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Phytochemical profiling and antidiabetic evaluation of *Peperomia pellucida* as a potential alpha-glucosidase inhibitor

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Abstract. *Hidayati S, Agustin AT, Sari EK, Sari SM, Destiawan RA, Silvana WA. 2023. Phytochemical profiling and antidiabetic evaluation of* Peperomia pellucida *as a potential alpha-glucosidase inhibitor. Biodiversitas 24: 5972-5978.* One strategy in post-prandial hyperglycemic control can be done by inhibiting the digestion of dietary carbohydrates through the inhibition of alpha-glucosidase enzymes. This study was conducted to determine the antidiabetic activity of *Peperomia pellucida* in inhibiting the alpha-glucosidase. An inhibitory activity analysis test was carried out on alpha-glucosidase in vitro and in silico using alpha-glucosidase. Molecular docking between receptors and ligands was performed using Hex 8.0 software. The setting column is set in Shape+Electro+DARS mode. The results showed ethanol extract and ethyl acetate fraction in vitro showed the ability to inhibit the activity of alpha-glucosidase enzyme with C50 values of 13.43 mg/mL and 9.73 mg/mL respectively with IC50 positive control acarbose values of 8.11 mg/mL. The results of in silico analysis showed that the Patuloside A component was able to bind to the binding site of alpha-glucosidase and gave the smallest binding energy value with a value of -321.4 kcal/mol compared to isovitexin, isoswertisin, pellucidatin, and caryatin-7-O-β-rhamnoside compounds. *P. pellucida* has the potential to be developed as an antidiabetic agent that has activity in inhibiting the work of the enzyme alpha-glucosidase so that it can reduce blood glucose levels.

Keywords: Alpha-glucosidase, antidiabetic, in silico, in vitro, *Peperomia pellucida*

INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic condition, characterized by an increase in blood glucose levels with a heterogeneous clinical condition and disease development. This condition causes delays in diagnosis, some pathophysiological abnormalities, and varying susceptibility to complications. Complications of type 2 diabetes can be classified into microvascular complications, such as retinopathy, neuropathy, and nephropathy, or macrovascular complications, including cardiovascular, cerebrovascular, and peripheral vascular diseases (Mansour et al. 2023). Diabetes is generally caused by chronic inflammation and apoptosis of the islets of Langerhans of the pancreas. Chronic damage caused by oxidative and continuous inflammatory activity in tissues will lead to diabetes complications including neuropathy, retinopathy, nephropathy and foot ulcers (Giri et al. 2018).

The sudden increase in blood glucose, which causes hyperglycemic in type 2 diabetes occurs due to the hydrolysis of starch by alpha-amylase and absorption in the intestine by alpha-glucosidase (Proença et al. 2022). One strategy in post-prandial hyperglycemic control can be done by inhibiting the digestion of dietary carbohydrates through inhibition of alpha-amylase and alpha-glucosidase enzymes (Pasmans et al. 2022). Pancreatic alpha-amylase (EC 3.2.1.1) is a key enzyme that breaks down carbohydrate-like foods as starch into simple monosaccharides in the digestive system. It is further degraded by alpha-glucosidase into glucose which, upon absorption, enters the bloodstream. Therefore, inhibiting alpha-amylase and alpha-glucosidase enzymes can suppress carbohydrate digestion, delay glucose uptake and consequently, lower blood sugar levels (Alqahtani et al. 2020).

Many herbal remedies are recommended as alternative treatments for diabetes. Traditional herbal medicine is used all over the world to treat various symptoms of diabetes. Traditional medicine is believed to be safer when compared to synthetic conventional drugs. This is evidenced by the few side effects that arise from the use of traditional medicine. In addition, traditional medicine is also seen as more economical, so this encourages health organizations and research in general to conduct testing to make natural medicine an alternative to synthetic drugs. Another advantage of using natural medicine is that it often has a synergistic effect as many plants have been shown to have more than one pharmacological effect, making it suitable for use in the treatment of metabolic and degenerative diseases. The disadvantages of traditional medicine are weak pharmacological effects, non-standard raw materials and lack of research to guarantee the effectiveness and safety of drug dosages. Therefore, the study of compounds obtained from traditional medicinal plants is becoming increasingly important (Yuan et al. 2016).

