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Research Article

Identification of Potential Antioxidant and Antidiabetic α-Glucosidase Inhibitors from *Pometia pinnata* Through *In Vitro*, Metabolite Profiling, and Molecular Docking

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Abstract
Oxidative stress induced by reactive oxygen species is critical in the progressing of type 2 diabetes mellitus. Thus, early intervention using plant-derived secondary metabolites with antioxidant and α-glucosidase inhibitory (AGI) activities is essential for adequate glycemic control. *Pometia pinnata* has demonstrated antidiabetic potential, though scientific evidence remains limited. This study aimed to determine the *in vitro* antioxidant and AGI activities of *P. pinnata* extracts (fruit peel, root bark, and stem bark). Metabolites from selected stem bark extracts were identified via LC-HRMS and further validated through molecular docking. Sequential maceration using *n*-hexane, ethyl acetate, methanol, and water was employed to obtain extracts. Ethyl acetate extracts of stem and root bark showed significantly higher total phenolic and flavonoid contents with higher antioxidant activity than other solvent extracts. The stem bark extract yielded the highest compound content (2.74%) and AGI activity (62.78 ± 0.85% inhibition at 15 µg/mL), outperforming the standard drug acarbose (14.66% at the same dose). LC-HRMS profiling of the stem bark extract identified 26 metabolites, with cianidanol exhibiting the strongest binding affinity (ΔG°bind = –7.63 kcal/mol) in molecular docking, surpassing acarbose (–2.51 kcal/mol). These findings confirm the presence of bioactive compounds in *P. pinnata* that contribute to α-glucosidase inhibition and are correlated with antioxidant capacity.

Keywords: α-Glucosidase inhibitors, cianidanol, LC-HRMS metabolite profiles, *Pometia pinnata* stem bark, type 2 diabetes mellitus

1. INTRODUCTION

Globally, the number of individuals who have diabetes continues to rise each year. In 2021, healthcare costs due to diabetes reached nearly one trillion USD, a figure that nearly exceeds the projected number of individuals with diabetes in 2030, estimated to reach 643 million—an increase of 16.5% from 2021 [1]. Early and appropriate treatment is urgently needed to mitigate these high costs. The metabolic condition known as type 2 diabetes mellitus (T2DM) impacts the body's defense mechanisms and organs, leading to chronic and long-lasting complications. It is believed that patients with diabetes mellitus suffer from oxidative stress, which plays a role in the emergence of various issues [2]. Many diseases are thought to be caused by oxidative stress, including diabetes mellitus [3]. Glucose metabolism generates reactive oxygen species (ROS) essential for specific physiological functions. However, an excess of ROS leads to oxidative stress. Hyperglycemia triggers alternative glucose metabolic pathways, resulting in increased ROS production, cellular damage, insulin resistance, and exacerbation of diabetic complications [4]. Acarbose, a commercial drug for T2DM, functions as an α-glucosidase inhibitory (AGI) but is commonly associated with gastrointestinal side effects [5]. The development of natural product-based inhibitors provides an alternative approach to managing hyperglycemia [6][7] and offers valuable sources of natural antioxidants, which enhance plasma antioxidant capacity and reduce disease risk [8]. Essential antioxidants, including phenolics and flavonoids, are secondary metabolites found in various plant parts—leaves, fruits, seeds, roots, and bark—each with unique chemical compositions and biological activities [9][10]. Phenolic compounds can donate hydrogen atoms to radicals, stabilizing them [11]. Additionally, natural polyphenols have improved insulin resistance and reduced inflammation and oxidative damage in obesity-associated diabetes [12].

Pometia pinnata, commonly known as matoa,

Table 1. Yield of extracts from different parts of *P. pinnata* using various solvents.

Mass of Dried Plant Material (g)	Solvents	Extract Mass (g)	Yield (%)
785 g of Stem barks	*n*-Hexane	0.95	0.12
	Ethyl acetate	21.52	2.74
	Methanol	31.04	3.95
	Water	23.92	3.05
905 g of Root barks	*n*-Hexane	0.66	0.07
	Ethyl acetate	1.08	0.12
	Methanol	1.43	0.16
	Water	10.82	1.20
369 g of Fruit peels	*n*-Hexane	0.77	0.21
	Ethyl acetate	9.39	2.55
	Methanol	30.46	8.25
	Water	8.18	2.22

belongs to the Sapindaceae family and has long been used in traditional Indonesian medicine to reduce and manage hyperglycemia [13][14]. Flavonoids and tannins in the leaf extract indicate vigorous antioxidant activity [15]. *In vivo* studies have provided initial evidence supporting the antidiabetic properties of *P. pinnata*. In alloxan-induced diabetic mice, administration of leaf extract at 200 mg/kg body weight significantly reduced fasting blood glucose levels and improved insulin sensitivity [16]. Another study reported that ethanol extract of the leaves—containing flavonoids, alkaloids, saponins, tannins, and steroids—effectively reduced blood glucose levels in streptozotocin-induced diabetic rats [17]. The bark extract has also effectively decreased blood glucose and glycosylated hemoglobin levels, underlining its potential as an antidiabetic substance [18]. Flavone-rich plants exhibit antidiabetic properties, especially when flavonoids are present as aglycones at the *C*-glycoside position. In contrast, they do not display antihyperglycemic activities if the aglycone is attached to *O*-glycosides [19].

This study aimed to determine the *in vitro* antioxidant and AGI activities of *P. pinnata* (fruit peel, root bark, and stem bark extracts). Extracts were obtained through successive maceration using solvents of increasing polarity, from non-polar to polar, to maximize the range of extracted phytochemicals [20]. Secondary metabolites from selected stem bark extracts were identified by LC-HRMS and further validated via molecular docking. Untargeted metabolite profiling was employed to characterize secondary metabolites without prior selection of compound classes. Although *P. pinnata* shows promising therapeutic potential, integrated studies combining metabolite profiling and molecular docking remain limited. This study offers a new perspective by linking the phytochemical profile of *P. pinnata* with its biological activities, supporting its use as a complementary strategy for managing T2DM.

2. MATERIALS AND METHODS

2.1. Materials

Pometia pinnata J.R.Forst. & G.Forst was the plant species used in this study, taxonomically verified at the Traditional Medicine Raw Materials Laboratory, BRIN (Certificate No. SHU 227920). Stem bark, root bark, and fruit peel were collected in October 2023 from the KST B.J. Habibie Provincial Botanical Garden, BRIN, Banten, Indonesia. The samples were cleaned, oven-dried at 45 °C for 48 h, and then ground into 60-mesh powder using a mechanical grinder for 2–5 min. These following chemicals were purchased from Merck Millipore: 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate diammonium salt (ABTS), Folin & Ciocalteu's phenol reagent, 2,4,6-tris(2-pyridyl)-s-triazine, acarbose, quercetin, bovine serum albumin, potassium persulfate

($K_2S_2O_8$), aluminum chloride ($AlCl_3$), gallic acid, sodium carbonate (Na_2CO_3), dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), and methanol. Phosphate buffer pH 6.8 and *p*-nitrophenyl-α-D-glucopyranoside (pNPG) were purchased from Sigma Aldrich. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Tokyo Chemical IndustryThe α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) was obtained from Wako Pure Chemical Corporation. LC-MS grade solvents, *i.e.,* acetonitrile, methanol, and water, were purchased from Fisher Scientific. Aquabidest and sterile distilled water were purchased from Ikapharmindo Putramas Tbk. Distilled technical solvents, *i.e.*, ethanol, methanol, *n*-hexane, and ethyl acetate, were supplied by PT Brataco.

The instruments used included a UV-Vis microplate spectrophotometer (Thermo Scientific Multiskan SkyHigh), LC-HRMS (Thermo Scientific™, Germany), analytical balance (Kern, Germany), rotary vacuum evaporator (Buchi, Switzerland), and micropipettes (Eppendorf, Germany).

