Antidiabetic, antioxidant and *in silico* molecular docking of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa phytochemical compounds on human pancreatic α-amylase and human lysosomal acid-α-glucosidase

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Key Words

Phytochemicals, antidiabetic, antioxidant, docking, diabetes mellitus, polyphenols, flavonoids

ABSTRACT

Millions of people in developing nations rely on herbal traditional medicine for the treatment of ailments such as diabetes mellitus, stomach disorders and respiratory diseases. *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa is a medicinal plant used traditionally in Zimbabwe to treat diabetes mellitus and its complications. However, there is no scientific evidence to support its role as an antidiabetic medicinal plant. Here we hypothesized that *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa contain bioactive phytochemicals that can scavenge free radicals, and inhibit digestive enzymes that contribute to type 2 diabetes mellitus (T2DM). To test this hypothesis, we examined the free radical scavenging potential of crude extracts using the diphenyl-2-picrylhydrazyl assay *in vitro*. Furthermore, we carried out *in vitro* antidiabetic activity of crude extracts using chromogenic 3,5-dinitrosalicylic acid and p-nitrophenyl-alpha-D-glucopyranoside substrates on alpha-amylase and alpha-glucosidase. In addition, we used molecular docking approaches to screen for bioactive phytochemical compounds targeting the digestive enzymes. Our results showed that phytochemicals in *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts scavenged free radicals with IC_{50} values ranging from 0.011-0.013 micrograms/mL. Further, the crude extracts significantly inhibited alpha-amylase and alpha-glucosidase with IC_{50} values of 12.9-21.1 micrograms/mL and 8.8-16.0 micrograms/mL, respectively. *In silico* molecular docking findings and pharmacokinetic predictions showed that myricetin is a novel inhibitor of the digestive enzymes that contributes to high blood glucose. Collectively, our findings suggest pharmacological targeting of digestive enzymes by *Xeroderris stuhlmannii* (Taub). Mendonca & E.P. Sousa crude extracts could lesion type 2 mellitus complications in humans.

INTRODUCTION

Diabetes mellitus or high blood glucose is a major epidemic of the 21st century that affects millions of people worldwide and contributes to high morbidity and mortality in adult populations [1–3]. There are two major forms of diabetes mellitus: type 1 and type 2 diabetes mellitus. Type 2 diabetes mellitus (T2DM) is the most common form, and affects 462 million people (about 6.28% of the world's population) [4]. In Africa, there is an increase in the incidence of T2DM in urban areas because of rural to urban migration and lifestyle changes [5]. In the case of Zimbabwe, it was estimated that approximately 5.7% of its population are living with T2DM [6]. However, the cost of healthcare is extremely high, and many people are turning their attention to herbal traditional medicine for the treatment and management of diabetes mellitus and its complications [7].

One effective therapeutic approach of reducing postprandial glucose in T2DM patients involves modulating the activities of digestive enzymes using α-glucosidase inhibitors such as acarbose, miglitol and voglibose [8]. These α-glucosidase inhibitors reduce intestinal glucose absorption by delaying carbohydrate digestion [8]. However, a number of side effects have been reported in patients using α-glucosidase inhibitors [9]. This has led to the search for new plant derived natural product inhibitors targeting digestive enzymes with fewer side effects.

For many years, herbal traditional medicines have been clinically used to treat chronic metabolic diseases such as diabetes mellitus [10,11]. Phytochemical compounds in plants such as polyphenols and flavonoids have antioxidative properties, and are capable of scavenging free radicals and reduce oxidative stress, which in-turn lowers blood glucose [12]. In addition, the phytochemical compounds in plant extracts improve insulin resistance and glucagon production by binding to β-cells and α-cells in response to high blood glucose [13]. Additionally,

phytochemical compounds reduce glucose absorption in the gastrointestinal tract by inhibiting digestive enzymes (such as α-glucosidases) [14]. High blood glucose is associated with many comorbidities such as heart disease, stroke, kidney disease, eye problems, dental disease, nerve damage and feet problems that reduce the quality of life and life expectancy of diabetic patients [15]. However, despite the widespread use of medicinal plants by many African people to treat diabetes mellitus, many plants native to Zimbabwe such as *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa have not received much attention to evaluate their *in vitro* antidiabetic activity.

Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa from *Xeroderris* Roberty genus of the Fabaceae family is a medicinal plant widely used traditionally to treat many ailments across Zimbabwe [16]. Different parts of the plant are used to treat diabetes mellitus, bacterial wound infections, coughs, diarrhea, malaria, colds, rheumatoid arthritis, stomachache, dysentery and eye infections. *In vitro* antibacterial studies showed that the bark extracts effectively fight bacterial pathogens that cause gastrointestinal disorders in humans [16]. Ethnobotanical studies of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and leave extracts in the Central Region of Togo showed that the decoctions were effective in the management of diabetes mellitus and hypertension [17]. Spectrochemical characterization of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa by Gas chromatography mass spectroscopy (GC MS) and liquid chromatography tandem mass spectroscopy (LC MS/MS) studies showed the presence of thirty-six (36) important bioactive phytochemical compounds categorized into phenolic, flavonoid and alkaloid compounds [16]. Whether some of the phytochemical compounds in *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa bark extracts have antidiabetic properties remains to be studied. In Zimbabwe, decoctions of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa

barks and roots are traditionally used to treat diabetes mellitus and its complications. However, no scientific evidence supports its use as an antidiabetic agent. For this reason, this study aims to determine the inhibitory effects of bark and root extracts of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa on α-amylase and α-glucosidase and thus assess their effectiveness in high blood glucose in humans with T2DM.

MATERIALS AND METHODS

Collection of plant materials and preparation of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and bark extracts

Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa root and bark samples were obtained from Kwekwe in Zimbabwe, and the voucher specimen for the plant is *Luckmore Kazingizi Number 1*. The specimen is stored at National Herbarium & Botanic Garden in Harare, Zimbabwe. Plant samples were pre-washed with running tap water and rinsed with distilled water, followed by sun drying for several days at ambient temperatures. The dried samples were grinded and sieved with a 1 mm sieve to obtain finely divided powdered samples. Crude root and bark extracts were obtained by the maceration method using methanol and ethyl acetate and filtered through (Whatman No. 1) filter paper. Following extraction, the solvents were removed to complete dryness using a rotary evaporator and the percentage yields of extracts were recorded (see Table 1).

Determination of total phenolic content

The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu method [18]. Briefly, 0.2 mL of crude plant extracts was added to 0.2 mL of 10%

methanol or ethyl acetate, followed by 5 mL of Folin-Ciocalteau phenol reagent (diluted 10 times) and 4.0 mL of 7.5% Na_2CO_3 solution. The mixture was incubated at room temperature in the dark for 30 min. After 30 min, the absorbance of the solution was determined at 765 nm. Total phenolics were quantified by a calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard (0-200 μg mL^{-1}). All measurements were done in triplicates on two separate days. The data is expressed as mg of gallic acid equivalents/g of dry extract (mg GA/g DE).

Determination of total flavonoid content

The total flavonoid content in *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa was determined using the aluminum chloride ($AlCl_3$) colorimetric assay according to published procedures [18]. Briefly, 0.15 mL of plant extract was mixed with 0.45 mL methanol and 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature in the dark, followed by absorbance measurement at 420 nm. Quercetin was used as a standard for the calibration curve. The standard solutions of quercetin were prepared by serial dilutions using methanol (5–50 μg/mL). Total flavonoids content of the extract is expressed as mg quercetin equivalents (QE) per gram of sample (mg/g).

***In vitro* antioxidant activity of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts**

Free radical scavenging activity of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa crude extracts and garlic acid were determined *in vitro* by diphenyl-2-picrylhydrazyl assay according to published procedures [18]. Briefly, 1 mL of 0.1 mM DPPH in methanol was added

to 3 mL of various concentrations (0.0005-0.03 μg/mL) of plant extracts at ambient temperature. The samples were vigorously mixed and incubated in the dark for 30 min. After 30 min, absorbance of plant samples was measured at 517 nm. Measurements were carried out in triplicates and on two separate days to improve scientific accuracy of results. The percentage (%) DPPH free scavenging activity (RSA) was calculated as presented in equation 1, below and the IC_{50} values denote the concentration of the sample required to scavenge 50% DPPH free radicals. Gallic acid (Sigma-Aldrich, South Africa) was used as a positive control.

