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In vitro assessment of α-glucosidase inhibitory activity and compound prediction in *Phyllanthus niruri* from West Java Indonesia

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ABSTRACT

The aim of this study was to assess the *in vitro* α-glucosidase inhibitory activity of *Phyllanthus niruri* fractions (PNFs) and identify the chemicals involved. The ethanolic *P. niruri* extract was partitioned using medium-pressure liquid chromatography. The PNFs were examined using ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS), the α-glucosidase inhibitory activity was assessed using yeast α-glucosidase, and molecular docking simulations were performed via Molegro Virtual Docker version 6.0. The extract had a water-soluble content of 37.26%, ethanol-soluble content of 17.16%, total phenol concentration of 167.65 mg GAE/g extract, total flavonoid concentration of 10.07 mg QE/g extract, water content of 2.72%, loss on drying of 33.39%, total ash content of 4.1%, an acid-insoluble ash content of 0.42%, and an *in vitro* α-glucosidase inhibitory activity with an IC_{50} of 1.48 µg/ml. Also, at concentrations of 1 and 3 µg/ml, PNF-1 to PNF-6 had a much stronger inhibitory effect on α-glucosidase than PNF-7 and PNF-8. The UPLC-QTOF/MS analysis of the active fraction identified significant chemicals, notably corilagin **(7)** and gallic acid dimethyl ester **(8)**. The docking analysis revealed advantageous docking scores of −152 and −81. This indicates that these chemicals may serve as effective α-glucosidase inhibitors. The results of this study support the use of *P. niruri* as a good natural product for treating diabetes through the α-glucosidase inhibitory mechanism.

INTRODUCTION

Diabetes mellitus, a chronic disorder marked by hyperglycaemia, ranks among the top 10 causes of death globally. According to the International Diabetes Federation, diabetes currently affects over 537 million people. Diabetes mellitus in adults aged 20 years and over in Indonesia reached 12.5% in 2021 and is predicted to increase to 28.6% in 2045, positioning Indonesia as the fifth country with the highest number of diabetes sufferers in the world. Over 90% of diabetic patients have type 2 diabetes mellitus or T2DM [1]. T2DM is defined by persistently high blood glucose levels, increased blood insulin, dyslipidaemia, and low-grade inflammation [2–4]. A treatment for T2DM involves controlling postprandial hyperglycaemia to reduce glucose absorption by blocking carbohydrate-hydrolyzing enzymes in the digestive system, including α-glucosidase [5].

The α-glucosidase enzyme is a hydrolase that catalyses the hydrolysis of non-reducing terminal carbohydrates into α-glucose [6]. Glucosidase inhibitors impede carbohydrate absorption in the small intestine, unabsorbed complex carbohydrates into absorbable simple carbohydrates by

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competitively blocking glucosidase. By postponing carbohydrate absorption, they mitigate the postprandial metabolic acceleration in blood glucose levels. The α-glucosidase inhibitors may postpone the onset of diabetes in people with diminished glucose tolerance or impaired fasting plasma glucose level [7]. Therefore, a crucial technique for managing postprandial hyperglycemia associated with diabetes mellitus is the suppression of intestinal *α*-glucosidase.

Phyllanthus niruri Linn. (Euphorbiaceae), also referred to as Meniran, as traditional medicine, extensive utilization has been observed in various regions of Indonesia. These therapeutic plants encompass several phytochemical elements, including flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, saponins, steroids, phenols, and glycosides [8,9]. Preclinical studies demonstrated that extracts of *P. niruri* possess antidiabetic properties [10–13]. A recent study on the antidiabetic potential of *Phyllanthus* plants has focused on the α-glucosidase inhibition. The component of corilagin and repanducinic acid A present in the aqueous extract of *P. niruri* showed activity of glucosidase inhibitory by using high-performance liquid chromatography (HPLC)–high-resolution mass spectrometry and nuclear magnetic resonance (NMR) investigations [14]. A study by Mediani *et al* [15] found that flavonoids, phenolic acids, and strong α-glucosidase inhibition were all linked in ethanolic extracts of *P. niruri* at different ratios and water extracts.

The measurement of constituents in *P. niruri* by HPLC has been published, indicating that the phenolics in *P. niruri* may exert antidiabetic activity through *α*-glucosidase inhibitory pathways [12]. *Phyllanthus urinaria*, a different type of *Phyllanthus*, has been shown to help treat diabetes using α-glucosidase inhibitors. The NMR analysis showed that corilagin, mallotinin, and repandusinic acid A were the active ingredients in the water extract [11]. The α-glucosidase inhibition method has been successfully found using bioassay-guided fractionation. However, it is a time-consuming process that needs a lot of preparative-scale separations, which could mean that small parts with important bioactivity are missed [16].

This study aims to evaluate the potential of *P. niruri* fractions (PNFs) as an antidiabetic agent using an *in vitro* α-glucosidase enzyme inhibition approach. The partitioning of *P. niruri* ethanolic extract was performed using medium-pressure liquid chromatography (MPLC). The examination and identification of chemical and biological substances in each fraction employed ultra-HPLC with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS). The UPLC-QTOF/MS delivers good mass accuracy during a brief run, establishing itself as a preferred approach for impartial compound screening and yielding substantial global constituent and metabolite profiling data [17]. The molecular docking simulation method was employed to predict the bioactive inhibitory potential of these drugs *in silico*. We performed molecular docking studies to elucidate the precise interactions between the bioactive components of *P. niruri* and the α-glucosidase enzyme model.