2.2. Methods

2.2.1. Preparation of Plant Materials

The plant materials (stem bark, root bark, and fruit peel) were extracted using a solid-state maceration method, employing a series of solvents with increasing polarity: *n*-hexane (non-polar), ethyl acetate (semi-polar), methanol (polar), and water (polar). The extraction was conducted at a powder-to-solvent ratio of 1:5 (w/v) at room temperature in three stages, each lasting 24 h. The extraction procedure was adapted from previous work [21] with minor modifications to the solvent type. These extracts from all parts of the plant were concentrated using a rotary evaporator under vacuum at 50 °C. All dried extract samples were weighed, and the yield was determined and stored for further analysis.

2.2.2. Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

TPC was determined using the Folin-Ciocalteu reagent with gallic acid as the standard, following the previous method [22]. The absorbance was measured at 750 nm, and TPC was expressed as mg GAE/g. TFC was quantified using the $AlCl_3$ colorimetric method, with absorbance measured at 510 nm using a UV-Vis spectrophotometer. Quercetin was used as the standard for the

Table 2. TPC and TFC of *P. pinnata* extracts from different parts using various solvents.

Parts	Solvents	TPC		TFC	
		mg GAE/g extract	Mean ranks	mg QE/g extract	Mean ranks
Stem barks	*n*-Hexane	50.81 ± 2.84	23.67 ^{ab}	107.02 ± 13.73	23.00 ^{abc}
Stem barks	Ethyl acetate	66.27 ± 3.92	30.33 ^{ab}	241.80 ± 4.46	32.00 ^{bc}
Stem barks	Methanol	51.33 ± 3.04	25.33 ^{ab}	134.89 ± 18.18	25.67 ^{abc}
Stem barks	Water	3.03 ± 0.19	5.00 ^{a}	13.30 ± 1.17	8.00 ^{abc}
Root barks	*n*-Hexane	4.20 ± 0.28	10.33 ^{ab}	63.27 ± 6.26	17.00 ^{abc}
Root barks	Ethyl acetate	72.33 ± 0.69	35.00 ^{b}	279.42 ± 14.20	35.00 ^{c}
Root barks	Methanol	66.81 ± 2.74	30.67 ^{ab}	225.25 ± 11.87	29.00 ^{abc}
Root barks	Water	4.12 ± 0.42	10.00 ^{ab}	8.75 ± 1.26	5.00 ^{ab}
Fruit peels	*n*-Hexane	4.45 ± 0.21	12.67 ^{ab}	85.18 ± 11.90	20.33 ^{abc}
Fruit peels	Ethyl acetate	11.63 ± 0.78	17.00 ^{ab}	33.39 ± 4.23	13.33 ^{abc}
Fruit peels	Methanol	25.09 ± 1.41	20.00 ^{ab}	29.59 ± 1.29	11.67 ^{abc}
Fruit peels	Water	1.54 ± 0.01	2.00 ^{a}	3.24 ± 0.34	2.00 ^{a}

Significant differences were detected for TPC mean ranks (K = 34.207, asymptotic $p < 0.0003$, and df = 11) and TFC mean ranks (K = 34.598, asymptotic $p < 0.0003$, df = 11) data. Different superscript letters within a column indicate values significantly differ at $p < 0.05$.

Table 3. Inhibition (%) of extracts obtained from different parts of *P. pinnata* using various solvents, tested at 10 µg/mL and 25 µg/mL against ABTS and DPPH radicals, respectively.

Parts	Solvents	ABTS		DPPH	
		% Inhibition	Mean ranks	% Inhibition	Mean ranks
Stem barks	*n*-Hexane	Not Active	-	Not active	-
Stem barks	Ethyl acetate	76.48 ± 0.76	16.67 ^{ab}	55.11 ± 1.41	23.67 ^{ab}
Stem barks	Methanol	77.17 ± 0.05	22.00 ^{ab}	30.68 ± 0.35	20.00 ^{ab}
Stem barks	Water	53.98 ± 0.03	4.00 ^{a}	10.59 ± 1.95	14.00 ^{ab}
Root barks	*n*-Hexane	Not active	-	7.10 ± 0.15	11.00 ^{ab}
Root barks	Ethyl acetate	77.53 ± 0.17	26.00 ^{b}	77.51 ± 1.07	29.00 ^{b}
Root barks	Methanol	76.82 ± 0.05	16.83 ^{ab}	57.64 ± 4.05	25.33 ^{ab}
Root barks	Water	50.36 ± 3.33	3.00 ^{a}	1.60 ± 0.09	2.00 ^{a}
Fruit peels	*n*-Hexane	Not Active	-	Not Active	-
Fruit peels	Ethyl acetate	71.26 ± 0.43	11.00 ^{ab}	2.85 ± 0.48	6.67 ^{ab}
Fruit peels	Methanol	77.00 ± 0.20	18.50 ^{ab}	16.33 ± 2.79	17.00 ^{ab}
Fruit peels	Water	59.75 ± 3.42	8.00 ^{ab}	2.81 ± 0.28	6.33 ^{ab}

Significant differences were detected for ABTS mean ranks (K = 24.264, asymptotic p = 0.002, and df = 8) and DPPH mean ranks (K = 28.449, asymptotic p = 0.001, and df = 9). Different superscript letters within a column indicate values significantly differ at $p < 0.05$.

calibration curve, and TFC was expressed as mg QE/g. All measurements were performed in triplicate.

2.2.3. Antioxidant Activity Assays using ABTS Method

The ABTS assay was performed according to the previous protocol [23] with modifications. All extracts were dissolved in a methanolic environment at a concentration of 20 µg/mL, which resulted in a final testing concentration of 10 µg/mL. Then, 5 mL of 7 mM ABTS reagent solution in deionized water was mixed with 88 µL of 2.45 mM $K_2S_2O_8$ solution. The mixture was stored in the dark at room temperature for 16 h to allow radical generation. The resulting ABTS solution was diluted with deionized water at a ratio of 1:44 (v/v) to produce an ABTS reagent with an absorbance reached 0.70 ± 0.02 at 734 nm. This ABTS reagent was ready for the subsequent ABTS assay. The ABTS inhibitory activity assay was divided into a sample and a blank. The sample was assessed by mixing 1 mL of the dissolved extract and 1 mL of the diluted ABTS solution, followed by incubation for 6 min at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at 734 nm. The blank was prepared similarly with the diluted ABTS solution being substituted by deionized water. The percentage of inhibition was calculated using Equation (1). All measurements were performed in triplicate.

$$Inhibition\ (\%) = \left[1 - \frac{A_{sample}}{A_{blank}}\right] \times 100\% \quad \textbf{(1)}$$

2.2.4. Antioxidant Activity Assays using DPPH Method

The DPPH assay was performed according to the previous method [23]. All extracts were dissolved in a methanolic environment at a concentration of 250 µg/mL, which resulted in a final testing concentration of 25 µg/mL. The DPPH inhibitory activity assay consisted of a sample and a blank. The sample was assessed by mixing 500 µL of 1 mM DPPH radical solution in methanol with 250 µL of dissolved extract and 1750 µL of methanol. The mixture was incubated in the dark for 30 min at room temperature. The absorbance was measured at 515 nm using a UV-Vis spectrophotometer. The blank was prepared similarly, with the DPPH radical solution being substituted by methanol. The assay was performed in triplicate and the percentage of inhibition was also calculated using

Equation (1).