RSA (%) = $\frac{A_{control}-A_{sample}}{A_{control}} x100$……..……..Equation 1

Where, A_{sample} is the absorbance of DPPH and crude extract; $A_{control}$ is the absorbance of DPPH without crude extract.

Inhibition of α-amylase by crude *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts

The inhibition of α-amylase by crude root and bark extracts of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa was performed using the 3,5-dinitrosalicylic acid (DNSA) as reported elsewhere [19]. Briefly, crude root and bark extracts of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa were dissolved in 20 mM sodium phosphate buffer, pH 6.9 to give concentrations ranging from 0 to 100 μg/mL. 0.2 mL of bacterial α-amylase, Phillip Harris Manufacturing Ltd, United Kingdom (Cat no: F55885) (2 units/mL) was mixed with 0.2 mL sample extract and incubated for 10 minutes at 30 °C. After 10 minutes, 0.2 mL of the starch solution (1% in water w/v) was added to each tube and incubated for a further 3 min. The reaction was terminated by adding 0.2 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and boiled for 10 min in a

water bath at 85°C. The mixture was cooled to ambient temperature and diluted with 5 mL of distilled water. Absorbance was immediately measured at 540 nm using a UV-visible spectrophotometer. The control experiment was carried out with enzyme and starch solution only. Acarbose (Fisher Scientific, USA) was used as a positive control. The percentage (%) inhibition was calculated as follows:

Inhibition (%) = $\frac{A_{540nm}\ (control) - A_{540nm}\ (extract)}{A_{540nm}(control)} \times 100$……Equation 2

Inhibition of α-glucosidase by crude *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts

The α-glucosidase inhibitory activity was measured using p-nitrophenyl-α-d-glucopyranoside (pNPG) and various concentrations of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa crude extracts according to published procedures [19,20]. Briefly, 50 µL of crude root and bark extracts at varying concentrations (0-400 µg/mL) was mixed with 10 µL of α-glucosidase from Aspergillus, Niger (Sigma-Aldrich, South Africa) (1 U/mL) and 125 µL of 20 mM sodium phosphate buffer, pH 6.9. The resultant mixture was incubated at 37 °C for 20 min. After 20 min, the reaction was initiated by adding 20 µL of 1 M pNPG and the mixture was incubated for 30 min at room temperature. The reaction was terminated by addition of 0.1M of Na_2CO_3 (50 µL) and final absorbance was measured at 405 nm. Acarbose (Fisher Scientific, USA) was used as a positive control. The percentage (%) inhibition was calculated as follows:

Inhibition (%) = $\frac{A_{540nm}\ (control) - A_{540nm}\ (extract)}{A_{540nm}(control)} \times 100$……..……Equation 3

Molecular docking

Molecular docking of phytochemical compounds was carried out on human lysosomal acid-α-glucosidase, hGAA (PDB: 5NN8) and human pancreatic α-amylase, HPA (PDB: 2QV4) ([21,22]. The X-ray crystallographic structures of hGAA and HPA were retrieved from the protein data bank (https://www.rcsb.org/), visualized and prepared for docking using Bovia Discovery studio visualizer v21.1.0.20298. Prior to docking, co-crystallized water molecules and non-essential small organic molecules were removed from the crystal structures. The retained protein-ligand complexes were then protonated, optimized and typed using Charmm and MMFF94 forcefields.

A dataset of thirty-six phytochemical compounds from *Xeroderris stuhlmannii* Taub. Mendonca & E.P. Sousa was used for molecular docking [16]. The structures of *Xeroderris stuhlmannii* Taub. Mendonca & E.P. Sousa were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format, protonated using Discovery Studio visualizer and energetically optimized with MMFF94 forcefield using RDkit optimizer node in KNIME Analytics v4.3.3 [23]. Molecular docking simulations were performed using Autodock Vina (Vina) in Pyrx v0.8 [24,25]. Co-crystalized acarbose ligand (positive control) in human lysosomal acid-α-glucosidase and pancreatic α-amylase was used for validating the docking procedures. The docking scores from Autodock Vina are reported as binding affinity (kcal/mol). Discovery studio visualizer was used to analyze the binding interactions of the docked protein-ligand complexes.

The physicochemical, drug-likeness, medicinal, ADME and toxicity properties of the selected *Xeroderris stuhlmannii (Taub.)* Mendonca & E.P. Sousa compounds

The physicochemical properties (molecular weight (MW), hydrogen bond donor count (HBD), hydrogen bond acceptor count (HBA), rotatable bond count (RB), lipophilicity and water solubility) were computed using SwissADME (http://www.swissadme.ch/). Additionally, the drug-likeness, oral bioavailability and medicinal chemistry properties of the phytochemical compounds were evaluated using SwissADME. Finally, the absorption, distribution, metabolism, elimination (ADME) and toxicity parameters of the selected phytochemical compounds were estimated using ADMETlab 2.0 (https://admetmesh.scbdd.com/).

Statistical analysis

All experiments were performed in triplicates and on two separate days in order to increase the scientific consistency of the results. Data analysis was performed by one-way analysis of variance (ANOVA) using GraphPad Prism version 8.0. The results are expressed as means of three replicate determinations ± standard deviation.

RESULTS

Crude extract yields, total phenolic and total flavonoid content of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and bark extracts

The percentage (%) yield of crude root and bark *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts are summarized in Table 1. The yield of plant extracts ranged from 4.2 to 13.6% for bark and roots. Methanol extracted more secondary plant metabolites/phytochemicals compared to ethyl acetate (13.6% versus 5.2% for barks and 9.4% versus 4.2% for roots). The quantified total phenolics of bark and roots varied between methanol and ethyl acetate (Table 1). Using methanol as a solvent for extraction of secondary plant metabolites, the total phenolic content of *Xeroderris stuhlmannii (Taub)* Mendonca & E.P. Sousa root extract was 11.5±0.02 mg of gallic acid equivalents (GAE) per gram of dry plant extract (mg GAE/g dry weight) whereas for bark extract, the total phenolic content was 7.5±0.02 mg GAE per gram of dry weight. The phenolic content of *Xeroderris stuhlmannii (Taub)* Mendonca & E.P. Sousa bark and root extracts were 19.6 ± 0.04 and 25.7 ± 0.05 mg GAE/g, respectively, with ethyl acetate as the solvent for extraction. Ethyl acetate extracted more polyphenols compared to methanol.

The total flavonoid content of bark and root extracts of Xeroderris *stuhlmannii (Taub)* Mendonca & E.P. Sousa are expressed as mg quercetin equivalents/g of dry weight extract (Table 1). The total flavonoid content of the root extract was 15.6±0.2 mg and 21.3±0.4 mg quercetin equivalent per gram of weight dry extract (QE/g dry weight extract) with bark methanol and ethyl acetate. For bark extract the total flavonoid content were 17.4±0.2 and 19.5±0.5 mg of QE/g dry weight extract for methanol and ethyl acetate, respectively. The quantified total flavonoid results showed that the total flavonoid contents of bark and root extracts were not significantly different from each other.

DPPH Free Radical Scavenging Activity

The *in vitro* antioxidant activity assay was carried out to assess the ability of crude *Xeroderris stuhlmannii* (Taub) Mendonca & E.P. Sousa root and bark extracts to scavenge free radicals such as 2,2-di-(4-tert-octylphenyl)-1-picrylhydrazyl free radical (DPPH·). Figure 1 and Table 2 summarize the antioxidant results obtained for the crude methanolic and ethyl acetate root and bark extracts. Gallic acid was used as a positive control. As shown in Table 2, both root and bark extracts significantly scavenged DPPH radicals (IC_{50} values ranged from 0.011-0.013 µg/mL for methanolic and ethyl acetate extracts). The lower the IC_{50} value of a plant extract, the higher its antioxidant activity [18]. Gallic acid displayed a good scavenging effect against the DPPH radical with a calculated IC_{50} value of 0.215 µg/mL. However, its inhibitory activity was moderately weaker than that of root and bark extracts.

