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Inhibition of α-glucosidase and α-amylase by herbal compounds for the treatment of type 2 diabetes: A validation of in silico reverse docking with in vitro enzyme assays

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Abstract

Background: α-Amylase and α-glucosidase are important therapeutic targets for the management of type 2 diabetes mellitus. The inhibition of these enzymes decreases postprandial hyperglycemia. In the present study, compounds found in commercially available herbs and spices were tested for their ability to inhibit α-amylase and α-glucosidase. These compounds were acetyleugenol, apigenin, cinnamic acid, eriodictyol, myrcene, piperine, and rosmarinic acid.

Methods: The enzyme inhibitory nature of the compounds was evaluated using in silico docking analysis with Maestro software and was further confirmed by in vitro α-amylase and α-glucosidase biochemical assays.

Results: The relationships between the in silico and in vitro results were well correlated; a more negative docking score was associated with a higher in vitro inhibitory activity. There was no significant ($P > .05$) difference between the inhibition constant (K_i) value of acarbose, a widely prescribed α-glucosidase and α-amylase inhibitor, and those of apigenin, eriodictyol, and piperine. For α-amylase, there was no significant ($P > .05$) difference between the K_i value of acarbose and those of apigenin, cinnamic acid, and rosmarinic acid. The effect of the herbal compounds on cell viability was assessed with the sulforhodamine B (SRB) assay in C2C12 and HepG2 cells. Acetyleugenol, cinnamic acid, myrcene, piperine, and rosmarinic acid had similar ($P > .05$) IC_{50} values to acarbose.

Conclusions: Several of the herbal compounds studied could regulate postprandial hyperglycemia. Using herbal plants has several advantages including low cost, natural origin, and easy cultivation. These compounds can easily be consumed as teas or as herbs and spices to flavor food.

KEYWORDS

α-amylase, α-glucosidase, herbal compounds, in vitro cytotoxicity, reverse molecular docking, type 2 diabetes

Highlights

- There is a positive relationship between in silico docking and in vitro assays.
- Several of the herbal compounds studied regulate postprandial hyperglycemia.
- Apigenin is a monotherapeutic agent, inhibiting both α-amylase and α-glucosidase.
- Most herbal compounds are not more toxic than acarbose.

1 | INTRODUCTION

Diabetes mellitus (DM) is one of the major health challenges of the 21st century. It is a chronic metabolic disorder characterized by hyperglycemia, caused by insufficient insulin secretion and/or insulin resistance.[1,2] According to the International Diabetes Federation (IDF), nearly half a billion people have DM worldwide.[3] Type 2 DM (T2DM) accounts for about 90% of all diabetic cases[4,5] and is primarily caused by various genetic, environmental, and behavioral factors.[4] Prolonged hyperglycemia causes various complications, including stroke, cardiovascular diseases, neuropathy, retinopathy, and nephropathy.[5-7] One of the best treatment strategies for T2DM involves hyperglycemic control[8-10] through the inhibition of carbohydrate hydrolyzing enzymes, such α-amylase and α-glucosidase.[9]

Pancreatic α-amylase is secreted once carbohydrates reach the intestinal lumen and is responsible for the hydrolysis of carbohydrates into smaller oligosaccharides, such as maltotriose, maltose, and glucose.[11] These oligosaccharides are further broken down into glucose by α-glucosidase, found in the brush border of the intestine.[8,9,11] The released glucose molecules are absorbed into the bloodstream, resulting in hyperglycemia.[9,12,13] Therefore, inhibition of α-amylase and α-glucosidase decreases the rate of starch hydrolysis and prevents a sudden surge in glucose, resulting in lower postprandial hyperglycemia.[8,12,14,15] The oral drug, acarbose is a widely prescribed α-amylase and α-glucosidase inhibitor, despite causing various gastrointestinal adverse effects such as flatulence, diarrhea, and abdominal pain.[11,16-18] There is an increasing effort to discover effective α-amylase and α-glucosidase inhibitors from natural sources.[8,16-18]

Herbs and spices have been used for centuries to flavor and preserve food.[19] They possess a wide variety of bioactivities, including anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antioxidant effects.[19,20] Using herbal compounds as an alternative treatment strategy for T2DM is beneficial in many developing countries where conventional medicine is logistically unavailable and/or cannot be afforded. Herbs and spices are widely available and fairly inexpensive.

In the present study, we examined the α-amylase and α-glucosidase inhibitory activity of seven herbal compounds found in commercially available herbs and spices. The compounds were found in herbs that had three characteristics according to the literature,[20] α-amylase inhibitory activity,[20] α-glucosidase inhibitory activity,[20] and insulin mimicking effects.[20] Acetyleugenol is a phenol ester[21] found abundantly in *Syzygium aromaticum* (cloves).[22] The flavone[23] apigenin is found in *Petroselinum crispum* (parsley).[24] Cinnamic acid, a phenylpropanoid acid,[25] is found in *Cinnamomum loureiroi* (Vietnamese cinnamon).[26] The flavanone[27] eriodictyol is found in *Lippia graveolens* (Mexican oregano).[27] Myrcene is a monoterpene[28] found in *Myristica fragrans* (nutmeg).[29] Piperine is an alkaloid[30] found abundantly in *Piper nigrum* (black pepper),[29] and rosmarinic acid is a phenolic acid[31] found abundantly in *Mentha piperita* (peppermint).[31] Previous studies have shown that herbs and spices such as cinnamon, cloves, mint, nutmeg, oregano, parsley, and pepper alleviate abdominal pain, diarrhea, and flatulence,[33] counteracting the side effects commonly caused by α-amylase and α-glucosidase inhibitors.

