biology and life sciences forum

MDPI

Proceeding Paper

α-Amylase Inhibitory Secondary Metabolites from *Artemisia pallens* Wall ex DC—Biochemical and Docking Studies †

Anjali A. Kulkarni * and Rohit P. Kamble

Department of Botany, Savitribai Phule Pune University, Ganeshkhind Road, Pune 411 007, India; rohitkamble97@gmail.com

* Correspondence: anjali.uop@gmail.com or akulkarni@unipune.ac.in

† Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021; Available online: https://iecps2021.sciforum.net/.

Abstract: Diabetes Mellitus Type-2 (DM-2) has become a challenging disease worldwide as many young adults are also getting affected by it due sedentary lifestyle and wrong diets. Multiple studies have shown that control over α-amylase enzyme in the gut could be a better approach to treat DM-2. The secondary metabolites that are produced by plants have various biological properties and many are used as drugs. In the current study, we isolated secondary metabolites from acetone leaf and bud extracts of *Artemisia pallens* Wall ex DC (Family: Asteraceae) and tested them for their porcine pancreatic α-amylase (PPA) inhibitory activity in vitro and in silico. This extract exhibited good PPA inhibition, with IC_{50} value of 388.05 µg/mL. The IC_{50} value of Acarbose (a known pancreatic α-amylase inhibitor drug/positive control) was 9.71 µg/mL. Various secondary metabolites that were detected from acetone leaf and bud extract by LC-MS analysis were used for the molecular docking studies using AutoDock 4.2.6. The co-crystallized structure of PPA and acarbose was retrieved from Protein Data Bank (PDB ID: 1OSE). The binding energies of few metabolites were (kcal/mol): isoquercetin (−11.57), cryptochlorogenic acid (−11.17), cirsilineol (−10.24), kaempferide (−9.99), fustin (−9.86), 6-demetroxycapillarisin (−9.82), piperine (−9.45), ergometrine (−9.43), apigenin (−9.38), and artemisinin (−9.27). Acarbose had a binding energy of −17.58 kcal/mol. All the metabolites looked highly promising as α-amylase inhibitors and most of them interacted with PPA via hydrogen bonding with crucial amino acid residues: Asp197, Asp300, and Glu233. Thus, the acetone extract of *A. pallens* leaf and buds can potentially inhibit PPA (strong amino acid sequence similarity with human pancreatic α-amylase) and hence extrapolation of these inhibitory results could be valid for human pancreatic α-amylase as well.

Keywords: diabetes mellitus; α-amylase; in silico docking; *Artemisia pallens* Wall ex DC

Citation: Kulkarni, A.A.; Kamble, R.P. α-Amylase Inhibitory Secondary Metabolites from *Artemisia pallens* Wall ex DC—Biochemical and Docking Studies. *Biol. Life Sci. Forum* **2022**, *11*, 73. https://doi.org/10.3390/IECPS2021-11978

Academic Editor: Giedre Samuoliene

Published: 30 November 2021

1. Introduction

Diabetes Mellitus Type-2 (DM-2) is a lifestyle-induced metabolic disease. Prolonged high blood sugar level (chronic hyperglycaemia), dyslipidaemia, and impaired protein metabolism are the usual symptoms of this disease which leads to liver, pancreas, eye, nerve, and kidney damage. The worldwide survey by WHO [1], showed that globally 422 million people are affected by DM and it may soon become a leading cause of death by 2030. According to International Diabetes Federation, 72.9 million people in India are suffering from DM-2 [2]. These patients show impaired insulin production, secretion, activity of insulin receptors, or overall metabolism [3]. Many therapeutic treatments of DM-2 are in use and they trigger endogenous insulin secretion as well as stimulate the action of insulin at target organs or tissue. An additional target is to correct the imbalance of a number of metabolic enzymes in diabetic patients. Pancreatic alpha amylase plays a key role in the breakdown of starch and produces simple oligosaccharides. These simple oligosaccharides pass through the small intestine where these are acted upon by further

digestive enzymes and degraded into glucose which is absorbed into the bloodstream leading to post-prandial hyperglycemia. If phytochemicals are able to inhibit such digestive enzymes, the degradation of dietary starch to simple monomeric glucose is slowed down, preventing the harmful post-prandial glucose surge [4].

Synthetic drugs are widely used for the treatment of DM but they have negative side effects on humans. Hence, the search for new anti-hyperglycemic molecules of plant origin is still on; such phytochemicals are envisaged to have negligible side effects [5]. *Artemisia* is a large and economically important genus containing more than 500 species. Most of the species are perennial herbs with a few annual or biannual exceptions [6]. This genus came into limelight when Prof. Tu Youyou was awarded the Nobel Prize in Physiology or Medicine (2015) for conclusively showing that artemisinin is the effective secondary metabolite from *Artemisia annua* L., exhibiting strong anti-malarial activity against *Plasmodium* spp. [7]. In traditional herbal systems of medicine, this genus has also been used to treat diabetes for many years [8]. Nofal et al. observed that aqueous and ethanolic extracts of *A. judaica* significantly reduced blood glucose levels in diabetic rats but had no effect on normal rats [9]. Anaya-Eugenio et al. isolated eupatilin and salvinine from *A. ludoviciana* and fully revealed their hypoglycemic and anti-hyperglycemic effects along with enzyme-binding studies [10]. In Japan, *A. campestris* has been used as a folk medicine for liver and kidney complications. The investigation that was done by Aniya etal. proved that the aqueous extract of *A. campestris* possessed strong antioxidant and hepatoprotective activity [11]. One of the few reports about *A. dracunculoides* indicated the presence of coumarins [12]. Their anti-diabetic activity has been reported [13]. Apart from the α-amylases and α-glucosidases, other enzymes such as aldose reductases also play crucial role in glucose metabolism. The ethanolic extract of *A. dracunculus* exhibited excellent aldose reductase inhibitory activity. At the concentration of 3.75 µg/mL of extract, about 40% of the enzyme activity was retarded. It was quite comparable to the quercitrin (a known aldose reductase inhibitor) which retarded the enzyme activity by 54% at the same concentration [14].