2.2.5. Antioxidant Activity Assays using FRAP Method

The ferric reducing antioxidant power (FRAP) assay was conducted following the previous method [23]. The assay is based on reducing Fe^{3+}-tripyridyltriazine (colorless) to Fe^{2+}-tripyridyltriazine (blue), with the absorbance measured at 593 nm. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3$ solution at a volumetric ratio of 10:1:1. Prior to assay, extracts were dissolved in a methanolic environment at a given dilution factor. Subsequently, 40 µL of the dissolved extract was mixed with 1.2 mL of the FRAP reagent. The mixture was incubated at 37 °C in a water bath for 30 min. After incubation, the absorbance was measured at 593 nm. The FRAP values were calculated using a Trolox standard calibration curve, corrected with the dilution factor, and expressed as mg TE/g. The assay was performed in triplicate.

2.2.6. AGIs Activity

Antidiabetics were measured by AGIs activity using the *in vitro* assay method as previously reported [24][25], with slight modifications in the concentration and number of reagents added. All extracts were dissolved in a 10% DMSO solution at a concentration of 100 µg/mL, resulting in a final 15 µg/mL testing concentration. The inhibitory activity assay was divided into four groups comprising a blank (A1), a control (A2), a sample blank (A3), and a sample test (A4) [26]. The sample test (A4) was prepared by mixing 30 µL of dissolved extract, 36 µL of phosphate buffer (pH 6.8), and 17 µL of 3 mM pNPG solution, followed by 5 min of incubation at 37 °C. Then, 17 µL of 0.1 U/mL α-glucosidase solution was added, and the incubation was continued for 15 min at 37 °C. The second incubation was stopped by adding 100 µL of 267 mM Na_2CO_3 solution. The absorbance was measured at 405 nm using a microplate reader. The sample blank (A3) was assessed similarly to the sample test (A4), but the sodium carbonate solution was added before the α-glucosidase solution at 15 min of incubation. Control (A2) was evaluated similarly to the sample test (A4); however, the phosphate buffer replacing the dissolved extract. Finally, the blank (A1) was prepared similarly to the control (A2), by adding the enzyme inactivation solution first as in step A3. The percentage of inhibition was calculated using Equation (2). All measurements were performed in

Table 4. Antioxidant activity of extracts obtained from different parts of *P. pinnata* using various solvents as assessed using FRAP and expressed as Trolox equivalent (mg/g).

Parts	Solvents	mg TE/g extract	Mean ranks
Stem barks	*n*-Hexane	22.72 ± 0.64	2.00 ^{a}
Stem barks	Ethyl acetate	86.58 ± 1.50	29.67 ^{ab}
Stem barks	Methanol	86.19 ± 0.67	29.00 ^{ab}
Stem barks	Water	73.69 ± 5.21	18.33 ^{ab}
Root barks	*n*-Hexane	25.54 ± 2.24	5.00 ^{ab}
Root barks	Ethyl acetate	86.84 ± 0.87	32.33 ^{b}
Root barks	Methanol	86.63 ± 0.47	31.00 ^{b}
Root barks	Water	56.66 ± 3.90	12.67 ^{ab}
Fruit peels	*n*-Hexane	30.13 ± 1.27	8.00 ^{ab}
Fruit peels	Ethyl acetate	75.99 ± 3.29	18.67 ^{ab}
Fruit peels	Methanol	84.30 ± 0.28	23.00 ^{ab}
Fruit peels	Water	57.06 ± 1.46	12.33 ^{ab}

Significant differences were detected for FRAP data (K = 33.505, asymptotic p = 0.0004, and df = 11). Different superscript letters within a column indicate values are significantly different at the level of $p < 0.05$.

Table 5. Inhibition (%) of extracts obtained from different parts of *P. pinnata* using various solvents, tested at 15 µg/mL, against α-glucosidase from *S. cerevisiae*.

Parts	Solvents	AGIs	
		% Inhibition	Mean ranks
Stem barks	*n*-Hexane	8.06 ± 1.35	20.33 ^{ab}
Stem barks	Ethyl acetate	62.78 ± 0.85	41.00 ^{b}
Stem barks	Methanol	20.94 ± 0.57	29.00 ^{ab}
Stem barks	Water	3.42 ± 0.98	4.67 ^{a}
Root barks	*n*-Hexane	Not Active	-
Root barks	Ethyl acetate	59.15 ± 0.24	37.67 ^{ab}
Root barks	Methanol	58.41 ± 0.60	35.33 ^{ab}
Root barks	Water	5.80 ± 1.51	13.33 ^{ab}
Fruit peels	*n*-Hexane	2.13 ± 0.35	3.00 ^{a}
Fruit peels	Ethyl acetate	4.65 ± 0.86	9.17 ^{ab}
Fruit peels	Methanol	9.32 ± 0.70	24.33 ^{ab}
Fruit peels	Water	8.23 ± 2.18	20.00 ^{ab}

Significant differences were detected for AGIs mean ranks ($K = 30.560$, asymptotic $p = 0.001$, and $df = 10$). Different superscript letters within a column indicate values are significantly different at the level of $p < 0.05$. In comparison, acarbose at the same concentration displayed 14.66% of inhibition.

triplicate.

$$Inhibition\ (\%) = \left[1 - \frac{(A4 - A3)}{(A2 - A1)}\right] \times 100\% \quad (2)$$

2.2.7. Statistical Analysis

A normality test was performed on all datasets, revealing that they did not follow a normal distribution. Consequently, the Kruskal-Wallis test, a non-parametric method, was applied to determine significant differences among groups. Subsequently, a Spearman correlation analysis was conducted to examine the relationship between TPC or TFC and antioxidant activities and antidiabetic activity. All statistical analyses were conducted using XLSTAT version 2023.1.2.1406 software (Addinsoft, New York, NY, USA), an add-on for Microsoft Excel (Microsoft, Redmond, WA, USA).

2.2.8. Metabolite Profiling by LC-HRMS Analysis

Sample preparation and analysis were performed following the previous method [27]. For LC-HRMS analysis, 50 mg of the ethyl acetate fraction from *P. pinnata* stem bark was dissolved in 1 mL of LC-MS grade acetonitrile in a 2 mL microtube. The mixture was vortexed for 30 s and sonicated at room temperature for 30 min. Afterward, the sample was centrifuged at 1400xg for 5 min to separate undissolved pellets. The supernatant was then filtered through a 0.22 µm PTFE filter and prepared for injection into the LC-HRMS system. MS-grade methanol was used as the blank.

Sample analysis was conducted using a Thermo Scientific Vanquish UHPLC system coupled with a Q Exactive™ Orbitrap™ High-Resolution Mass Spectrometer. Separation was achieved on a Thermo Scientific™ Accucore™ phenyl-hexyl column (100 mm × 2.1 mm ID × 2.6 µm) using MS -grade water (A) and methanol (B), both containing 0.1% formic acid, in a gradient elution at a flow rate of 0.3 mL/min. Mobile phase B was increased from 5% to 90% over 16 min, held for 4 min, and then returned to 5% for 25 min. The column temperature was maintained at 40 °C, and the injection volume was 3 µL. Untargeted screening was performed in full MS/dd-MS2 mode with positive ionization, using nitrogen as sheath, auxiliary, and sweep gases, a spray voltage of 3.30 kV, and a capillary temperature of 320 °C. The scan range was 66.7–1000 m/z, with a resolution of 70,000 for full MS and 17,500 for dd-MS2. XCalibur 4.4 software was used to control the system, and calibration was carried out with Pierce ESI Ion Calibration

Solution. Metabolites in the ethyl acetate extract of *P. pinnata* stem bark were identified using Compound Discoverer® software. Compound peak analysis was conducted using the MzCloud and ChemSpider databases, selecting only those that fully matched, with mass annotations within a range of -5 to 5 ppm. The peak intensities were then normalized to represent the overall spectrum intensity [28].