The aim of this study was to find new inhibitors (Figure 1) of α-amylase and α-glucosidase in commercially available herbs and spices, using in silico and in vitro relationship studies. To the best of our knowledge, this is the first study documenting the inhibition constants (K_i) and mode of inhibition of these herbal compounds on α-amylase and α-glucosidase.

2 | MATERIALS AND METHODS

2.1 | Chemicals

The following analytical grade reagents were purchased from Sigma-Aldrich Co (St Louis, Missouri): 3,5-dinitrosalicylic acid (DNSA), acarbose, acetyleugenol, α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, apigenin, cinnamic acid, Dulbecco's Modified Eagle Media (DMEM), eriodictyol, maltose monohydrate, myrcene, piperine, *p*-nitrophenol, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), porcine pancreatic α-amylase

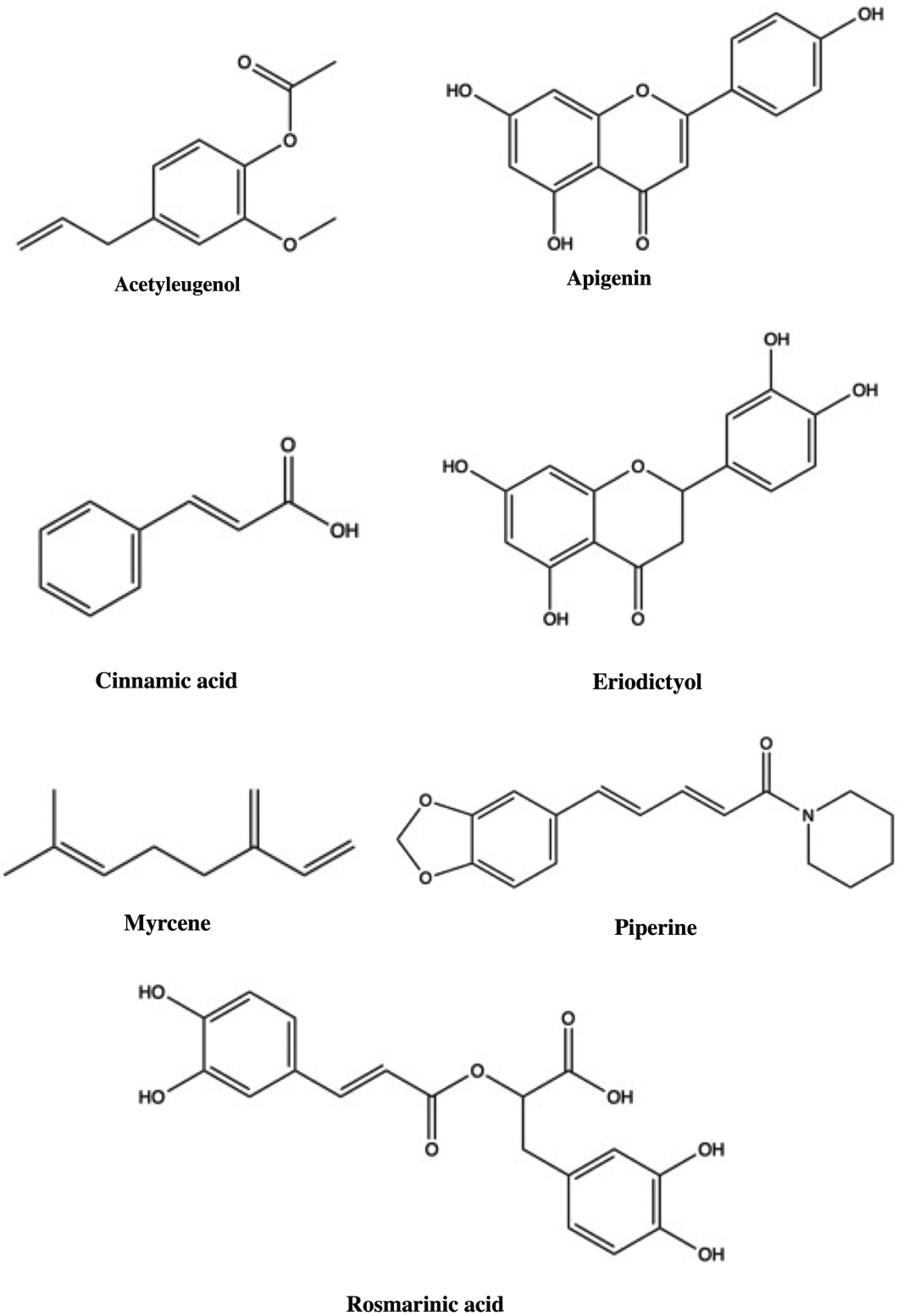

FIGURE 1 Chemical structures of the herbal compounds

(EC 3.2.1.1), rosmarinic acid, starch from potato, and sulforhodamine B (SRB). C2C12 myotubes (American Type Culture Collection [ATCC] CRL-1772) and HepG2 hepatocarcinoma cells (ATCC HB-8065) were obtained from the ATCC.

