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In vitro assessment of the antihyperglycemic property of Prosopis farcta J. F. Macbr and Rheum ribes L. and phytochemical profiling of the most active extract

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Original Research

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Abstract:

This study aimed to evaluate the α -glucosidase inhibition potential of *Prosopis farcta* and *Rheum ribes*. For this purpose, various plant parts-including the root bark, roots, fruits, and leaves of *P. farcta*, as well as the roots and stalks of *R. ribes*-were sequentially extracted employing *n*-hexane, ethyl acetate, and methanol. The α -glucosidase inhibition potential of the extracts was assessed. Subsequently, the phytochemical investigation was carried out on the ethyl acetate extract derived from the root of *P. farcta*, which exhibited the most pronounced inhibitory effect. This analysis resulted in the isolation and identification of six compounds, including four steroids, one pentacyclic triterpenoid, and one flavonoid. The isolated compounds underwent further evaluation for their α -glucosidase inhibition potential. Among them, daucosterol (3) demonstrated the highest potency, with an IC₅₀ value of 18.6 μ M.

 $\textbf{Keywords:} \ \alpha\text{-Glucosidase; Flavonoid; Phytochemical analysis; } \textit{Prosopis farcta; Rheum ribes; } \textbf{Terpenoid}$

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, primarily resulting from insulin resistance, impaired insulin secretion, or inappropriate glucagon production (Singh, 2023). The condition is broadly classified into two major types. Type 1 diabetes is an autoimmune disorder in which the immune system targets and destroys pancreatic β -cells responsible for insulin production, leading to severely reduced insulin levels. Type 2 diabetes, which is more prevalent than type 1, is characterized by the pancreas producing insulin; however, the body fails to utilize it effectively due to functional impairment and insulin resistance. The American Diabetes Association (ADA) defines this condition as one marked by hyperglycemia, resulting from the body's diminished ability to utilize glucose as an energy source-either due

to insufficient insulin secretion or improper insulin utilization (Lewis, 2007; Ignatavicius et al., 2015). Diabetes is a multifactorial disorder that necessitates diverse therapeutic strategies (Mahdavi et al., 2021; Mousavi et al., 2025). One of the key approaches involves regulating blood glucose levels and mitigating postprandial hyperglycemia by inhibiting carbohydrate digestion through the use of glucosidase inhibitors. Glucosidases are essential intestinal enzymes responsible for breaking down complex carbohydrates into simple monosaccharides. Among them, α glucosidase facilitates the hydrolysis of oligosaccharides to release glucose within the epithelial lining of the small intestine. α -Glucosidase inhibitors, recognized as modulators of postprandial hyperglycemia, have been employed for therapeutic management of type 2 diabetes mellitus since the 1980s (Vardanyan et al., 2016). α -Glucosidase inhibitors