Artemisia pallens is a small aromatic herb with excellent essential oil content. Few scientists have reported its anti-hyperglycemic activity. Hence there is still scope to explore this species for its therapeutic properties. In 1996, Subramoniam et al. reported the significant dose-dependent blood glucose lowering effect of methanol extract of aerial plant parts of *A. pallens* [15], but no active compounds were described. The present study was done to explore the anti-hyperglycemic potential of *A. pallens*. The crude acetone extract of the leaf and buds showed α-amylase inhibitory activity in primary starch-iodide assay. This was then quantified as IC_{50} value with DNSA assay. The secondary metabolites from the acetone crude extract were identified by the LC-MS technique. These results of α-amylase inhibition were then cross confirmed with in silico docking studies.

2. Methods

2.1. Collection of Plant Material

The plant material of *Artemisia pallens* Wall. ex DC. was collected from Jejuri, district Pune, Maharashtra (Latitude 18.292450° N and Longitude 74.144680° E). The plant was identified and authenticated by Botanical Survey of India, Western regional centre, Pune-7. (Voucher number: BSI/WEC/Iden.Cer./2021/1603210007747).

2.2. Preparation of Plant Extracts

Fresh leaves and buds were cleaned and used for extraction. The extracts were prepared by a hot extraction method i.e., by using Soxhlet apparatus (Borosil Ltd., Mumbai, India). For extraction, 50 g of cleaned fresh leaf and buds were weighed and wrapped in a muslin cloth. The sequential extraction was carried out with 250 mL of each of six different solvents with ascending polarity such as pet ether, chloroform, ethyl acetate, acetone, methanol and water. A total of three consecutive hot extraction cycles were run for each solvent. Each of the solvent extracts were collected separately, filtered, and then

all the extracts were dried under reduced pressure with the help of the rotary vacuum evaporator (Buchi Labortechnik, Essen, Germany) to yield crude powder extracts for further experiments. These concentrated extracts were then stored at 4 °C until further use.

2.3. Qualitative Phytochemical Analysis of Extracts

The phytochemical screening of six different extracts were carried out by standard methods that were reported by Trease and Evans for the presence of alkaloids, tannins, saponins, glycosides, steroids, flavonoids, phenols, terpenoids, and quinines [16].

2.4. α-Amylase Inhibition Assay

The standard assay that was reported by Sudha et al. was referred to perform the α-amylase inhibition assay [17]. For the primary screening of the inhibitor from the extracts, pure α-amylase enzyme from porcine pancreas (PPA) (Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India, Catalogue No. A6255) was used. The assay was based on a color reaction that was produced by starch iodine complex, also known as starch-iodide assay. The assay mixture consisted of phosphate buffer (pH 6.9 containing 6.7 mM sodium chloride), 0.2 units of porcine pancreatic amylase (PPA) solution and 20 µL of plant extract in DMSO (1 mg/mL *w*/*v*). The reaction mixture was incubated at 37 °C for 15 min. Then soluble starch (1%, *w*/*v*) was added to each reaction well and again incubated at 37 °C for 15 min. 20 µL of 0.2 M Tris solution was used to stop the enzymatic reaction and then 20 µL of Iodine reagent was added. The color change of the reaction mixture was observed. The positive drug/substrate control (SC) was a synthetic, known PPA inhibitor, Acarbose (Glucobay tablets, Bayer AG, Leverkrusen, Germany).

2.5. 3,5-Dinitrosalicylic Acid (DNSA) Assay

The inhibition assay was executed by using a method that was described by Miller [18] with slight modifications. The total assay mixture that was composed of 500 µLof 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.2 units of PPA solution and extract of concentration 2.5 mg/mL (25, 50, 75, 100 and 125 µL). All the reactants were incubated at 37 °C for 10 min. After this pre-incubation, 500 µL of 1% (*w*/*v*) starch solution in the above buffer was added to each tube and incubated further at 37 °C for 15 min. The reaction was terminated with the addition of 1.0 mL DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature, diluted, and the absorbance was measured at 540 nm. The control PPA at 0.2 U·mL^{-1} represented 100% enzyme activity and did not contain any plant extract. To eliminate the absorbance that was produced by plant extract, appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included. The known PPA inhibitor, Acarbose, was used as a positive control. The other quantifiers were calculated as follows:

$$\% \text{ Relative PPA activity} = (\text{enzyme activity of test/enzyme activity of control}) \times 100 \quad (1)$$

$$\% \text{ inhibition in the PPA activity} = (100 - \% \text{ relative enzyme activity}). \quad (2)$$

2.6. Determination of Total Flavonoid Content of Acetone Extract

For determination of the total flavonoid content, aluminum chloride colorimetric method was followed with slight modifications [19]. The stock solution of 5 mg/mL of acetone extract was used. The stock solution of acetone leaf extract (25–125 µL) was mixed with 1.25 mL distilled water and 75 µL 5% $NaNO_2$ solution prior to a 5 min incubation period. A total of 150 µL of 10% $AlCl_3$ was added and again the test solution was incubated for 6 min. Then 500 µL of 1 M NaOH and 275 µL distilled water was added to the test solution. The absorbance was read at 510 nm using UV-VIS spectrophotometer (UV-1800, SHIMADZU, Kyoto, Japan). The total flavonoid content was calculated using a calibration curve. The results were expressed as a Quercetin (Sigma, St. Louis, MO, USA) equivalent in µg/mg of sample.

2.7. Determination of Total Phenol Content of Acetone Extract

The total phenol content was determined by the Folin–Ciocalteu method with slight modifications [19]. A total of 5 mg/mL stock solution of acetone extract was prepared. From this sample, 10–50 µL was then mixed with 0.5 mL of a 10-fold diluted Folin–Ciocalteu reagent (HiMedia Laboratories Pvt. Ltd., Mumbai, India) and 1 mL of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 min at room temperature. Then the absorbance was read at 760 nm spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan). The total phenolic content was calculated using a calibration curve. The concentration of phenolic content was expressed as gallic acid (Sigma-Aldrich Inc., St. Louis, MO, USA) equivalent in µg/mg of sample.