Since the 1990s, anti-diabetic drugs with glucosidase-inhibitory abilities, such as acarbose, miglitol and voglibose, have been commercially available for the treatment of postprandial hyperglycemia. Because its molecular structure is comparable to that of disaccharides or oligosaccharides, such antidiabetic drugs can bind to the binding enzyme alpha-glucosidase. The complexes resulting from such binding have a higher value of affinity than carbohydrate-glucosidase complexes, which consequently causes delays in the digestion and absorption of carbohydrates and thus reduces postprandial hyperglycemia. Despite this, repeated consumption of them leads to flatulence, severe abdominal discomfort, allergic reactions, etc.

Several studies have reported that the class of secondary metabolite compounds in plants has an important role in providing alpha-glucosidase inhibitor activity, namely the class of alkaloid compounds from the stem of *Tinospora cordifolia*, the class of triterpenoid compounds from the leaves and twigs of *Fagus hayatae*, the class of polyphenolic compounds such as flavonoids. The hydroxyl group of the polyphenol compound forms a complex by occupying the active side of the enzyme so that the activity of the alpha-glucosidase enzyme is inhibited, and there is no breakdown of oligosaccharides into monosaccharides (Tijjani et al. 2020). Therefore, this study was conducted to determine the antidiabetic activity of *P. pellucida* in inhibiting the enzyme alpha-glucosidase both in vitro and in silico, so that it can be predicted that compounds containing *P. pellucida* are potentially active as inhibitors of alpha-glucosidase enzymes.

MATERIALS AND METHODS

Study area

The materials used in silico included the 3D chemical structure of isovitexin (CID: 162350), isoswertisin (CID: 44258317), and patuloside A (CID: 53883544) obtained from NCBI's PubChem database (https://www.ncbi.nlm.nih.gov/) and 3D human alpha-glucosidase structure (PDB ID: 5kzx) obtained from RSCB GDP database (https://www.rcsb.org/). Materials in vitro studies include *P. pellucida* obtained from the Jember Curahkalong area, 96% ethanol solvent, ethyl acetate solvent, and quercetin. Equipment used for in silico studies includes hardware with Intel Core i5 specifications, ChemDraw Ultra software, PyRx software, Discovery software, Hex 8.0 studio software. Equipment used for in vitro studies includes ovens, blenders, and UV-VIS Spectrophotometer instruments.

Extraction and fractionation of *P. pellucida*

Peperomia pellucida is obtained from the Jember Curahkalong area, then wet sorting is carried out and cleaning and washing with running water. Next, clean herbs are chopped with a size of ±2 cm and aerated to dry. Dried herbs in the oven at 50°C until a stable weight is obtained. Then blended to obtain *P. pellucida* powder. The extraction was carried out by ultrasonication method for 1 h. *Peperomia pellucida* powder was extracted using 96% ethanol solvent with a ratio of 1:7.5 and the resulting extract was then fractionated with ethyl acetate solvent (Hidayati 2021).

Phytochemical screening of extraction and fractionation of *P. pellucida*

Phytochemical screening was carried out by testing 4 compounds in *P. pellucida*, namely flavonoids, alkaloids, polyphenols and saponins. Identification of flavonoid content is carried out using the ammonia vapor method. The positive result of flavonoids is characterized by the appearance of yellow color on the filter paper. Alkaloid Identification Test is performed using dragendrof reagent. The positive result of the alkaloid is indicated by the orange color and the formation of orange deposits. Identification of polyphenol content is done by dripping FeCl3 reagent on the sample. Positive results of polyphenols are indicated in blue-black, green or turquoise color. Identification of saponin content is carried out by adding hot aquadest to the sample, after cooling strong shaking is carried out for 10 seconds. A positive result of saponins is characterized by the formation of foam 1-10 cm high. The foam will disappear if 1 drop of HCl 2N is added (Sulistyawati et al. 2017).

in vitro test of alpha-glucosidase enzyme inhibition

The alpha-glucosidase inhibitory activity test refers to the method used Daud et al. (2019). The test solution was prepared from 10 µL of sample solution plus 120 µL of phosphate pH 6.8 and 20 µL of 0.1 U/mL α-glucosidase enzyme solution in a microwell. Then incubated for 15 minutes at a temperature of 37°C. After that, 20 µL of 10 mM PNPG substrate was added and then incubated for 60 minutes at 37°C. The reaction was stopped with the addition of 80 µL sodium carbonate 0.2 M. The resulting P-nitrophenol was read its absorbance at a wavelength of 415 nm using a spectrophotometer instrument. All tests were performed three times of replication.