In vitro antidiabetic activity of *Xeroderris stuhlmannii* (Taub) Mendonca & E.P. Sousa crude extracts on α-amylase and α-glucosidase

Polyphenols in plants have antioxidant properties and are reported to exert anti-hyperglycemic effects through non-specific binding to glucose transporters and competitively inhibit digestive enzymes (α-amylase and α-glucosidase). The inhibition of α-amylase and α-glucosidase by *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and bark extracts is described in Figure 2 and Table 2. *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa methanol and ethyl acetate bark extracts inhibited α-amylase with IC_{50} values of 12.8±1.4 µg/mL and 13.9±1.6 µg/mL respectively. Inhibition of α-amylase by methanol and ethyl acetate root extracts occurred with IC_{50} values of 21.1±1.9 and 18.8±1.2 µg/mL, respectively. In contrast, acarbose inhibited α-amylase with an IC_{50} value of 54.4±6.4 µg/mL. We next tested the *in vitro*

inhibition of α-glucosidase by *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa methanol and ethyl acetate bark and root extracts. Inhibition of α-glucosidase by methanolic and ethyl acetate bark extracts occurred with IC_{50} values of 13.2±2.8 µg/mL and 16.0±4.5µg/mL, respectively. The calculated IC_{50} values for methanolic and ethyl acetate root extracts were 10.5±1.6 µg/mL and 8.8±1.5µg/mL. In contrast, a-glucosidase inhibition by acarbose occurred with an IC_{50} value of 92.2±15.2 µg/mL.

Table 1 Extraction yields, total phenolic and flavonoid contents of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa roots and bark extracts

Plant species	Plant part used	Solvent used	Yield of the plant (%w/w)	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
Xeroderris stuhlmannii (Taub)	root	Methanol	09.4	11.5±0.02	15.6±0.2
		Ethyl acetate	04.2	25.7±0.05	21.3±0.4
	bark	Methanol	13.6	07.5±0.02	17.4±0.2
		Ethyl acetate	05.2	19.6 ±0.04	19.4±0.5

Table 2 **Concentrations of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and bark extracts that causes 50% inhibition (IC_{50}) values in DPPH radical scavenging, α-amylase and α-glucosidase inhibitory assays**

Plant species/ standard	Plant part used	Solvent used	DPPH scavenging activity (IC_{50} µg/mL)	α-Amylase inhibition (IC_{50} µg/mL)	α-Glucosidase inhibition (IC_{50} µg/mL)
Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa	root	Methanol	0.011±0.001	21.1±1.9	10.5±1.6
		Ethyl acetate	0.012±0.001	18.8±1.2	8.8±1.5
	bark	Methanol	0.011±0.001	12.8±1.4	13.2±2.8
		Ethyl acetate	0.013±0.002	13.9±1.6	16.0±4.5
Gallic acid (positive control)			0.215±0.015	-	-
Acarbose (positive control)			-	54.1±2.9	107.4±7.7

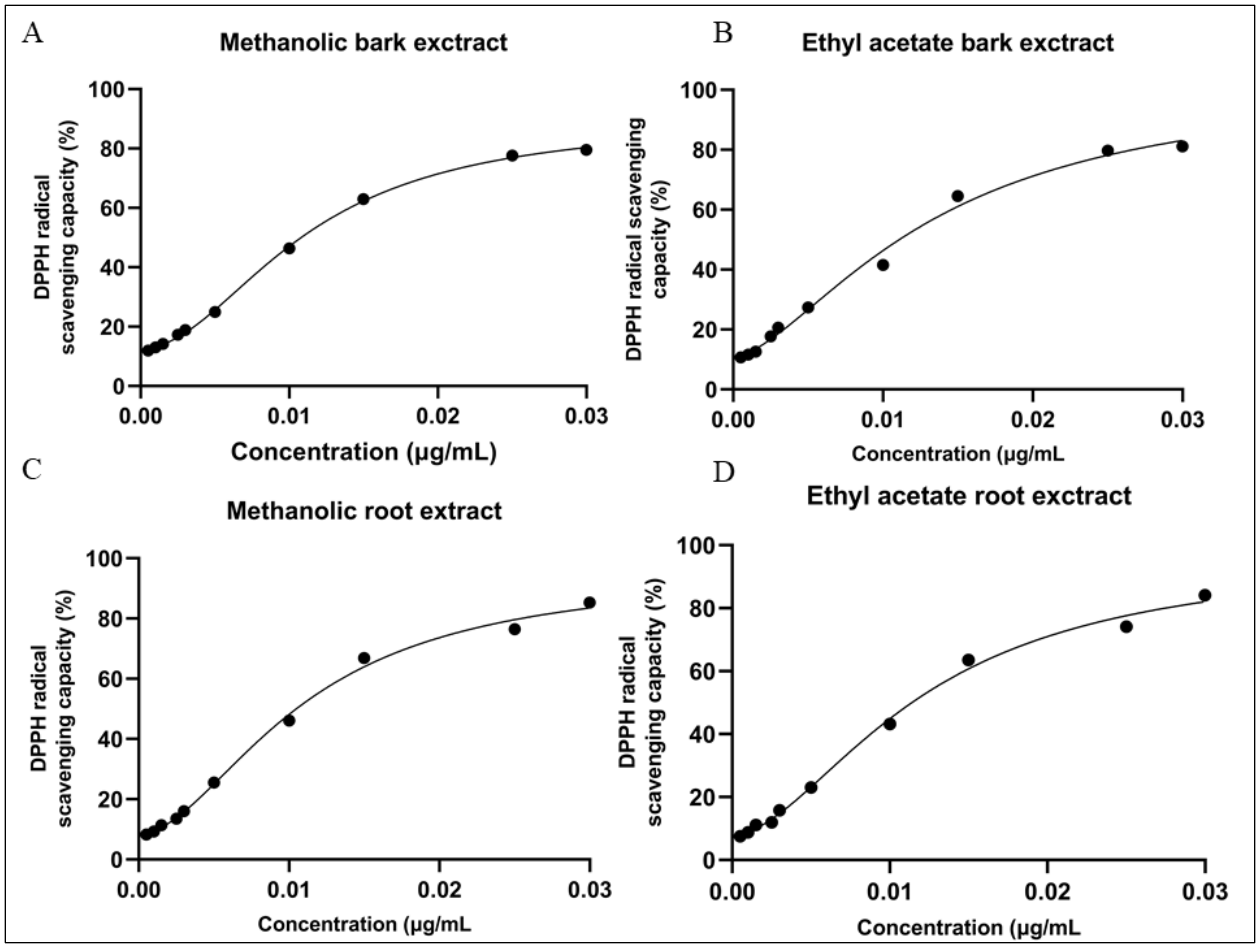

Figure 1 The ability of root and bark extracts to scavenge the DPPH radical was determined *in vitro* by diphenyl-2-picrylhydrazyl (DPPH) assay at 517 nm. The DPPH radical scavenging capacity of methanolic bark extract, (A), ethyl acetate bark extract, (B), methanolic root extract, (C) and ethyl acetate root extract, (D). Each data point represents the mean of three (3) experiments.

