



Chemical profiling, antioxidant capacity, and potential antihyperglycemic activity of *Farsetia stylosa* R.Br. methanolic extract

[Perfil químico, capacidad antioxidante y posible actividad antihiper glucémica de *Farsetia stylosa* R.Br. extracto metanólico]

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Abstract

Context: In recent years, there has been significant focus on exploring antihyperglycemic drugs that target enzymes involved in carbohydrate metabolism. *Farsetia stylosa* has been used in folk medicine in the management of diabetes and has been studied once for its anti-diabetic effect on rats.

Aims: To evaluate the biological potentials of *F. stylosa* extract through *in vitro* testing of its antihyperglycemic, antioxidant, and antimicrobial activities. Furthermore, the phytochemical content of the plant and virtual assessment of its antihyperglycemic action were also examined.

Methods: Antihyperglycemic activity was evaluated using α -amylase and α -glucosidase inhibition assays, antioxidant capacity was evaluated using ABTS, DPPH, TAC, and H_2O_2 radical scavenging assays, antimicrobial activity was determined using the agar well diffusion method, and *in silico* studies were performed using molecular docking simulation into the active sites of α -amylase and α -glucosidase enzymes.

Results: *F. stylosa* extract showed strong activity against α -amylase and α -glucosidase enzymes ($IC_{50} = 13.45 \pm 0.39$ and 0.614 ± 0.02 μ g/mL, respectively) compared to the standard drug, acarbose ($IC_{50} = 27.2 \pm 0.78$ and 0.375 ± 0.01 μ g/mL, respectively). Investigation of the antioxidant capacity indicated mild activity in four assays. LC-HRMS analysis enabled the detection of 23 metabolites, including glucosinolates, flavonoids, amino acids, organic acids, and fatty acids. A molecular docking simulation study pointed out remarkable interactions of flavonoids with the catalytic residues of both enzymes.

Conclusions: *F. stylosa* growing in Saudi Arabia contains variable metabolites with notable antihyperglycemic activity. These metabolites are recommended for further purification and investigation of their antidiabetic efficacy.

Keywords: diabetes mellitus; *Farsetia stylosa*; LC-MS/MS; molecular docking simulation.

Resumen

Contexto: En los últimos años, se ha prestado mucha atención a la exploración de fármacos antihiper glucemiantes que se dirigen a las enzimas implicadas en el metabolismo de los carbohidratos. *Farsetia stylosa* se ha utilizado en la medicina popular para el tratamiento de la diabetes y se ha estudiado una vez por su efecto antidiabético en ratas.

Objetivos: Evaluar los potenciales biológicos del extracto de *F. stylosa* mediante pruebas *in vitro* de sus actividades antihiper glucémicas, antioxidantes y antimicrobianas. Además, también se examinó el contenido fitoquímico de la planta y la evaluación virtual de su acción antihiper glucemiante.

Métodos: La actividad antihiper glucémica se evaluó mediante ensayos de inhibición de α -amilasa y α -glucosidasa, la capacidad antioxidante se evaluó mediante ensayos de eliminación de radicales ABTS, DPPH, TAC y H_2O_2 , la actividad antimicrobiana se determinó mediante el método de difusión en pozos de agar y se realizaron estudios *in silico*. realizado mediante simulación de acoplamiento molecular en los sitios activos de las enzimas α -amilasa y α -glucosidasa.

Resultados: El extracto de *F. stylosa* mostró una fuerte actividad contra las enzimas α -amilasa y α -glucosidasa ($IC_{50} = 13,45 \pm 0,39$ y $0,614 \pm 0,02$ μ g/mL, respectivamente) en comparación con el fármaco estándar, acarbose ($IC_{50} = 27,2 \pm 0,78$ y $0,375 \pm 0,01$). μ g/mL, respectivamente). La investigación de la capacidad antioxidante indicó una actividad leve en cuatro ensayos. El análisis LC-HRMS permitió la detección de 23 metabolitos, incluidos glucosinolatos, flavonoides, aminoácidos, ácidos orgánicos y ácidos grasos. Un estudio de simulación de acoplamiento molecular señaló interacciones notables de los flavonoides con los residuos catalíticos de ambas enzimas.

Conclusiones: *F. stylosa* que crece en Arabia Saudita contiene metabolitos variables con notable actividad antihiper glucémica. Estos metabolitos se recomiendan para una mayor purificación e investigación de su eficacia antidiabética.

Palabras Clave: diabetes mellitus; *Farsetia stylosa*; LC-MS/MS; simulación de acoplamiento molecular.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic multifactorial metabolic disorder characterized by persistent elevated blood glucose levels as a result of a defect in pancreatic production or cellular uptake of insulin in the body (Al-Goblan et al., 2014). Diabetic patients are classified into three main categories: type 1 (insulin-dependent) diabetes, where the pancreas secretes little or no insulin; type 2 diabetes, which occurs when secreted insulin is relatively deficient or not effectively used by the cells (insulin resistant), and this represents 90–95% of diabetic cases. In addition to the third type (gestational diabetes), pregnant women (Neagu et al., 2023). Individuals with DM face a higher mortality risk compared to the general population due to the progressive development of severe complications over time. The current conventional antidiabetic drugs, mainly insulin and oral antihyperglycaemic agents, possess various drawbacks, including limited effectiveness, side effects, and hypoglycaemic risk (Neagu et al., 2023). Accordingly, there is a growing interest in developing natural alternatives for the management of diabetes. In this concern, medicinal plants are favored alternatives over synthetic drugs due to reduced side effects, the presence of synergistic effects of biologically active compounds, and lower costs (Jacob and Narendhirakannan, 2019). One therapeutic perspective to manage diabetes is the inhibition of the carbohydrates metabolizing enzymes, α -amylase, and α -glucosidase enzymes. Considering the biochemical roles of these two enzymes, α -amylase catalyzes the hydrolysis of α -1,4-glycosidic linkages in polysaccharides to lower molecular weight products, which are further degraded by α -glucosidase to α -glucose as one of the main products (Neagu et al., 2023). Inhibiting these enzymes slows down the postprandial elevation in blood glucose levels. Accordingly, many attempts have been made to explore new and effective natural inhibitors of these enzymes.

Consumption of cruciferous vegetables has a protective effect against type 2 DM, and this may be associated with their content of isothiocyanates, indole-3-carbinol, and their precursors, glucosinolates (Connolly et al., 2021). An *in vivo* study on the antidiabetic activity of isothiocyanates resulted in inhibition of liver gluconeogenesis, which in turn improved glucose tolerance and increased insulin signaling and sensitivity (Waterman et al., 2015).

Farsetia stylosa Royle belonging to the *Brassicaceae* family (formerly known as *Cruciferae*), is an erect or ascending, simple, annual desert herb.

The plant has sessile, oblong linear leaves, racemes of terminal white to pale pink small flower, a little longer fruit that is rounded toward both ends, a brownish-black winged seed, and a thick woody root (Quattrocchi, 2016; Singh and Shrivastava, 2007). Previous phytochemical screening of the aerial parts of the plant revealed the presence of flavonoids, sterols, saponins, glycosides, and tannins, while GC/MS analysis detected quercetin, urs-12-en-3-one, betulins, lanosterin, dihydrotroscol, and phytol (El-Sharkawy et al., 2017). The plant has been reported to have tonic, astringent, anti-rheumatic, anti-inflammatory, and antioxidant activities (El-Sharkawy et al., 2017; Quattrocchi, 2016). Additionally, several folk healers stated that *F. stylosa* could be used to treat diabetic patients. This claimed efficacy has been *in vivo* investigated on alloxan-induced diabetic rats, and the study revealed that 300 mg/kg of the methanol extract significantly lowered the fasting blood glucose level (Hayat et al., 2014).