MATERIALS AND METHODS

Plant material and reagents

Phyllanthus niruri herb were sourced from Bogor, West Java, Indonesia. The plant was identified at the Research Centre for Biology, Indonesia Institute for the Science, with number identified 2094/IPH.1.01/if.07/XII/2018. Ethanol food grade for the extraction process (PT Acidatama, Indonesia). Dimethyl sulfoxide, p-nitrophenyl-alpha-D-glucopyranoside (pNPG), α-glucosidase derived from *Saccharomyces cerevisiae*, sodium phosphate monobasic dihydrate, sodium phosphate dibasic, corosolic acid, gallic acid, quercetin, aluminium chloride, sodium carbonate, sodium hydroxide, and Folin-Ciocalteu reagent (Sigma-Aldrich, USA). The waters were purified utilizing Milli-Q Academic manufactured by Merck Millipore (Burlington, MA). Methanol for MPLC and acetonitrile, along with formic acid for UPLC-DAD (Diode Array Detector)-QToF/MS analysis (Merck Millipore and Sigma-Aldrich, USA).

Extraction

Phyllanthus niruri herbs powder (1,000 g) was mixed with 70% ethanol (10 l) and extracted using the maceration method for 4 hours at a temperature of 60ºC with shaking. The filtrate was evaporated until a semisolid crude extract (200.39 g) was obtained.

Characterization of extract

Organoleptic qualities

We observed the colour, aroma, and shape of the leaves of the 70% ethanol extract of *P. niruri*.

The water-soluble extract content

In a glass-stopped flask, 5 g of ethanol extract of *P. niruri* were macerated for 24 hours with 100 ml of chloroform-saturated water, while being shaken for the first 6 hours, left for 18 hours. Filtered and subsequently dried in a shallow flat-bottomed dish at 105ºC. Heat the remainder at 105ºC until the weight remains constant. Calculate the content in % of air-soluble extract [18].

The ethanol-soluble extract content

In a glass-stopped flask, 5 g of ethanol extract of *P. niruri* were macerated for 24 hours with 100 ml of 96% ethanol, while being shaken for the first 6 hours, left for 18 hours. Filtered and subsequently dried in a flat-bottomed dish at 105ºC. Heat the remainder at 105ºC until the weight remains constant. Calculate the content in % of ethanol-soluble extract [18].

Determination of water content

The water content of *P. niruri* extract was determined using Karl–Fischer titration method. An amount of 50 mg of extract with glass weight and the transfer into the titration chamber, which contains combiMethanol-5. Titrate the extract using the CombiTitran-5 reagent automatically. Water content expressed as a percentage of the *P. niruri* extract weight [19].

Determination of loss on drying

The loss on drying of *P. niruri* extract was determined using a water balance. Approximately 0.5 g of extract was accurately weighed into an aluminium weighing pan and heated at 105°C. The extract was evenly distributed over the entire surface of the pan, then placed into the moisture balance chamber. Drying was carried out at a predetermined

temperature until the instrument automatically displayed a stabil final reading [19].

Total ash content

Approximately 1 g of extract was added to the crucible. It was then placed in a furnace and heated at 450ºC until the weight was constant. The sample was removed, cooled in a desiccator, and weighed. If the charcoal could not be removed, boiling water should be added and filtered through ash-free filter paper. The residue and filter paper in the same crucible were burned. The filtrate was placed in a crucible, evaporated, and burned until constant, then weighed. The total ash content was calculated against the weight of the extract [18].

Acid insoluble ash content

The ash obtained in the determination of the ash total content, heated with 25 ml of diluted sulfuric acid solution for 5 minutes. The insoluble component was collected, filtered through a glass crucible or ash-free filter paper, rinsed in hot water, and burned until the weight constant, weigh. The acid-insoluble ash content was calculated against the dried material [18].

Total phenol content

The total phenol in *P. niruri* extract was measured using the Folin–Ciocalteu method of Abd-Ghafar *et al.* [20], with slight modifications. The extract of *P. niruri* was dissolved in ethanol (1,000 µg/ml). The assay was conducted by adding 25 µl of sample to the 96-well microplate. To this, 75 µl of Folin–Ciocalteu reagent (10-fold dilution with deionized water) was added and mixed thoroughly at room temperature for 5 minutes. Then, 75 µl of sodium carbonate (6%) solution was added to the mixture. The solution was incubated for 90 min in the dark at room temperature. Absorbance was measured at 725 nm using a microplate reader (BioTek Instruments, Germany). The total phenolic content was determined using a standard curve of gallic acid at 6.25–200 µg/ml concentrations. Total phenolic content was calculated and expressed as mg of gallic acid equivalent in 1 g of the extract (mg GAE/g extract).

Total flavonoid content

The flavonoid content of *P. niruri* extract was measured using the aluminium chloride colorimetry method as described by Singh *et al*. [21], with slight modifications. Briefly, 50 µl of *P. niruri* extract (1,000 µg/ml) was added into a microplate containing 50 µl of 95% ethanol. Then, 20 µl of 10% aluminium chloride hexahydrate, 20 µl of 1 M sodium acetate, and 100 µl of water were added to the 96-well microplate. The mixture was incubated for 30 minutes at room temperature, and the absorbance was read at 425 nm using a microplate reader (BioTek Instrument, Germany). Quercetin was used as the standard to determine the total flavonoid content values of the samples. A standard curve was used with concentrations of quercetin in the 10–160 µg/ml range, and the total flavonoid content values are presented as mg quercetin equivalent in 1 g of the extract (mg QE/g extract).

Fractionation

The extract (1.02 g) was fractionated by MPLC, which was equipped with an ultraviolet (UV) and evaporative light scattering detector (ELSD). The sample was run in the YMC-Pack ODS column AQ-HG 250 × 20 mm, 10 µm, Kyoto, Japan with the water (A) and methanol (B) gradient elution as follows: 0%–100% (B) for 105 minutes, flow rate = 20 ml/min, and injection volume = 5 ml.