2.2 | In silico docking analysis

The docking studies were performed using Schrödinger's Maestro (Maestro v 11.5; Schrödinger LLC, New York, New York) program.

2.2.1 | Ligand preparation

In this study, the isomeric simplified molecular-input line-entry system (SMILES) of roughly a thousand compounds, identified from 30 commercially available herbs and spices,[20] were imported onto Maestro. Acarbose was selected as a standard drug reference molecule. The 3D structures[34] of the ligands were prepared using the LigPrep function, which generates several poses from each input structure.[35] The default parameters, including "Retain specified chiralities," were kept.

2.2.2 | Protein preparation

The crystal structure of the two enzymes (Protein Data Bank [PDB] entries: 3L4Y and 4GQR for α-glucosidase and α-amylase, respectively) were downloaded from the PDB (www.rscb.org) and imported into Maestro. The protein preparation wizard was used to prepare the proteins for in silico experimentation. The imported 3D structures were made fit to study the docking.[34] Therefore, all the cofactor and water molecules were removed from the proteins, and the hydrogen bonding was optimized, followed by an energy minimization step.[36]

2.2.3 | Molecular docking

Grid representations of the active sites of both proteins were created using the receptor grid generation tool.[37] The default parameters were kept.[38] Protein docking was carried out using the Glide high-throughput virtual screening (HTVS) protein docking module of the virtual screening workflow function of Maestro. The interactions between the ligands and protein were quantified with the GlideScore.[34] The best docked pose with the lowest GlideScore value was recorded for each ligand.

Research done by Pereira et al[20] identified herbs and spices with known α-amylase and α-glucosidase inhibition. We validated their findings using Schrödinger's Maestro program and identified seven bioactive compounds found in these herbs and spices to test for their inhibitory activity in vitro. Acarbose was used as a positive control. We chose four compounds with stronger docking than acarbose and three compounds with weaker docking as negative controls.

2.2.4 | Calculation of physiochemical properties

Schrödinger's Canvas (Canvas v 3.5.011; Schrödinger LLC, New York, New York) program was used to determine the bioavailability and toxicity of the eight compounds. The SMILES of each compound was imported into Canvas, after which the physiochemical properties were calculated. Each compound-structure was minimized to obtain 3D structures before the Qikprop descriptors were computed. Once the calculations were completed, the percentage human oral absorption, QPlogHERG, and #stars for each compound were noted.

2.3 | In vitro enzyme analysis

2.3.1 | Kinetics of α-glucosidase inhibition

The in vitro α-glucosidase activity was measured using previously described methods.[15,39] For the enzymatic assay, the compounds were dissolved in 100 mM phosphate buffer (pH 6.8). Briefly, in a 96-well plate (Greiner, clear F-bottom) 25 μL enzyme (0.2 U/mL) diluted in phosphate buffer (100 mM, pH 6.8) was preincubated with 100 μL inhibitor (250, 500, and 1000 μM) or the positive control acarbose (250, 500, and 1000 μM) for 10 minutes at 37°C. Thereafter, 25 μL *p*NPG (0, 0.15, 0.3, 0.6, 0.9, 1.15, and 1.5 mM) was added, and the reaction mixtures were incubated at 37°C for 30 minutes. The reaction was stopped by adding 50 μL NaOH (0.1 M). Enzyme activity was quantified by measuring the absorbance at 405 nm using an ultraviolet-visible (UV-VIS) spectrophotometer (Spectramax paradigm; Molecular Devices Inc, San Jose, California).

2.3.2 | Kinetics of α-amylase inhibition

The α-amylase inhibitory activity of the compounds was evaluated using a colorimetric assay described in previous literature[18] with slight modifications. In short, 100 μL porcine pancreatic amylase enzyme (2 U/mL) dissolved in 20 mM phosphate buffer (pH 6.9) with 6.7 mM NaCl was preincubated with 100 μL herbal compounds (2.5, 5, and 10 μM) or the positive control, acarbose (2.5, 5, and 10 μM) for 10 minutes at 25°C. One hundred microliter of the substrate (0, 1.3, 2.6, 4.0, 5.3, and 6.6 mg/mL) starch was added before the reaction was stopped after 10 minutes by adding 100 μL DNSA (96 mM). The reaction mixtures were heated at 85°C for 10 minutes. After the final reaction mixtures were diluted with 1.1 mL double-distilled H_2O, 200 μL was pipetted into 96-well plates to read the absorbance at 540 nm using the UV-VIS spectrophotometer.