As mentioned above, the drawbacks of the currently used oral antihyperglycemic drugs strongly encourage the research for natural alternatives. In this regard, and because *F. stylosa*, as well as several other members of the *Brassicaceae* family, have a history of antidiabetic activity, this plant species was chosen for the study. The *in vitro* testing about the ability of plant extracts to inhibit the carbohydrate metabolic enzymes α -amylase and α -glucosidase represents a simple, reliable, and rapid method to evaluate antihyperglycemic activity. Moreover, oxidative stress is one of the complications associated with diabetes, and most diabetes management strategies include treatment with antioxidants. Hence, the study aims to evaluate the inhibitory activity of *F. stylosa* crude extract against both enzymes and its antioxidant capacity as well.

To shed light on the phytoconstituents that might be responsible for the antihyperglycemic effects, the study also involved examining the chemical content of the alcoholic extract through LC-MS/MS analysis followed by molecular docking simulation of detected metabolites into the active sites of α -amylase and α -glucosidase enzymes.

MATERIAL AND METHODS

Chemicals and reagents

Methanol was purchased from El-Nasr company for pharmaceuticals and chemicals (Egypt). Acetonitrile, water, and formic acid as solvents for HPLC analysis were purchased from Sigma Aldrich (Saint Louis, Mo, USA).

Plant material and extract preparation

The aerial parts of *F. stylosa* were collected from the Qassim region (KSA) during February 2022 (26°20'52.6"N, 43°44'40.4"E), the plant was identified by the area botanical expert, Mr. Ibrahim Aldkhail and a voucher, specimen with number QPP-108 was kept at the college of pharmacy, Qassim University. The collected plant was washed, dried, and then crushed into a fine powder. The powdered plant (200 g) was macerated in 80% aqueous methanol (5 × 600 mL), the extract was then filtered, and the filtrate was evaporated under reduced pressure. The dried extract was kept at -5°C till further analysis.

Phytochemical analysis

Preliminary phytochemical screening

Hydroalcoholic extract of *F. stylosa* was subjected to qualitative testing for carbohydrates, sterols and/or triterpenes, flavonoids, tannins, saponins, cardiac glycosides, alkaloids, and anthraquinones using previously reported tests (Amin et al., 2022).

Evaluation of total phenolic content (TPC) and total flavonoid content (TFC)

TPC in the crude extract was assessed using Folin-Ciocalteu reagent and gallic acid standard (Amin et al., 2023). Briefly, each of the plant extract (0.5 mL, 1 mg/mL) and serial dilutions of the standard dilutions (0.5 mL) were mixed with 2.25 mL of Folin-Ciocalteu reagent (10% aqueous solution). Then, 2 mL of 7.5% w/v anhydrous Na₂CO₃ was added, vortexed, and incubated for 30 min at room temperature. Finally, absorbance was recorded in a spectrophotometer (UV-1601 PC, Shimadzu, Japan) at 750 nm.

TFC in the crude extract was assessed using aluminum chloride and rutin standard (Amin et al., 2023). Briefly, each of the plant extract (0.5 mL, 1 mg/mL) and serial dilutions of the standard dilutions (0.5 mL) were diluted with 2 mL of distilled water and mixed with 0.15 mL of 5% NaNO₂, vortexed, and left to stand for 6 min. Then, 0.15 mL AlCl₃ (10%) was added; 5 min later, 1 mL of 1 M NaOH was added and completed the solution up to 5 mL with distilled water, followed by incubating test tubes for 30 min and recording the absorbance at 430 nm in a spectrophotometer (UV-1601 PC, Shimadzu, Japan).

Chemical profiling

Methanol extract of *F. stylosa* was subjected to LC-HRMS analysis for qualitative identification of secondary metabolites. The system consists of HPLC (Shimadzu ExionLC) coupled with a mass spectrometer (X500 QTOF). The chromatographic separation

was carried out using a column (GL-Science; 100*2.1 mm, 3 μm). The mobile phase used comprises solvent A (0.1% formic acid in water) and solvent B (acetonitrile). Gradient elution with 5% B (0–5 min), 5–95% B (5–25 min), 95–95% B (25–30 min), 95–5% B (30–40 min) was then used. 2 μL of the sample was injected, and the column temperature was set at 50°C; Flow rate: 0.35 mL/min.

Antihyperglycemic activity

α-Amylase inhibition assay

The alpha-amylase assay of the tested sample was conducted using the α-amylase inhibitor screening kit (K482-100, BioVision® Inc.) according to the standard method with slight modification (Etsassala et al., 2020). Fifty microlitres were added from each serial dilution of the sample (S), inhibitor control (IC), α-amylase assay buffer (enzyme control, EC), and 100 μL α-amylase assay buffer (background control, BC). For each well, 50 μL were added of diluted α-amylase enzyme solution (10 μL of α-amylase with 490 μL of α-amylase assay buffer) and incubated at 25°C for 10 min. Then 50 μL of diluted α-amylase substrate solution (490 μL of α-amylase assay buffer, 10 μL of α-amylase substrate) was added into each well and well mixed in a 96-well microplate. After that, the absorbance was measured kinetically at 405 nm for 20–25 min in a microplate reader (BioTek, Winooski, VT, USA). The calculated % relative inhibition was determined by the equation [1].

$$\text{Relative inhibition (\%)} = \frac{(\text{Slope of EC} - \text{Slope of S})}{\text{Slope of EC}} \times 100 \quad [1]$$

The IC₅₀ values of *F. stylosa* extract and standard drug were calculated from the dose-response curve via interpolation from the linear regression analysis.

α-Glucosidase inhibitory assay

To measure the α-glucosidase inhibitory of *F. stylosa* alcoholic extract, the colorimetric assay was performed by using the α-glucosidase inhibitor screening kit (K938-100, BioVision®, Inc.). According to the manufacturer's instructions (Indrianingsih and Tachibana, 2016), 10 μL of the tested sample [extract in serial dilutions (sample, S), acarbose (inhibitor control, IC), or α-glucosidase assay buffer (enzyme control, EC)] was added to 20 μL α-glucosidase assay buffer (background control, BC) in a 96-well microplate. For each well, 10 μL of diluted α-glucosidase enzyme solution (2 μL of α-glucosidase with 38 μL of α-glucosidase assay buffer) was added and incubated at 25°C for 20 min. Then 20 μL of reaction mix solution (3 μL α-glucosidase substrate mix, 17 μL α-glucosidase assay buffer) was added into each well

and was mixed well. After that, the absorbance was measured kinetically at 410 nm for 60 min in a microplate reader (BioTek, Winooski, VT, USA). The calculated % relative inhibition was determined by the equation [1].

The IC₅₀ values were then calculated from the dose-response curve via interpolation from the linear regression analysis.

Antioxidant capacity

ABTS radical scavenging method

The ABTS scavenging capacity method examines the ability of antioxidants to react directly with ABTS cation radicals produced by a chemical process. It is a decolorization assay (Sánchez et al., 2007). The ABTS radical cation was created by combining 0.63 mM potassium persulfate with 1.8 mM ABTS stock solution and letting the mixture to sit undisturbed at room temperature for 12 to 16 hours before use. After that, ethanol was added to the solution to dilute it until the absorbance at 734 nm was 0.700 ($\pm$ 0.030). Using methanol (80%), various concentrations ranging from 0.5 to 1000 g/mL for each chemical were made. In a microtitre plate, 190 μ L of ABTS cation solution was mixed with 10 μ L of the tested sample. The absorbance was read at 734 nm every 1 min till 13 min following initial mixing in a microplate reader (BioTek, Winooski, VT, USA). Ascorbic acid was used as the standard antioxidant, while methanol (80%) was used as the negative control. The percent free radical scavenging activity was calculated according to the equation [2].

$$\text{Scavenging activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad [2]$$

The IC₅₀ value was calculated from the curve representing scavenging activities (%) versus concentrations of the sample.