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exert their effect by binding to the enzyme and thereby hindering the breakdown of disaccharide and oligosaccharide substrates into absorbable monosaccharides. Several agents, including acarbose, miglitol, and voglibose, have been employed in the management of type 2 diabetes; however, their clinical use is limited by various adverse effects, such as cardiovascular complications, hypertension, and gastrointestinal disturbances-including abdominal pain, diarrhea, and flatulence (Aronson, 2010). Consequently, the pursuit of novel α -glucosidase inhibitors remains a topic of significant interest. Medicinal plants serve as abundant sources of bioactive constituents with potential therapeutic applications, including enzyme inhibition and disease management (Olaoluwa et al., 2022). The empirical strategy of systematically screening plant extracts or phytochemicals-guided by ethnopharmacological insights-continues to be a valuable approach to identifying new lead compounds (Barboza et al., 2009; Vogl et al., 2013; Yang et al., 2015). Numerous plant extracts have been evaluated globally for their effectiveness as α -glucosidase inhibitors (Bhandari et al., 2008; Tundis et al., 2010; Kumar et al., 2011). Prosopis farcta J. F. Macbr. and Rheum ribes L. have a well-documented history of traditional use in the management of diabetes (Said et al., 2002; Chen et al., 2015). Accordingly, this study aimed to identify natural compounds with α -glucosidase inhibitory potential by assessing the enzyme-inhibitory activity of various extracts obtained from these plants using a spectrophotometric assay on an ELISA microplate reader, followed by phytochemical analysis of the most active extract. P. farcta, commonly referred to as Syrian mesquite in Arabic-speaking regions and known locally in Iran as Kahurak and Jeghjegheh, is a woody perennial dwarf legume distributed across regions spanning from India to Jordan. Various species of the Prosopis genus have historically been utilized for diverse applications, including the production of gum, paint, and cordage (Orozco-Villafuerte et al., 2003), as well as dietary supplements for ruminant nutrition (Andrade-Montemayor et al., 2011) and traditional medicinal practices. The beans and leaves of *P. farcta* have been traditionally employed in treating a range of disorders, including diabetes (Hamdan et al., 2004; Al-Aboudi et al., 2011; Feyzmand et al., 2018; Shahbazi et al., 2020; Heidari et al., 2025), wound healing (Ranjbar-Heidari et al., 2012), diarrhea, and common colds (Saidi et al., 2016). R. ribes (commonly known as rhubarb) has been referred to as "the wondrous drug" due to its broad spectrum of medicinal applications (Foust, 1992). R. ribes has demonstrated a wide range of biological activities, including antidiarrheal effects (Oi et al., 2002), reduction of blood urea nitrogen levels (Yokozawa et al., 1991), as well as antibacterial (Park et al., 2002; Keser et al., 2020) and antiviral properties (Kim et al., 2001). It is recognized as a rich source of anthraquinones and other pharmacologically active constituents, including emodin, chrysophanol, physcion, aloe-emodin, sennosides, fatty acids, fatty acid esters, and simple hydrocarbons (Singh et al., 2010; Geçibesler, 2024).

As part of an ongoing project focused on the screening of plants for structurally novel bioactive metabolites (Farimani et al., 2014; Asghari et al., 2015; Farimani et al., 2015; Fari

mani et al., 2016), the α -glucosidase inhibition potential of various extracts derived from different parts of two medicinal plants-*Prosopis farcta* and *Rheum ribes*-was evaluated. Based on these results, the ethyl acetate extract obtained from the roots of *P. farcta*, which demonstrated the most potent inhibitory effect, was selected for the isolation and characterization of its active constituents.

2. Experimental

2.1 General experimental procedures

Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance III spectrometer operating at 500.13 MHz for ¹H and 125.77 MHz for ¹³C nuclei, using a 1 mm TXI probe. Spectral data were processed using TopSpin 2.1 software (Bruker). Sample absorbance was measured with a BioTek PowerWave XS2 spectrophotometer. Thinlayer chromatography (TLC) spots were detected under UV light at 254 nm or after spraying with 5.0% phosphomolybdic acid in ethanol, followed by heating at 110 °C for 10 minutes. Column chromatography (CC) was carried out using silica gel (70 - 230 mesh, Merck) as the stationary phase. α-Glucosidase enzyme (EC 3.2.1.20) Type I from Saccharomyces cerevisiae, along with p-nitrophenyl- α -Dglucopyranoside (pNPG) and acarbose, were obtained from Sigma-Aldrich (USA). All solvents and additional reagents were purchased from local commercial suppliers.

2.2 Plant material

Fresh roots and stalks of *Rheum ribes* were collected from Qalladze in the Kurdistan Regional Government, Iraq, in March 2015. The roots, root bark, leaves, and fruits of *Prosopis farcta* were gathered from Koya, Erbil Governorate, Iraq, in July 2015. Plant identification was confirmed by Dr. Ali Sonboli, and voucher specimens were deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, under the accession numbers MPH-1296 and MPH-1342, respectively.