2.3.3 | In vitro cytotoxicity

The cytotoxicity of the herbal compounds was evaluated using the method described by Vichai and Kirtikara.[40] The cells were seeded (100 μL) at 1×10^5 per well and left overnight at 37°C to allow attachment. A volume of 100 μL herbal compounds (0.005-500 μM) was added, and the plates were further incubated for 72 hours at 37°C. Saponin was used as a positive control. Wells containing media only served as blanks, while wells containing cells and media served as the negative control. The cells were fixed by adding 50 μL 50% (w/v) trichloroacetic acid and incubating the plates at 4°C for 24 hours. The plates were washed with water and dried overnight before 100 μL SRB dye (0.057% w/v) was added. After a 30-minute incubation period, the plates were washed with 1% acetic acid. The plates were dried overnight before 200 μL tris (10 mM) was added. The plates were shaken gently (550 rpm) for 1 hour, and the absorbance was read at 540 nm.

2.4 | Data analysis

All of the results represent at least three independent experiments and are expressed as mean ± SD. The kinetic parameters of the compounds and the type of inhibition they exert were studied on GraphPad Prism (version 8.3.0; GraphPad Software, San Diego, California) using Michaelis-Menten kinetics and the corresponding Lineweaver-Burk double reciprocal plots. The K_i values were obtained with secondary plots and were analyzed using a one-sided unpaired Student's *t* test. The IC_{50} of the compounds was calculated by plotting the percentage cell viability against the log drug (compound) concentration on GraphPad Prism. The kinetic parameters and IC_{50} values were subjected to a

TABLE 1 Glide HTVS docking scores of the herbal compounds docked to α-amylase

Compound	Glide score (Kcal/mol)
Rosmarinic acid	−7.9
Eriodictyol	−7.4
Apigenin	−6.6
Piperine	−5.8
Acarbose (positive control)	−5.2
Acetyleugenol	−4.3
Cinnamic acid	−4.2
Myrcene	−1.6

Abbreviation: HTVS, high-throughput virtual screening.

TABLE 2 Glide HTVS docking scores of the herbal compounds docked to α-glucosidase

Compound	Glide score (Kcal/mol)
Eriodictyol	−5.5
Rosmarinic acid	−5.4
Apigenin	−5.3
Piperine	−4.2
Acarbose (positive control)	−4.1
Cinnamic acid	−3.4
Acetyleugenol	−3.4
Myrcene	−1.4

Abbreviation: HTVS, high-throughput virtual screening.

TABLE 3 Selected molecular properties of herbal compounds

Compound	Human oral absorption[a] (%)	QPlogHERG[b]	#Stars[c]
Acarbose (positive control)	0	−5.6	13
Eriodictyol	63	−4.9	0
Piperine	100	−4.8	1
Acetyleugenol	100	−4.6	0
Myrcene	100	−3.8	5
Apigenin	71	−3.8	3
Rosmarinic acid	35	−3.7	2
Cinnamic acid	45	−3.7	4

[a]Predicted human oral absorption on a 0% to 100% scale. The prediction is based on a quantitative multiple linear regression model. A value of >80% is considered high, and <25% is considered poor.[46]
[b]Predicted IC_{50} value for blockage of HERG K+ channels. A value below −5 is a concern.[46]
[c]Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. A large number of stars suggests that a molecule is less drug-like than molecules with few stars. The recommended range is 0 to 5, where 0 indicates no violation or best candidate.[46]

two-sided, unpaired Student's t test. Differences were considered significant at $P < .05$.

3 | RESULTS

3.1 | Molecular docking studies

We investigated roughly a thousand herbal compounds in silico for α-amylase and α-glucosidase inhibition through docking analysis. To reduce the number of compounds for in vitro analysis, we used a literature review[20] to identify compounds in our docking study with confirmed inhibitory activity of both α-amylase and α-glucosidase. Identifying compounds with both α-amylase and α-glucosidase inhibitory activity increased our chances of finding monotherapeutic targets.

The results in Tables 1 and 2 show the docking scores of these seven herbal compounds docked to α-amylase and α-glucosidase, respectively. The compounds with better docking scores than acarbose to both enzymes were apigenin, eriodictyol, piperine, and rosmarinic acid. Acetyleugenol, cinnamic acid, and myrcene had weaker docking scores than acarbose.

3.2 | In silico physiochemical properties

Canvas was used to evaluate the toxicity, bioavailability, and druggability of the chosen herbal compounds in silico. These parameters were tested independently, without the enzymes. The results are purely based on the structure of the compound itself. Acetyleugenol, myrcene, and piperine had the highest percentage human oral absorption (100%), while acarbose had the lowest at 0% (Table 3). Cinnamic acid and rosmarinic acid had the lowest HERG toxicity, while acarbose had the highest. Acarbose had the lowest druggability score at 13 stars. The herbal compounds had no more than five stars, substantially less than acarbose.

TABLE 4 Inhibitory activity of herbal compounds against porcine pancreatic α-amylase

Compound	Type of inhibition	K_i (μM)	*P* value
Acarbose (positive control)	Competitive	3.8 ± 1.9	
Rosmarinic acid	Noncompetitive	4.5 ± 2.9	.364
Apigenin	Mixed	7.8 ± 2.7	.054
Cinnamic acid	Noncompetitive	8.0 ± 4.5	.094
Eriodictyol	Noncompetitive	10.5 ± 3.6[a]	.023
Piperine	Competitive	10.9 ± 5.5[a]	.042
Acetyleugenol	Mixed	12.1 ± 5.8[a]	.039
Myrcene	None	49.0 ± 33.7[a]	.038

Note: Data are represented as mean ± SD (n = 3).
Abbreviation: K_i, inhibition constant.
[a]Values significantly different ($P < .05$) from acarbose as determined by a one-sided Student's t test.