2.8. Determination of Total Terpenoid Content of Acetone Extract

The total terpenoid content was determined according to Wei et al. with slight modifications [20]. A total of 5 mg/mL stock solution of acetone extract was prepared and a sample from this (10–50 µL) was mixed with 500 µL of 5% (*w*/*v*) vanillin-acetic solution and 300 µL sulfuric acid. This mix was incubated at 70 °C for 30 min. Then the mixed solution was cooled and diluted to 3.25 mL with acetic acid. Then the absorbance was read at 573 nm spectrophotometrically (UV-1800, Shimadzu, Kyoto, Japan). The blank consisted of all the reagents and solvents without sample solution. The concentration of the terpenoid content was determined using the standard Ursolic Acid (Sigma-Aldrich Inc., St. Louis, MO, USA) calibration curve.

2.9. LC-MS/MS Analysis of Acetone Extract

The LC-MS/MS method was developed and used for identification of secondary metabolites that were present in the crude acetone extracts of leaf and buds of *A. pallens* in this study. All the MS acquisitions were performed in the positive electrospray ionization mode. The capillary voltage, cone voltage, and fragmenter voltage were 4 kV, 45 V, and 170 V, respectively. The gas temperature was set at 325 °C. The data were acquired at a scan rate of 3 Hz in mass range of 100–1000 *m*/*z*. Further data was analyzed with Mass Hunter qualitative software and METLIN database.

The LC- MS/MS experiments were performed on Agilent 1260 binary LC System (Agilent Technol. Inc., Santa Clara, CA, USA). An Agilent Zorbax Extend C18 RRHT column (50 × 2.1 mm, 1.8 µm) was used. The mobile phase was composed of (A) water + 0.1% formic acid and (B) acetonitrile. A gradient program was set as: 0–5 min (95% A: 5% B), 5–18 min (5% A: 95% B), 18–27 min (5% A: 95% B), 27–27.10 min (95% A: 5% B), and 27.10–30 min (95% A: 5% B). The flow rate of the mobile phase was 0.30 mL/min. The injection volume was 1 µL. The compounds were characterized by their MS, MS/MS spectra, as well as UV spectra.

2.10. Molecular Docking Studies

2.10.1. Softwares and Tools

The Protein Data Bank (PDB), PubChem, Discovery Studio Visualizer, and AutoDock Version 4.2.6 were used.

2.10.2. Ligand Preparation

The secondary metabolites that were detected in the LC-MS analysis were used as the ligands for docking studies. Their 3-D structures were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 16 March 2021) database in SDF format. This SDF format was converted into PDB format using Discovery Studio Visualizer software.

2.10.3. Protein Preparation

The crystal structure of PPA (PDB ID: 1OSE) was obtained from the Protein Data Bank (www.rcsb.org Accessed on: 16 March 2021) and further prepared for docking by removing water molecules and ligand molecules which co-crystallized at protein structure and active site. Polar hydrogen atom and Kollman charges were added to protein by using AutoDock Version 4.2.6 program. This complex was saved as a pdbqt format file for docking.

2.10.4. Docking Studies

For molecular docking studies, the site where acarbose got bound to the 1OSE, was selected as the PPA active site and a grid box was set at the centre of the co-crystallized ligand with dimension of 45 Å × 40 Å × 44 Å (X: 37.909; Y: 38.049; Z: −1.869) with a grid spacing of 0.375 Å.

2.10.5. Protein-Ligand Interactions

Discovery studio software (https://discover.3ds.com/discovery-studiovisualizer-download, accessed on 18 March 2021) was used to visualize the docked conformations of the ligand-protein complex. The lowest binding energy that was showed by the ligand molecules indicated the highest binding affinity for PPA.

3. Results

3.1. Qualitative Phytochemical Analysis of A. pallens Leaf and Buds Extracts

Qualitative phytochemical analysis was done for all the six extracts. In the present work, we are presenting the data that are related to acetone extract, as it showed the best PPA inhibition. Acetone extract showed presence of tannins, flavonoids, phenols, terpenes, and quinines.

3.2. Determination of Total Phytochemicals Content

The total flavonoid, phenol, and terpenoid content that was calculated by using calibration curves of respective standards was 275.5 ± 0.01 µg/mg, 9.1 ± 0.004 µg/mg, and 68.5 ± 0.01 µg/mg dry weight of leaf and bud acetone extract, respectively (detailed data not shown).

3.3. LC-MS/MS Analysis of Leaf and Bud Acetone Extract

The identification of secondary metabolites in leaf and bud acetone extract was carried out by LC-MS/MS. This revealed the presence of total 24 secondary metabolites belonging to different classes. Among these, 10 compounds were selected for insilico study. These would be discussed in detail.

3.4. Qualitative Starch-Iodide Assay for PPA Inhibition

Qualitative screening (color reaction) of PPA inhibition was performed based on starch-iodine complex formation. In this assay, among the six prepared extracts, petroleum ether, chloroform, ethyl acetate, acetone, and methanol extracts showed PPA inhibition (Figure 1). The blue color of the starch-iodide complex due to acetone extract was persistent even after 24 h. Therefore, acetone extract was carried forward for further analysis.

Figure 1. PPA inhibitory activity of various plant extracts using qualitative starch iodide assay. B: Blank, C: Control, EC: Enzyme Control, SC: Substrate Control (drug/Acarbose), 1: Pet Ether, 2: Chloroform, 3: Ethyl Acetate, 4: Acetone, 5: Methanol, 6: Water Extract. The three numbered rows indicate assays done in triplicate.

3.5. Quantitative DNSA Assay for PPA Inhibition

The crude acetone extract of *A. pallens* showed persistent inhibition of PPA qualitatively. The DNSA assay was performed to measure the PPA inhibition quantitatively. A dose-dependent percent inhibition activity of PPA was observed due to acetone leaf and bud extract. PPA inhibition increased as the concentration of the acetone extract increased. The calculated IC_{50} value for plant extract was 338.05 µg/mL. The increasing concentrations of plant extract as 62.5 µg/mL, 125 µg/mL, 187.5 µg/mL, 250 µg/mL and 312.5 µg/mL showed 28.36%, 35.05%, 38.93%, 43.45%, and 46.19% inhibition activity respectively (Figure 2).