DPPH radical scavenging activity

DPPH stock solution (0.5 Mm in methanol) was prepared. The absorbance of the DPPH working solution was adjusted to 0.98 ± 0.02 at 517 nm by dilution of the DPPH stock solution with MeOH. In a test tube, 300 μ L plant extract or ascorbic acid (different concentrations between 0.025-0.4 mg/mL) was mixed with three mL DPPH working solution. The mixture was incubated for 30 min. The absorbance was measured at 517 nm in a microplate reader (BioTek, Winooski, VT, USA). The percent antioxidant capacity was calculated using the same formula mentioned before in the ABTS assay (Amin et al., 2022). Results were expressed as mean IC₅₀ value $\pm$ SEM. The IC₅₀ values

were then calculated from the dose-response curve via interpolation from the linear regression analysis.

Total antioxidant capacity (TAC) assay

TAC was estimated by the method described by Alam et al. (2013). The assay was based on the formation of a green phosphate/MO (V) complex at an acidic pH due to the reduction of MO (VI) to MO (V). 0.1 mL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was mixed with 0.2 mL of methanol sample solution. In a thermal block, the mixture was incubated at 95°C for 90 min. The sample absorbance was read at 695 nm after cooling in a spectrophotometer (UV-1601 PC, Shimadzu, Japan). TAC was calculated (as mg equivalents of ascorbic acid).

H₂O₂ radical scavenging method

A solution of H₂O₂ (43 mM) was prepared in phosphate buffer (pH 7.4). 0.2 mL of extract (different concentrations in distilled water) was mixed with the H₂O₂ solution (600 μ L), and the absorbance was recorded after 10 min at 230 nm in a spectrophotometer (UV-1601 PC, Shimadzu, Japan). The absorbance was compared with a control solution containing phosphate buffer without H₂O₂ (Alam et al., 2013). The percentage scavenging was calculated using equation [3].

$$\text{H}_2\text{O}_2 \text{ scavenging (\%)} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 \quad [3]$$

Results were expressed as mean IC₅₀ value $\pm$ standard error (SEM).

Antimicrobial activity

Six microbial strains were obtained from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The diameter of inhibition zones served as an indicator of the antibacterial and antifungal activity. The previously published agar well diffusion method was applied (Abo-Ashour et al., 2018). On the other hand, MIC was carried out using the serial dilution method of Abo-Ashour et al. (2018), as indicated by the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Ketoconazole and gentamycin were used as antifungal and antibacterial positive controls, respectively. The results reported were means of at least three separate experiments.

Statistical analysis

Results of TPC and TFC were expressed as mean values $\pm$ standard deviation (SD), while all other results were expressed as mean values $\pm$ standard error

of mean (SEM) from three separate experiments. The IC₅₀ values for antihyperglycemic and antioxidant assays were calculated from the dose-response curves using linear regression analysis, and statistical analyses were done utilizing Graph Pad Prism 6 statistical software (Graph Pad Software, San Diego, CA, USA). Statistical significance was considered when $p < 0.05$.

Molecular docking

Detected metabolites in the plant were docked into the active sites of α -glucosidase and α -amylase enzymes using pdb entries of 5NN8 (Roig-Zamboni et al., 2017) and 4W93 (Williams et al., 2015), respectively, that were retrieved from Protein Data Bank (<https://www4.rcsb.org/>). Structures of all compounds were downloaded from PubChem [October 2023], and the compounds' energies were minimized by Chem Bio 3D (Chem Bio Office Ultra 12.0 suite). Molecular docking investigation was accomplished using Autodock Vina in Pyrx (Trott and Olson, 2010). XYZ coordinates were set as 5NN8: -12.58, -28.63, 94.67; 4W93: -9.85, -6.96, -21.72. Pymol software (Lill and Danielson, 2011) was used to visualize and analyze the docked ligand poses.

ADME and toxicity

A predictive analysis of the metabolic and toxicity profiles of the metabolites that exhibited the highest binding affinities to the targeted enzymes has been performed using the ADMET SAR online server (<http://lmmd.ecust.edu.cn/admetSar1>) (Cheng et al., 2012). Molecular information on the compounds was retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) in the form of SMILES.

RESULTS AND DISCUSSION

Phytochemical analysis

The powdered plant material was macerated in aqueous methanol (80%), and the extraction process yield was calculated as 5.8%. The prepared extract was investigated for the presence of variable metabolites, e.g., alkaloids, glycosides, and carbohydrates. The concentrations of the total phenolic and total flavonoid contents were quantified, and then LC-HRMS analysis was assessed.

Preliminary phytochemical screening

Preliminary testing of the variable metabolites' classes in the hydroalcoholic extract of *F. stylosa* collected from the Saudi Arabia desert revealed the presence of carbohydrates, sterols and/or triterpenes, flavonoids, tannins, and saponins as well as the ab-

sence of cardiac glycosides, alkaloids, and anthraquinones. These results greatly matched the previous results reported by El-Sharkawy et al. (2017).

Evaluation of total phenolic content (TPC)

The phenolic content of the plant was quantified utilizing the Folin-Ciocalteu method, and gallic acid was used as a standard phenolic compound. The results estimated the phenolic content in the studied plant extract as 12.8 ± 0.6 mg GAE/g extract (Table 1). This result was similar to that reported by Hayat et al. (2014).

Table 1. Contents of total phenolic (TPC) and flavonoid (TFC) in *F. stylosa* methanol extract.

Metabolites	Concentration
TPC (mg GAE/g extract)	12.8 ± 0.6
TFC (mg RE/g extract)	203.3 ± 1.9

Data represent mean $\pm$ SEM (n = 3).

Evaluation of total flavonoid content (TFC)

In order to quantify the flavonoid content, the aluminum chloride method was used, where rutin was the standard reference compound. The flavonoid content was calculated as 203.3 ± 1.9 mg RE/g extract. Comparing our result to the previous report (Hayat et al., 2014), we concluded an appreciably higher flavonoid concentrations in the plant species collected from KSA.

The above-mentioned results of preliminary phytochemical screening, TPC, and TFC noted the presence of variable metabolites, which in turn, recommended subsequent testing of the possible bioactivities of this plant species.

LC-HRMS analysis of the methanolic extract of *F. stylosa*

LC-HRMS investigation of the methanol extract of *F. stylosa* using both positive and negative modes enabled the annotation of twenty-three constituents representing several chemical classes: glucosinolates (three metabolites), flavonoids (four metabolites), phenolic, organic acids and their esters (seven metabolites), fatty acids (three metabolites), and amino acids and their derivatives (four metabolites), in addition to one lignan compound and one trisaccharide (Table 2, Fig. 1). The chromatograms of the identified compounds are shown in the supplementary data (Figs. S1 and S2).

Glucosinolates (β -thioglucoside-*N*-hydroxy-sulfates) are a class of secondary metabolites unique to the family *Brassicaceae*. Their structures are composed of two moieties, such as β -D-thioglucose and a sulfonated aldoxime, in addition to a variable side

chain that causes their classification into three groups such as aliphatic, indole, and aromatic glucosinolates (Connolly et al., 2021). Herein, three glucosinolates, including glucoraphanin, progoitrin, and glucoerucin, were detected in the methanol extract of *F. stylosa*. Glucoraphanin has been previously reported in several *Brassicaceae* vegetables, e.g., broccoli and kale, progoitrin in Chinese cabbage, Taicai, Mizuna, and Swede, while glucoerucin was detected in Kohlrabi (Zhu et al., 2023). Remarkably, several other *Farsetia* species were reported to contain a large number of glucosinolates, such as glucoiberin, sinalbin, sinigrin,

glucocheirolin, and glucoerysolin that were detected in *F. aegyptia* and *F. longisiliqua* (Marzouk et al., 2020).