TABLE 5 Inhibitory activity of herbal compounds against yeast α-glucosidase

Compound	Type of inhibition	K_i (μM)	*P* value
Eriodictyol	Mixed	130 ± 70	.284
Apigenin	Mixed	160 ± 50	.452
Acarbose (positive control)	Mixed	170 ± 80	
Piperine	Mixed	280 ± 120	.115
Cinnamic acid	Noncompetitive	620 ± 380[a]	.043
Acetyleugenol	Noncompetitive	950 ± 240[a]	.002
Myrcene	None	1580 ± 650[a]	.011
Rosmarinic acid	Uncompetitive	2580 ± 550[a]	.008

Note: Data are represented as mean ± SD (n = 3).
Abbreviation: K_i, inhibition constant.
[a]Values significantly different ($P < .05$) from acarbose as determined by a one-sided Student's t test.

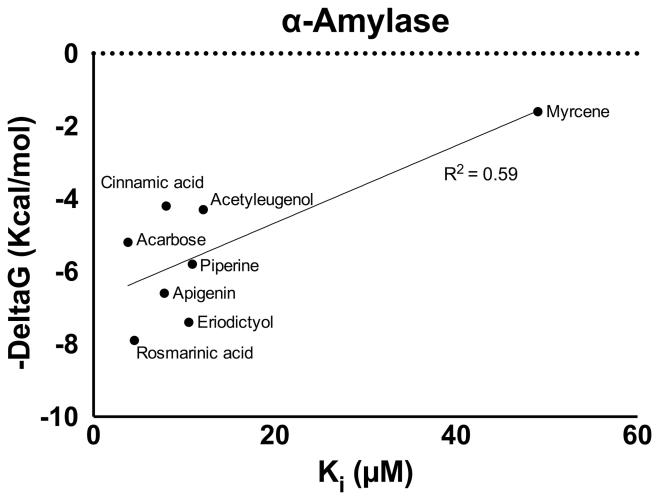

FIGURE 2 Graph of negative delta G vs the K_i value of the herbal compounds against α-amylase

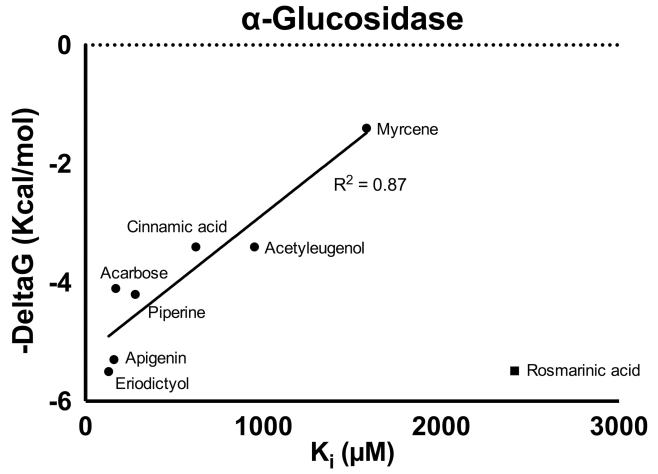

FIGURE 3 Graph of negative delta G vs the K_i value of the herbal compounds against α-glucosidase. Rosmarinic acid (shown with a square symbol) was identified as an outlier with undue influence on the slope

3.3 | Kinetics of the in vitro α-amylase and α-glucosidase inhibition

The ability of the herbal compounds to inhibit α-amylase and α-glucosidase was investigated in vitro. The inhibitory characteristics of the herbal compounds were explored by performing kinetic assays, with double reciprocal Lineweaver-Burk plots to calculate the kinetic parameters. The type of inhibition exerted by the herbal compounds was deduced from the calculation of the Michaelis constant (K_m) and maximum enzyme velocity (V_{max}). Regarding the inhibition of α-amylase, it can be concluded that acarbose and piperine were competitive inhibitors, whereas cinnamic acid, eriodictyol, and rosmarinic acid were noncompetitive inhibitors. Although the Lineweaver-Burk graphs (Figure S1) of cinnamic acid and rosmarinic acid do not clearly differentiate between mixed and noncompetitive inhibition, there is no statistically significant difference between their K_m values (Table S1) and the K_m value of the uninhibited reaction, corresponding to noncompetitive inhibition. Acetyleugenol and apigenin showed mixed inhibition. Acetyleugenol and cinnamic acid inhibited α-glucosidase noncompetitively, while acarbose, apigenin, eriodictyol, and piperine showed mixed inhibition (Figure S2 and Table S2). Rosmarinic acid showed uncompetitive inhibition. Myrcene showed no statistically significant inhibition of α-amylase or α-glucosidase.

The inhibition potential of each compound was evaluated and compared based on their K_i values. The K_i was calculated with secondary graphs by plotting the reciprocal of the Lineweaver-Burk plot slope against the inhibitor concentration. There was no significant difference between the K_i value of acarbose and those of apigenin, cinnamic acid, and rosmarinic acid when inhibiting α-amylase. Acetyleugenol eriodictyol, myrcene, and piperine had significantly higher K_i values than acarbose. For α-glucosidase inhibition, there was no significant difference between the K_i value of acarbose and those of apigenin, eriodictyol, and piperine, while acetyleugenol, cinnamic acid, myrcene, and rosmarinic acid had a statistically significantly higher K_i than acarbose.