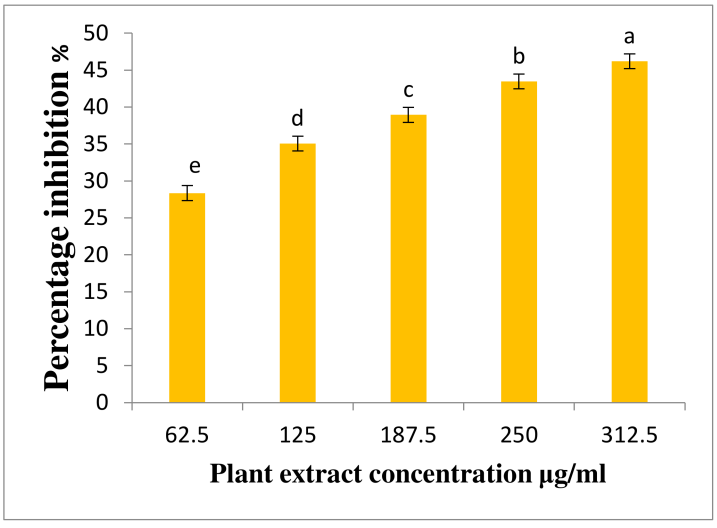


Figure 2. The percent inhibition of PPA in the presence of different concentrations of acetone leaf and bud extract. The data is represented as the mean ± SEM; ($n - 3$). A one way ANOVA test was used and the error bars with superscripts a,b,c,d,e show significant difference with respect to the control ($p < 0.05$).

3.6. Molecular Docking Studies

Ligand Preparation

The 3D ligand structures of 10 compounds were obtained from the PubChem database in SDF format and converted to PDB format using Discovery Studio software (Figure 3). The structures were saved in PDBQT format.

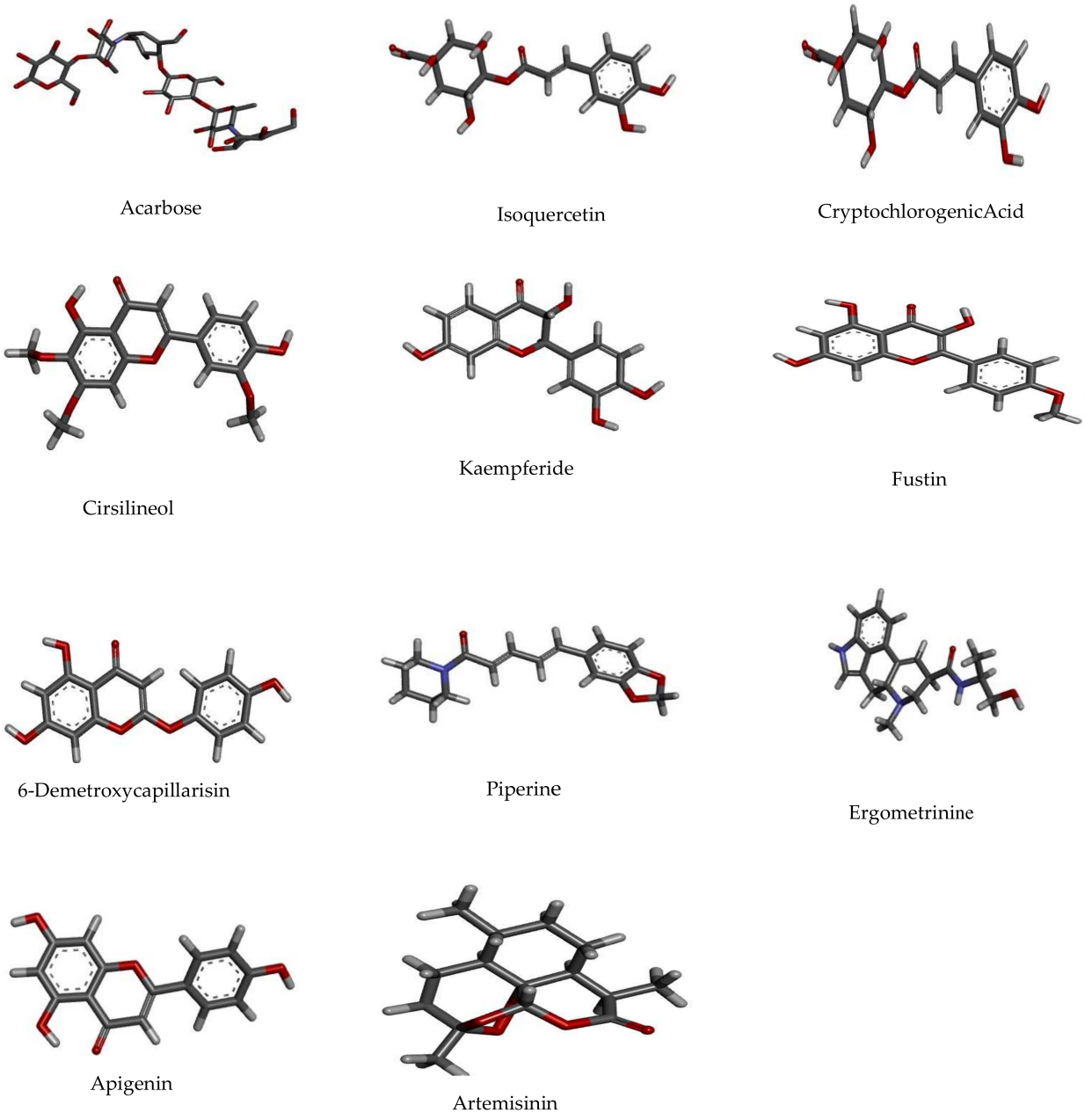


Figure 3. The 3-D ligand structures of 10 secondary metabolites from acetone leaf and bud extract of *A. pallens* that were used in docking studies for targeting PPA. (Acarbose is the drug/positive control).

3.7. Protein Preparation

The crystal structure of PPA (PDB ID: 1OSE) was obtained from the Protein Data Bank. The protein structure prepared by using AutoDock 4.2.6 program (Figure 4).