Flavonoids were reported to be successful chemosystemic marker for differentiation between *Farsetia* species. These flavonoids were found to be derivatives of luteolin, kaempferol, quercetin, isorhamnetin, apigenin, and rhamnocitrin (Marzouk et al., 2020). In the current study two flavonol glycosides: quercetagen-7-O-glucoside and datiscin together with two flavone aglycons: apigenin and 6-methoxyluteolin were detected in the methanol extract of the studied species.

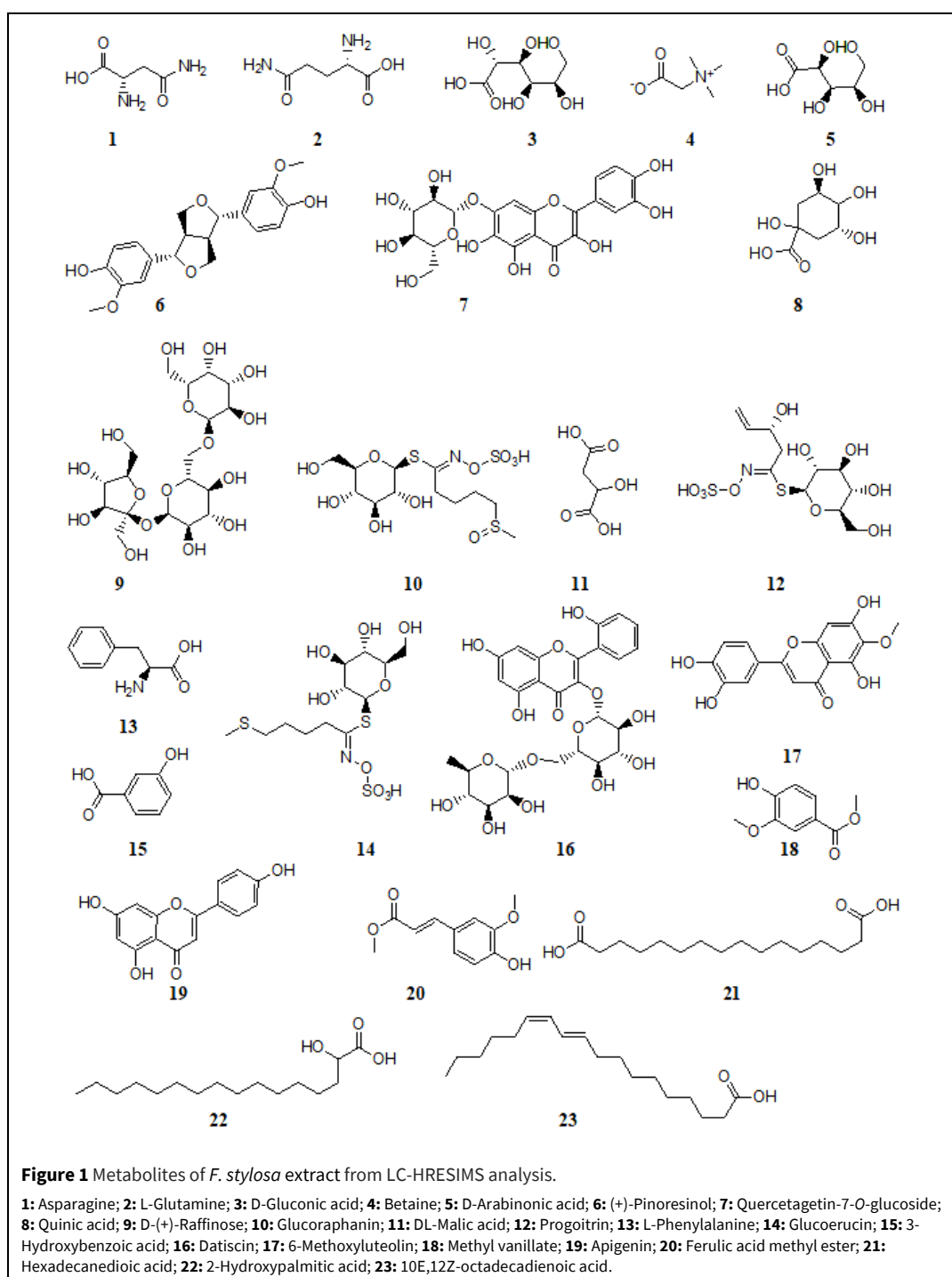


Table 2. LC-HRMS analysis of the methanol extract of *F. stylosa*.

No.	RT (min)	Metabolites name	Chemical	MF	m/z	Fragments*	Polarity
1	2.65	Asparagine	Amino acid	C ₄ H ₈ N ₂ O ₃	131.0503	131.0503, 114.0240, 70.0320	N
2	2.74	L-Glutamine	Amino acid	C ₅ H ₁₀ N ₂ O ₃	145.0656	127.0548, 109.0439, 84.0481	N
3	2.96	D-Gluconic acid	Organic acid	C ₆ H ₁₂ O ₇	195.0564	195.0564, 129.023, 99.0119, 87.011, 75.0106, 59.0156	N
4	2.99	Betaine	Amino acid derivative	C ₅ H ₁₁ NO ₂	118.0844	58.06, 59.07, 118.08	p
5	3.01	D-Arabinonic acid	Organic acid	C ₅ H ₁₀ O ₆	165.0455	129.0236, 99.0121, 85.032, 75.0368, 59.0157	N
6	3.08	(+)-Pinoresinol	Lignan	C ₂₀ H ₂₂ O ₆	357.1143	357.1151	N
7	3.19	Quercetagenin-7-O-glucoside	Flavonoid glycoside	C ₂₁ H ₂₀ O ₁₃	479.1200	479.123, 317.063	N
8	3.39	Quinic acid	Organic acid	C ₇ H ₁₂ O ₆	191.0605	191.0624, 93.079	N
9	3.45	D-(+)-Raffinose	Trisaccharide	C ₁₈ H ₃₂ O ₁₆	503.1755	503.1766, 89.0280	N
10	3.57	Glucoraphanin	Glucosinolate	C ₁₂ H ₂₃ NO ₁₀ S ₃	436.0508	436.0506, 437.0544, 438.0638, 178.0225, 96.9618, 95.9542	N
11	3.67	DL-Malic acid	Organic acid	C ₄ H ₆ O ₅	133.0179	133.0179, 115.0071, 71.016	N
12	7.70	Progoitrin	Glucosinolate	C ₁₁ H ₁₉ NO ₁₀ S ₂	388.0472	388.0480, 96.9620, 95.9545, 74.9925	N
13	11.09	L-Phenylalanine	Amino acid	C ₉ H ₁₁ NO ₂	164.0763	72.012, 91.0587, 103.0591, 147.0497, 164.0763	N
14	11.87	Glucorucin	Glucosinolate	C ₁₂ H ₂₃ NO ₉ S ₃	420.0568	420.0568, 96.962, 95.9554, 74.9935	N
15	12.05	3-Hydroxybenzoic acid	Organic acid	C ₇ H ₆ O ₃	137.0287	137.0287, 93.0377, 93.0192, 65.0422	N
16	12.49	Datiscin	Flavonoid glycoside	C ₂₇ H ₃₀ O ₁₅	595.1637	287.056, 595.1637	P
17	12.59	6-Methoxyluteolin	Flavonoid	C ₁₆ H ₁₂ O ₇	315.0600	315.0600, 300.0367	N
18	15.65	Methyl vanillate	Organic acid ester	C ₉ H ₁₀ O ₄	183.0635	183.0635, 151.04, 124.05, 59.0431	N
19	16.23	Apigenin	Flavonoid	C ₁₅ H ₁₀ O ₅	269.0527	269.0527, 117.0380	N
20	16.47	Ferulic acid methyl ester	Organic acid	C ₁₁ H ₁₂ O ₄	207.0724	192.0478, 177.0248, 134.0034, 133.0334, 105.037	N
21	19.31	Hexadecanedioic acid	Fatty acid	C ₁₆ H ₃₀ O ₄	285.2139	285.2139, 267.2047, 223.2135	N
22	23.61	2-Hydroxypalmitic acid	Fatty acid	C ₁₆ H ₃₂ O ₃	271.2351	271.2351, 225.2272, 223.213, 197.1956, 55.0213	N
23	24.38	10E,12Z-octadecadienoic acid	Fatty acid	C ₁₈ H ₃₂ O ₂	581.4681	581.4681, 280.2445, 279.2404	N