The relationship between the docking scores and K_i were visualized with graphs by plotting negative delta G against the K_i of each compound. There is a positive relationship between the negative delta G score and the K_i values of the herbal compounds when inhibiting α-glucosidase and α-amylase. The K_i value of rosmarinic acid, inhibiting α-glucosidase, was excluded from the calculation of the slope in Figure 3 because it had undue influence on the line but is included in the graph with a different symbol.

TABLE 6 IC_{50} values of herbal compounds on C2C12 and HepG2 cells

Compound	C2C12 IC_{50} (μM)	HepG2 IC_{50} (μM)
Eriodictyol	11 ± 2[a]	41 ± 2
Apigenin	31 ± 4[a]	210 ± 41
Acarbose (positive control)	60 ± 15	>500
Piperine	79 ± 8	>500
Rosmarinic acid	83 ± 2	>500
Myrcene	84 ± 3	>500
Cinnamic acid	87 ± 2	>500
Acetyleugenol	96 ± 24	>500

Note: Data are represented as mean ± SD (n = 3).
[a]Values significantly different ($P < .05$) from acarbose determined with a two-sided Student's *t* test.

TABLE 7 Herbal dosage required to relate to a daily dose of acarbose

Compound	Main source	Amount in herb/spice (mg/100 g)	Amount (g) needed to relate to daily dose of acarbose
Acetyleugenol	*Syzygium aromaticum* (cloves)	2075	7.2
Apigenin	*Petroselinum crispum* (parsley, dried)	1263	11.9
Cinnamic acid	*Cinnamomum loureiroi* (Vietnamese cinnamon)	1697	8.8
Eriodictyol	*Lippia graveolens* (Mexican oregano, dried)	85	176
Myrcene	*Myristica fragrans* (nutmeg)	333	45
Piperine	*Piper nigrum* (black pepper)	5350	2.8
Rosmarinic acid	*Mentha piperita* (peppermint, dried)	1734	8.6

Note: Data for acetyleugenol, apigenin, eriodictyol, myrcene, and piperine from foodb.ca.[31]
Data for acetyleugenol, eriodictyol, and rosmarinic acid from phenol-explorer.eu.
Data for cinnamic acid from Lee et al.[26]
All based on a 150-mg acarbose dose per day.

3.4 | In vitro cytotoxicity

The cytotoxic effects of the herbal compounds on the cell lines were quantified with IC_{50} values (Table 6). All herbal compounds, except for apigenin and eriodictyol, displayed limited cytotoxicity in the HepG2 cell line, where concentrations up to 500 μM did not induce 50% cell death (Figure S4); their IC_{50} values could not be calculated accurately. Eriodictyol displayed significant toxicity, while apigenin displayed milder toxicity against this cell line.

In C2C12 cells, acetyleugenol, cinnamic acid, myrcene, piperine, and rosmarinic acid had similar IC_{50} values to acarbose. Thus, the toxicity of these compounds was not statistically more significant ($P > .05$) than the toxicity of acarbose at the same concentration (500 μM). Eriodictyol and apigenin were significantly ($P < .05$) more toxic than acarbose.

3.5 | Herbal dosage related to daily acarbose dose

The FooDB[32] and Phenol Explorer[31] databases were used to search for natural sources of each herbal compound. The databases give the amount (mg/100 g) of each compound found in many herbs and spices. This was used to calculate the amount (g) of each herb required to relate to the average daily dose (150 mg) of acarbose (Table 7).

4 | DISCUSSION

The control of postprandial hyperglycemia is important in the treatment of T2DM and the prevention of short- and long-term complications.[41] Inhibition of enzymes, such as α-amylase and α-glucosidase, involved in the metabolism of carbohydrates is an important therapeutic approach for reducing postprandial hyperglycemia.[41] Interest in using herbal plants has grown recently due to their low cost, natural origin, and easy cultivation.[42] This study highlights the inhibitory effect of acetyleugenol, apigenin, cinnamic acid, eriodictyol, myrcene, piperine, and rosmarinic acid on the activity of yeast α-glucosidase and porcine pancreatic amylase.

The predicted binding of the herbal compounds to α-amylase and α-glucosidase were studied in silico before any studies were performed in the lab. Herbal compound docking scores for α-amylase and α-glucosidase were generated through Maestro. Maestro uses Schrödinger's GlideScore function; this algorithm recognizes favorable hydrophobic, hydrogen-bonding, and metal-ligation interactions and penalizes steric clashes between the ligand and the protein.[43] The herbal compounds were docked into the catalytic site of both enzymes where they interacted with the amino acids through a negative binding energy, indicating a spontaneous binding and potential inhibition. With α-amylase, the decreasing order of the positive binding and potential inhibition was

rosmarinic acid > eriodictyol > apigenin > piperine > acarbose > acetyleugenol > cinnamic acid > myrcene (Table 1). With α-glucosidase it was eriodictyol > rosmarinic acid > apigenin > piperine > acarbose > cinnamic acid > acetyleugenol > myrcene (Table 2). The docking scores of these herbal compounds were compared with the interactions of acarbose with α-amylase and α-glucosidase, whose binding energies were −5.2 and −4.1 Kcal/mol, respectively. Evidently, apigenin, eriodictyol, piperine, and rosmarinic acid had better docking scores to α-amylase and α-glucosidase than acarbose. Acetyleugenol, cinnamic acid, and myrcene showed weaker docking scores than acarbose. The results of the docking analysis encouraged us to further investigate the enzyme inhibitory activity using enzyme assays.