UPLC-QTOF/MS examination

The sample of PNFs was studied using a Waters Acquity UPLC system that has a photodiode array and a QTOF mass detector (Waters, Milford, MA). The system has an Acquity UPLC® BEH C18 column that is 1.7 µm, 2.1 × 100 mm, and is kept at 40ºC. The mobile phase eluent system consisted of A (0.1% formic acid in demineralized water) and B (0.1% formic acid in acetonitrile) at a flow rate = 0.4 ml/min, injection volume = 2 µl, and concentration of sample of 1,000 µg/ml. The sample analysis concentrated on phenolic and tannin chemicals, including flavonoids, utilizing gradient elution as follows: 5% (B) for 0.0–1.0 minutes, 5%–100% (B) for 1.0–20.0 minutes, 100% (B) for 20.0–22.3 minutes, 100%–5% (B) for 22.3–22.4 minutes, and 5% (B) for 22.4–25.0 minutes.

***In vitro* of α-glucosidase inhibition assay**

The *α*-glucosidase inhibition assay was measured using the method adopted previously by Hou *et al.* and Ni *et al*. [22,23], with minor modifications. The assay utilized pure enzymes obtained from yeast (*S. cerevisiae*). To do the test, 50 µl of 100 mM phosphate buffer (pH 6.8), 10 µl of α-glucosidase (1 U/ml), and 20 µl of *P. niruri* extract or PNF at different concentrations (0.3, 1, 3, 10, 13, and 100 µl/ml) were put into a 96-well plate. We incubated the reaction mixture at 37°C for 15 minutes. Subsequently, 20 µl of 5 mM pNPG was introduced, and then incubated for 20 minutes at 37ºC. The reaction was stopped by introducing 50 µl of 0.1 M sodium carbonate. At 405 nm, the absorbance was measured using a microplate reader (Epoch, BioTek Instruments, Germany). The control utilized dimethyl sulfoxide, while the positive control employed corosolic acid. To determine α-glucosidase inhibition, the following equation was used: Inhibitory activity (%) = (1 − A405 of sample/A405 of control) × 100. We determined the IC_{50} value using linear regression analysis.

Molecular docking simulation

The simulation of molecular docking was performed using Molegro Virtual Docker (MVD) ver. 6.0 to estimate the inhibitory action mechanism of *P. niruri* bioactive compounds against α-glucosidase. For the 3D structure of α-glucosidase from the *Saccharomyces* model, homology modelling was constructed using the Swiss model (swissmodel.expasy.org) as the structure is not available in the Protein Data Bank (www.rscb.org). The α-glucosidase structure used in the assay is an enzyme with a sigma product number G0660 and commission number 3.2.1.20. Based on this information, the appropriate sequence was selected in the UniProt P53341-MAAL12-yeast, which consists of 584 amino acids. The model was built by

copying and pasting the fasta of the sequence using the Swiss model (swissmodel.com) with the 3AXH.1A as a template, which has a sequence identity of 72.12% and a GMQE of 0.94. The 3D crystal structure of α-glucosidase model was used as a receptor in the docking study then it was imported to the MVD program. As an initial approach, validation of docking protocol settings was done through isomaltase (ligand reference of 3AXH.1A) at some cavities of the enzyme structure. We are confirming the confidence in our study used the docking protocol reproduces the bound structure closely with root mean square deviation (RMSD) values less than 2Å [24]. Then, active compounds of *P. niruri* herb were selected to be docked into the receptor at the position, and the interactions were investigated.

Statistical analysis

The data are presented as mean ± SD of three observations. The IC_{50} values of were statistically analyzed using the slope of the scatter plot in Excel.

RESULTS AND DISCUSSION

Extraction and characterization of *P. niruri* extract

We assessed the extraction and characterization of *P. niruri* extract (Table 1). The organoleptic evaluation of the ethanol extract of *P. niruri* indicated a blackish-green hue, absence of odor, and a bitter flavor, with a yield of 20.03%. The subsequent characterization parameter involved quantifying the concentrations of the chemical dissolved in water and ethanol. This parameter represents the amount of chemicals that a specific solvent can extract or dissolve. The results indicated that the extract containing a water-soluble solvent produced higher quantities than the ethanol-soluble extract. The solubility of *P. niruri* extract in water and ethanol was 37.86% ± 0.08% and 17.16% ± 0.28%, respectively. In this finding, *P. nirurri* extract was predicted to contain high levels of polar compounds. Meanwhile, based on the research results of Jayani *et al*. [25], the levels of water-soluble and ethanol extracts of *P. nirurri* leaves powder showed values of 17.65% ± 0.93% and 11.10% ± 0.16%, respectively.

Based on analysis, *P. niruri* extract had a water content of 2.72% ± 0.14%. *Phyllanthus niruri* extract is a thick extract preparation that is in accordance with standards for *P. niruri* herbs, which must have a moisture content of less than 17% [18]. The water content in the extract can vary depending on the humidity of the storage area and the amount of water still present in the extract. The water content is critical for preserving the extract's quality, inhibiting the proliferation of bacteria, fungus, or insects, as well as preventing degradation from hydrolysis [26].

Table 1. Characterization of *Phyllanthus niruri* extract.

Parameters	Result obtained
Water soluble extract content	37.86% ± 0.08%
Ethanol soluble extract content	17.16% ± 0.28%
Water content	2.72% ± 0.14%
Loss on drying	33.39% ± 0.14%
Total ash	4.01% ± 0.41%
Acid insoluble ash	0.42% ± 0.01%
Total phenolic content	167.65 ± 4.80 mg GAE/g of extract
Total flavonoid content	10.07 ± 0.22 mg QE/g of extract

GAE = gallic acid equivalent; QE = quercetin equivalent.

The drying loss aims to determine the maximum limit of the number of compounds lost in the form of water and volatile components in the drying process. To calculate the loss on drying, we must weigh the substance after it has dried at 105°C to a constant weight and express it as a percentage. The assessment of loss on drying of the *P. niruri* extract yielded a value of 33.39% ± 0.14%. The loss on drying of the extract shows that the remaining volatile substances in the *P. niruri* extract are a maximum of 33.39% ± 0.14% [19].