in silico test of alpha-glucosidase enzyme inhibition

Ligand and receptor preparation

Ligands (isovitexin, isoswertisin, pellucidatin, caryatin-7-O-β-rhamnoside, and patuloside A) were prepared using PyRx software to minimize energy. Preparation of alpha-glucosidase receptors (PDP ID: 5kzx) by removing water molecules or ligands bound to the receptor using Discovery Studio software. After the preparation is complete, all of them are saved in .pdb format using open Babel (Agustin et al. 2020).

Molecular docking and visualization

Molecular docking between receptors and ligands was performed using Hex 8.0 software. The setting column is set in Shape+Electro+DARS mode. The docking results are then saved with the .pdb extension. Docking results were visualized and analyzed using Discovery Studio 2016 software.

Data analysis

The antidiabetic activity of the sample is determined by the magnitude of the alpha-glucosidase enzyme inhibition which can be calculated by:

$$\% \text{ Inhibition} = \frac{\text{Ab Control} - \text{Ab Sample}}{\text{Ab Control}} \times 100\%$$

The IC_{50} value of the sample was calculated using the linear regression equation formula between the sample concentration and % inhibition. The IC_{50} was determined from the regression equation (Li et al. 2018).

RESULTS AND DISCUSSION

Peperomia pellucida plant is taken from Curahkalong Region, Jember. The plant has wide adaptability to various climatic conditions, soil types, and highland environments, and is also widely cultivated for ornamental purposes. Most Peperomia species have leaves with specialized tissues for water storage, with succulent levels varying greatly according to leaf morphology and several geophytic species have been described. The main form of seed dispersal occurs through resinous seeds that can stick to the legs, feathers, and feathers of birds, bats, and insects. This plant is a small, shallow-rooted annual, that is easy to find growing wild on the shores of waterways or ripens and parks. Its size is 15 to 45 cm. The stem is succulent (juicy), bright, fleshy, as are the rather thick but soft leaves (Figure 1).

Phytochemical screening of *P. pellucida* extract and fraction

Phytochemical screening is carried out by the color reaction method. The screening results show that the ethyl acetate fraction contains more flavonoids, alkaloids and polyphenols, but does not contain steroids can be seen in Table 1.

Errand plants produce a variety of primary and secondary metabolites. Isolation of dill-apiol, acetate aurantiamide, and pachypophyllin from *P. pellucida* leaf extract has been performed and explained its structure by NMR studies. Pellucidin A, a new ArC2 dimer compound, along with dill-apiol have been isolated from the aerial parts of *P. pellucida*. Isolation of compounds such as secolignans, tetrahydrofuran lignans, highly methoxylated dihydronaphthalenone, peperomins, sesamin, and isoswertisin from whole plants from *P. pellucida* and marked as Patuloside A compounds (3-β-D-glucopyranosyloxy-1,5,6 trihydroxy-9H-xanthene-9-1) (Xu et al. 2006; Alves et al. 2019). The compound isolated flavone glycosides from the whole plant from *P. pellucida* and characterized the compound as vitexin by chromatographic and spectral analysis. This study was conducted by Amarathunga and Kankanamge (2017) identified compounds namely stigmasterol, an analogue of pheophytin and β-sitosterol-D-glucopyranoside in the solvent extract of *P. pellucida.*

In the ethanol extract of the herb, there is a quercetin flavonoid content of 88.24 mgQE/g extract (Hidayati et al. 2022). Flavonoids are polyphenol group compounds that are polar so they tend to dissolve in polar solvents and slightly soluble in semipolar solvents (Dias et al. 2021). Flavonoids have been widely studied for their broad bioactive benefits including anti-oxidation, cardioprotection, anti-bacteria, and anti-inflammation activities (Wang et al. 2017; Ullah et al. 2020). Flavonoids are recognized to regulate animals, food, digestion, and processes, through interaction with digestive enzymes. Docking analysis showed that flavonoids bound closely to the enzyme's active site, while acarbose bound to other sites behind the catalytic triad. Extrinsic fluorescence analysis, along with docking analysis showed that hydrophobic interactions regulate flavonoid-α-amylase interactions (Martinez-Gonzalez et al. 2019). Suppression of starch digestion by flavonoids by forming starch-flavonoid complexes through hydrophobic interactions, and starch formation that is not easily digested by alpha amylase with the formation of covalent bonds between flavonoids and starch during ripening and in the stomach (Takahama and Hirota 2018).