2.3 Preparation of extracts

The plant materials were air-dried, ground into a fine powder using a mechanical grinder, and weighed precisely (10 g per sample) on a digital balance. Each sample underwent successive extraction with n-hexane (3 \times 50 mL), ethyl acetate (3 \times 50 mL), and methanol (3 \times 50 mL) by maceration on a magnetic stirrer for 1 hour at ambient temperature. Each solvent fraction was separately combined and concentrated to dryness under reduced pressure at 45 °C, yielding solvent-free extracts of n-hexane, ethyl acetate, and methanol. The resulting extracts were subsequently assessed for α -glucosidase inhibitory activity through in vitro enzymatic assays.

2.4 Assay for α -glucosidase inhibitory activity

The α -glucosidase inhibition potential of the plant extracts was evaluated spectrophotometrically following the method described by Mccue et al. (2005), with minimal modifications. In brief, a reaction mixture comprising 90 μ L of 4-nitrophenyl- α -D-glucopyranoside (pNPG, 5 mM), 150

μL of 0.1 M phosphate buffer (pH 6.8), and 1 μL of the test sample at different concentration levels was prepared in 96-well microplates. Following a 15-minute incubation at room temperature, the enzymatic reaction was initiated by adding 9 μL of α -glucosidase (1 unit/mL). Absorbance was recorded eight times at 15-second intervals. Reactions lacking the test sample served as blanks to correct for background absorbance. Acarbose was employed as the standard reference treatment (positive control) to validate the experimental outcomes. All assays were performed in triplicate. The percentage of α -glucosidase inhibition was calculated using Eq. 1.

$$\alpha$$
 – Glucosidase inhibition(%) = $(E - S)/E \times 100$ (1)

Where E denotes the enzyme activity in the absence of the test sample, and S corresponds to the activity measured in the presence of the sample. To account for spontaneous substrate hydrolysis or extract-induced absorbance fluctuations, the rate of reaction prior to enzyme addition was subtracted from the post-addition rate.

2.5 Statistical analysis

All assays were performed in triplicate, and the data are expressed as mean \pm standard deviation (SD). Statistical analyses were conducted using SPSS software via one-way ANOVA, with significance determined at p < 0.05.

2.6 Isolation process

Air-dried roots of P. farcta (3.0 kg) were powdered and sequentially extracted with *n*-hexane (3 \times 15 L) and ethyl acetate (5 \times 15 L) by maceration at ambient temperature for 7 days each. The resulting extracts were concentrated under reduced pressure, affording dark, gummy residues. The ethyl acetate extract (80 g) was subjected to silica gel column chromatography (5 \times 80 cm, 800 g), eluted with a solvent gradient starting from pure *n*-hexane to pure ethyl acetate (100:0 to 0:100), followed by ethyl acetate enriched with methanol concentrations up to 20%. Based on TLC profiling, fractions with comparable compositions were combined, resulting in 18 pooled fractions (1-18). Fraction 1 (50 mg), eluted with *n*-hexane-ethyl acetate (90:10), was further purified by silica gel column chromatography (30 g, 1.0×30 cm) using *n*-hexane-acetone (99:1) as the mobile phase, affording six subfractions (1a-1f). Subfraction 1d was recrystallized from chloroform to yield β-sitosterol-