*References for mass fragments are supplied in supplementary data.

The recorded results also indicated the presence of three amino acids: asparagine, L-glutamine, and phenylalanine, as well as the amino acid derivative betaine. Reviewing the relevant literature, glutamic acid, isoleucine, histidine, glycine, cysteine, tyrosine, and tryptophan amino acids were previously detected in *F. hamiltonii* and *F. aegyptia* (Marzouk et al., 2020). Gluconic and quinic acids were detected in the alcoholic extract of the studied species. These acids were also previously reported in *F. aegyptia* and *F. longisiliqua*. In addition, arabinonic acid, malic acid, *p*-

hydroxybenzoic acid, and methyl vanillate ester were detected in the tested extract of *F. stylosa*. Organic and phenolic acids, e.g., malic, *p*-hydroxybenzoic acid, and vanillic acid, were previously reported in many *Brassicaceae* vegetables (Cartea et al., 2010).

Several lignans, e.g., pinoresinol and lariciresinol were reported as abundant metabolites in many *Brassicaceae* plants (Milder et al., 2005). In the current study, the lignan compound pinoresinol was detected in the tested extract. As per fatty acids, hexadecanedi-

oic acid is a dicarboxylic fatty acid detected herein and was also previously reported in some *Brassicaceae* seeds (Molina et al., 2006).

It is worth mentioning that the methanol extract of *F. hamiltonii* was previously investigated using GC-MS analysis and was reported to contain vanillin, betulin, urs-12-en-3-one, lanosterin, cyclolaudenol, globulol, 3-O-acetyl-delta-24-cycloartenol, dihydrocoumarone, dihydrotorulosol, quercetin, chrysene, hexa-hydrothunbergol, neophytadiene, coniferol, phytol, and juniper camphor (El-Sharkawy et al., 2017). Furthermore, another study analyzed the metabolic content of different fractions of *F. hamiltonii* and identified nine compounds belonging to hydrocarbons, fatty acids, and fatty alcohols (Hayat and Uzair, 2019).

In vitro testing of antihyperglycemic activity and antioxidant capacity

Antihyperglycemic activity

α -Amylase and α -glucosidase inhibitors from natural sources are considered promising tools for diabetes management. Hence, research adopting *in vitro* testing of inhibition activity against these carbohydrate metabolizing enzymes is now considered a rapid and reliable strategy for testing many plant extracts. In view of traditional uses, *F. stylosa* is commonly known for its anti-diabetic and anti-spasmodic activity. The current study was devoted to evaluating the *in vitro* antihyperglycemic potential of the alcohol extract of this plant species as well as to exploring its metabolic content for potential antihyperglycemic constituents. *In vitro* testing of the α -amylase and α -glucosidase inhibition effects of the methanolic extract of *F. stylosa* indicated a strong α -amylase inhibitory activity with IC_{50} value of $13.45 \pm 0.39 \mu\text{g/mL}$ compared to the standard drug, acarbose ($IC_{50} = 27.2 \pm 0.78 \mu\text{g/mL}$). In addition, the α -glucosidase inhibition was estimated as $IC_{50} = 0.614 \pm 0.02 \mu\text{g/mL}$ for *F. stylosa* extract and $IC_{50} = 0.375 \pm 0.01 \mu\text{g/mL}$ for acarbose (Table 3). A previous *in vivo* experiment revealed the antidiabetic activity of *F. hamiltonii* extract in alloxane-induced diabetic rats (Hayat et al., 2014). Furthermore, another study reported the potential anti- α -glucosidase activity of the methanolic extract of the same *F.* species (Hayat and Uzair, 2019). These authors justified the folkloric therapeutic potential of *F. hamiltonii* root in treating diabetes by studying the anti- α -glucosidase activity of the methanol extract of *F. hamiltonii* root. Antihyperglycemic activity may be attributed to plant contents of phenolics and flavonoids. Different compounds from these classes were reported to have potential antihyperglycemic activity, and this activity was attributed to various mecha-

nisms. One of these mechanisms is α glucosidase/ α amylase inhibition (Neagu et al., 2023). It is worth mentioning that the antihyperglycemic activity of cruciferous vegetables may be associated with the content of glucosinolates (Connolly et al., 2021).

Table 3: Enzyme inhibitory activity of *F. stylosa* methanol extract against α -amylase and α -glucosidase enzymes.

Extract/ standard	IC_{50} ($\mu\text{g/mL}$)	
	α -Amylase	α -Glucosidase
<i>F. stylosa</i> extract	$13.45 \pm 0.39^{***}$	$0.614 \pm 0.02^{***}$
Acarbose	27.20 ± 0.78	0.375 ± 0.01

Data represent mean $\pm$ SEM (n = 3), where ***p<0.001 is considered statistically significant compared to the standard drug acarbose using an unpaired student t-test.

Antioxidant capacity

The methanol extract was tested for antioxidant potential using several methods (Table 4). The results of the four employed methods displayed a weak antioxidant capacity of *F. stylosa* compared to ascorbic acid and butylated hydroxytoluene (BHT) standards. Reviewing the relevant literature, similar results were found indicating low activity of *F. hamiltonii* in comparison to *Launaea nudicaulis* using DPPH and FRAP assay methods (El-Sharkawy et al., 2017). The antioxidant activity of the plant may be attributed to its content of phenolic and flavonoids.

Antimicrobial activity

As previously noted, the current study was devoted to investigating the phytochemical content and the biological potentials of *F. stylosa* collected from Saudi Arabia flora. In this regard, the plant extract was tested for antimicrobial activity, where results revealed no antibacterial or antifungal activity against the tested pathogenic Gram-positive and Gram-negative bacterial strains and fungal species (Table 5). These findings confirmed the previous statement of El-Sharkawy et al. (2017), while on the other hand, Hayat and Uzair. (2019) reported moderate antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*.

Molecular docking simulation

A molecular docking study was conducted against both α -amylase and α -glucosidase enzymes to investigate the antihyperglycemic potential of the detected compounds in the plant. Analysis of docking outcomes revealed that the antihyperglycemic potential of *F. stylosa* extract could be attributable to flavonoids; their binding diagram unveiled interactions with key

Table 4. Antioxidant capacity of *F. stylosa* methanol extract.