Test of inhibitory activity of alpha-glucosidase extract and *P. pellucida* fraction

Determination of the antidiabetic activity of *P. pellucida* was carried out in vitro by the method of inhibition of the enzyme alpha-glucosidase. Based on the test results showed that the ethyl acetate fraction of the herb *suruhan* had an inhibitory effect on the enzyme alpha-glucosidase better than the ethanol extract of the herb *P. pellucida* with a value comparable to the positive control of acarbose. Therefore, the ethyl acetate fraction of the herb has very potential to be developed as an antidiabetic drug.

Table 1. Phytochemical screening results of *P. pellucida* extract and fraction

Phytochemical screening	Ethanol extract	Ethyl acetate fraction
Flavonoids	+	++
Alkaloids	+	++
Polyphenols	+	++
Steroids	+	-

Table 2. In vitro activity of the herb against the inhibition of alpha-glucosidase enzyme

Concentration (mg/mL)	Ethanol extract	Ethyl acetate fraction	Acarbose
6	10.16	31.99	33.08
8	22.38	44.29	48.06
10	34.55	52.10	65.92
12	42.15	60.50	82.67
14	52.06	68.30	96.95
IC50 (mg/mL)	13.43	9.73	8.11

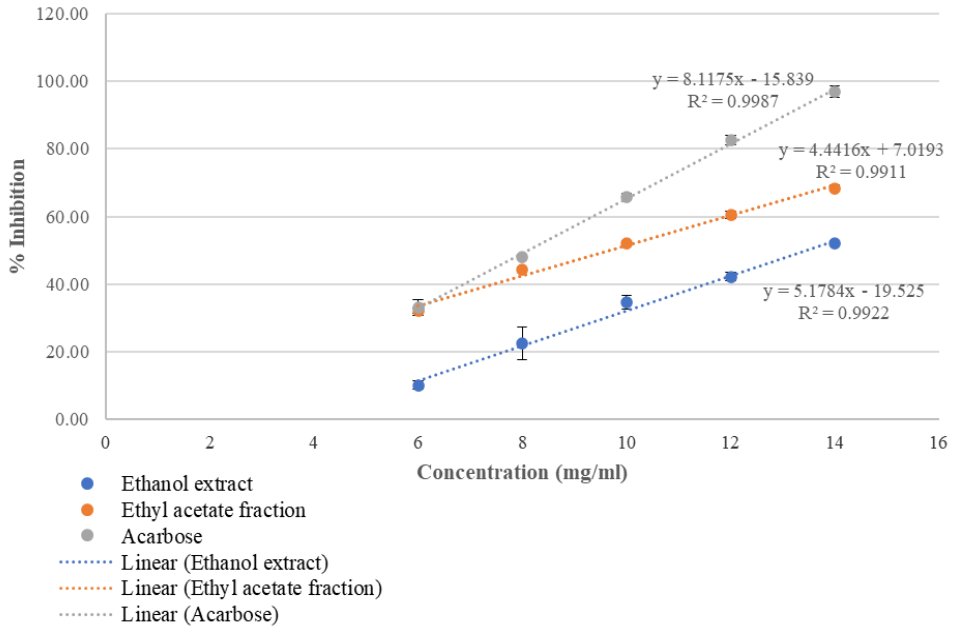

Figure 2. Linear regression curve of ethanol extract, ethyl acetate fraction of *P. pellucida*, and positive controf of acarbose with % inhibition to calculate of IC50