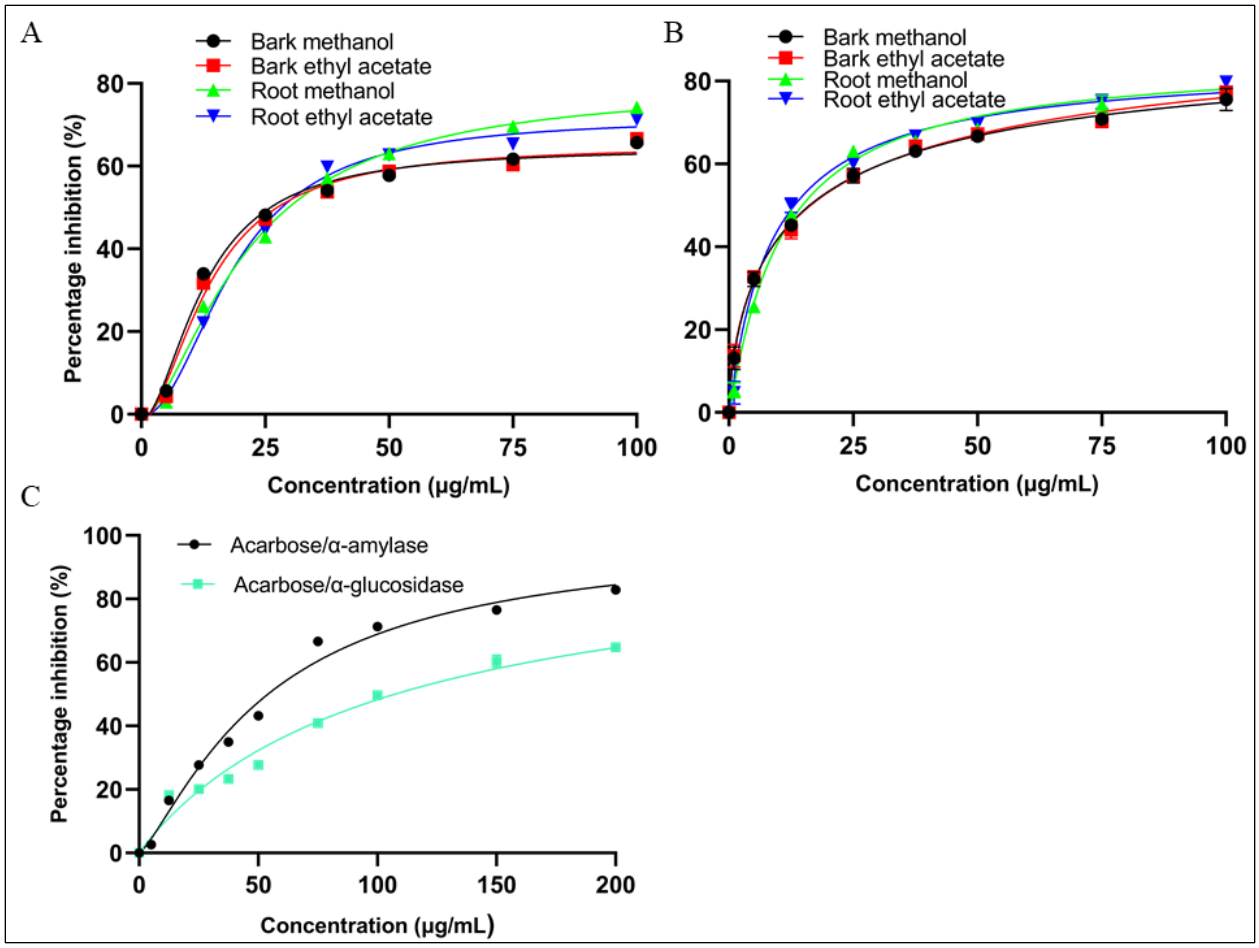

Figure 2. The *in vitro* inhibitory effects of crude root and bark extracts against a-amylase, A and a-glucosidase, B in vitro inhibitory effects of the acarbose standard against a-amylase and a-glucosidase, C were determined according to the procedures in Materials and Methods. Each data point represents the mean of three (3) experiments.

***Molecular docking of the phytochemical compounds of Xeroderris stuhlmannii* Taub.**

Thirty-six phytochemical compounds from *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa were virtually screened using Autodock Vina to identify novel inhibitors of HPA and GAA. The docking method was validated by redocking co-crystallized acarbose and an acarbose derivative to the active site of the enzymes. Figures 3 shows the graphical summary of binding affinities of the thirty-six phytochemical compounds and acarbose (positive control) against HPA and hGAA enzymes. As shown in Figures 3, the binding affinities of the phytochemical compounds against HPA ranged from -10.3 to -5.6 kcal/mol, whereas those for hGAA ranged from -8.4 to -5.6 kcal/mol. Khasianine, brassinolide, oleanolic aldehyde and castasterone had binding affinities lower (more negative) than that of acarbose in HPA. -Only khasianine, oleanolic aldehyde and apiin had lower binding affinities than that of acarbose in hGAA. The phytochemical compounds with lower binding affinities than acarbose in both HPA and hGAA were included in the pharmacokinetic prediction studies. In addition, a few other compounds with binding affinities below that of acarbose such as ursolic acid, myricetin, and myricitrin were also included in pharmacokinetic studies because they are known to inhibit the digestive enzymes that contributes to diabetes mellitus [26–28].

Intermolecular interactions between selected ligands (those with lower binding affinities than acarbose) and active site residues of HPA and hGAA are shown in Table 3 and Figures 4&5. The major intermolecular interactions observed between the ligands and digestive enzymes were hydrogen-bonding (H-bonding), π-π, electrostatic and hydrophobic-interactions. Furthermore, the majority of selected compounds formed H-bonds with bond lengths less than 3 Å. When the co-crystallized acarbose derivative was redocked on the binding site of the human pancreatic amylase (HPA), the root mean square deviation (RMSD) was 1.62Å (Figure 4). In this orientation, the

acarbose derivative formed two strong H-bonds with the catalytic residues, GLU233 and ASP300. In addition, the acarbose derivative was also stabilized by eight extra H-bonds with neighboring amino acids (Figure 4). All other docked compounds, except myricetin formed a H-bond with at least one of the three key catalytic residues (ASP197, GLU233 and ASP300) of HPA (see Figure 4). Brassinolide, khasianine and apiin formed more H-bonds than other phytochemical compounds leading to low binding affinities (Figure 4). To get insight into the interaction of hGAA with the phytochemical compounds, we docked the selected phytochemical compounds with low binding affinities into the active site of hGAA (Figure 5). Acarbose is stabilized within the active site by four strong H-bonds with ASP282, ASP404, ASP600 and ASP616. When apiin was docked into the substrate binding site of hGAA, it was more stabilized by H-bonds compared to acarbose and other compounds with similar binding affinities (Figure 5). Together, the docking scores presented here showed that phytochemical compounds inhibited digestive enzymes by directly interfering with the catalytic residues or indirectly by forming strong intermolecular forces with neighboring residues in active site of the enzymes.

Table 3 Hydrogen bonding (H-bonding) and binding affinity of selected phytochemical compounds against HPA and GAA enzymes.

Enzyme	**Phytochemical compound**	**Hydrogen bond (H-bond) interacting residues and the H-bond distance (Å)**	**Binding affinity ($kcalmol^{-1}$)**
HPA	Acarbose derivative	TYR62 (3.09), GLN63 (2.41), ALA106 (2.77), VAL107 (2.48), THR163 (1.92), GLY164 (2.48), ARG195 (2.96), GLU233, HIS299 (2.18), (2.43), ASP300 (2.06)	-9.3
	Brassinolide	ASP197 (2.20), GLU233 (2.55), ASP300 (2.12)	-10.0
	Khasianine	ASN53 (2.40), ASP197 (2.40), ALA198 (2.94), ASP300 (2.75)	-10.3
	Oleanolic aldehyde	GLU233	-9.4
	Castasterone	ASP197 (2.83), THR163(2.09)	-9.3
	Apiin	ASP197 (2.22), GLU233 (2.46), ILE235 (2.36)	-9.2
	Myricitin	GLN63 (2.39), ASP197 (2.97),	-8.5
	Myricetin	TYR62 (2.66), GLN63 (2.13)	-9.0