2.2.9. Molecular Docking

The metabolites identified through LC-HRMS analysis were evaluated computationally using molecular docking. This technique predicts potential antidiabetic activities by modeling the interactions between ligands and target proteins associated with diabetes, evaluating the binding affinity and stability relative to natural ligands. The ligand's binding affinity is evaluated using Gibbs free binding energy (-ΔG). A lower binding energy relative to natural ligands indicates a higher binding affinity and more significant potential for biological activity. This study also used acarbose as the reference drug for molecular docking analysis. The molecular docking was performed using AutoDock 1.5.6 software. The ligand was flexible, while the protein was fixed, and the resulting binding energy reflected the most favorable configuration of the ligand within the protein's binding site. The target protein was a-glucosidase from *S. cerevisiae* (PDB ID: 3A4A). The protein was downloaded from the Protein Data Bank (PDB, https://www.rcsb.org/). Metabolites were optimized using GaussView 6.0 software. Protein validation was performed using AutoDock Tools 1.5.6, comparing the docking results with co-crystallized natural ligand data from

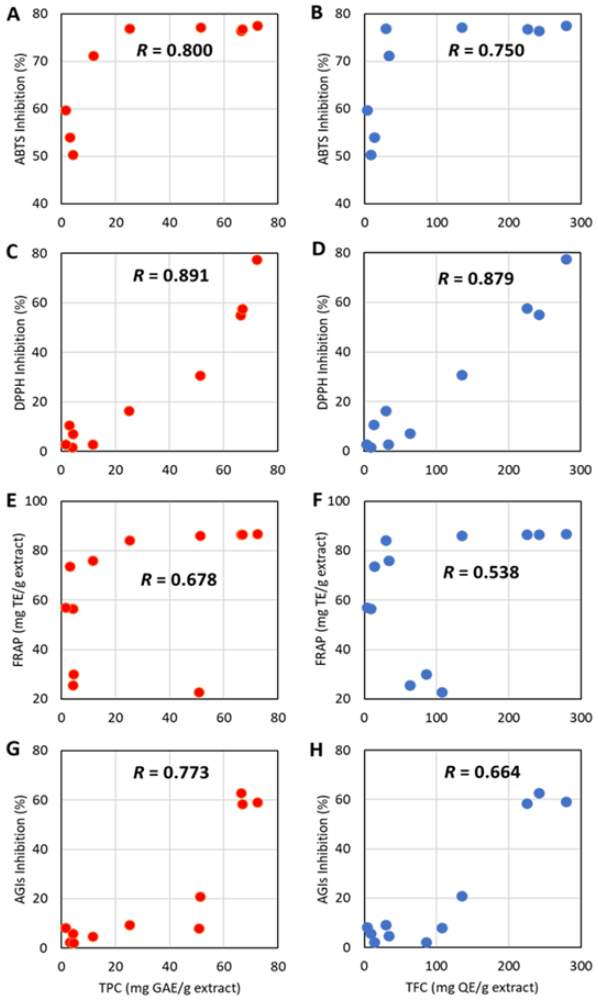


Figure 1. Correlation between bioactivities of extracts obtained from different parts of P. pinnata using various solvents for (A) ABTS and TPC, (B) ABTS and TFC, (C) DPPH and TPC, (D) DPPH and TFC, (E) FRAP and TPC, (F) FRAP and TFC, (G) AGIs and TPC, and (H) AGIs and TFC.

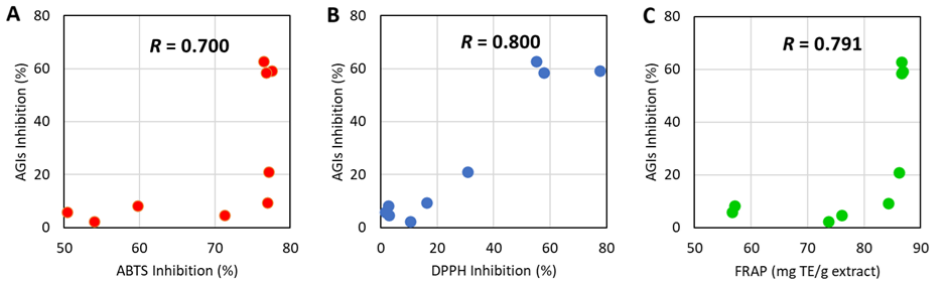


Figure 2. Correlation between AGIs and antioxidant activities of extracts obtained from different parts of *P. pinnata* using various solvents for (A) AGIs and ABTS, (B) AGIs and DPPH, and (C) AGIs and FRAP.

the PDB to achieve an RMSD value of ≤ 2 [29].

3. RESULTS AND DISCUSSIONS

3.1. Extraction of Metabolite Compound using Solvents

Secondary metabolites predominantly accumulate in plant cell vacuoles, and solvent extraction facilitates their release by disrupting cellular membranes. Both ethanol and methanol are known to compromise membrane integrity, enhancing the diffusion of intracellular compounds into the solvent, with ethanol reported to be slightly more efficient [30]. Table 1 presents the extraction yields from various parts of *P. pinnata* using solvents of increasing polarity: *n*-hexane, ethyl acetate, methanol, and water. Overall, methanol and water—both highly polar solvents—consistently produced higher yields across most plant parts, attributable to their ability to solubilize polar compounds [20]. The highest yield was observed in the methanol extract of fruit peel (8.25%), followed by the water extract of stem bark (3.05%).

Among the ethyl acetate extracts, the stem bark produced the highest yield (2.74%), suggesting a greater abundance of semi-polar compound metabolites in this plant tissue. In contrast, all extracts obtained using *n*-hexane yielded less than 1%, indicating a minimal presence of non-polar constituents. These findings highlight the critical role of solvent polarity in the extraction efficiency of secondary metabolites, consistent with the previous observations [31]. Quantifying extraction yield is valuable for estimating the concentration of secondary metabolites extracted by a given solvent; however, it does not provide information on the specific types or identities of the compounds obtained [32]. Supporting this trend, Utari et al. [33] reported extraction yields of 1.22% and 1.14% from *P. pinnata* leaves using ethyl acetate and *n*-hexane, respectively. Similarly, Chua et al. [34] observed increasing extraction yields by solvent polarity: *n*-hexane (1.17%) < ethyl acetate (1.67%) < ethanol (2.51%) < water (4.81%). In another study, methanolic extraction of *P. pinnata* fruit flesh yielded 21.65%, notably higher than those obtained with ethyl acetate (2.92%) and *n*-hexane (0.91%) [35]. These results are consistent with Baehaki et al. [36], who demonstrated superior extraction from water hyacinth flowers using methanol (26.06%) compared to *n*-hexane (4.95%) and ethyl acetate (1.69%). These data confirm that polar solvents are significantly more effective than less polar alternatives in extracting polar compound metabolites from plant matrices.

3.2. TPC and TFC

Phenolic compounds are organic molecules that contain an aromatic ring with one or more hydroxyl groups. Simple phenolics have a single hydroxyl group, while polyphenols contain multiple hydroxyl groups on one or more aromatic rings. Plant-derived phenolics are classified into phenolic acids, flavonoids, and non-flavonoids [37]. As a typical class of secondary metabolites in plants, phenolic compounds are known for their potent antioxidant properties and their role in preventing oxidative stress-related diseases such as high blood pressure, neurodegenerative disorders, and cancer [9][38]. The TPC and TFC of *P. pinnata* extracts obtained through successive maceration with *n*-hexane, ethyl acetate, methanol, and water are presented in Table 2.

In this study, phytochemical extractions from *P. pinnata* stem bark, root bark, and fruit peel were conducted using solvents of varying polarity: *n*-

hexane, ethyl acetate, methanol, and water. Among these, the ethyl acetate extracts of the root and stem bark exhibited the highest TPC and TFC, with the root bark extract yielding 72.33 mg GAE/g and 279.42 mg QE/g, and the stem bark extract 66.27 mg GAE/g and 241.80 mg QE/g, respectively. These results suggest that ethyl acetate, a semi-polar solvent, is particularly effective at extracting polyphenolic and flavonoid compounds, likely due to the moderate polarity of these metabolites.