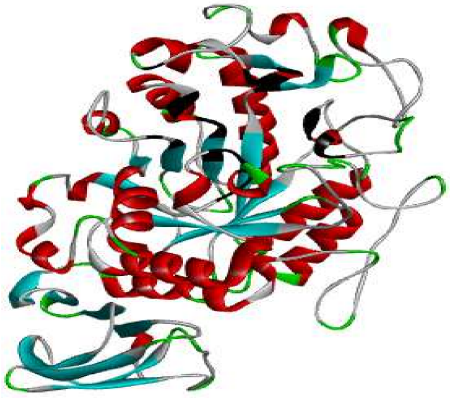


Figure 4. The prepared protein structure of PPA.

3.8. Docking Studies by Using AutoDock 4.2.6

The results are shown in Table 1 indicating the docking scores for the 10 selected ligands that were bound with PPA. The binding energies of ligands with PPA enzyme are represented as kcal/mol. Docking studies were done in comparison with positive control acarbose.

Table 1. The Binding Energy of Ligands and Acarbose with PPA.

No.	Pubchem ID	Compound Name	Binding Energy Kcal/mol	Interaction	Amino Acid Residues
1	41774	Acarbose	−17.58	5-H Bonds	Tyr59, Arg195, His201, **Glu233, Asp300**
				Van Der Waal	Trp58, Tyr62, Tyr151, **Asp197**, Lys200, Ile235, Gly306, His305, Ala307
2	5280804	Isoquercetin	−11.57	4-HBond	Trp59, Gln63, **Glu233**, Gly306
				Van Der Waal	Trp58, Arg195, His299
				Pi-Pi	His201, Ile235, Leu162, Val163, Ala198
3	9798666	Cryptochlorogenic Acid	−11.17	3-H Bonds	Trp59, Tyr62, Tyr151
				Van Der Waal	Glu60, Gln63, Trp58, Val163, Leu165, His101, Arg195, **Asp197**, Lys200, His201, Ile235, His299, **Asp300**
4	162464	Cirsilineol	−10.24	1-H Bond	Tyr62
				Van Der Waal	Gln63, Val98, His101, Tyr151, Val163, Ser199, Val234
				Pi-Pi	His201
5	5281666	Kaempferide	−9.99	4-H Bonds	Gln63, **Glu233, His299, Asp300**
				Van Der Waal	Trp58, Leu162, Arg195
				Pi-Pi	Trp59, Tyr62
6	5317435	Fustin	−9.86	4-H Bonds	Trp62, Leu162, **Asp197, Asp197**
7	5316511	6-Demethoxy-capillarisin	−9.82	4-H Bond	His101, Val163, **Asp197, Asp300**
				Van Der Waal	Trp58, Trp59, Gln63, Leu162
				Pi-Pi	Tyr62, Lue165, Ala198
8	638024	Piperine	−9.45	Van Der Waal	Gln63, His101, Val163, Leu165, **Asp197**, Ser199, Val234
				Pi-Pi	His201

Table 1. *Cont.*

No.	Pubchem ID	Compound Name	Binding Energy Kcal/mol	Interaction	Amino Acid Residues
9	443884	Ergometrine	−9.43	3-H Bond	**Asp197, Glu233, Asp300**
				Van Der Waal	Trp58, Arg195
				Pi-Pi	Tyr62, His201, Ala198, Lys200
10	5280443	Apigenin	−9.38	3 H Bonds	Gln63, **Glu233, His299**
				Van Der Waal	Trp58, Leu162, Val163, Leu165, Arg195, His201
				Pi-Pi	Tyr62, Trp59
11	68827	Artemisinin	−9.27	H bond	Gln63
				Van Der Waal	**Asp300**, Asp197, Alu162, Alu165
				Pi-Pi	Trp59, Tyr62, His101, Val163, His305

4. Discussion

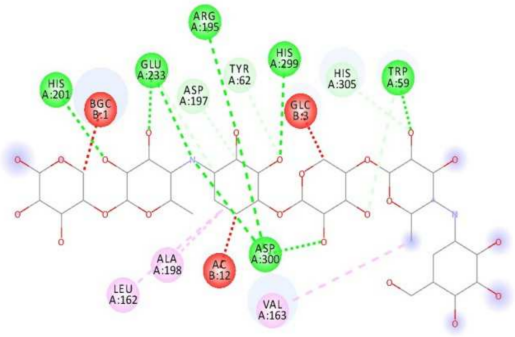
For last few decades, secondary metabolites have been investigated for their therapeutic properties. They have proved effective against many diseases such as cancer, arthritis, malaria, Alzheimer's, and diabetes [21–24].

In this study, 10 compounds belonging to different secondary metabolite classes were studied for their PPA inhibitory activity. These were isolated from crude acetone extract of flower leaf and buds of *A. pallens*. A previous study of methanol extract of aerial parts of *A. pallens* reported total phenolic and flavonoid content as 127.16 µg/mg and 13.57 µg/mg, respectively [25]. In our study, the total phenol, flavonoid, and terpene content of the acetone extract of leaf and buds was 9.1 ± 0.004 µg/mg, 275.5 ± 0.01 µg/mg, and 68.5 ± 0.01 µg/mg, respectively; that was quite different from the previous study.

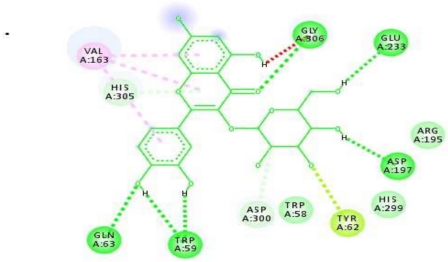
LC-MS/MS analysis identified the presence of total 24 major secondary metabolites. Among them, 10 compounds showed good affinity and binding score for PPA. These 10 compounds were selected for detailed in-silico study.

A-amylase uses active site carboxylic acid to catalyze its hydrolytic reaction. Among the amino acid residues that are present at the active site, Asp197, Glu233, and Asp300 are proven for their importance in catalytic activity. These are completely conserved and essential amino acids for the efficient hydrolysis of oligosaccharides [26]. Rydberg et al. reported that amino acid residue Asp197 is a catalytic nucleophile and Glu233 is a general acid catalyst by using site-directed mutagenesis and kinetic and structure determination techniques [27]. They also suggested that Asp300 plays a key role in the catalysis of amylase. It is also seen our docking studies that the catalytic amino acid residue Asp197 is involved in an interaction with all the 10 selected secondary metabolite ligands.