The determination of ash content entails heating the extract until organic molecules and their derivatives decompose and evaporate, resulting in residual mineral and inorganic elements. This provides a comprehensive overview of the internal and exterior mineral composition from the early stages of the process until extract generation. The extract of *P. niruri* contains a total ash concentration of 4.0% ± 0.41% and an acid-insoluble ash level of 0.42% ± 0.01%. The amount of acid-insoluble ash shows that there are extra contaminants such as soil and sand, while the amount of total ash in the extract shows that overall minerals, including plant minerals and possible external residues, are present during the maceration process. The maximum ash content range indicates the level of pollution and cleanliness. It is utilized to assess the mineral composition in extracts and the mineral content that is insoluble in acids [26].

According to the study, the total phenol content of the 70% ethanol extract of *P. niruri* herb has a phenol content of 167.65 ± 4.80 mg GAE/g extract. Phenolic compounds are secondary metabolite and have anti-diabetes and antioxidant activity. The analysis of phenol levels showed higher results than previous studies. Research by Rusmana *et al*. [27] reported that 70% ethanol extract of *P. niruri* extracted for 24 hours using the maceration method obtained a total phenol content of 61.36 ± 0.42 µg EGA/mg extract. Mediani *et al*. [15] studied changes in metabolites of 70% ethanol extract of *P. niruri* after three freeze drying, oven drying, and air drying, showing phenol content values of 0.145 ± 0.006, 0.107 ± 0.003, and 0.113 ± 0.004 mg GAE/mg extract, respectively.

In our research results, the total flavonoid content of the 70% ethanol extract of *P. niruri* was obtained at 10.07 ± 0.22 mg QE / g extract. Several researchers have reported the flavonoid content of *P. niruri*, Carmagnani *et al*. [28] found that the extract obtained by the maceration method for 7 days using 50% and 96% ethanol solvents obtained total flavonoid values of 35.67 ± 6.139 and 55.72 ± 3.137 mg QE/g extract. *Phyllanthus niruri* extracted with 80% ethanol for 3 × 24 hours obtained a value of 60.1 ± 1.95 mg QE/g extract [29]. Meanwhile, the flavonoid content in the 70% ethanol extract of *P. niruri* obtained through maceration is 12.32 ± 0.53 mg of rutin equivalent per gram of extract (mg RE/g extract) [30]. Phenolic compounds are known to have antidiabetic activity by inhibiting the α-glucosidase enzyme, which plays a role in breaking down carbohydrates into glucose. Inhibition of this enzyme can

slow down the absorption of glucose in the intestine, so that it can reduce postprandial blood glucose levels. Some phenolic compounds, such as flavonoids and phenolic acids, show a high affinity for the α-glucosidase enzyme and have the potential as natural therapeutic agents for type 2 diabetes [31–33].

Inhibitory activities of the extract *P. niruri* against α-glucosidase

The evaluation of inhibitory activities of the extract of *P. niruri* was examined using yeast glucosidase. The strongest inhibitory effect of the ethanolic extract of *P. niruri* was 98.85% at 10 µg/ml, while the strongest effect of the positive control, corosolic acid, was 99.25% at 30 µg/ml. Figure 1 shows that the percentage inhibition profiles relative to sample concentration indicate that the extract of *P. niruri* exhibited the most potent glucosidase inhibition with an IC_{50} value of 1.48 ± 0.51 µg/ml, than corosolic acid (IC_{50} = 2.72 ± 0.72 µg/ml).

According to previous research, the *α*-glucosidase inhibitory activity of *P. niruri* in our study was higher than that of a number of known extracts. The ethanol extract of *Phyllanthus amarus*, obtained through maceration, demonstrated activity with an IC_{50} value of 362.33 µg/ml [34]. After 30 minutes of ultrasonication and overnight maceration, the IC_{50} value of 6.3 ± 4.8 mg/ml of *P. niruri* ethanol extract was found to have α-glucosidase inhibitory [35]. The 70% ethanol extract of *P. niruri* was made by freeze drying, oven drying, and air drying, had α-glucosidase inhibitory effects with the IC_{50} values of 11.43 ± 1.06, 12.89 ± 1.41, and 11.62 ± 1.32 µg/ml, respectively [15]. The ethanol extracts of *P. amarus* and *P. urinaria*, which were made by sonicating the plants for 30 minutes and then macerating them overnight, had IC_{50} values of 926.5 ± 39.0 and 39.7 ± 97 µg/ml, respectively, for glucosidase inhibition [11]. Nevertheless, compared to other extract types, our result's efficacy was marginally inferior. *Phyllanthus niruri* methanol extract exhibited α-glucosidase inhibition with an IC_{50} value of 0.2 ± 0.02 mg/ml [12]. The IC_{50} of *Phyllanthus emblica* was found to be 0.66 ± 0.01 µg/ml for α-glucosidase inhibition [36]. Corosolic acid inhibited α-glucosidase with an IC_{50} value of 1.35×10^{-5} mol/l [23]. Corosolic acid is a triterpenoid molecule with significant potential for glucosidase inhibitory action. Corosolic acid is present in *Lagerstroemia speciosa* leaves, ethyl acetate fraction with an IC_{50} = 3.53 pg/ml and in *Psidium guajava* leaves, methanol extract with an IC_{50} = 1.33 µg/ml [37,38]. Meanwhile, acarbose was used as a standard control of the α-glucosidase inhibitor, with an IC_{50} value of 41.23 µg/ml [39]. Acarbose, a common drug prescribed an α-glucosidase inhibitor, has been approved for the management of T2DM [39,40]. These results indicate that the extract of *P. niruri* exhibited stronger α-glucosidase inhibitory activity, as reflected by its lower IC_{50} value compared to both acarbose and gallic acid.