palmitate (2, 1.5 mg) (Sun et al., 2003). Fraction 3, eluted with *n*-hexane-ethyl acetate (15:85), provided a crude solid that was recrystallized in acetone to afford lupeol (5, 3 mg) (Nguyen et al., 2011). Recrystallization of fraction 4, eluted with *n*-hexane-ethyl acetate (80:20), in *n*-hexane, afforded β-sitosterol (1, 8 mg) (Moghaddam et al., 2007). Fraction 12, obtained through elution with n-hexane-ethyl acetate (40:60), was triturated with acetone to isolate an insoluble solid, which was subsequently recrystallized from methanol to yield sitoindoside I (4, 4 mg) (Ghosal et al., 1984). Fraction 13, eluted with n-hexane-ethyl acetate (30:70), was further purified by silica gel column chromatography (50 g, 1.5×70 cm), using chloroform followed by increasing concentrations of methanol (up to 5%) as the eluent, resulting in six subfractions (13a-13f). A brown powder obtained from subfraction 13c was recrystallized from methanol, yielding (-)-epicatechin (6, 9 mg) (Usman et al., 2016). Fraction 14, eluted with *n*-hexane-ethyl acetate (10:90), was further purified by silica gel column chromatography (70 g, 2.0×60 cm), using a gradient of dichloromethane-methanol (98:2 to 90:10) as the mobile phase, yielding five subfractions (14a-14e). From subfraction 14b, a crude residue was purified by recrystallization in methanol to afford daucosterol (3, 10 mg) (Esmaeili et al., 2014).

3. Results and discussion

Diabetes is characterized by elevated blood glucose levels, and treatment strategies primarily aim to regulate glycemia by controlling carbohydrate metabolism. Historically, P. farcta and R. ribes have been used in traditional medicine for the management of diabetes, demonstrating potential therapeutic properties. To evaluate the anti-diabetic potential of the plant extracts, an *in vitro* α -glucosidase inhibition assay was conducted on various extracts of P. farcta and R. ribes. As presented in Table 1, the ethyl acetate extract of P. farcta roots exhibited the highest α -glucosidase inhibitory activity with an IC $_{50}$ value of 10.9 μ g/mL-surpassing the activity of the positive control, acarbose ($IC_{50} = 18.5 \,\mu\text{g/mL}$). Notably, the ethyl acetate and methanol extracts of *P. farcta* root bark also demonstrated considerable inhibitory effects, with IC₅₀ values of 14.7 and 17.1 μ g/mL, respectively. The remaining extracts exhibited α-glucosidase inhibition potential with IC₅₀ values ranging from 26.4 μg/mL to over 125 μ g/mL. Based on its superior inhibitory potency, the ethyl acetate extract of *P. farcta* roots-showing the lowest

Table 1. The IC ₅₀ (μ g/mL) values of α -Glucosidase inhibitory of diffe	ferent extracts obtained from <i>P. farcta</i> and <i>R. ribes</i> .
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Sample	IC ₅₀ (μg/mL)		
Sample	<i>n</i> -Hexane	Ethyl acetate	Methanol
Roots barks of P. farcta	> 125	$14.7 \pm 1.1 \text{ a}$	17.1±1.1 f
Roots of <i>P. farcta</i>	> 125	$10.9 \pm 1.0 \text{ b}$	26.4±1.4 g
Fruits of <i>P. farcta</i>	> 125	$68.1\pm2.1 \text{ c}$	59.8±1.9 h
Leaves of P. farcta	> 125	$54.7 \pm 2.0 \text{ d}$	40.1±1.1 i
Roots of <i>R. ribes</i>	> 125	90.6±2.1 e	92.2±2.1 e
Stalks of <i>R. ribes</i> Acarbose (positive control)	> 125	> 125	> 125
4,			18.5

Data are presented as mean \pm SD values of triplicate determination. Different superscripts letters for a given value within a column are significantly different from each other (P < 0.05). S.D. Standard Deviation.