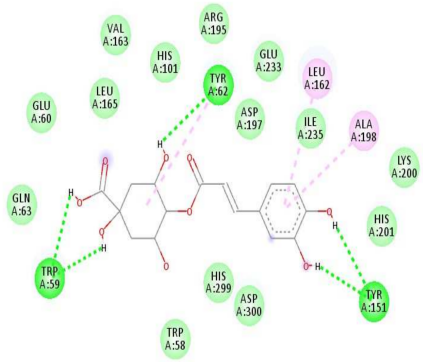
In the literature, the flavonoid class of compounds is shown to be the potent α-amylase inhibitor [28] due to unique structure. The ability of flavonoids to inhibit PPA is based on the formation of conjugated π-system which maintains the stability of the receptor-ligand complex [29]. In the present study, we observed that in molecular docking study, secondary metabolites belonging to the flavonoid group such as, isoquercetin, cirsilineol, apigenin, fustin, and kaempferide formed π-interactions as well as hydrogen bonds (Figure 5) with different amino acid residues present at the active site of PPA enzyme, stabilizing the interactions.



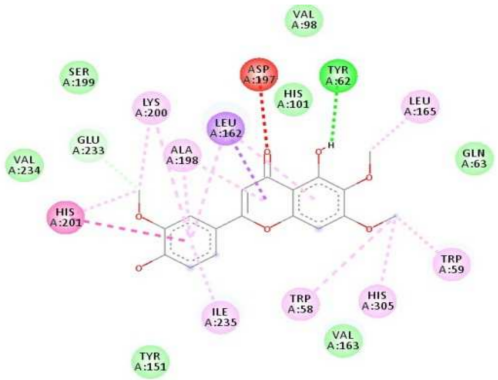
(A) Acarbose



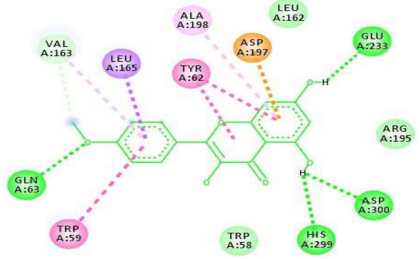
(B) Isoquercetin



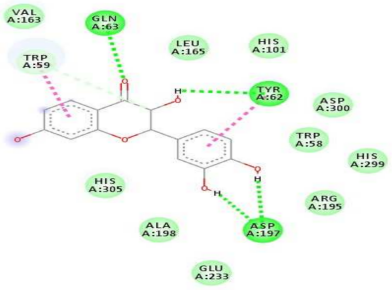
(C) Cryptochlorogenic Acid



(D) Cirsilineol

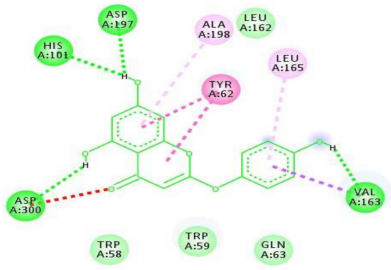


(E) Kaempferide

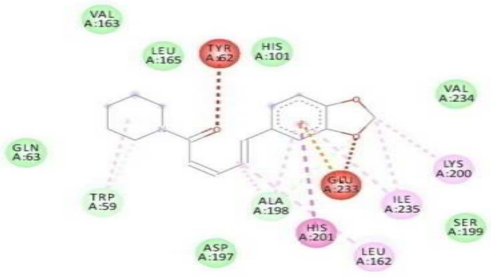


(F) Fustin

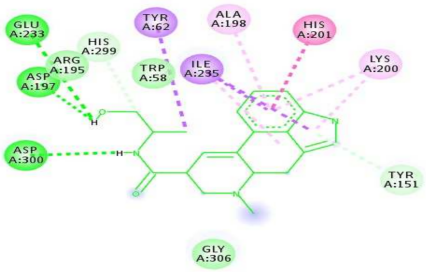
Figure 5. *Cont.*



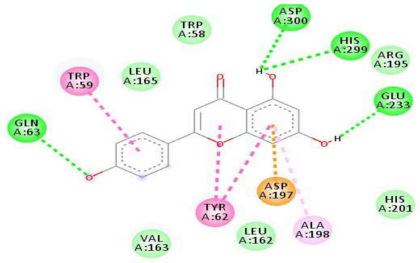
(**G**) **6-Demetroxy-capillarisin**



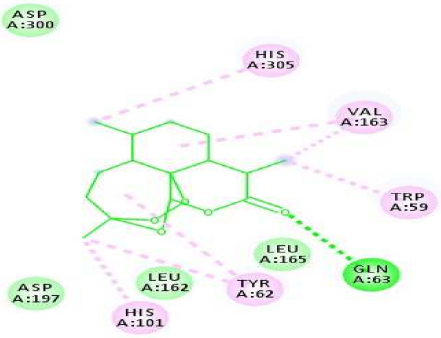
(**H**) **Piperine**



(**I**) **Ergometrine**



(**J**) **Apigenin**



(**K**) **Artemisinin**

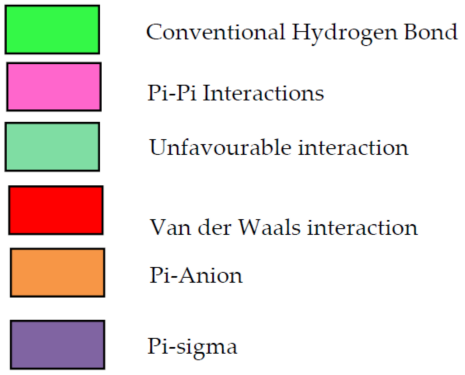


Figure 5. 2-D Representation of PPA protein-ligand interactions. (**A**) Acarbose (**B**) Isoquercetin (**C**) Cryptochlorogenic Acid (**D**) Cirsilineol (**E**) Kaempferide (**F**) Fustin (**G**) Demetroxycapillarisin (**H**) Piperine (**I**) Ergometrine (**J**) Apigenin (**K**) Artrmisinin. The neon green color represents conventional hydrogen bond. The light green colour represents Van der Waals interaction. The dark and light pink colors represent Pi-Pi interactions. The yellow colour represents Pi-Anion and the red colour represents unfavorable interactions.