Extract/ standard	ABTS (IC ₅₀ µg/mL)	DPPH (IC ₅₀ µg/mL)	TAC (mg GAE/g)	H ₂ O ₂ (IC ₅₀ µg/mL)
<i>F. stylosa</i>	116.49 ± 2.34***	122.10 ± 0.61***	34.72 ± 0.49***	130.77 ± 3.59***
Ascorbic acid	10.66 ± 0.51	15.31 ± 0.49	75.30 ± 1.70	--
BHT	--	--	--	12.51 ± 0.35

Data represent mean ± SEM (n = 3), where ***p<0.001 is considered statistically significant compared to standard drugs using the unpaired student t-test.; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; TAC: total antioxidant capacity; BTH: Butylated hydroxytoluene.

Table 5. Mean zone of inhibition (mm) of *F. stylosa* and standard drugs produced on a range of pathogenic microorganisms.

Tested microorganisms	<i>F. stylosa</i>	Standard
Fungi		Ketoconazole
<i>Aspergillus fumigates</i>	NA	17
<i>Candida albicans</i>	NA	20
Gram positive bacteria		Gentamycin
<i>Staphylococcus aureus</i>	NA	24
<i>Bacillus subtilis</i>	NA	26
Gram negatvie bacteria		Gentamycin
<i>Escherichia coli</i>	NA	30
<i>Proteus vulgaris</i>	NA	25

NA: No activity.

amino acids in both enzymes. Such interactions may be responsible for their stabilization inside the binding pockets, and their binding scores were found to be comparable and, in certain cases, higher than those of the co-crystallized ligands.

Four flavonoids and one lignan exhibited comparatively high binding affinities (from -9.4 to -8.5 kcal/mol) to α-amylase enzyme that are very close to that of the co-crystallized ligand; montbretin A (-9.6 kcal/mol), a natural flavonol glycoside of potent inhibition activity on human pancreatic α-amylase enzyme (Williams et al., 2015). On the other hand, seven metabolites, including four flavonoids, one lignan, one glucosinolate, and one trisaccharide, showed binding affinities to α-glucosidase (from -8.0 to -6.8 kcal/mol) that exceeded the co-crystallized ligand; acarbose (-6.4 kcal/mol) (Table 6). Interestingly, results revealed that the most active compounds in the tested series were datiscin, which disclosed the highest binding affinities to both α-amylase and α-glucosidase enzymes (-9.4 and -8.0 kcal/mol, respectively), followed by quercetagenin-7-O-glucoside (-9.4 and -7.4 kcal/mol, respectively).

Previous studies on the catalytic mechanism of human pancreatic α-amylase declared the paramount importance of three amino acids (Asp197, Glu233, and Asp300) for the hydrolysis of starch and similar α-1,4-

linked polysaccharides (Pinto et al., 2015). Moreover, three other residues, Arg195, Asp298, and Arg337, have a substantial impact on the allosteric activation of the enzyme via binding to chloride ions (Maurus et al., 2008). Datiscin was found to bind tightly in the α-amylase active site. As shown in Fig. 2A, the two sugar moieties were imbedded in the binding pocket where rhamnose moiety was involved in hydrogen bonds with the catalytic nucleophile residue Asp197 and the catalytic acid-base residue Asp300, in addition to hydrogen bonds with Arg195 and His299. Two hydrogen bond interactions were recognized between glucose moiety and the catalytic residue Glu233 along with Ile235. Moreover, the carbonyl oxygen at C-4 and the hydroxyl group at C-5 of the flavonoid nucleus each formed H-bonds with both Lys200 and Ile235, and the hydroxyl group at C-2' was involved in a hydrogen bond with Ala307. Quercetagenin-7-O-glucoside displayed high binding affinity to α-amylase (-9.4 kcal/mol), which is in agreement with previously reported data (Mohamed et al., 2023). Six hydrogen bonds contributed to the overall binding affinity; the hydroxyl groups at C-3' and C-4' interacted with the catalytic residues Asp197 and Arg195, respectively. Moreover, the hydroxyl group at C-3 and carbonyl oxygen at C-4 were connected to Tyr62 and Gln63, respectively. Additionally, the glucose

Table 6. Docking scores (kcal/mol) of detected compounds by LC-MS analysis of the methanol extract of *F. stylosa* against α -amylase and α -glucosidase enzymes.

Compound name	Docking scores (kcal/mol)	
	α -Amylase	α -Glucosidase
DL-Malic acid	-4.7	-4.9
Asparagine	-4.8	-4.9
Glutamine	-4.6	-5.1
Arabinonic acid	-5.5	-4.9
Betaine	-3.3	-3.6
Gluconic acid	-5.6	-5.5
3-Hydroxybenzoic acid	-5.4	-5.4
Quinic acid	-5.9	-5.6
Methyl vanillate	-5.7	-5.6
L-Phenylalanine	-5.7	-5.7
Ferulic acid methyl ester	-6.4	-5.6
Progoitrin	-6.6	-6.8
Glucoerucin	-5.9	-5.9
Glucoraphanin	-6.4	-6.3
Apigenin	-8.6	-6.8
6-Methoxyluteolin	-8.6	-7.1
Hexadecanedioic acid	-5.1	-5.5
2-Hydroxypalmitic acid	-5.1	-5.5
10E,12Z-Octadecadienoic	-5.4	-5.5
D-(+)-Raffinose	-6.7	-6.8
(+)-Pinoresinol	-8.5	-6.9
Quercetagenin-7-O-glucoside	-9.4	-7.4
Datiscin	-9.4	-8.0

moiety contributed to interactions via two hydrogen bonds with Trp59 and His305 (Fig. 2B).

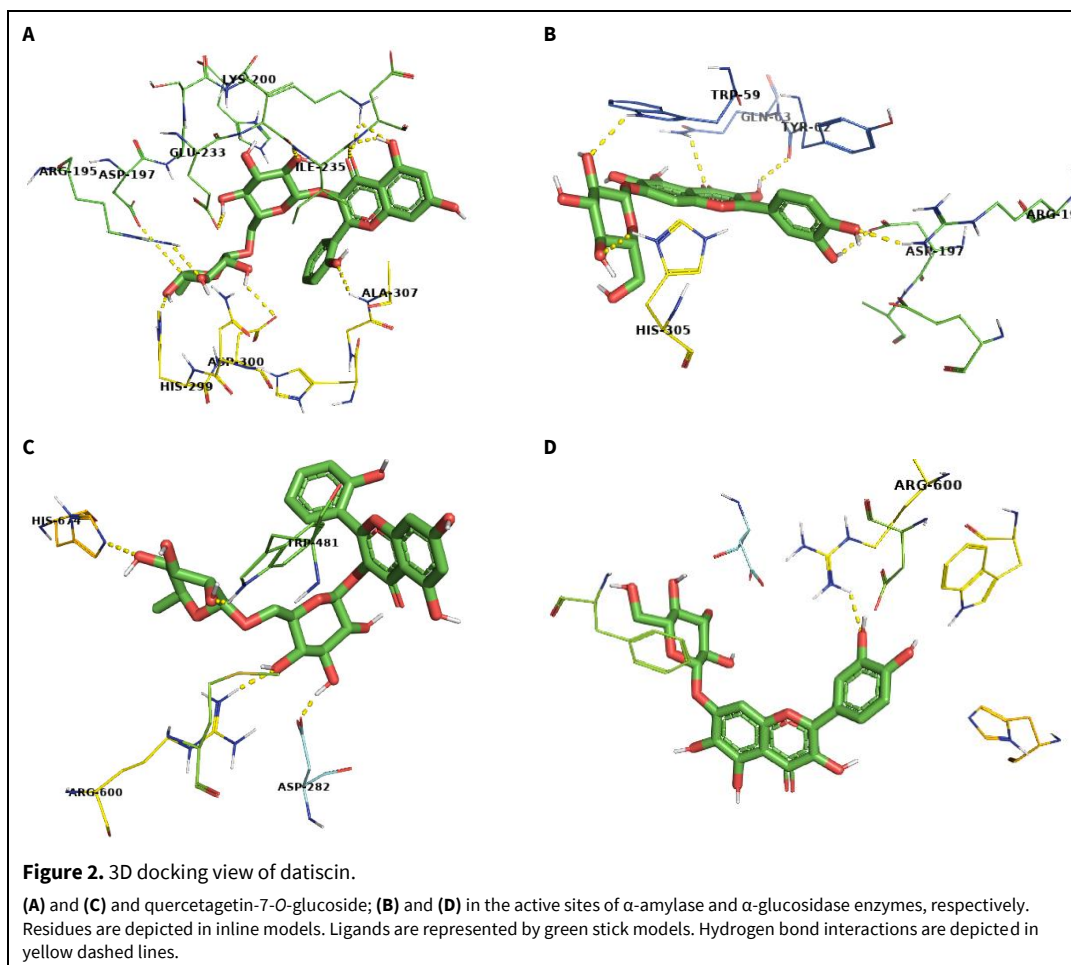
On the other hand, the observed docking results against α -glucosidase enzyme highlighted the importance of the sugar moieties to the binding affinity of datiscin since glucose moiety established two hydrogen bonds with the conserved Arg600 and with Asp282, while the peripheral rhamnose moiety involved in hydrogen bond interactions with Trp481 and His674 (Fig. 2C), similarly, quercetagenin-7-O-glucoside anchored itself in the active site of α -glucosidase and was hydrogen bonded to the conserved amino acid Arg600 (Fig. 2D).