Fractionation and characterization of the phytochemicals in *P. niruri* extract

Phyllanthus niruri extract was fractionated utilizing a gradient methanol–water system by MPLC. The gradient system was rendered highly polar during the initial 10 minutes to isolate all chemicals present in the aqueous fraction. Subsequently, the polarity level transitions gradually to the non-polar phase. Compounds with analogous polarity will reside in the same fraction. Subsequently, eight fractions (PNF-1 to PNF-8) were derived, exhibiting a dispersion of components with varying polarity.

UPLC was employed to analyze the phytochemicals in the ethanol extract of *P. niruri* to ascertain its chemical makeup and potential active constituents. Figure 2 illustrates the performance of profiling mass spectrometry on the fraction using negative ion modes. The UV and MS spectrum data primarily demonstrated the presence of derivatives of phenolic acid, tannin, and flavonoids in the fraction (Table 2). The

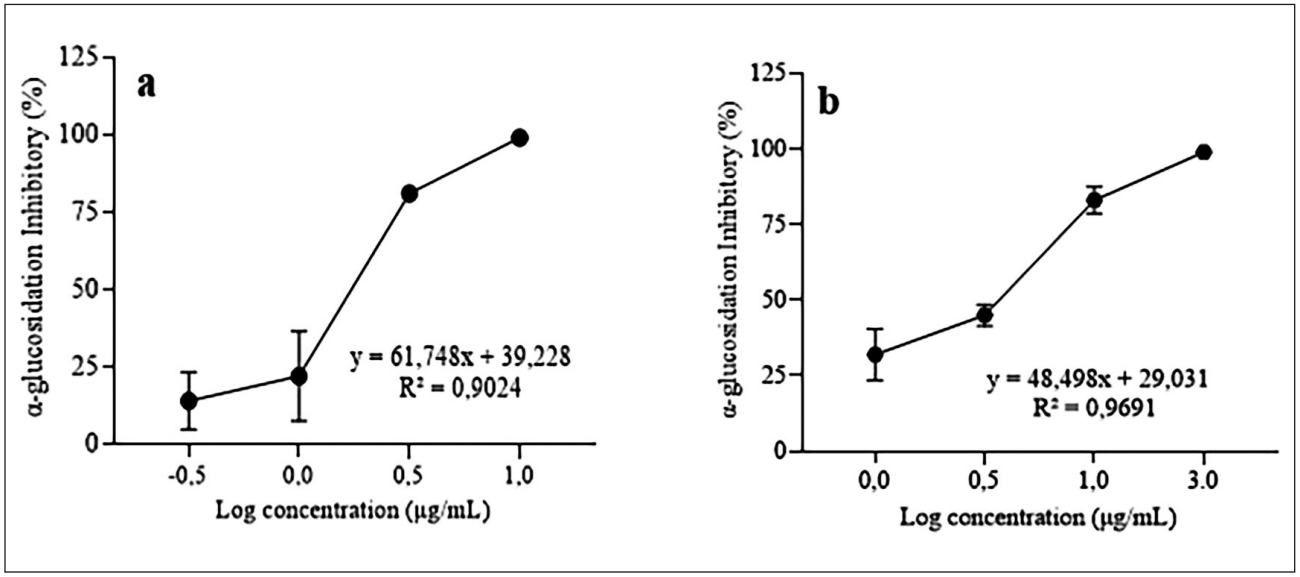

Figure 1. Profile of concentration inhibition of α-glucosidase activity. *Phyllanthus niruri* extract (a) and corosolic acid (b). The IC_{50} values for the *P. niruri* extract and corosolic acid on α-glucosidase inhibition are 1.48 ± 0.51 and 2.72 ± 0.72 µg/ml, respectively.