Few studies have also investigated various alkaloids for their anti-diabetic potential. These alkaloids could inhibit α-amylases and α-glucosidase enzymes [30,31]. The alkaloid Ergometrine (Figure 5I) studied here also showed good binding energy of −9.43 kcal/mol with involvement of Asp197 in the hydrogen bonding. Piperine, a well-known alkaloid (Figure 5H) interacted with PPA through key amino acids and retarded the enzyme action [32]. Our docking results with Piperine also showed that it interacted with crucial amino acid Asp197 through van der Waals interactions.

Previous studies showed that chlorogenic acid, an isomer of cryptochlorogenic acid reported here, exhibited a good level of PPA inhibition resulting in low post-prandial hyperglycemia [33]. In our study too, cryptochlorogenicacid (Figure 5C) showed a binding energy of −11.17 kcal/mol which could form stable receptor-ligand complex. The terpene

class of secondary metabolites have shown good alpha-amylase inhibitory potential [34]. Another terpene, artemisinin (Figure 5K) formed π-interactions and hydrogen bonds while interacting with PPA, suggesting a stable receptor-ligand complex development.

A. dracunculoides contained coumarins [12] and in the literature, coumarins have been reported for their alpha-amylase inhibitory property. The identified coumarin: 6-Demethoxycapillarisin interacted via hydrogen bonding (Figure 5G) as well as π interaction with PPA in our studies, thus proving earlier studies.

5. Conclusions

A rapid and efficient method involving LC-MS/MS analysis and molecular docking was established and effectively used for the screening of α-amylase inhibitors from *A. pallens*. A total of 24 major compounds were identified from acetone extract of *A. pallens* by LC-MS profiling and among them, 10 compounds were found to have good binding affinity for α-amylase as determined by molecular docking. All the ligand molecules interacted with the important catalytic residues Asp197, Glu233, and Asp300 of PPA through hydrogen bonds, van der Waal forces, and pi-pi interactions. The present work reveals a new insight towards the α-amylase inhibitory secondary metabolites of *A. pallens* which might be useful for application in functional therapeutics for management of DM-2.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECPS2021-11978/s1. Poster: α-Amylase Inhibitory Secondary Metabolites from Artemi-sia pallens Wall ex DC—Biochemical and Docking Studies.

Author Contributions: A.A.K. established the workflow, designed the experiments, standardized the experimental protocols, contributed in results analysis and manuscript writing. R.P.K. performed the in vitro and in silico experiments and contributed in manuscript writing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data is presented in the manuscript.

Acknowledgments: R.P.K. would like to acknowledge CSIR-UGC for providing research fellowship.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

DM2	Diabetes mellitus Type-II
LC-MS	Liquid Chromatography-Mass Spectrometry
PPA	Porcine Pancreatic Amylase
DMSO	Dimethyl Sulfoxide
SC	Substrate Control
DNSA	3,5-Dinitrosalicylic acid

References

1. World Health Organization (WHO). Available online: https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed on 5 November 2020).
2. Cho, N.H.; Shaw, J.E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J.D.; Ohlrogge, A.W.; Malanda, B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* **2018**, *138*, 271–281. [CrossRef] [PubMed]
3. Bogan, J.S. Regulation of Glucose Transporter Translocation in Health and Diabetes. *Annu. Rev. Biochem.* **2012**, *81*, 507–532. [CrossRef] [PubMed]
4. Tarling, C.A.; Woods, K.; Zhang, R.; Brastianos, H.C.; Brayer, G.D.; Andersen, R.J.; Withers, S.G. The search for novel human pancreatic α-amylase inhibitors: High-throughput screening of terrestrial and marine natural product extracts. *ChemBioChem* **2008**, *9*, 433–438. [CrossRef] [PubMed]

