the paper on <https://www.acgpubs.org/journal/records-of-agricultural-and-food-chemistry>

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