IC50 value-was selected for further investigation to identify its active constituents. To further investigate the active constituents, a large-scale extraction was carried out, yielding 80 g of dried extract. The extract underwent silica gel column chromatography and 18 combined fractions were obtained based on TLC analysis. Further purification of the active fractions led to the isolation of six compounds, whose structures were elucidated through spectroscopic techniques, including ESI-MS, 1D and 2D NMR, and subsequently confirmed by comparison with literature-reported spectral data. The structures of the isolated compounds were identified as β -sitosterol (1), β -sitosterol palmitate (2), daucosterol (3), sitoindoside I [6-O-palmitoyl-sitosteryl-β-D-glucose] (4), lupeol (5), and (-)-epicatechin (6) (Fig. 1). Of these, the first four compounds are classified as steroids, while lupeol and (-)-epicatechin fall under the categories of pentacyclic triterpenoid and flavonoid, respectively. All isolated compounds were evaluated for their α -glucosidase inhibitory activity using a 96-well microplate assay, and each demonstrated dose-dependent inhibition. As indicated in Table 2, daucosterol (3) displayed the most potent α -glucosidase inhibitory activity (IC₅₀ = $18.6 \mu M$), followed by sitoindoside I (4, $IC_{50} = 21.8 \,\mu\text{M}$) and β -sitosterol palmitate (2, $IC_{50} = 33.5$ μM). In this study, acarbose served as the positive control, exhibiting an IC₅₀ value of 28.7 μ M. The α -glucosidase inhibitory activity observed in the isolated compounds suggests a link to the presence of steroidal and terpenoidal constituents. A preliminary structure-activity relationship (SAR) analysis was conducted on the purified compounds. The results indicate that the glucosidal moiety plays a crucial role in inhibitory activity (Shahbazi et al., 2020). While the long-chain ester attached to the sugar moiety has minimal direct effect, its linkage to the steroidal aglycone-such as in β -sitosterol palmitate (2)-enhances inhibitory potency nearly two-fold compared to β -sitosterol (1). Moreover, the reduced activity of compound 5 suggests that the presence of a double bond is not essential for enzyme inhibition.

4. Concluding remarks

In the present study, the α -glucosidase inhibitory activity of two Iraqi plants-Prosopis farcta and Rheum ribes-was evaluated using the enzymatic method described by Mccue et al. (2005). Given that inhibition of α -glucosidase is a validated therapeutic strategy for managing diabetes, this investigation focused on identifying active constituents responsible for the observed effects. Phytochemical analysis of the ethyl acetate extract of P. farcta-which exhibited the highest inhibitory activity- led to the isolation of four steroidal compounds, one pentacyclic triterpenoid, and one flavonoid. Among these, daucosterol demonstrated the most potent inhibition of α -glucosidase, with an IC₅₀ value of 18.6 μ M. To the best of our knowledge, this is the first report detailing the isolation and structural elucidation of chemical constituents from P. farcta. These findings suggest that *P. farcta* roots possess promising anti-diabetic potential due to the presence of potent α -glucosidase inhibitors.

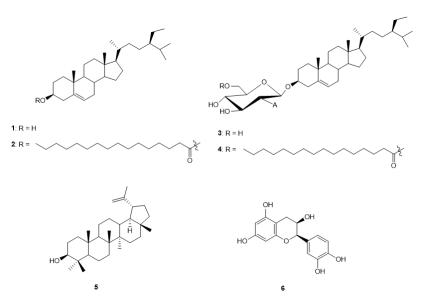


Figure 1. Structure of compounds 1-6.

Table 2. The α -glucosidase inhibitory effect of isolated compounds from the ethyl acetate extract of roots of *P. farcta*.

compound	IC ₅₀ (μM)
β-Sitosterol (1)	64.8
β-Sitosterol palmitate (2)	33.5
Daucostrol (3)	18.6
Sitoindoside I (4)	21.8
Lupeol (5)	79.3
(-)-Epicatechin (6)	47.0
Acarbose (positive control)	28.7

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Authors contributions

Ismael Hasan Mohammed and Vahed Zarial conducted the extraction, isolation, and structural identification of compounds, performed the biological assays, and prepared the original draft of the manuscript. Mahdi Moridi Farimani conceived and oversaw the project, reviewed the manuscript, and supervised the study. Maryam Kharatha was responsible for conducting the literature search, implementing experimental procedures, and writing, reviewing, and editing the manuscript. Marzieh Tabefam measured the NMR spectra. Conceptualization and validation were carried out by Esmail Salih Ibrahim Kakey. Final approval was granted by all contributing authors.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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