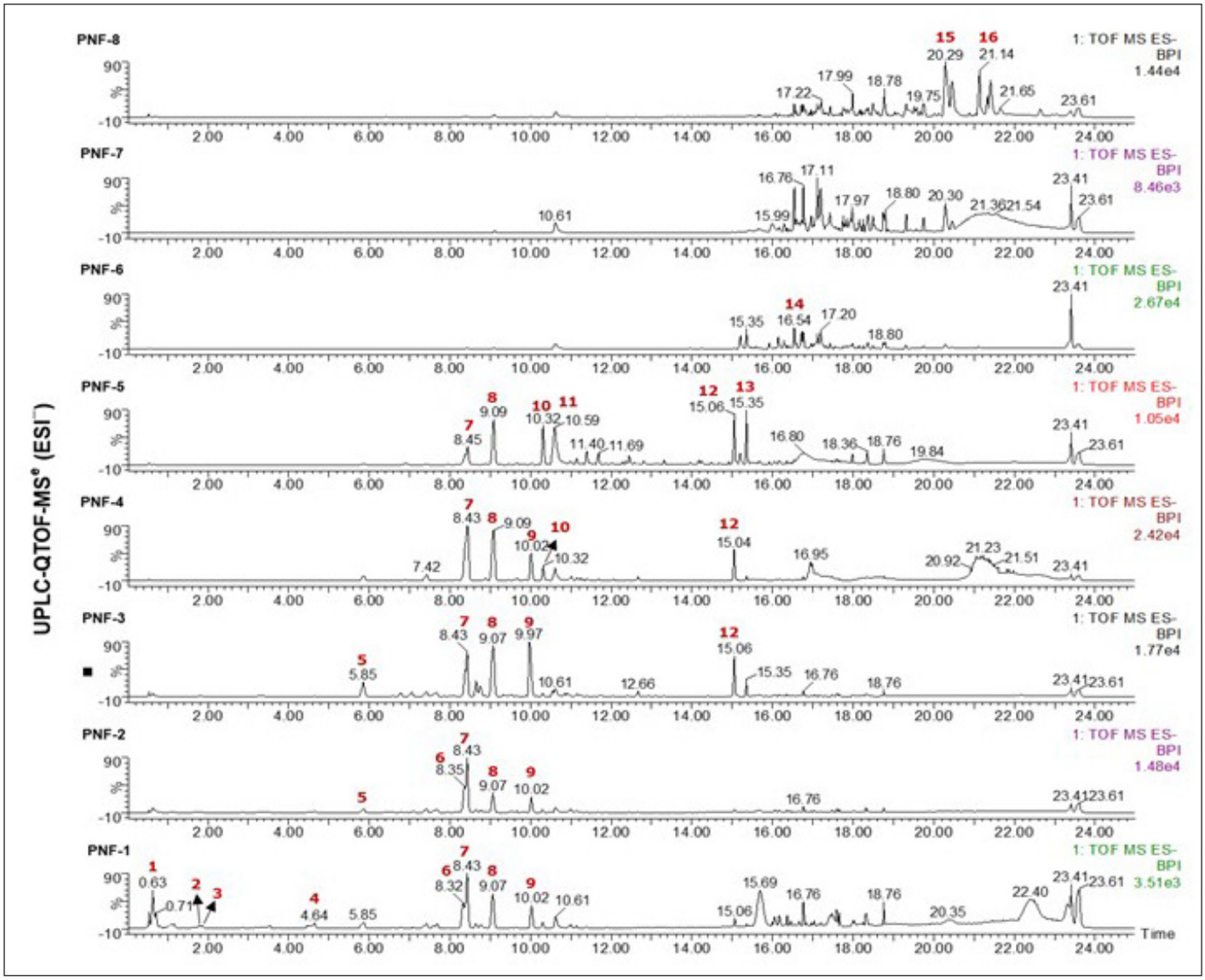

Figure 2. The MS chromatograms of the *Phyllanthus niruri* fractions in negative mode.

primary constituents identified were geraniin (**6**), corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin derivatives (**9**), quercetin (**10**), hydroxyflavonol (**11**), and galangin-8-sulfonate (**12**). In addition, there are unidentified compounds (**4**), (**13**), and (**14**). However, according to the molecular formula and fragmentation pattern derived from UPLC-QTOF-MS data, the finding is ellagitannin class (**4**), phenolic acid derivatives (**13**), and flavonoids (**14**) are found.

In vitro inhibition of *α*-glucosidase by PNFs

In vitro investigations demonstrated that the inhibitory potency of the α-glucosidase enzyme is closely correlated with the compound's polarity, as seen by the peak in the UPLC chromatogram (Table 3). The results showed that fractions PNF-1 to PNF-6 significantly blocked *α*-glucosidase, with doses of 1 and 3 µg/ml showing more than 90% inhibition. The inhibitions were markedly elevated ($p < 0.05$) in comparison to fractions PNF-7 and PNF-8. The UPLC study reveals that the primary constituents of the active fraction of *P. niruri* include geraniin, corilagin, gallic acid dimethyl ester, and quercetin derivatives. Corilagin and gallic acid dimethyl ester were identified as consistent components in fractions PNF-1 to PNF-5. While fraction PNF-6 to PNF-8 lacks these chemicals, it remains unclear whether the drug inhibits the glucosidase enzyme. Fraction PNF-6 to PNF-8 showed a decrease of α-glucosidase inhibitory activity when compared to fraction PNF-1 to PNF-5, which could be due to the synergistic effect of active compounds present in fraction PNF-1 to PNF5 in inhibiting *α*-glucosidase [40–42].

The loss of synergy in the fraction reported in studies of α-glucosidase inhibition activity. Mugaranja *et al*. [40] founded that the α-glucosidase inhibition activity of the purified fraction-14 from *Simarouba glauca* was slightly lower than that of the crude extract, likely because the phenolic compounds

Table 2. Tentative identification of *Phyllanthus niruri* components using UPLC-QTOF/MS.

No.	tR, min	Molecular formula	UV	Negative					Tentative ID	Class	References
				MS RT (min)	Detected ion $[M-H]^-$	Calculated ion $[M-H]^-$	Error (µg/ml)	Major fragment ions			
1	0.63	C12H14O10	201	0.63	317.0487	317.0509	−6.9	317, 209, 191, 162	Mucic acid derivatives	Galactaric acid	[72]
2	1.75	C13H16O10	207, 275	1.76	331.0661	661.0665	−1.2	331, 169	Gallic acid hexoside	Phenolic acid	[58,59]
3	1.82	C7H6O5	207, 270	1.86	169.1021	169.0137	−9.5	169, 125	Gallic acid	Phenolic acid	[57–59]
4	4.55	C18H14O13	207, 300	4.57	437.0337	437.0356	−4.3	437, 409, 137	Unknown	Ellagitannin	*
5	5.81	C8H8O5	208, 275	5.85	184.0290	184.0400	−1.1	183, 125	Gallic acid methyl ester	Phenolic acid	[57–59]
6	8.32	C41H28O27	215, 265	8.32	951.0736	951.0740	−0.4	951, 633	Geraniin	Tannin	[58,73,74]
7	8.38	C27H22O18	215, 270	8.41	633.0681	633.0728	−7.4	633, 631, 387, 137	Corilagin	Ellagitannin	[58,72]
8	9.02	C9H10O5	211, 272	9.04	197.0434	197.0450	−8.1	197, 169, 125	Gallic acid dimethyl ester	Phenolic acid	[58,72]
9	9.86	C24H16O13	211	9.92	511.0510	511.0513	−0.6	511, 301, 137	Quercetin derivatives	Flavonoid	[69,75]
10	10.28	C15H10O7	211	10.29	301.0374	301.0348	8.6	301, 271, 255, 229	Quercetin	Flavonoid	[60]
11	10.56	C15H10O7	211, 253	10.61	317.0026	317.0041	−2.0	635 [2M-H], 317, 301	Hydroxyflavonol	Flavonoid	[76]
12	14.96	C15H9O8S	218	14.99	348.9966	348.9987	−7.7	348 [M-Na], 269	Galangin-8-sulfonate	Flavonol Sulfonates	[77]
13	15.32	C6H20N2O4	218	15.35	183.1343	183.1345	−1.1	183	Unknown	Phenolic acid	*
14	16.50	C30H60O16	220	16.53	675.3790	675.3803	−1.9	721 [M+FA], 675, 397	Unknown	Flavonoid	*

*Based on fragmentation pattern.