The results of linear regression between % inhibition of alpha-glucosidase enzyme with sample concentration of ethanol extract of *P. pellucida*, ethyl acetate fraction and positive control of akarbose *P. pellucida* according to Figure 2. From the Figure 2, it can be seen that the r value in ethanol extract is 0.9922, ethyl acetate fraction is 0.9911 and acarbose positive control is 0.9987. After obtaining a linear equation, then by entering the value 50 in the equation will get the value of IC50 in each sample. The results of IC50 values can be seen in (Table 2) showing IC50 ethanol extract of 13.43 mg/mL and IC50 ethyl acetate fraction smaller at 9.73 mg/mL (Table 2). This can be possible because the chemical compound content of ethyl acetate fraction is more than ethanol extract (Table 1). This showed that the ethyl acetate fraction of *P. pellucida* was more effective in inhibiting alpha-glucosidase enzymes than the ethanol extract of *P. pellucida*. Acarbose as an antidiabetic drug that has been proven to inhibit the activity of the enzyme alpha-glucosidase is used as a positive control and obtained IC50 value results of 8.11 mg/mL. The active components of extracts with inhibitory activity of alpha-glucosidase are quercetin-3-O-glucuronide, quercetin, kaempferol-3-O-rhamnoside, kaempferol, genistein and asiatic acid. Studies related to molecular docking have revealed that these components can occupy the active site of α-glucosidase more easily than acarbose (Ning et al. 2019).

Based on Table 1, the content of flavonoids, alkaloids and polyphenols in the ethyl acetate fraction of *P. pellucida* is greater than that of ethanol extract of *P. pellucida*. Some studies report that flavonoids can inhibit the enzyme alpha-glucosidase (Barber et al. 2021). Adequately substituted flavonoids are effective inhibitors of alpha-glucosidase. The structure of flavonoids, the position and the number of OH groups are determinants of the desired effect (Shen et al. 2022). The most active flavonoid is quercetin, which indicates hydroxylation at positions 5 and 7 or 8 of the A ring, at positions 3′ and 4′ of the B ring, and at position 3 of the C ring, as well as the double bond of C2 = C3 at the C ring, are essential for the inhibitory activity of flavonoids. The results obtained in this study provide a series of potentially effective flavonoids that can be used as an alternative to alpha-glucosidase inhibitors commonly administered in diabetes mellitus therapy (Tang et al. 2020; Safe et al. 2021). Research related to the determination of quercetin levels in the ethyl acetate fraction of *P. pellucida* showed results of 80.45±2.81 mgQE/g (Hidayati et al. 2022).

in silico study of *P. pellucida* extract and fraction against alpha-glucosidase receptors

The initial step of in silico studies is the preparation of ligands (isovitexin, isoswertisin, pellucidatin, caryatin-7-O-β-rhamnoside, and patuloside A) using PyRx software to minimize energy. Furthermore, receptor preparation (alpha-glucosidase) is carried out by removing water molecules or ligands bound to the receptor using Discovery Studio software. After the preparation is complete, all of them are saved in .pdb format using open babel. The next step is molecular docking between receptors and ligands using Hex 8.0 software. The setting column is set in Shape+Electro+DARS mode. The docking results are then saved with the .pdb extension. Docking results were visualized and analyzed using Discovery studio 2016 software. The results of antidiabetic docking through inhibition of alpha-glucosidase can be seen in Figure 3.

Recently the use of bioactive alpha-glucosidase inhibitors for the treatment of diabetes has proven to be the most efficient drug solution to control postprandial hyperglycemia and adverse physiological complications, especially in type 2 diabetes (Hossain et al. 2020). Alpha-glucosidase is an

exoenzyme that works in a similar way to glucoamylase in di- and oligo-saccharides as well as aryl glucosides and produces glucose (Bhatnagar and Mishra 2022). All plants contain alpha-glucosidase as an endocellular enzyme, and are in germinated and non-germinated cereals (Shao et al. 2021). This enzyme hydrolyzes maltooligosaccharides, phenyl α-maltosides, nigerosa, soluble starch, amylose, amylopectin, and β limit dextrin (Ćorković et al. 2022).

The molecular docking results of the five compounds containing *P. pellucida* showed that isovitexin was able to bind to the alpha-glucosidase binding site on GLU866, HIS717, and GLU869 residues. Isoswertisin compounds are able to bind to alpha-glucosidase binding sites in SER265, LEU264, GLY288, THR274, ARG275, and ILE276 residues. Pellucidatin compounds are able to bind to alpha-glucosidase binding sites in HIS717 and LEU865 residues. Caryatin-7-O-β-rhamnoside is able to bind to alpha-glucosidase binding sites on TYR360, GLY359, HIS717, VAL718 and PRO198 residues. Patuloside A is able to bind to alpha-glucosidase binding sites on SER265, SER270, GLY288 and MET122 residues (Figure 3). The result of molecular bonding of phytochemical compounds from *P. pellucida* with alpha-glucosidase which produces the smallest binding energy, namely petuloside A compounds with a value of -321.4 kcal/mol (Table 3.). Molecules need energy called binding affinity to form chemical bonds with other molecules. The smaller the binding affinity indicates the less energy is needed between the two molecules to interact. Therefore, they can interact with each other easily (Rafi et al. 2015; Desantis et al. 2022).