Conversely, the water extract of the fruit peel exhibited the lowest TPC (1.54 mg GAE/g) and TFC (3.24 mg QE/g), possibly due to a higher proportion of water-soluble non-phenolic constituents such as sugars or terpenoids, which are not detected in TPC and TFC assays. The extraction efficiency followed the trend: ethyl acetate > methanol > *n*-hexane > water, although fruit peel extracts deviated from this pattern. Interestingly, *n*-hexane, despite being non-polar, was able to extract moderate levels of TPC and TFC from the stem bark (50.81 mg GAE/g and 107.02 mg QE/g, respectively), indicating the presence of lipophilic phenolics and flavonoids. Comparable findings were reported by Aziz et al. [39], who identified substantial phenolic content (490 mg GAE/100 g) in the *n*-hexane fraction of *Heterotrigona itama* propolis, and by Bomfim et al. [40], who found the highest TPC (205.95 ± 4.14 mg/g) in *Clidemia capitellata* and the highest TFC (143.99 ± 4.18 mg/g) in *Clidemia hirta* from their respective *n*-hexane extracts. These results support that certain phenolic compounds possess sufficient lipophilicity to be extracted using non-polar solvents. Comparative studies also support the efficiency of ethyl acetate in extracting phenolics. Al-Juhaimi et al. [41] demonstrated that ethyl acetate extracts of *Rosmarinus officinalis* contained higher TPC (41.8 mg GAE/g) than methanol extracts of *Lavandula angustifolia* (12.7 mg GAE/g). Similarly, Topuzović et al. [42] reported that among different plant parts of *Sambucus ebulus* L., the fruit ethyl acetate extract had the highest TPC (126.10 mg GAE/g), underscoring the importance of solvent selection and plant part in maximizing phenolic compound recovery.

3.3. Antioxidant Activities

The assessment of antioxidant capacity is inherently complex, as no single method can fully replicate the intricate reactions that occur *in vivo* [38]. The diverse range of available methodologies

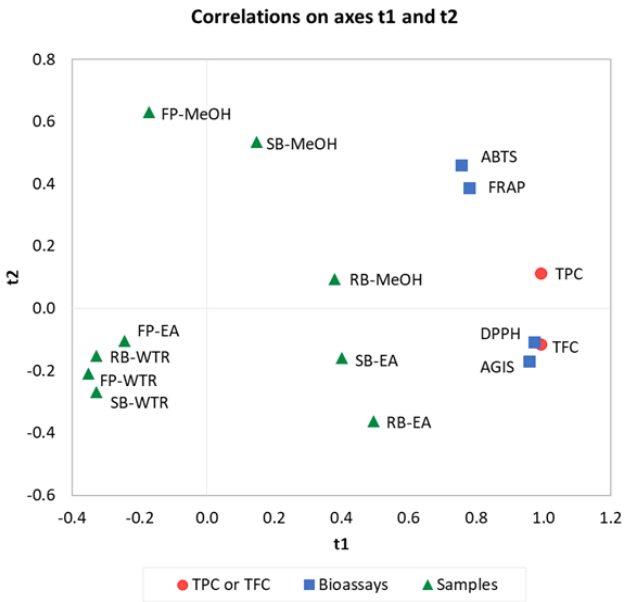


Figure 3. PLSR biplot of either TPC or TFC (Xs) and the bioassay data (Ys) of extracts obtained from different parts of *P. pinnata* using various solvents. Samples were coded accordingly, indicating their parts and solvents. SB, RB, and FP refer to stem bark, root bark, and fruit peel, respectively. EA, MeOH, and WTR refer to ethyl acetate, methanol, and water, respectively. Only nine samples with complete data were subjected to PLSR analysis.

Table 6. Chemical constituents of the stem bark ethyl acetate extract of *P. pinnata* and molecular docking result.

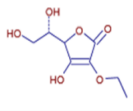
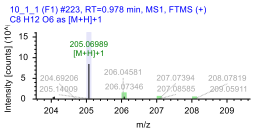
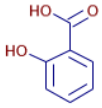
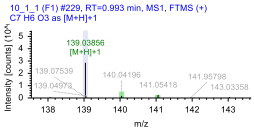
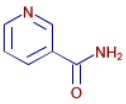
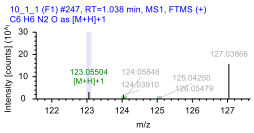
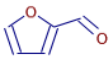
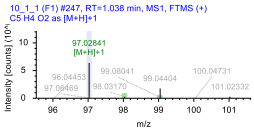
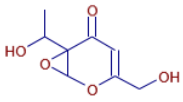
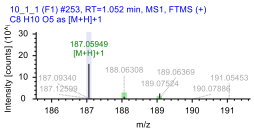
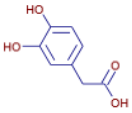
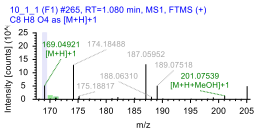
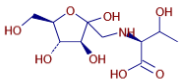
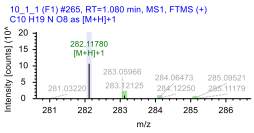
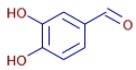
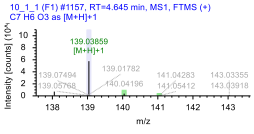
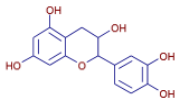
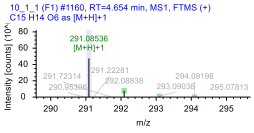
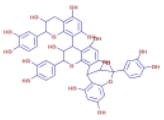
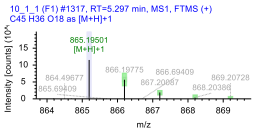
RT (min)	Compound Name	Structure	$[M+H]^+$ Mass Spectrum	Binding Energy (kcal/mol)
0.981	2-*O*-Ethyl ascorbate ($C_8H_{12}O_6$)			-5.61
0.996	Salicylic acid ($C_7H_6O_3$)			-2.82
1.039	Nicotinamide ($C_6H_6N_2O$)			-4.96
1.041	Furfural ($C_5H_4O_2$)			-3.92
1.048	6-(1-Hydroxyethyl)-3-(hydroxymethyl)-2,7-dioxabicyclo [4.1.0]hept-3-en-5-one ($C_8H_{10}O_5$)			-5.45
1.074	3,4-Dihydroxyphenylacetic acid ($C_8H_8O_4$)			-3.23
1.075	(2S)-3-Hydroxy-2-({[(3S,4S,5R)-2,3,4-trihydroxy-5-(hydroxymethyl) tetrahydro-2-furanyl] methyl}amino) butanoic acid ($C_{10}H_{19}NO_8$)			-6.60
4.641	3,4-Dihydroxybenzaldehyde ($C_7H_6O_3$)			-5.67
4.653	Cianidanol ($C_{15}H_{14}O_6$)			-7.63
5.303	Lindetannin ($C_{45}H_{36}O_{18}$)			(+) 418.36