	Ursolic acid	GLU233 (2.29), ASP300 (2.60)	-8.9
hGAA	Acarbose	ASP282 (1.72), ASP404 (2.30), ASP616 (1.67), ASP600 (2.87)	-8.1
	Brassinolide	SER523 (2.64), ASN524 (2.64), SER676 (1.93), LEU677 (2.38), LEU678 (2.01)	-7.1
	Khasianine	ARG281 (2.10), ASP616 (2.04)	-8.1
	Oleanolic aldehyde	None	-8.4
	Castasterone	ASP282 (1.96), LEU677 (2.86), LEU678 (2.26)	-7.6
	Apiin	ASP282 (2.11), ASP404 (2.45), ARG600 (2.80), ASP616 (2.35), LEU677 (2.62)	-8.3
	Myricitin	ARG281 (2.70), ASP282 (2.52)	-7.4
	Myricetin	ASP404 (2.27), HIS674 (2.56)	-7.1
	Ursolic acid	ARG600 (2.28), ASP282 (2.13)	-7.8

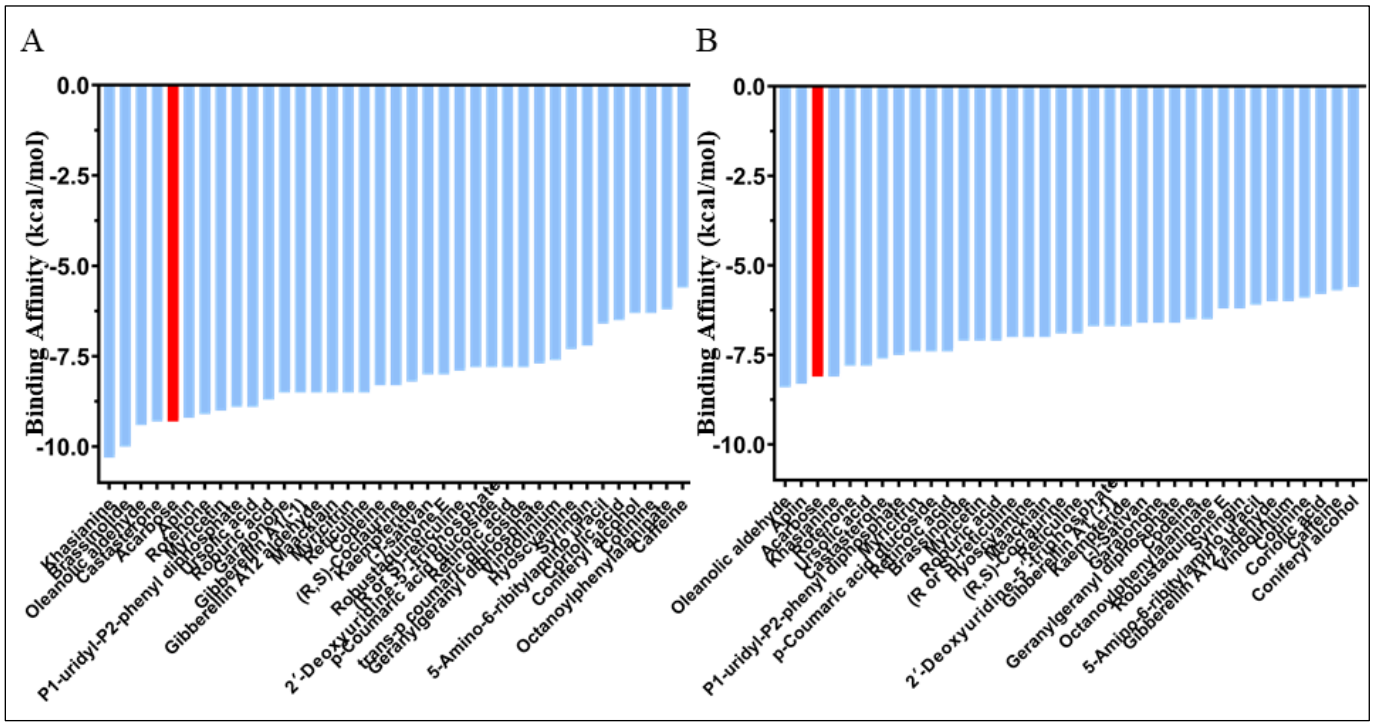

Figure 3. The binding affinities of thirty-six phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa and acarbose against: A. human pancreatic α-amylase (HPA) and B. human lysosomal acid-α-glucosidase (hGAA).

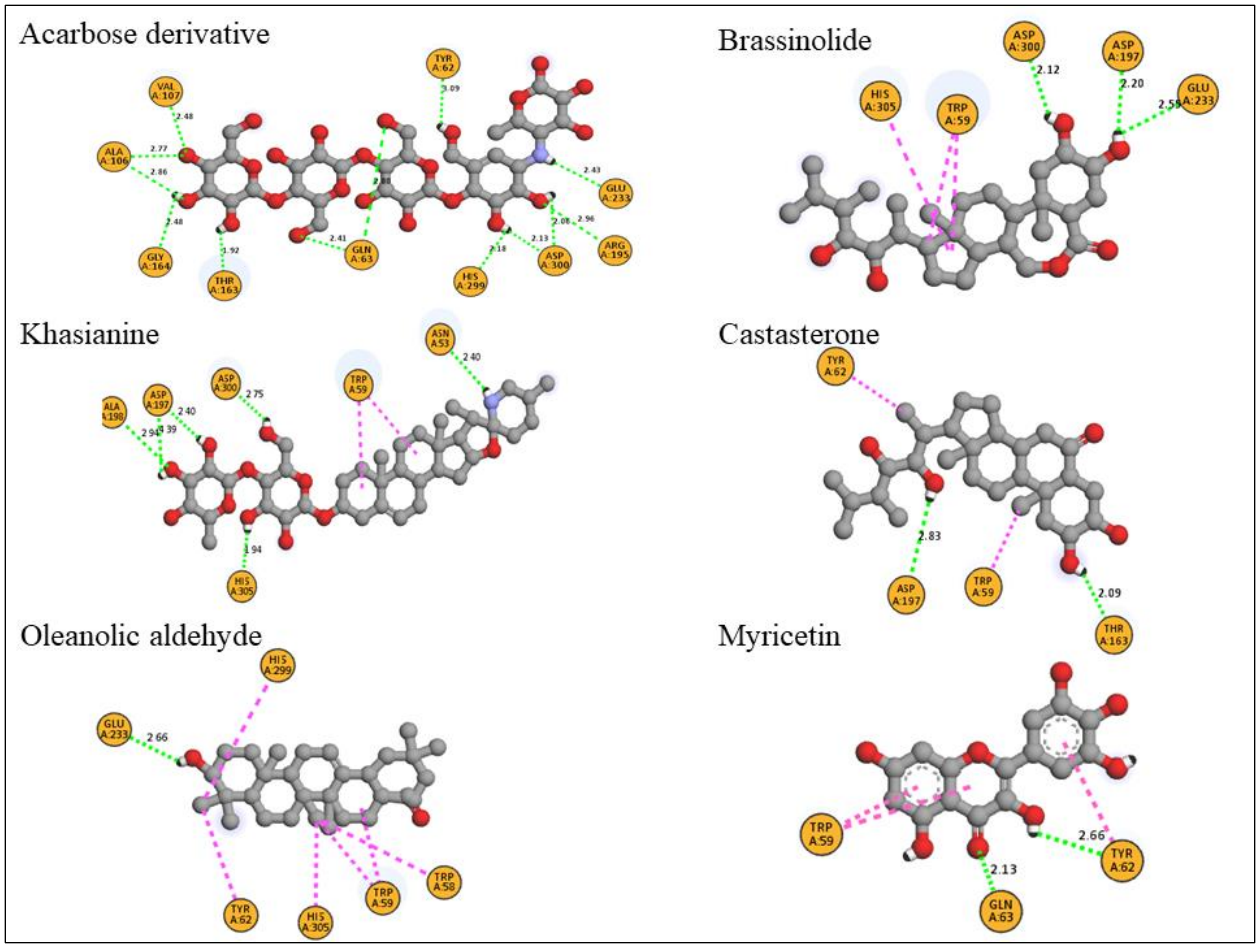

Figure 4. The interaction of human pancreatic α-amylase (HPA) with selected phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa and acarbose. Hydrogen bonds are represented in green dotted lines. The purple dotted lines represent hydrophobic interactions (π-alkyl, π-σ, π- π stacking and π- π T-shaped).