Patuloside A (3-beta-D-glucopyranosyloxy-1,5,6-trihydroxy-9H-xanthene-9-one, 1) belongs to the group of xanthon glycosides isolated from *P. pellucida* using chromatographic techniques (TLC, PTLC, GC) and the structure was confirmed on the basis of spectral data (liquid chromatography/electrospray-mass spectroscopy, 1H and 13C NMR including JMOD, COSY, NOESY, HMBC, HSQC) (Wakhidah et al. 2021). Xanthones are undeniably a class of heterocyclic compounds with a special motive as alpha-glucosidase inhibitors that deserve special attention for future investigations in the development of new and improved anti-diabetic agents (Santos et al. 2018; Malik et al. 2020). These novel xanthone triazole derivatives exhibited dual therapeutic effects of α-glucosidase inhibition and glucose uptake promotion, thus they could be used as antidiabetic agents for developing novel drugs against type 2 diabetes (Ye et al. 2019).

Table 3. Molecular interaction of isovitexin, isoswertisin, pellucidatin, caryatin-7-O-B-rhamnoside, and patuloside A complexes with alpha-glucosidase

Compounds	Point interaction	Chemistry bond	Type	Energy binding (kcal/mol)
Isovitexin-alpha-glucosidase	A:GLU866:HN - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-281.8
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:HIS717:NE2	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:GLU869:OE1 - :LIG1	Electrostatic	Pi-Anion	
	A:GLU869:OE2 - :LIG1	Electrostatic	Pi-Anion	
Isoswertisin-alpha-glucosidase	A:SER265:HG - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-317.3
	:LIG1:H - A:LEU264:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	:LIG1:H - A:LEU264:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:THR274:CG2 - :LIG1	Hydrophobic	Pi-Sigma	
	A:ARG275:O - :LIG1	Other	Pi-Lone Pair	
	:LIG1 - A:ILE276	Hydrophobic	Pi-Alkyl	
Pellucidatin-alpha-glucosidase	:UNK1:H - A:HIS717:NE2	Hydrogen Bond	Conventional Hydrogen Bond	-279.8
	:UNK1:H - :UNK1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK1:H - :UNK1:O	Hydrogen Bond	Carbon Hydrogen Bond	
Caryatin-7-O-β-rhamnoside-alpha-glucosidase	A:TYR360:HN - :UNK1:O	Hydrogen Bond	Conventional Hydrogen Bond	-315.9
	A:TYR360:HN - :UNK1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:GLY359:CA - :UNK1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK1:H - A:TYR360:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK1:H - :UNK1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK1:H - A:HIS717:O	Hydrogen Bond	Carbon Hydrogen Bond	
	:UNK1:H - :UNK1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:VAL718:CA - :UNK1	Hydrophobic	Pi-Sigma	
	A:TYR360 - :UNK1	Hydrophobic	Pi-Pi Stacked	
	A:TYR360 - :UNK1	Hydrophobic	Pi-Pi Stacked	
	A:HIS717 - :UNK1	Hydrophobic	Pi-Pi T-shaped	
	:UNK1:C - A:PRO198	Hydrophobic	Alkyl	
Patuloside A-alpha-glucosidase	A:MET122:HN1 - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	-321.4
	:LIG1:H - :LIG1:O	Hydrogen Bond	Conventional Hydrogen Bond	
	A:SER270:CB - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	
	A:GLY288:CA - :LIG1:O	Hydrogen Bond	Carbon Hydrogen Bond	