Table 3. The *α*-glucosidase inhibitory activity and tentative compounds of *Phyllanthus niruri* fractions

Fractions	Weight (mg)	α-glucosidase inhibitory (%)		Tentative compounds of *P. niruri* fractions
		1 µg/ml	3 µg/ml	
PNF-1	301.0	99.25 ± 0.19	98.36 ± 0.00	Mucic acid derivatives (**1**), gallic acid hexoside (**2**), gallic acid (**3**), geraniin (**6**), corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin derivatives (**9**)
PNF-2	384.6	99.25 ± 0.19	99.69 ± 0.05	Gallic acid methyl ester (**5**), geraniin (**6**), corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin derivatives (**9**)
PNF-3	10.1	96.21 ± 0.15	99.52 ± 0.00	Gallic acid methyl ester (**5**), corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin derivatives (**9**), galangin-8-sulfonate (**12**)
PNF-4	24.2	96.55 ±0.05	99.59 ± 0.00	Corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin derivatives (**9**), quercetin (**10**), galangin-8-sulfonate (**12**)
PNF-5	36.4	96.51 ± 0.00	99.49 ± 0.15	Corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin (**10**), hydroxyflavonol (**11**), galangin-8-sulfonate (**12**)
PNF-6	17.5	94.10 ± 1.64	99.45 ± 0.00	Unknown
PNF-7	12.9	47.83 ± 3.56	75.15 ± 5.85	Unknown
PNF-8	9.2	7.62 ± 1.55	21.16 ± 0.68	Unknown

in the crude extract work together to enhance the activity. The ethanol extract of *S. glauca* and fraction-14 had activity of α-glucosidase inhibition with an IC_{50} value of 0.5 ± 0.04 and 2.4 ± 0.4 µg/ml. Black tea and green tea crude extracts exhibited lower α-glucosidase inhibition activity than semi-purified extracts. The black tea of 70% ethanol extract and semi-purified extract was observed to have activity of α-glucosidase inhibition with an IC_{50} value of 30.01 ± 1.50 and 46.02 ± 1.71 µg/ml. Meanwhile, a small reduction in α-glucosidase inhibition activity was detected in 70% ethanol of green tea and semi-purified extract, with IC_{50} values of 26.04 ± 1.20 µg/ml and 38.07 ± 1.40 µg/ml, respectively [41]. Suzlin Sulaiman *et al*. [42], reported the alkaloid separation process from *Uncaria longiflora*, showing pteropodine and isopteropodine inhibited α-glucosidase with IC_{50} values of 226.70 ± 2.82 and 98.06 ± 1.98 µg/ml, while the crude extract methanol IC_{50} value was 138.10 ± 1.32 µg/ml. This pattern suggests that isopteropodine and its other chemical constituents could synergistically contribute to the extract's activity. In addition, the presence of antagonistic compounds in a mixture of natural ingredients can result in decreased *α*-glucosidase enzyme activity. Researchers Rahayu *et al*. [43] reported that the combined infusion extract of bay leaves and soursop or guava and soursop showed antagonistic effects. The combination of bay and soursop and guava and soursop infusions had IC_{50} values of 0.063 ± 0.005 microgram gallic acid equivalent per ml (µg GAE/ml) and 0.094 ± 0.002 µg GAE/ml. But, the infusion of bay, guava, and soursop showed lower 0.025± 0.007, 0.083 ± 0.01, and 0.533 ± 0.039 µg GAE/ml of α-glucosidase activity inhibitory. These findings correlated with their total phenolic content, where bay, guava, and soursop infusions contained 18.48 ± 2.27, 17.84 ± 2.48, and 21.43 ± 0.13 µg GAE/ml. In addition, the effect of the active ingredient is covered by other compounds in a complex mixture, which also occurs in natural ingredient mixtures. Complexity can also occur because the levels of compounds contained are very small, causing the active compound to have low activity, which results in decreased activity [43,44].

Our investigation suggests that the four principal substances (geraniin, corilagin, gallic acid dimethyl ester, and a quercetin derivative) substantially inhibited the α-glucosidase enzyme. Our findings are corroborated by prior research, which demonstrated that these chemicals can block α-glucosidase [11,22,38,45–66].

Geraniin is an ellagitannin, a category of hydrolyzable tannin, that constitutes a significant component in various plant species, including *Phyllanthus, Geranium*, *Nephelium lappaceum*, and *Erodium glaucophyllum*. Geraniin has been recognized for its antioxidant, anti-hyperglycemic, antiviral, and anticancer properties [46,48,49,66]. According to a study by Agyare *et al*. [50], the primary component was an aqueous extract of *Phyllanthus muellerianus* leaves, which contained 2.9% geraniin. LC-MS analysis of a cocktail extract of *Phyllanthus* species (a mixed of *Phyllanthus watsonii, P. amarus, P. niruri,* and *P. urinaria* in a 2:2:1:1 ratio) revealed geraniin as a predominant constituent. The rind extracts of *N. lappaceum* identified geraniin as a significant constituent, accounting for 56.8% of the methanolic extract [51]. According to Palanisamy *et al*. [49], geraniin in *N. lappaceum* rind ethanol extract was around 37.9 mg/g. This extract demonstrated the ability to inhibit the α-glucosidase enzyme with an IC_{50} value of 0.92 µg/ml, while geraniin had an IC_{50} value of 16.12 µg/ml [46].