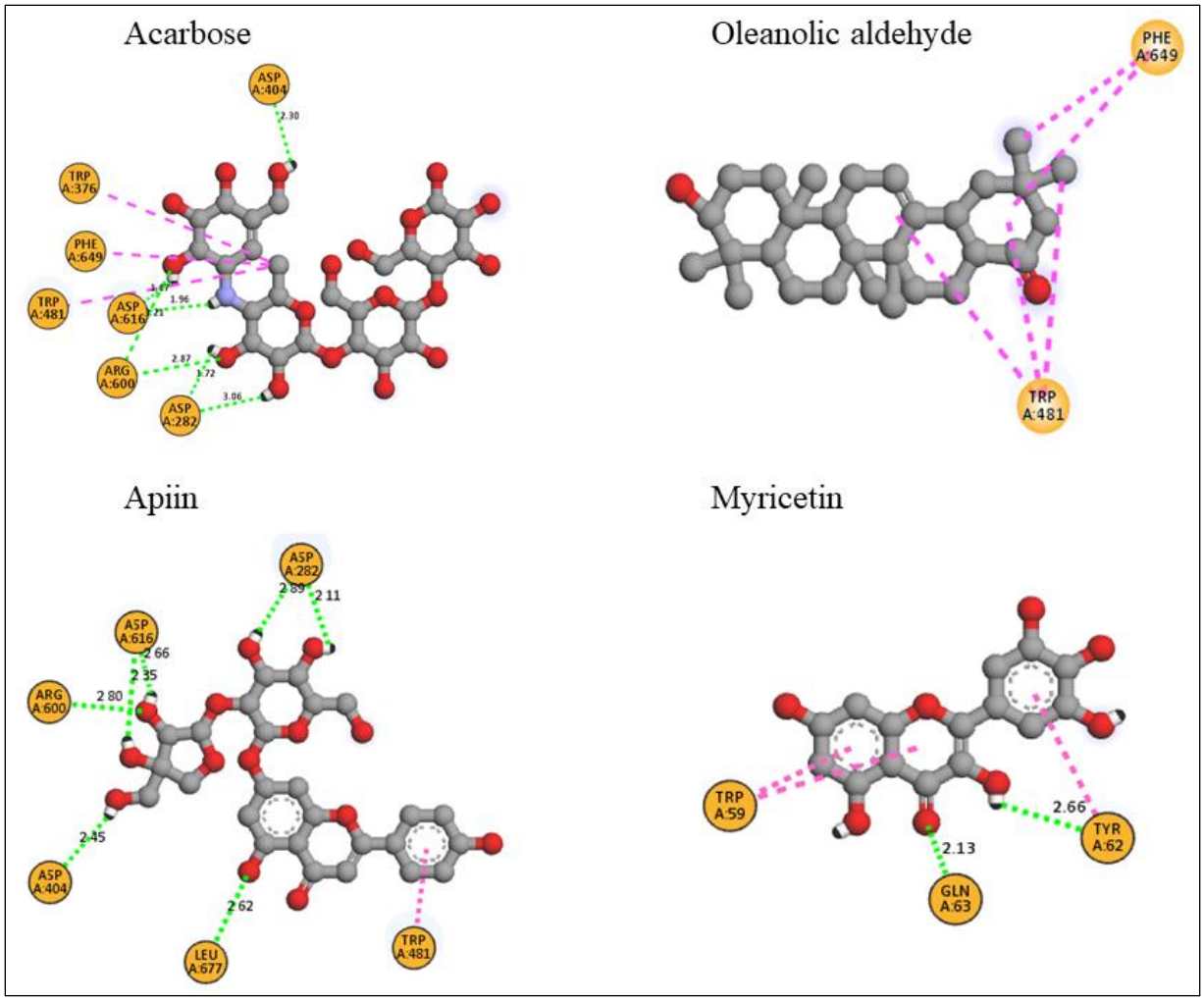

Figure 5. The interaction of human lysosomal acid-α-glucosidase (hGAA) with selected phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa and acarbose. Hydrogen bonds are represented in green dotted lines. The purple dotted lines represent hydrophobic interactions (π-alkyl, π-σ, π- π stacking and π- π T-shaped).

The physicochemical, drug-likeness, ADME and toxicity properties of Xeroderris stuhlmannii Taub. phytochemical compounds

The physicochemical, drug-likeness, bioavailability and medicinal properties of the selected compounds were evaluated using Swiss ADME, and are shown in Tables 4-7. The fraction of sp^3 carbon atoms (Fsp^3) were greater than 0.9 except apiin (0.42), myricitin (0.29) and myricetin (0). The polarity index of the selected compounds was assessed by the topological surface area (TPSA) descriptor, and ranged from 37.3 to 225.1. The solubility property, consensus LogP of the docked compounds ranged from -6.22 to 6.32 (Table 5). Only acarbose (cLogP=-6.22) is highly soluble in water, whereas apiin (cLogP= -0.72), myricitin (cLogP= -0.23) and myricetin (cLogP= 0.79) are less soluble. Ursolic acid and oleanolic acid are highly insoluble (cLogP>5). The druglikeness properties of the compounds showed that only brassinolide and castasterone did not violate the Lipinski's Rule of Five with a bioavailability score of 0.55. Oleanolic aldehyde, myricetin and ursolic acid violated one Lipinski's Rule of Five with bioavailability scores of 0.55, 0.55 and 0.85, respectively. Myricitin, acarbose, apiin and khasianine had at least two Lipinski's rule violations, and were predicted to have poor bioavailability. Only myricetin had good bioavailability and solubility properties with fewer violations of the Lipiski, Ghose, Veber, Egan and Muegge rules, and was best described as a drug-like compound. Myricitin, and myricetin were flagged to have at least one PAINS substructure whereas, brassinolide, castasterone and apiin were not flagged to have a BRENK substructure (see Table 7). The predicted synthetic accessibility values of the phytochemical compounds are shown in Table 7, and ranged from 3.29 to 9.1. The synthetic accessibility values presented here demonstrate that *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa phytochemical compounds have moderate to complex synthetic route.

Collectively, the results presented here indicate that myricetin has drug-likeness properties, and can be a good candidate for diabetes mellitus treatment.

To explore the pharmacokinetic properties (ADME) of the selected compounds, we computed the human intestinal absorption (HIA), caco-2 permeability (caco-2), P-glycoprotein (P-gp) inhibitor and substrate parameters in order to predict the absorption of the compounds in the gastrointestinal tract (Table 8). As shown in Table 5, khasianine, apiin, myricitin, and acarbose have low human intestinal absorption (HIA) probabilities. The computed caco-2 permeability parameters ranged from -4.85 cm/s to -6.35 cm/s. Caco-permeabilities greater than -5.15 are considered optimal [29]. Our results showed that only castasterone and brassinolide have better intestinal permeabilities compared to other phytochemical compounds. None of the compounds are considered as P-gp inhibitors but apiin and acarbose are categorized as P-gp substrates. Furthermore, we predicted that oleanolic aldehyde and ursolic acid are capable of crossing the blood brain barrier. Brassinolide, castasterone, castasterone and myricetin have low therapeutic index (>90% Plasma Protein Binding). The widespread microsomal cytochrome P450 (CYP) enzymes play an important role in phase 1 biotransformation of endogenous and exogenous compounds including many pharmaceutical drugs and phytochemical compounds [30]. We therefore evaluated the inhibition of the major drug metabolizing CYPs by the phytochemical compounds, and showed that myricetin is an inhibitor of CYP1A2, while oleanolic aldehyde was predicted to be an inhibitor of CYP2D6 and CYP3A4. All other compounds were not classified as inhibitors of the CYP enzymes. Finally, castasterone and brassinolide have higher drug clearance than that of acarbose and other compounds. Overall, the ADME results showed none of the phytochemical compounds have a complete set of pharmacokinetic properties needed for their disposition in the blood stream.