To the best of our knowledge, this is the first time to evaluate datiscin inhibition against both α -amylase and α -glucosidase enzymes using an *in silico* approach, where it possessed significant polar interactions with key amino acids in both enzymes that shed light on plausible antidiabetic activity. Previous stud-

ies have outlined α -glucosidase inhibition activity of the aglycone quercetagenin (Barber et al., 2021), but no reports concerning α -glucosidase inhibition activity of the glycoside, quercetagenin-7-O-glucoside.

ADME and toxicity

ADMET criteria for the two compounds that depicted the highest binding affinities toward the targeted enzymes were predicted using ADMET SAR online server. Both compounds, datiscin and quercetagenin-7-O-glucoside, could be absorbed via the human intestine and have aqueous solubility (LogS 2.7724 and 2.4489, respectively). They could not penetrate the blood-brain barrier or Caco-2 cells. Both compounds were shown to be substrates for P-glycoprotein but non-substrates for human cytochrome P450s that efflux compounds for further metabolism and clearance (Goncalves et al., 2020). Datiscin and quercetagenin-7-O-glucoside did not reveal



any acute toxicity concerning AMEs test and have no carcinogenic risk. Moreover, they showed low acute oral toxicity (category III that constitutes compounds with LD₅₀ values greater than 500 mg/kg but less than 5000 mg/kg based on criteria of US EPA (Cheng et al., 2012) (Table 7).

CONCLUSION

The current research is a comprehensive study of alcoholic extract of *Farsetia stylosa* growing in Saudi Arabia deserts, revealing its promising antihyperglycemic activity as indicated by its inhibitory effects on the two carbohydrates metabolizing enzymes: α -amylase and α -glucosidase, in addition to its antioxidant capacity. Chemical analysis revealed total phenolic and total flavonoid contents of 12.8 ± 0.6 mg GAE/g and 203.3 ± 1.9 mg RE/g, respectively, while LC-HRMS analysis of this species enabled the detection of 23 metabolites representing various chemical structures. Finally, molecular docking simulation demonstrated the high contribution of flavonoids to

the antihyperglycemic activity, particularly datiscin and quercetagenin-7-O-glucoside, where they established binding interactions with catalytically essential amino acids in both enzymes, resulting in the greatest binding affinities among the tested compounds. Reviewing the literature, investigation of the inhibitory effect on α -amylase and α -glucosidase enzymes, antioxidant capacity, and chemical analysis followed by virtual estimation using molecular docking simulation concluded that the plant extract might have antihyperglycemic and antioxidant actions. Hence, further chemical and biological investigations are recommended to prove the activities.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Table 7. ADMET profile of datiscin and quercetageitin-7-O-glucoside.

Model	Datiscin		Quercetageitin-7-O-glucoside	
	Result	Probability	Result	Probability
Absorption				
Blood-brain barrier	BBB -	0.8542	BBB -	0.6975
Human intestinal absorption	HIA+	0.8041	HIA+	0.7855
Caco-2 permeability	Caco2 -	0.9172	Caco2 -	0.9398
P-glycoprotein substrate	Substrate	0.6901	Substrate	0.5905
P-glycoprotein inhibitor	Non-inhibitor	0.8759	Non-inhibitor	0.8782
Renal organic cation transporter	Non-inhibitor	0.8977	Non-inhibitor	0.8921
Aqueous solubility	-2.7724 (LogS)		-2.4489 (LogS)	
Distribution				
Subcellular localization	Mitochondria	0.7639	Mitochondria	0.6068
Metabolism				
CYP450 2 C9 substrate	Non-substrate	0.7639	Non-substrate	0.8116
CYP450 2 D6 substrate	Non-substrate	0.8962	Non-substrate	0.8923
CYP450 3 A4 substrate	Non-substrate	0.5374	Non-substrate	0.6035
CYP450 1 A2 inhibitor	Non- inhibitor	0.8673	Non- inhibitor	0.9084
CYP450 2 C9 inhibitor	Non- inhibitor	0.9071	Non- inhibitor	0.9296
CYP450 2 D6 inhibitor	Non- inhibitor	0.9545	Non- inhibitor	0.9513
CYP450 2 C19 inhibitor	Non- inhibitor	0.9025	Non- inhibitor	0.9289
CYP450 3 A4 inhibitor	Non- inhibitor	0.9249	Non- inhibitor	0.9193
CYP inhibitory promiscuity	Low	0.6787	Low	0.7728
Toxicity				
AMES toxicity	Non-AMES toxic	0.5118	Non-AMES toxic	0.5776
Carcinogens	Non-carcinogen	0.9608	Non-carcinogen	0.9589
Acute oral toxicity	III	0.5971	III	0.4045

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AUTHOR CONTRIBUTION:

Contribution	Ahmed H	Abouzeid H	Mohamed E	Sebak M	Alhumaid M	Aljasser R	Amin E	Afifi N
Concepts or ideas							x	
Design	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x					x	x
Literature search	x	x					x	x
Experimental studies	x	x					x	x
Data acquisition	x	x					x	x
Data analysis	x	x	x	x			x	x
Statistical analysis	x	x					x	x
Manuscript preparation	x	x	x	x			x	x
Manuscript editing	x	x					x	x
Manuscript review	x	x	x	x	x	x	x	x

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Supplementary data

Table S1. LC-HRMS analysis of the methanol extract of *Farsetia stylosa*.