Table 6. *Cont.*

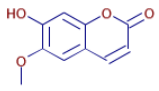
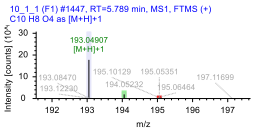
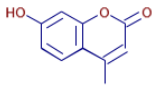
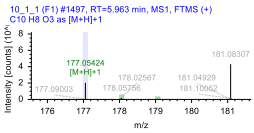
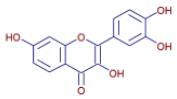
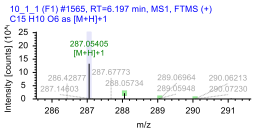
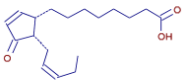
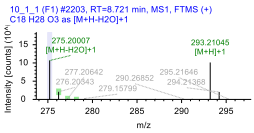
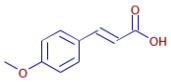
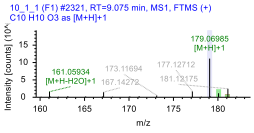
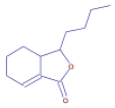
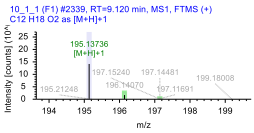
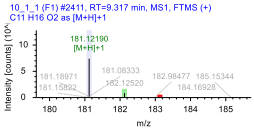
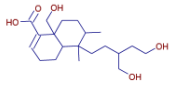
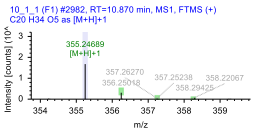
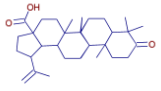
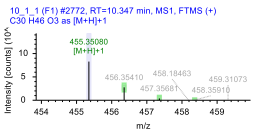
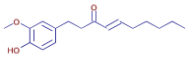
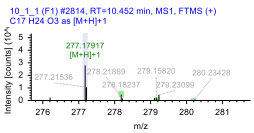
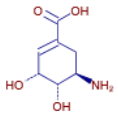
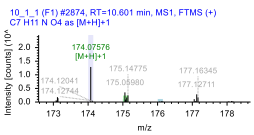
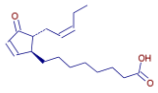
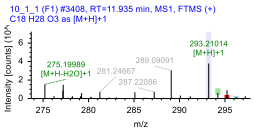
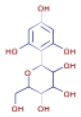
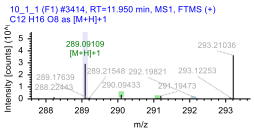
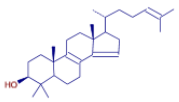
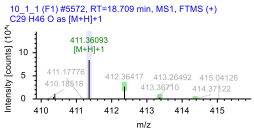
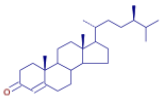
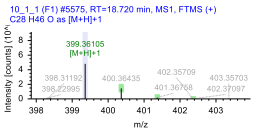
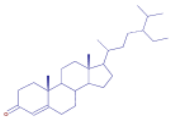
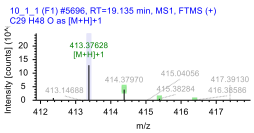
RT (min)	Compound Name	Structure	$[M+H]^+$ Mass Spectrum	Binding Energy (kcal/mol)
5.791	7-Hydroxy-6-methoxy-2*H*-chromen-2-one ($C_{10}H_8O_4$)			-5.47
5.911	Hymecromone ($C_{10}H_8O_3$)			-5.47
6.197	Fisetin ($C_{15}H_{10}O_6$)			-7.74
8.727	12-Oxo-phytodienoic acid ($C_{18}H_{28}O_3$)			-6.05
9.082	4-Methoxycinnamic acid ($C_{10}H_{10}O_3$)			-3.77
9.119	Sedanolide ($C_{12}H_{18}O_2$)			-6.61
9.320	3-*tert*-Butyl-4-hydroxyanisole ($C_{11}H_{16}O_2$)			-4.97
10.871	5-[5-hydroxy-3-(hydroxymethyl) pentyl]-8a-(hydroxymethyl)-5,6-dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphtha-lene-1-carboxylic acid ($C_{20}H_{34}O_5$)			-6.79
10.342	Betulonic acid ($C_{30}H_{46}O_3$)			-6.25
10.454	Shogaol ($C_{17}H_{24}O_3$)			-6.22
10.599	5-Deoxy-5-aminoshikimic acid ($C_7H_{11}NO_4$)			-6.88

Table 6. *Cont.*

RT (min)	Compound Name	Structure	[M+H]⁺ Mass Spectrum	Binding Energy (kcal/mol)
11.934	9*S*,13*R*-12-Oxophytodienoic acid ($C_{18}H_{28}O_3$)			-5.09
11.953	1,5-Anhydro-1-(2,4,6-trihydroxyphenyl) hexitol ($C_{12}H_{16}O_8$)			-7.08
18.711	4,4-Dimethyl-5α-cholesta-8,14,24-trien-3β-ol ($C_{29}H_{46}O$)			-8.52
18.722	Campest-4-en-3-one ($C_{28}H_{46}O$)			-11.31
19.134	Sitostenone ($C_{29}H_{48}O$)			-11.49

is limited in accurately modeling and measuring these biological processes. Each method operates based on specific reaction mechanisms and experimental systems, making them selective for specific antioxidant components and types of reactions as well. Consequently, only some approaches can accurately quantify the total antioxidant capacity [43]. Tables 3 and 4 present the antioxidant capacity measurements obtained via the ABTS, DPPH, and FRAP assays. Each method employs a distinct mechanism to assess a compound's ability to neutralize free radicals, providing valuable insights about its potential benefits.

The ethyl acetate, methanol, and water extracts from various parts of *P. pinnata* demonstrated significant antioxidant activity in the ABTS assay, with inhibition values ranging from 50.36 ± 3.33% to 77.53 ± 0.17% (Table 3). In contrast, pronounced DPPH radical scavenging was evident primarily in the stem and root bark extracts prepared with ethyl acetate and methanol, showing inhibition between 30.68 ± 0.35% and 77.51 ± 1.07%. FRAP results also revealed strong antioxidant capacity across all ethyl acetate, methanol, and water extracts (56.66 ± 3.90 to 86.84 ± 0.87 mg TE/g), regardless of the plant part used (Table 4).

Conversely, nearly all *n*-hexane extracts were inactive in ABTS, DPPH, and FRAP assays. This lack of activity among the *n*-hexane extracts is likely due to the limited solubility and reduced interaction of non-polar (lipophilic) compounds within the polar assay environments. Although the *n*-hexane stem bark extract exhibited relatively high total phenolic content (50.81 mg GAE/g) and total flavonoid content (107.02 mg QE/g), its antioxidant performance was poor. These findings highlight that antioxidant capacity is not solely dictated by the quantity of phenolics and flavonoids but also by the structural characteristics of the bioactive compounds. Phenolics that lack key functional groups necessary for hydrogen donation or radical stabilization may exhibit limited radical scavenging capacity despite being present in significant amounts. Comparable findings were reported in *Passiflora edulis f. edulis* fruit peel extracts, where the methanol extract demonstrated high phenolic (35.95 mg GAE/g) and flavonoid (3.25 mg QE/g) contents, along with superior DPPH scavenging activity (IC_{50} = 14.63 mg/L), relative to the *n*-

hexane extract, which had negligible phenolic levels and significantly lower activity (IC_{50} = 37.39 mg/L) [44]. Similarly, in *Trema macrophylla*, the ethyl acetate extract showed the highest TPC and TFC levels and a strong correlation with antioxidant capacity in the FRAP assay. Although the DPPH assay moderately correlated with phenolic and flavonoid levels, ethyl acetate and methanol extracts outperformed the *n*-hexane extract [45].

These results emphasize the importance of solvent polarity and compatibility with the assay system. Polar solvents like methanol and ethyl acetate are more effective at extracting hydrophilic antioxidant compounds, which are better detected in polar assay media such as ABTS and DPPH. Lipophilic antioxidants in *n*-hexane extracts may be underestimated in these systems due to poor solubility and reduced radical interaction. This phenomenon has also been documented in *Licuala spinosa*, where phenolic-rich *n*-hexane extracts displayed weak antioxidant activity due to solubility issues [46]. In *Capsicum frutescens* seed extracts, despite moderate levels of phenolics (7.95–26.15 mg GAE/g) and flavonoids (4.64–12.84 mg RU/g), the *n*-hexane extract exhibited only 26.9% radical scavenging activity at 1 mg/mL in the DPPH assay [47]. Therefore, similar solvent-related limitations may account for the weak antioxidant activity observed in the *n*-hexane extracts of *P. pinnata*.