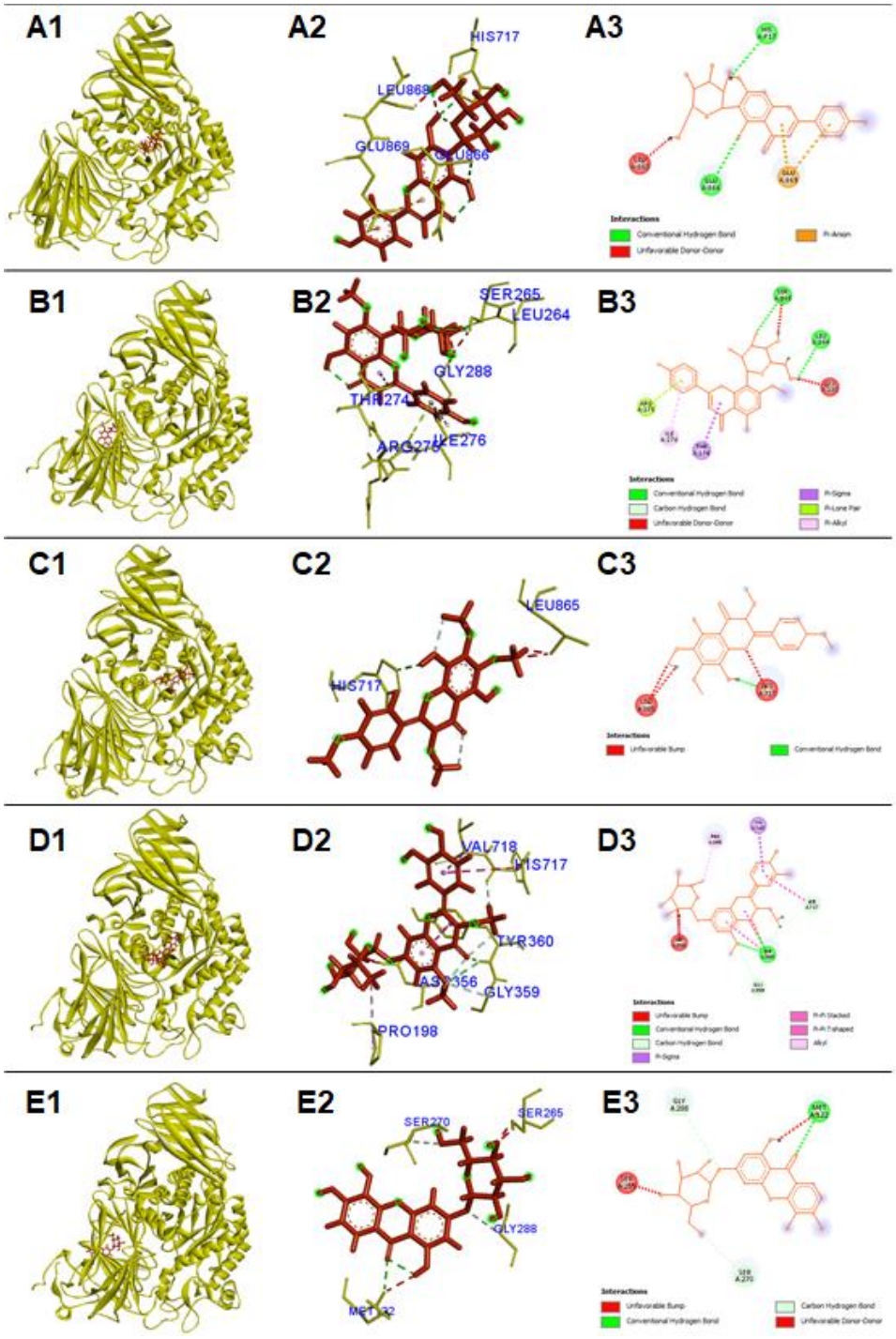

Figure 3. A. Molecular docking between isovitexin complexes, B. Isoswertisin, C. Pellucidatin, D. Caryatin-7-O-β-rhamnoside, E. Patuloside A with alpha-glucosidase. Numbers 1 and 2 show a 3D view of the ligand-receptor complex. The number 3 indicates a 2D view

In conclusion, the research results showed that the ethanol extract and ethyl acetate fraction of *P. pellucida in vitro* showed the ability to inhibit the activity of the alpha-glucosidase enzyme and in silico analysis showed that the Patuloside A component was able to bind to the alpha-glucosidase binding site by providing the smallest binding energy value when compared to isovitexin, isoswertisin, pellucidatin, and caryatin- 7-O-β-rhamnoside compounds so that *P. pellucida* has the potential as an antidiabetic drug which has the activity of inhibiting the action of the alpha-glucosidase enzyme.

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