No.	RT (min)	Metabolites name	Chemical	MF	m/z	Fragments	Polarity	Ref.
1	2.65	Asparagine	Amino acid	C ₄ H ₈ N ₂ O ₃	131.0503	131.0503, 114.0240, 70.0320	N	(López-Cervantes et al., 2013)
2	2.74	L-Glutamine	Amino acid	C ₅ H ₁₀ N ₂ O ₃	145.0656	127.0548, 109.0439, 84.0481	N	(Jahangir et al., 2009)
3	2.96	D-Gluconic acid	Organic acid	C ₆ H ₁₂ O ₇	195.0564	195.0564, 129.023, 99.0119, 87.011, 75.0106, 59.0156	N	(Farag et al., 2013)
4	2.99	Betaine	Amino acid derivative	C ₅ H ₁₁ NO ₂	118.0844	58.06, 59.07, 118.08	p	(Wanasundara et al., 2012)
5	3.01	D-Arabinonic acid	Organic acid	C ₅ H ₁₀ O ₆	165.0455	129.0236, 99.0121, 85.032, 75.0368, 59.0157	N	(Pant et al., 2015)
6	3.08	(+)-Pinoresinol	Lignan	C ₂₀ H ₂₂ O ₆	357.1143	357.1151	N	(Cutillo et al., 2003)
7	3.19	Quercetagenin-7-O-glucoside	Flavonoid glycoside	C ₂₁ H ₂₀ O ₁₃	479.1200	479.123, 317.063	N	(Chellan et al., 2012)
8	3.39	Quinic acid	Organic acid	C ₇ H ₁₂ O ₆	191.0605	191.0624, 93.079	N	(Grosso et al., 2011)
9	3.45	D-(+)-Raffinose	Trisaccharide	C ₁₈ H ₃₂ O ₁₆	503.1755	503.1766, 89.0280	N	(Gu et al., 2013)
10	3.57	Glucoraphanin	Glucosinolate	C ₁₂ H ₂₃ NO ₁₀ S ₃	436.0508	436.0506, 437.0544, 438.0638, 178.0225, 96.9618, 95.9542	N	(Iori et al., 2011, Perocco et al., 2006)
11	3.67	DL-Malic acid	Organic acid	C ₄ H ₆ O ₅	133.0179	133.0179, 115.0071, 71.016	N	(Grosso et al., 2011)
12	7.70	Progoitrin	Glucosinolate	C ₁₁ H ₁₉ NO ₁₀ S ₂	388.0472	388.0480, 96.9620, 95.9545, 74.9925	N	(Al-Gendy et al., 2010)
13	11.09	L-Phenylalanine	Amino acid	C ₉ H ₁₁ NO ₂	164.0763	72.012, 91.0587, 103.0591, 147.0497, 164.0763	N	(López-Cervantes et al., 2013)
14	11.87	Glucoruciferin	Glucosinolate	C ₁₂ H ₂₃ NO ₉ S ₃	420.0568	420.0568, 96.962, 95.9554, 74.9935	N	(Lucarini et al., 2019, Argentieri and Avato, 2012)
15	12.05	3-Hydroxybenzoic acid	Organic acid	C ₇ H ₆ O ₃	137.0287	137.0287, 93.0377, 93.0192, 65.0422	N	(Mollica et al., 2018)
16	12.49	Datiscin	Flavonoid glycoside	C ₂₇ H ₃₀ O ₁₅	595.1637	287.056, 595.1637	p	(El-Aal et al., 2021)
17	12.59	6-Methoxyluteolin	Flavonoid	C ₁₆ H ₁₂ O ₇	315.0600	315.0600, 300.0367	N	(Saeed et al., 2016)
18	15.65	Methyl vanillate	Organic acid ester	C ₉ H ₁₀ O ₄	183.0635	183.0635, 151.04, 124.05, 59.0431		(Blažević and Mastelić, 2009)
19	16.23	Apigenin	Flavonoid	C ₁₅ H ₁₀ O ₅	269.0527	269.0527, 117.0380	N	(Fratianni et al., 2014, Hussein et al., 2017)

Table S1. LC-HRMS analysis of the methanol extract of *Farsetia stylosa* (continued...)

No.	RT (min)	Metabolites name	Chemical	MF	m/z	Fragments	Polarity	Ref.
20	16.47	Ferulic acid methyl ester	Organic acid	C ₁₁ H ₁₂ O ₄	207.0724	192.0478, 177.0248, 134.0034, 133.0334, 105.037	N	(Olsen et al., 2009)
21	19.31	Hexadecanedioic acid	Fatty acid	C ₁₆ H ₃₀ O ₄	285.2139	285.2139, 267.2047, 223.2135	N	(Ibrahim et al., 2023)
22	23.61	2-Hydroxypalmitic acid	Fatty acid	C ₁₆ H ₃₂ O ₃	271.2351	271.2351, 225.2272, 223.213, 197.1956, 55.0213	N	(Ibrahim et al., 2023)
23	24.38	10E,12Z-octadecadienoic acid	Fatty acid	C ₁₈ H ₃₂ O ₂	581.4681	581.4681, 280.2445, 279.2404	N	(Frag et al., 2013)

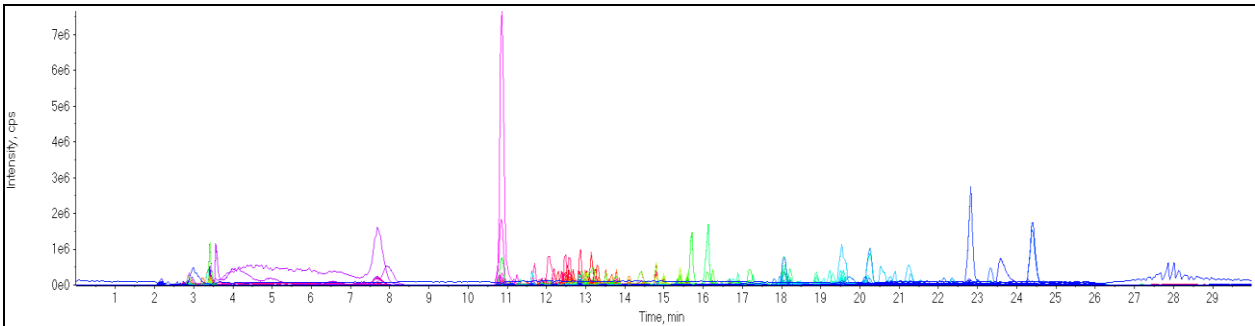


Figure S1. LC-MS/MS chromatogram of *Farsetia stylosa* extract in negative mode.

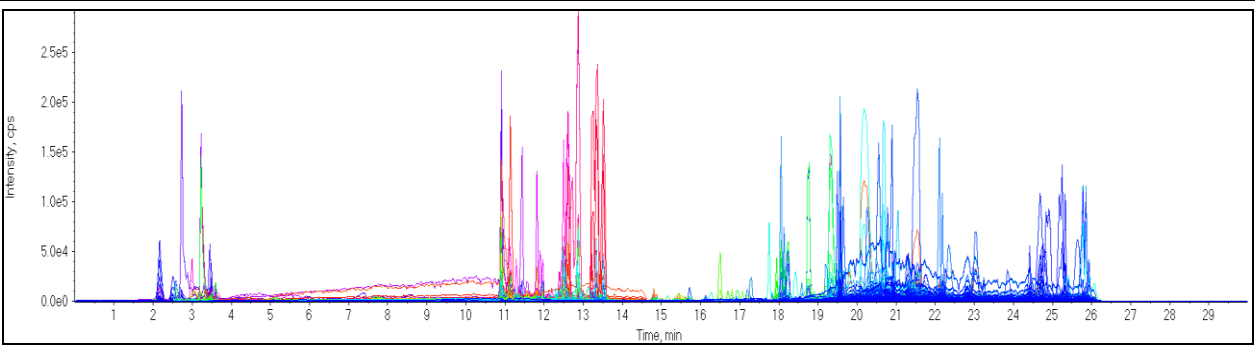


Figure S2. LC-MS/MS chromatogram of *Farsetia stylosa* extract in positive mode.