5. Grover, J.; Yadav, S.; Vats, V. Medicinal plants of India with anti-diabetic potential. *J. Ethnopharmacol.* **2002**, *81*, 81–100. [CrossRef]
6. Hayat, M.Q.; Ashraf, M.; Khan, M.A.; Yasmin, G.; Shaheen, N.; Jabeen, S. Diversity of foliar trichomes and their systematic implications in the genus *Artemisia* (Asteraceae). *Int. J. Agric. Biol.* **2009**, *11*, 542–546.
7. Su, X.-Z.; Miller, L.H. The discovery of artemisinin and the Nobel Prize in Physiology or Medicine. *Sci. China Life Sci.* **2015**, *58*, 1175–1179. [CrossRef]
8. Islam, N.; Jung, H.A.; Sohn, H.S.; Kim, H.M.; Choi, J.S. Potent α-glucosidase and protein tyrosine phosphatase 1B inhibitors from *Artemisia capillaris*. *Arch. Pharmacal Res.* **2013**, *36*, 542–552. [CrossRef]
9. Nofal, S.M.; Mahmoud, S.S.; Ramadan, A.; Soliman, G.A.; Fawzy, R. Anti-diabetic effect of *Artemisia judaica* extracts. *Res. J. Med. Med. Sci.* **2009**, *4*, 42–48.
10. Anaya-Eugenio, G.D.; Rivero-Cruz, I.; Rivera-Chávez, J.; Mata, R. Hypoglycemic properties of some preparations and compounds from *Artemisia ludoviciana* Nutt. *J. Ethnopharmacol.* **2014**, *155*, 416–425. [CrossRef]
11. Aniya, Y.; Shimabukuro, M.; Shimoji, M.; Kohatsu, M.; Gyamfi, M.A.; Miyagi, C.; Kunii, D.; Takayama, F.; Egashira, T. Antioxidant and hepatoprotective actions of the medicinal herb *Artemisia campestris* from the Okinawa Islands. *Biol. Pharm. Bull.* **2000**, *23*, 309–312. [CrossRef]
12. Herz, W.; Bhat, S.; Santhanam, P. Coumarins of *Artemisia dracunculoides* and 3′,6-dimethoxy-4′,5,7-trihydroxyflavone in *A. arctica*. *Phytochemistry* **1970**, *9*, 891–894. [CrossRef]
13. Li, H.; Yao, Y.; Li, L. Coumarins as potential antidiabetic agents. *J. Pharm. Pharmacol.* **2017**, *69*, 1253–1264. [CrossRef] [PubMed]
14. Logendra, S.; Ribnicky, D.M.; Yang, H.; Poulev, A.; Ma, J.; Kennelly, E.J.; Raskin, I. Bioassay-guided isolation of aldose reductase inhibitors from Artemisia dracunculus. *Phytochemistry* **2006**, *67*, 1539–1546. [CrossRef] [PubMed]
15. Subramoniam, A.; Pushpangadan, P.; Rajasekharan, S.; Evans, D.; Latha, P.; Valsaraj, R. Effects of *Artemisia pallens* Wall. on blood glucose levels in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.* **1996**, *50*, 13–17. [CrossRef]
16. Trease, G.E.; Evans, W.C. *Pharmacognosy*, 15th ed.; Saunders Publishers: London, UK, 2002; pp. 42–44.
17. Sudha, P.; Zinjarde, S.S.; Bhargava, S.Y.; Kumar, A.R. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. *BMC Complement. Altern. Med.* **2011**, *11*, 5. [CrossRef]
18. Miller, G.L. Use of Dinitrosalicylic Acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
19. Baba, S.A.; Malik, S.A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *J. Taibah Univ. Sci.* **2015**, *9*, 449–454. [CrossRef]
20. Wei, L.; Zhang, W.; Yin, L.; Yan, F.; Xu, Y.; Chen, F. Extraction optimization of total triterpenoids from *Jatropha curcas* leaves using response surface methodology and evaluations of their antimicrobial and antioxidant capacities. *Electron. J. Biotechnol.* **2015**, *18*, 88–95. [CrossRef]
21. Fakhri, S.; Moradi, S.Z.; Farzaei, M.H.; Bishayee, A. Modulation of dysregulated cancer metabolism by plant secondary metabolites: A mechanistic review. In *Seminars in Cancer Biology*; Academic Press: Cambridge, MA, USA, 2020. [CrossRef]
22. Saleem, S.; Khan, R.; Kazmi, I.; Afzal, M. Medicinal plants in the treatment of arthritis. In *Plant and Human Health*; Springer: Cham, Switzerland, 2019; Volume 3, pp. 101–137. [CrossRef]
23. Hien, D.F.D.S.; Dabiré, K.R.; Roche, B.; Diabate, A.; Yerbanga, R.S.; Cohuet, A.; Yameogo, B.K.; Gouagna, L.-C.; Hopkins, R.; Ouedraogo, G.A.; et al. Plant-mediated effects on mosquito capacity to transmit human malaria. *PLoSPathog.* **2016**, *12*, e1005773. [CrossRef]
24. Ota, A.; Ulrih, N.P. An overview of herbal products and secondary metabolites used for management of Type Two Diabetes. *Front. Pharmacol.* **2017**, *8*, 436. [CrossRef]
25. Ruikar, A.D.; Khatiwora, E.; Ghayal, N.A.; Misar, A.V.; Mujumdar, A.M.; Puranik, V.G.; Deshpande, N.R. Studies on aerial parts of *Artemisia pallens* Wall for phenol, flavonoid and evaluation of antioxidant activity. *J. Pharm. Bioallied Sci.* **2011**, *3*, 302–305. [CrossRef]
26. Svensson, B.; Søgaard, M. Mutational analysis of glycosylase function. *J. Biotechnol.* **1993**, *29*, 1–37. [CrossRef]
27. Rydberg, E.H.; Li, C.; Maurus, R.; Overall, C.M.; Brayer, G.D.; Withers, S.G. Mechanistic analyses of catalysis in human pancreatic α-amylase: Detailed kinetic and structural studies of mutants of three conserved carboxylic acids. *Biochemistry* **2002**, *41*, 4492–4502. [CrossRef] [PubMed]
28. Tadera, K.; Minami, Y.; Takamatsu, K.; Matsuoka, T. Inhibition of α-glucosidase and α-amylase by flavonoids. *J. Nutr. Sci. Vitaminol.* **2006**, *52*, 149–153. [CrossRef] [PubMed]
29. Piparo, E.L.; Scheib, H.; Frei, N.; Williamson, G.; Grigorov, M.; Chou, C.J. Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *J. Med. Chem.* **2008**, *51*, 3555–3561. [CrossRef]
30. Kidane, Y.; Bokrezion, T.; Mebrahtu, J.; Mehari, M.; Gebreab, Y.B.; Fessehaye, N.; Achila, O.O. In Vitro inhibition of α-amylase and α-glucosidase by extracts from *Psiadia punctulata* and *Meriandra bengalensis*. *Evid.-Based Complement. Altern. Med.* **2018**, *2018*, 2164345. [CrossRef]
31. Yin, Z.; Zhang, W.; Feng, F.; Zhang, Y.; Kang, W. α-glucosidase inhibitors isolated from medicinal plants. *Food Sci. Hum. Wellness* **2014**, *3*, 136–174. [CrossRef]
32. Magaña-Barajas, E.; Buitimea-Cantúa, G.V.; Hernández-Morales, A.; Torres-Pelayo, V.D.R.; Vázquez-Martínez, J.; Buitimea-Cantúa, N.E. In vitro α-amylase and α-glucosidase enzyme inhibition and antioxidant activity by capsaicin and piperine from *Capsicum chinense* and *Piper nigrum* fruits. *J. Environ. Sci. Health Part B* **2021**, *56*, 282–291. [CrossRef]

33. Zheng, Y.; Yang, W.; Sun, W.; Chen, S.; Liu, D.; Kong, X.; Tian, J.; Ye, X. Inhibition of porcine pancreatic α-amylase activity by chlorogenic acid. *J. Funct. Foods* **2019**, *64*, 103587. [CrossRef]
34. Jelenkovic, L.; Jovanovic, V.; Palic, I.; Mitic, V.; Radulovic, M. In vitro screening of α-amylase inhibition by selected terpenes from essential oils. *Trop. J. Pharm. Res.* **2014**, *13*, 1421. [CrossRef]