We next evaluated the toxicity of compounds, and showed that apiin, myricitin, myricetin and acarbose can induce liver damage, whereas apiin is a potential carcinogenic agent (see Table 9). Khasianine is predicted to inhibit the potassium channels encoded by the type 1 human Ether-à-go-go-Related Gene (hERG1), whereas none of the selected compounds are hepatotoxic. Additionally, the pharmacokinetic predictions showed that myricitin is Ames toxic, whereas ursolic acid, oleanolic aldehyde, khasianine and brassinolide can cause respiratory problems. Together, these toxicity results indicate that none of the hit compounds present in Xeroderris *stuhlmannii* (Taub.) Mendonca & E.P. Sousa are safe, and may require structural modifications to reduce the toxicity effects of the compounds.

Table 4 Physicochemical properties of the phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa determined using Swiss ADME.

Phytochemical compound	**Molecular Formula**	**Molecular Weight**	**#Heavy atoms**	**#Aromatic heavy atoms**	**#Rotatable bonds**	**#H-bond acceptors**	**#H-bond donors**	**Fraction Fsp^3**	**MR**	**TPSA**
Acarbose	$C_{25}H_{43}NO_{18}$	645.6	44	0	9	19	14	0.92	136.7	321.2
Brassinolide	$C_{28}H_{48}O_6$	480.68	34	0	5	6	4	0.96	133.7	107.2
Khasianine	$C_{39}H_{63}NO_{11}$	721.92	51	0	5	12	7	0.95	190.8	179.6
Oleanolic aldehyde	$C_{30}H_{48}O_2$	440.7	32	0	1	2	1	0.9	135.1	37.3
castasterone	$C_{28}H_{48}O_5$	464.68	33	0	5	5	4	0.96	132.6	98.0
Apiin	$C_{26}H_{28}O_{14}$	564.49	40	16	7	14	8	0.42	132.6	229.0
Myricitin	$C_{21}H_{20}O_{12}$	464.38	33	16	3	12	8	0.29	111.0	210.5
Myricetin	$C15H_{10}O_8$	318.24	23	16	1	8	6	0	80.1	151.6
Ursolic acid	$C_{30}H_{48}O_3$	456.7	33	0	1	3	2	0.9	136.9	57.5

Table 5 Lipophilicity and water solubility properties of the of the phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa determined using Swiss ADME.

Phytochemical compound	**Consensus Log P**	**ESOL Log S**	**ESOL Class**	**Ali Log S**	**Ali Class**	**Silicos-IT LogSw**	**Silicos-IT class**
Acarbose	-6.22	2.13	Highly soluble	2.56	Highly soluble	6.4	Soluble
Brassinolide	3.68	-5.54	Moderately soluble	-6.83	Poorly soluble	-2.7	Soluble
Khasianine	1.92	-5.34	Moderately soluble	-5.55	Moderately soluble	-1.77	Soluble

Oleanolic aldehyde	6.32	-7.18	Poorly soluble	-8.03	Poorly soluble	-6.71	Poorly soluble
castasterone	3.76	-5.35	Moderately soluble	-6.49	Poorly soluble	-2.97	Soluble
Apiin	-0.72	-2.95	Soluble	-3.99	Soluble	-1.92	Soluble
Myricitin	-0.23	-3.2	Soluble	-4.5	Moderately soluble	-1.49	Soluble
Myricetin	0.79	-3.01	Soluble	-3.96	Soluble	-2.66	Soluble
Ursolic acid	5.88	-7.23	Poorly soluble	-8.38	Poorly soluble	-5.67	Moderately soluble

Table 6 Drug-likeness properties and oral bioavailability of the phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa determined using Swiss ADME.

Phytochemical compound	**Lipinski violations**	**Ghose violations**	**Veber violations**	**Egan Violations**	**Muegge violations**	**Bioavailability Score**
Acarbose	3	4	1	1	5	0.17
Brassinolide	0	3	0	0	0	0.55
Khasianine	3	3	1	1	5	0.17
Oleanolic aldehyde	1	3	0	1	1	0.55
castasterone	0	2	0	0	0	0.55
Apiin	3	3	1	1	3	0.17
Myricitin	2	0	1	1	3	0.17
Myricetin	1	0	1	1	2	0.55
Ursolic acid	1	3	0	1	1	0.85

Table 7 Medicinal chemistry properties of the phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa determined using Swiss ADME.

Phytochemical compound	PAINS #alerts	Brenk #alerts	Leadlikeness #violations	Synthetic Accessibility
Acarbose	0	1	2	7.34
Brassinolide	0	0	2	6.14
Khasianine	0	1	1	9.1
Oleanolic aldehyde	0	2	2	5.99
castasterone	0	0	2	5.74
Apiin	0	0	1	6.08
Myricitin	1	1	1	5.32
Myricetin	1	1	0	3.27
Ursolic acid	0	1	2	6.21

Table 8 Pharmacokinetics properties of the phytochemical compounds of the *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa determined using ADMElab 2.0.

Phytochemical compound	**Human intestinal absorpt**	**Caco-permeability**	**Pgp inhibitor**	**Pgp substrate**	**PPB (%)**	**BBB penetration**	**CYP 1A2 inhibitor**	**CYP 2C19 inhibitor**	**CYP 2C9 inhibitor**	**CYP 2D6 inhibitor**	**CYP 3A4 inhibitor**	**CL (mL/min/kg)**

	ion (HIA)											
Acarbose	1.0	-6.35	0	0.85	8.2	0.39	No	No	No	No	No	0.37
Brassinolide	0.13	-4.85	0.1	0.06	92.7	0.23	No	No	No	No	No	19.35
Khasianine	0.92	-5.36	0	0.01	81.4	0.03	No	No	No	No	No	0.94
Oleanolic aldehyde	0.01	-5.09	0.03	0	80.7	0.93	No	No	No	Yes	Yes	6.24
Castasterone	0.27	-4.85	0.02	0.05	93.9	0.56	No	No	No	No	No	21.38
Apiin	0.96	-6.27	0	0.95	81.4	0.16	No	No	No	No	No	1.66
Myricitin	0.70	-6.27	0	0.58	87.7	0.01	No	No	No	No	No	5.26
Myricetin	0.04	-5.65	0	0.01	92.8	0.01	Yes	No	No	No	No	7.72
Ursolic acid	0.01	-5.22	0	0	98.8	0.72	No	No	No	No	No	3.67

Table 9 The toxicity probabilities of the phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa was determined using ADMElab 2.0.

Phytochemical compound	**hERG Blocker**	**Human hepatotoxicity**	**Drug induced liver injury (DILI)**	**Ames toxicity**	**Rat oral acute toxicity**	**FDAMDD probability**	**Skin sensitization**	**Carcinogenicity**	**Eye corrosion**	**Eye irritation**	**Respiratory toxicity**
Acarbose	0.04	0.22	0.98	0.10	0.04	0.0	0.0	0.048	0.003	0	0.03
Brassinolide	0.03	0.17	0.12	0.04	0.19	0.06	0.05	0.016	0.003	0.01	0.60
Khasianine	0.64	0.22	0.01	0.12	0.41	0.92	0.01	0.072	0.003	0	0.95
Oleanolic aldehyde	0.0	0.20	0.01	0.03	0.10	0.68	0.05	0.033	0.004	0.51	0.98
Castasterone	0.03	0.29	0.19	0.04	0.55	0.12	0.03	0.013	0.003	0.01	0.27
Apiin	0.08	0.12	0.95	0.50	0.03	0.01	0.05	0.718	0.003	0.01	0.02
Myricitin	0.02	0.16	0.98	0.73	0.06	0.23	0.60	0.034	0.003	0.17	0.05
Myricetin	0.15	0.10	0.98	0.48	0.02	0.56	0.94	0.028	0.008	0.93	0.07
Ursolic acid	0.0	0.21	0.01	0.01	0.18	0.61	0.01	0.031	0.003	0.14	0.97