Selected physiochemical parameters of the herbal compounds were evaluated in silico. The cardiotoxicity of each herbal compound was assessed using the QPlogHERG function, which is the projected log IC_{50} value for the blockage of HERG potassium (K^+) channels.[44,45] The Canvas software calculates the distances and angles between the carbon atoms of each herbal compound and predicts the pIC_{50}.[45] A value between 0 and −5 is desired.[44,45] A more negative value can lead to a disorder called long Q-T syndrome.[45] All the herbal compounds had more positive QPlogHERG values than acarbose, indicating that these compounds have a lower probability than acarbose of causing long Q-T syndrome. The percentage human oral absorption is calculated by studying the number of metabolites, logP, rotatable bonds, solubility, and cell permeability of each compound.

Acarbose is an orally administered drug, thus a low oral absorption is expected. In this case acarbose had a human oral absorption of 0%. No oral absorption is required since the target site for acarbose is the lumen of the gastrointestinal tract (GIT). Rosmarinic acid had a human oral absorption of 35%, which is the lowest of all the herbal compounds, while cinnamic acid and eriodictyol had an oral absorption of 45% and 63%, respectively. Apigenin had an oral absorption of 71%. These values are substantially higher than the oral absorption of acarbose. Acetyleugenol, myrcene, and piperine had an oral bioavailability of 100%, which means most of the administered drug gets absorbed into the systemic circulation. Thus, little drug remains in the GIT, where the drug's action is required.

The druggability of the compounds was evaluated using the #stars function. The QikProp function of Canvas includes 24 descriptors in the calculation of the #stars.[46] The #stars of each compound indicates the number of descriptor values that fall outside of 95% of similar values for known drugs.[47] Therefore, a lower #stars indicates a better drug-like molecule.[48] It is clear from Table 3 that all herbal compounds had no more than five stars, indicating that most of the 24 pharmaceutically relevant descriptors lie within the recommended range of known drugs.[48] All of the compounds have a lower #stars than acarbose, which means the herbal compounds are more drug-like than acarbose.

The kinetic parameters of the herbal compounds were calculated with Lineweaver-Burk double reciprocal plots. Acarbose was verified as a competitive inhibitor of α-amylase[17,49,50] and a mixed inhibitor of α-glucosidase.[17,51] Acetyleugenol, apigenin, cinnamic acid, eriodictyol, piperine, and rosmarinic acid displayed dose-dependent inhibition of α-amylase and α-glucosidase, using acarbose as positive control. Cinnamic acid, eriodictyol, and rosmarinic acid inhibited α-amylase noncompetitively, the K_m value remained constant, and the V_{max} value decreased (Table S1 and Figure S1). Acetyleugenol and apigenin inhibited α-amylase in a mixed fashion, the V_{max} was decreased, and the K_m increased. Piperine inhibited α-amylase competitively, leading to an increased K_m, while the V_{max} of the reaction stayed the same. Apigenin, eriodictyol, and piperine were mixed inhibitors of α-glucosidase, while acetyleugenol and cinnamic acid were noncompetitive inhibitors. Rosmarinic acid inhibited α-glucosidase uncompetitively, decreasing both the K_m and V_{max} (Table S2 and Figure S2). Myrcene presented no inhibitory activity against α-amylase and α-glucosidase.

K_i values can be a useful tool to compare the inhibitory activity of the herbal compounds, being an indicator of the binding affinity of the inhibitor. A lower K_i value suggests a higher binding affinity. The K_i values of the tested compounds against α-amylase were: acarbose < rosmarinic acid < apigenin < cinnamic acid < eriodictyol < piperine < acetyleugenol < myrcene. There was no statistically significant difference (Table 4, $P > .05$) between the K_i values of acarbose and those of rosmarinic acid, apigenin, and cinnamic acid. None of the herbal compounds had a K_i value lower than that of acarbose, which might be therapeutically desired. Mild inhibition of α-amylase is often preferred to avoid excessive bacterial fermentation, leading to gastrointestinal side effects.[11,52] Regarding α-glucosidase, the decreasing order of the K_i values were: eriodictyol < apigenin < acarbose < piperine < cinnamic acid < acetyleugenol < myrcene < rosmarinic acid. There was no statistically significant difference (Table 5, $P > .05$) in the inhibition effect of apigenin, eriodictyol, and piperine when compared with that of acarbose. This indicates that these herbal compounds inhibit α-glucosidase with the same efficacy as acarbose.