3.4. Antidiabetes by AGIs Activity

AGIs are a practical therapeutic approach for controlling hyperglycemia associated with T2DM. Current drugs, such as acarbose, miglitol, and voglibose, inhibit glucose absorption [48]. Acarbose, a known pharmacological inhibitor of α-glucosidase and pancreatic α-amylase, was used as a comparison in the assay [49]. The results of the current study on AGIs showed that the ethyl acetate extract of *P. pinnata* stem bark exhibited the most potent inhibitory activity, with an inhibition rate of 62.78%, followed by the ethyl acetate (59.15%) and methanol (58.41%) extracts of the root bark (Table 5). Among these, the ethyl acetate extract of stem bark demonstrated the highest activity, exhibiting approximately a four-times higher in AGI activity compared to acarbose, which showed only 14.66% inhibition at the same concentration. In contrast, the aqueous (3.42%) and *n* hexane extracts of the stem and root bark exhibited minimal AGI activity. These results indicate that these active constituents are neither highly hydrophilic nor non-polar in extracting α-glucosidase inhibitors. The fruit peel extracts, regardless of the solvent used, showed very low inhibition, ranging from 2.13±0.35% (*n*-hexane) to 9.32±0.70% (methanol). These results indicate that the fruit peel is a less promising source of AGIs than the stem bark.

Previous studies support the potential of ethyl acetate extracts in α-glucosidase inhibition in other plant. For example, ethyl acetate extract of *Catharanthus roseus* roots exhibited potent inhibitory activity (83.29% at 1000 µg/mL). This extract effectively lowered plasma glucose levels in vivo, demonstrating comparable efficacy to acarbose [50]. Similarly, Ngo et al. [51] successfully isolated and characterized ten compounds from the ethyl acetate extract of stems and roots of *Pseuderanthemum crenulatum*. One of these compounds showed α-glucosidase inhibitory activity up to ten times stronger than acarbose.

3.5. Correlation of Phenols, Flavonoids, Antioxidant Activity, and AGIs Activity

The correlation between antioxidant/antidiabetic activity and TPC/TFC of *P. pinnata* extracts is presented in Figures 1, 2, and 3. The correlation analysis used statistical methods to calculate the correlation coefficient (*R*). The *R* value can be interpreted as follows: A negative value signifies a negative correlation, while a positive value indicates a positive correlation. A value of 0 represents no correlation. Values ranging from 0 to 0.3 suggest a weak correlation, 0.3 to 0.7 indicate a moderate correlation, and 0.7 to 1 denote a strong correlation [52]. Correlation analysis revealed a strong positive correlation between TPC and antioxidant activity, as assessed via the ABTS assay (R = 0.800, Figure 1(A)) and DPPH assay (R = 0.891, Figure 1(C)). Similarly, a strong positive correlation was observed between TFC and ABTS (R = 0.740, Figure 1(B)) scavenging potential as well as between TFC and DPPH (R = 0.879, Figure 1(D)) scavenging potential. These findings indicated that higher TPC and TFC correspond to increased ABTS and DPPH antioxidant activities,

highlighting the role of phenolic and flavonoid compounds in reducing oxidative stress through efficient free radical scavenging. However, antioxidant activity assessed via the FRAP assay showed only a moderate positive correlation with either TPC (R = 0.678, Figure 1(E)) or TFC (R = 0.538, Figure 1(B)). Meanwhile, the correlation between antidiabetic activity and TPC was stronger (R = 0.773, Figure 1(G)) compared to that of AGIs and TFC (R = 0.664, Figure 1(H)). These results emphasized the significant contribution of phenolic and flavonoid compounds to the antidiabetic properties of *P. pinnata*. A further correlation study was proceeded between AGIs and antioxidant activities, assessed via three different methods, which revealed a strong correlation for each pair as follows: AGIs and ABTS (R = 0.700, Figure 2(A)), AGIs and DPPH (R = 0.800, Figure 2(B)), and AGIs and FRAP (R = 0.791, Figure 2(C)).

The biplot of PLSR analysis (Figure 3)

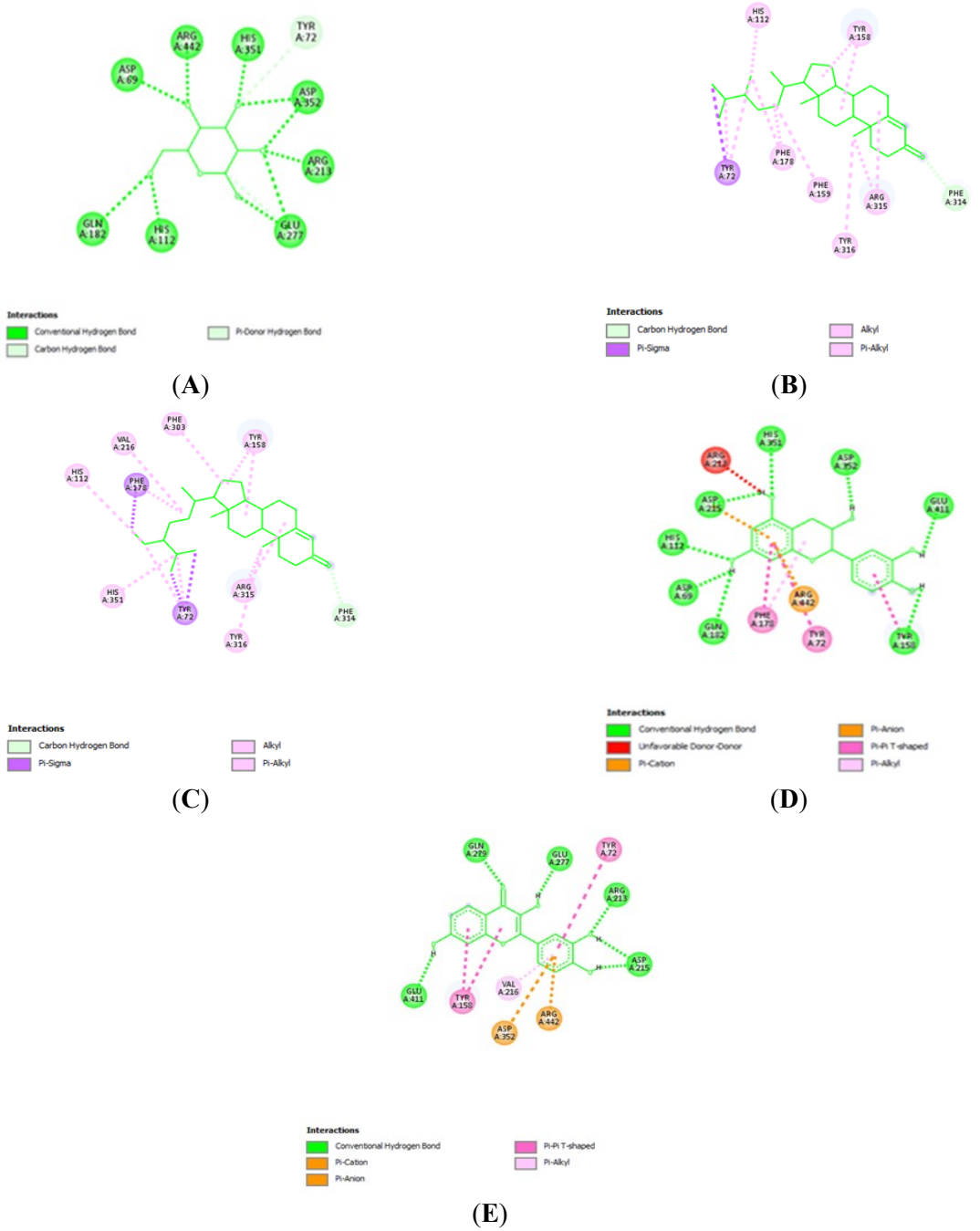


Figure 4. Visualization interaction of (A) native ligand, (B) campest-4-ene-3-one, (C) sitostenone, (D) cianidanol, and (E) fisetin with a-glucosidase from *S. cerevisiae.*

demonstrated nine samples distributed across all four quadrants. ABTS and FRAP had similar trends across the nine samples and thus they were anchored near to each other in the biplot. A similar phenomenon was seen for DPPH and AGIs. The position of TPC indicated its strong correlation with all bioactivity data, i.e. ABTS, FRAP, DPPH, and FRAP. Meanwhile, TFC had a stronger correlation with either DPPH and AGIs compared to its correlation with either ABTS or FRAP. Samples that showed parallel TPC, TFC, and bioactivity data were anchored in proximity as depicted by the three water extracts and the fruit peel ethyl acetate extract. These four extracts were located at the farthest location from TPC, TFC, and bioactivity since they showed low values across the observed variables. In contrast, the rest five samples were anchored closer to the observed variables, implying their higher values. They, however, were scattered, indicating their notable dissimilarity in TPC, TFC, and bioactivity data.