Discussion

Naturally occurring bioactive phytochemical compounds from medicinal plants have extensively been studied for potential health benefits and, in particular as therapeutics for diabetes mellitus [31]. These bioactive phytochemical compounds consist of flavonoids, terpenoids, saponins, polyphenols, alkaloids and glycosides. The polyphenolic compounds possess antioxidant activity, and can scavenge highly reactive free radicals within the biological system [12]. Highly reactive free radicals, often derived from oxygen (ROS) play a major role in the development and progression of diabetes mellitus and its complications [32–34]. During times of environmental stress and cell dysfunction, ROS levels increase dramatically, and may cause significant cellular damage in the body [32]. In order to prevent or reduce the ROS-induced oxidative damage, antioxidants in plants scavenge free radicals by accepting or donating electron(s) to eliminate the unpaired condition of the radical. In addition, antioxidants indirectly inhibit the activity or expression of free radical generating enzymes and enhance the activity or expression of intracellular antioxidant enzymes [35].

Plant extracts with electrons or hydrogen atoms donating abilities can convert the DPPH radical to its nonradical form, 1,10-diphenyl-2-picrylhydrazine [18]. In this study, we determined the antioxidant activity of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa root and bark extracts against DPPH radical, and showed that both root and bark extracts significantly scavenged DPPH radicals with low IC_{50} values (<0.013 μg/mL). The antioxidant activity of the extracts is attributed to the bioactive polyphenols and flavonoid compounds in the crude extracts. In addition, we showed that ethyl acetate extracted more polyphenols and flavonoids than methanol. This observation is in agreement with published results that showed that ethyl acetate fractions are rich in flavonoids [20]. These results showed *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa

extracts contain bioactive polyphenolic and flavonoid compounds with antioxidant properties that capable of scavenging free radicals that contribute to T2DM.

Inhibition of the activity of enzymes involved in carbohydrate metabolism including α-glucosidase and α-amylase is one of the novel approaches developed to treat type 2 diabetes mellitus and its complications. Novel α-glucosidase inhibitors delay the overall digestion of carbohydrates by increasing the digestion period, and reducing the rate of intestinal glucose absorption, which in turn diminishes postprandial hyperglycemia [36]. Here we investigated the *in vitro* inhibitory properties of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts on α-amylase and α-glucosidase enzymes, and showed that the half maximal inhibitory concentration (IC_{50}) of the extracts were significantly lower (ranged from 10.5 to 21.1 μg/mL) than that of acarbose (IC_{50} of acarbose with α-amylase was 54.4±6.4 and α-glucosidase was 107.4±7.7). Lower IC_{50} values corresponds to high activity of the extracts [18]. The reported IC_{50} values for acarbose are closer to those reported in literature [37]. The high potency of the extracts (low IC_{50} values) against digestive enzymes are likely due to the presence of a number of bioactive phytochemical compounds acting synergistically to inhibit the enzymes [16].

Molecular docking remains one of the most popular *in silico* approach used in drug discovery to virtually screen for hit compounds in virtual libraries containing millions of molecular structures against a variety of drug targets with known three-dimensional structures [38]. The molecular modelling approach provides important information on the ligands' binding affinity and can effectively predict different binding modes of ligand in the active site of target molecule [39]. Molecular docking approaches have successfully been utilized to identify novel natural product inhibitors of human α-amylase (HPA) and human α-glucosidase (hGAA) [40]. Here, we used Autodock Vina (Scripps Research, San Diego) to identify novel photochemical compounds of

Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa extracts that contributes to its antidiabetic activity of the plant. Among the thirty-six docked phytochemical compounds, only five (Khasianine, brassinolide, oleanolic aldehyde and apiin) had higher docking scores compared to acarbose, and were therefore predicted to inhibit α-glucosidases. These compounds likely contribute to the antidiabetic activity of the crude extracts by synergistically inhibiting the digestive enzymes. The inhibitory effects to the phytochemical ligands are consistent with the low IC_{50} values of the extracts against the digestive enzymes. In addition, we include myricetin, myricitrin and ursolic acid in molecular docking studies because they are known in literature to inhibit α-glucosidase enzymes [26–28].

Within the active site of HPA, three essential acidic residues (ASP197, GLU233 and ASP300) exist that catalyze the breakdown of glyosidic bonds [22,26]. Inhibitors that form strong H-bonds with these acidic residues can delay carbohydrate hydrolysis, and can ultimately reduce postprandial hyperglycemia. Our studies showed that all docked phytochemical compounds, with the exception of myricetin can form at least one H-bond with the catalytic residues. The inhibitory effects of the phytochemical compounds likely contribute to the antidiabetic activity of crude *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts. A closer look at the active site of hGAA showed that the catalytic nucleophile and acid/base residues are ASP518 and ASP616 [21]. We redocked acarbose in the active site of hGAA, together with eight other selected phytochemical compounds of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa. Only acarbose, apiin and khasianine formed a H-bond with the catalytic residue, ASP616. In addition, our results showed that apiin formed more H-bonds than other phytochemical compounds, and can be a novel inhibitor of hGAA. However, to the best of our knowledge the antidiabetic activity of

apiin have not yet been explored. Toxicity prediction studies showed that apiin is likely to be carcinogenic.

Evaluation of pharmacokinetic and pharmacodynamic properties of hits in drug discovery is important because they help us understand how drugs behave in the body and how the body reacts to drugs. Pharmacokinetic prediction studies presented in this work showed that none of the hit phytochemical compounds obeyed all laws of ADMET properties. Consistent with published results, our pharmacokinetic prediction studies also showed that myricetin had less violations than other compounds, and can be a novel inhibitor of α-glucosidases. The antidiabetic activity of myricetin and its derivatives has been reported in the last few years, and a high-resolution X-ray crystal structure of human pancreatic α-amylase (HPA) complexed with myricetin has been solved [26]. Myricetin binds at the active site and interacts directly with catalytic residues of HPA and reduces the normal conformational flexibility of the substrate binding cleft. The antidiabetic role of ursolic acid is mediated through insulin secretion and insulinomimetic effect on glucose uptake, synthesis and translocation of GLUT4 by a mechanism of cross-talk between calcium and protein kinases [27]. However, our results showed that ursolic acid is highly insoluble in aqueous and lipid environment, and may require structural modifications to improve its solubility. Myricitrin improves type 2 diabetes mellitus by significantly decreasing the fasting blood glucose levels, improving glucose intolerance and increasing pancreatic β-cell mass [28]. Together, these findings demonstrated *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa contain novel α-amylase and α-glucosidase inhibitors that synergistically act reduce the postprandial blood glucose in type 2 diabetic patients by inhibiting the digestive enzymes.

Conclusions

In this work, we identified novel α-amylase and α-glucosidase inhibitors present in *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa extracts capable delaying carbohydrate metabolism, which in turn can reduce postprandial hyperglycemia. These compounds likely inhibit carbohydrate metabolism as an individual or in combination by competitively binding to the active site of the enzymes, and preventing the substrates from accessing the active site. The synergistic effect of the compounds may have contributed significantly to the low IC_{50} values, and antidiabetic activity of the crude extracts. Molecular docking and pharmacokinetic prediction studies showed myricetin can be a novel inhibitor of α-glucosidases. Overall, our study demonstrated the remarkable inhibitory potential of *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa against α-glucosidases, and its role in the reduction of postprandial glucose in prediabetic and diabetic mellitus patients. Therefore, this study can present an opportunity to develop plant-based therapeutic medicines with fewer side effects for type 2 diabetes mellitus patients.

Author Contributions

FR, EM and GM were involved in concept development. BN and EM performed the *in vitro* experiments, and JTB performed the in-silico modeling experiments. FR, JTB, GM and BN wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare no competing financial interests.

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