One aim of this study was to determine the relationship between the in silico docking results and in vitro inhibitory strength. Figures 2 and 3 show the relationship

between the negative docking scores and K_i values. The slopes of both graphs were positive, indicating a positive relationship between the docking scores and the K_i values of the herbal compounds. A high negative docking score corresponds to a low K_i value, both indicating higher inhibition efficacy. The compounds with better docking scores than acarbose to both α-amylase and α-glucosidase were apigenin, eriodictyol, piperine, and rosmarinic acid. With regard to α-amylase, it can be seen that the in vitro results of rosmarinic acid and apigenin correspond well to the in silico results. For both enzymes, the herbal compounds that had weaker docking scores than acarbose showed a significantly weaker inhibition effect than acarbose in vitro, with the exception of cinnamic acid when inhibiting α-amylase. Concerning α-glucosidase, the docking scores of eriodictyol, apigenin, and piperine correlate well with the in vitro results, indicating a good overall correlation between the in silico docking results and in vitro inhibition results.

The cytotoxic effects of the herbal compounds on the cell lines were quantified with IC_{50} values. Cytotoxicity was only observed at concentrations larger than 50 μM (see Figures S3 and S4); this provides evidence of the preclinical safety of these compounds at culinary relevant concentrations. The HepG2 cells were overall more cytotoxic resistant than the C2C12 cells, likely due to the liver's detoxification capabilities. Acetyleugenol, cinnamic acid, myrcene, piperine, and rosmarinic acid did not induce a 50% decrease in cell viability at the highest tested concentration (500 μM) in HepG2 cells, confirming their low toxicity. Only two compounds, apigenin and eriodictyol, had toxic effects on both cell lines. These compounds have significantly lower IC_{50} values than acarbose. Apigenin and eriodictyol are both flavonoids and therefore have very similar structures. Various studies have reported the toxicity of flavonoids, including apigenin and eriodictyol, at high concentrations.[53] In C2C12 cells, the IC_{50} values of acetyleugenol, cinnamic acid, myrcene, piperine, rosmarinic acid, and acarbose were similar ($P > .05$), indicating that these compounds are not more toxic than acarbose, a widely prescribed drug. All of these compounds had higher IC_{50} values than acarbose, implying that they are less toxic than acarbose at the same dose. Neiro and Machado-Santelli[54] (2013) confirmed the low in vitro and in vivo cytotoxicity of cinnamic acid. Şahin, et al55 (2017) reported no cytotoxic activity when five different cell lines were treated with rosmarinic acid. The cytotoxicity of myrcene[56] and piperine[57,58] has been reported to be low in HepG2 and other cell lines, although the use of myrcene was recommended with caution. To the extent of our knowledge, our work is the first report of the cytotoxicity of acetyleugenol in these cell lines. Since each herbal compound tested in this study is currently found in commercially available herbs and spices, safety at culinary relevant levels is apparent.

The results have shown that various herbal compounds are effective α-amylase and α-glucosidase inhibitors. We wanted to estimate the daily dose of each herbal plant that would be equivalent to the average daily dose of acarbose, which is 150 mg daily for a 60 kg individual.[59,60,61] Since there is no statistically significant difference (Tables 4 and 5, $P > .05$) between the K_i of acarbose and those of apigenin, cinnamic acid, eriodictyol, piperine, and rosmarinic acid, we made the assumption that 150 mg of each compound will have the same effect as acarbose, without taking bioavailability into account. However, this assumption needs validation with animal studies in the future.

There are various simple ways to include herbs and spices in one's daily life. The most common way would be to add the herbs or spices to food. Another way would be to brew a herbal tea. Since a tea bag usually contains about 2.5 g dried leaves or herbs, it would be an easy way to incorporate a large amount of phytochemicals into one's diet. The amount (Table 7) of acetyleugenol, apigenin, piperine, and rosmarinic acid that needs to be ingested to relate to a daily dose of acarbose can thus be realistically achieved. Pepper is insoluble in water and should thus be used as a spice to be sprinkled over food; however, cloves, mint, and parsley can be brewed in a tea.[62-64]

5 | CONCLUSION

Due to the increasing prevalence of T2DM, there has been an ongoing effort to find natural compounds that can control hyperglycemia. Here we report that several herbal compounds possess potential antidiabetic activities due to their ability to inhibit both α-amylase and α-glucosidase. The strength of the enzyme inhibition was calculated in silico using docking analysis and compared with in vitro inhibition efficacy using enzymatic assays. The relationships between the in silico and in vitro results were well correlated, a more negative docking score translated to a higher in vitro inhibitory activity. Our results have shown that apigenin, cinnamic acid, and rosmarinic acid are effective α-amylase inhibitors, while apigenin, eriodictyol, and piperine inhibited α-glucosidase effectively. Apigenin was identified as a monotherapeutic agent, inhibiting both α-amylase and α-glucosidase. The present study provides in vitro evidence of the safety of each herbal compound tested at concentrations below 50 μM in C2C12 myotubes and HepG2 cells. These compounds are found in a wide variety of herbs and spices and can easily be incorporated

into one's daily life. In this study, we identified potentially effective herbal compounds that can be used as alternatives to the existing widely prescribed α-amylase and α-glucosidase inhibitors. Using herbs and spices has several advantages, including its widespread availability, affordability, and health benefits.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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