3.6. Metabolite Profiling and In Silico Evaluation of AGI Activity of the Ethyl Acetate Extract from the Stem Bark

LC-HRMS enables comprehensive metabolite profiling by generating mass spectra that include m/z values, molecular formulas, and signal intensities. The resulting mass spectrum plots ion intensity against *m/z* and facilitates compound identification [53]. In positive ion mode, metabolites are commonly detected as protonated ions $([M+H])^+$, with the added proton increasing the molecular mass by ~1 Da, aiding in accurate mass determination [54]. The large amount of m/z data on known and unknown compounds requires filtering by secondary metabolite class [55]. Compound peak analysis using the MzCloud database selects only fully matched compounds, resulting in 26 identified compounds (Table 6). Twenty-six secondary metabolites were identified from the ethyl acetate extract of *P. pinnata* stem bark were categorized into several classes: phenols, flavonoids, steroids, fatty acids, and alkaloids. Phenolic acids and flavonoids, key subclasses of plant-derived polyphenols, exhibit promising antidiabetic activity due to their potent antioxidant effects and their ability to inhibit α-glucosidase, making them valuable candidates for managing hyperglycemia in T2DM [56]. Flavonoids have been confirmed as AGIs through both experimental and *in silico* approaches [57].

Molecular docking of each ligand with the a-glucosidase protein (PDB ID: 3A4A) using AutoDock software through flexible-rigid docking. In this method, the ligand was flexible, while the protein was fixed, and the resulting binding energy reflected the most favorable configuration of the ligand within the protein's binding site [58]. The ligand's binding affinity was evaluated based on the ΔG, calculated by the AutoDock algorithm [59]. These findings suggested the presence of compounds with superior binding properties compared to natural ligands.

In selecting a suitable target for structural analysis, the crystal structure 3A4A was considered highly appropriate. This structure represents isomaltase in complex with maltose, a known competitive inhibitor, and shares 84% sequence similarity with *S. cerevisiae* α-glucosidase [60]. The high-resolution data of 1.60 Å and the lack of mutations within the active site further support its reliability as a model system [61]. Additionally, the amino acid sequence alignment between *S. cerevisiae* oligo-1,6-glucosidase and *S. cerevisiae* α -glucosidase MAL12 revealed a notable similarity of 71.92% [62], suggesting a conserved structural framework that may inform functional interpretations. Validation ensures that the docking software and parameters can correctly predict ligand binding to a target. This process was done by re-docking a known ligand (usually from a crystal structure) into its binding site and comparing the predicted pose with the experimentally determined one. Protein target validation yielded promising results, with an RMSD of 0.56 using a cubic grid box with a size of 50^3, coordinates x, y, z of 22.625, -8.069, and 24.158, and spacing of 0.375. These validation results confirmed the reliability of the docking protocol.

Each ligand conformation binding to the protein results in a distinct binding energy value. Variations in -ΔG can arise from differences in the interactions between the target protein's ligands and amino acids [63]. *In silico* analysis, 100 conformations are typically evaluated. As shown in Table 5, metabolites from *P. pinnata* were predicted to be potential AGIs, as their binding energies were lower

than those of the natural ligand and the reference drug acarbose. The natural ligand exhibited a binding energy of -6.39 kcal/mol, while acarbose had a binding energy of -2.51 kcal/mol. Additionally, several compounds from the stem bark of *P. pinnata* demonstrated even lower binding energies. These include cianidanol, fisetin, sitostenone, campest-4-en-3-one, 4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol, 1,5-anhydro-1-(2,4,6-trihydroxyphenyl)hexitol, 5-[5-hydroxy-3-(hydroxymethyl)pentyl]-8a-(hydroxymethyl)-5,6-dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalene-1-carboxylic acid, and 5-deoxy-5-aminoshikimic acid. Among these compounds, sitostenone and campest-4-en-3-one exhibited particularly low binding energies of -11.49 and -11.31 kcal/mol, respectively, suggesting they hold significant potential compared to the natural ligand and acarbose. The docking results of acarbose with α-glucosidase revealed that it aligned within the same pocket near the active site [64].

These compounds acted as competitive inhibitors, binding to the active site of the a-glucosidase enzyme and interacting with the amino acid residues within the active site pocket. We investigated the interactions of amino acids with two compounds showing the lowest binding energies from molecular docking result and compounds from the phenolic or flavonoid groups, comparing all interactions with those of the natural ligands. The visualization showed that although sitostenone and campest-4-en-3-one had the lowest binding energies, neither compound interacted with the amino acids at the active site of the α-glucosidase enzyme. In contrast, fisetin and cianidanol demonstrated multiple interactions with amino acids within the active site. The amino acid interaction visualizations are presented in Figure 4.

Figure 4(A) showed the native ligand formed hydrogen bonds with the amino acids Asp69, Arg442, His351, Asp352, Arg213, Glu277, and His112, which were key active site residues, including Asp69, His112, Arg213, Asp215, Glu277, His351, Asp352, and Arg441 [65]. Figures 4(B) and 4(C) showed compounds with the lowest binding energy campest-4-ene-3-one and sitostenone neither compound shares the same interaction pattern as the native ligand, suggesting that their binding occurs at a site distinct from the active site of α-glucosidase. While several selected flavonoid compounds showed in Figures 4(D) and 4 (E), cianidanol interacted with five amino acids: Asp215, His112, Asp69, Asp352, His351, and Arg213, and fisetin interacted with four similar amino acids: Glu277, Arg213, Asp215, and Asp352. Oligo-1,6-glucosidase contained amino acid residues responsible for breaking the glycosidic bond, namely His112, Asp215, Glu277, His351, and Asp352 [61]. These findings suggested that several phenolic and flavonoid compounds in *P. pinnata* stem bark contribute to its *in silico* antidiabetic activity. Among them, cianidanol was predicted to be the most promising candidate for further investigation. Several studies reported that cianidanol exhibited various medicinal properties, including antimicrobial, anticancer, antioxidant, and anti-inflammatory effects [66].

4. CONCLUSIONS

In this study, various plant parts of *P. pinnata* were extracted using solvents ranging from non-polar to polar, revealing the ethyl acetate stem bark extract as the most effective in terms of yield, AGI potency, and antioxidant capacity. LC-HRMS profiling identified 26 secondary metabolites, including phenols, flavonoids, steroids, fatty acids, and alkaloids. Notably, molecular docking studies highlighted that the flavonoid cianidanol demonstrated the strongest α-glucosidase binding affinity, exceeding that of acarbose. These results highlight the potential of *P. pinnata* stem bark, particularly cianidanol, as a promising natural agent for controlling postprandial hyperglycaemia in type 2 diabetes mellitus.

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Conflicts of Interest

The authors declare no conflict of interest.

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DECLARATION OF GENERATIVE AI

Not applicable.

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