Corilagin, a gallotannin, is predominantly located in the families Aceraceae, Combretaceae, Euphorbiaceae, Geraniaceae, and Sapindaceae. It is a significant active constituent of numerous plants, including *P. emblica, P. niruri,* and *P. urinaria* [52]. Notka *et al*. [53], reported isolating corilagin and geraniin from the water–alcohol extract of *P. amarus*, achieving concentrations of 2.28% and 1.10%, respectively, through HPLC analysis. Zheng *et al*. [54], found corilagin in plant alcoholic extracts. The amounts found were 67.9, 159, and 619 µg/g in *Phyllanthus debilis, Phyllanthus tenellus*, and *P. urinaria*, respectively. A study by Hou *et al.* [22], investigated extracts of *P. amarus, P. niruri, P. tenellus*, and *P. urinaria* utilizing an aqueous ionic liquid in conjunction with preparative HPLC and precipitation techniques; the

corilagin concentrations were determined to be 1111.68, 1157.87, 1684.08, and 144.09 µg/g, respectively. The inhibitory of α-glucosidase activity of corilagin derived from water extract of *P. urinaria* exhibited IC_{50} values of 1.70 and 0.9 µM [11,35]. A separate study identified corilagin in *Nymphaea stellata* flowers, methanol extract exhibited 24% (at a concentration of 1 mg/ml) of α-glucosidase inhibition. Corilagin, derived from *Macaranga tanarius* leaves ethyl acetate extract, has the ability to inhibit mammalian intestinal glucosidase, exhibiting an IC_{50} value of 2.63 mM [55]. Corilagin, also found derived from *Terminalia chebula* aqueous extract of was documented with an IC_{50} value of 2.64 µM [45].

Gallic acid and its methyl ester (gallic acid dimethyl ester), belonging to the phenolic acid class, are naturally occurring secondary metabolites present in several plants and herbs [56]. Gallic acid is abundantly sourced from medicinal plants within the *Phyllanthus* genus, including *P. amarus, P. urinaria, P. myrtifolius, P. multiflorus, P. debilis, P. embergeri, P. tenellus, P. emblica,* and *P. fraternus* [57–59]. Kumar *et al.* [58], demonstrated that *P. amarus* contained gallic acid at levels ranging from 0.45 to 32.80 mg/g and quercetin from 0.01 to 5.22 mg/g, as ascertained by UPLC-MRM. The gallic acid concentration in the aqueous extract of *P. amarus* is 135.08 mg/g. Moreover, gallic acid is found in other therapeutic plants, including *Momordica charantia* with 97.35 mg/g, *Achillea schischkinii* with 11.2 mg/g, and *Rhodiola crenulata* with 8.12 mg/g [60–62].

Gallic acid and alkyl gallates derived from winery by-products exhibited anti-α-glucosidase activity, with grape stems, grape pomace, and wine leaves demonstrating *α*-glucosidase inhibitory activity levels of 1.58, 1.46, and 123 units/l, respectively [63]. Simultaneously, 4-O-methyl gallic acid was identified in *Syzygium myrtifolium* ethyl acetate fraction, with an IC_{50} value of 25.19 µg/ml [64].

Quercetin is a flavonoid subclass seen in plants, fruits, vegetables, and leaves. Quercetin possesses multiple pharmacological characteristics, including the suppression of *α*-glucosidase. *P. urinaria* and *P.acidus* extract were obtained using 60% aqueous acetone and 50% ethanol, respectively, and were identified to contain the quercetin compound [67]. Quercetin is present in *P. emblica* fruit and *P. amarus* leaves extract at a measured level of 10.20 and 5.84 mg/g, respectively [60,68]. The callus culture of *P. niruri* comprises 1.72% quercetin [47]. At other plants, *M. charantia, Morus alba,* and *Ginkgo biloba* extracts contain quercetin at concentrations of 50.39, 6.29, and 3.49 mg/g, respectively [60,68]. According to Li *et al*. [69], reported quercetin and its derivative isoquercetin were identified as α-glucosidase activity inhibitors, IC_{50} = 0.017 and 0.185 mmol/l, respectively. Another report, as presented by Sofa *et al*. [65], quercetin derivatives (3',4'-dimethoxy quercetin, quercetin 3-O-α-L-rhamnoside, and quercetin-3-O-*α*-L-arabinopyranosyl (1→2) α-L rhamnopyranoside) from *Bryophyllum pinnatum* exhibited α-glucosidase inhibitory, IC_{50} = 103.20, 83.83, and 110.52 µg/ml, respectively.

Molecular docking analysis

A molecular docking study yields crucial insights into the framework of interactions between identified compounds and enzymes. Additionally, it provides binding energy data that can be used to assess the viability of specific substances as α-glucosidase inhibitors. Validation of the docking simulation was done by redocking the native ligand and then investigating its superimposition. The RMSD value obtained from the superimposition is 1.16 Å. The coordinates for docking at the specified place are $x = -19.80$, $y = -7.74$, and $z = -21.47$, with a radius of 15 Å. The simulation of molecular docking was conducted for the bioactive chemicals found in *P. niruri* plants, including mucic acid (**1**), gallic acid hexoside (**2**), gallic acid (**3**), gallic acid methyl ester (**5**), geraniin (**6**), corilagin (**7**), gallic acid dimethyl ester (**8**), quercetin (**10**), hydroxyflavonol (**11**), galangin-8-sulfonate (**12**). Each molecule was classified as either a ligand or an inhibitor, which had an impact on the receptor's functionality. The docking scores for each compound and amino acid that interacted were provided in Table 4, employing the MVD version 6.0 program.

In the present study, it was found that the α-glucosidase inhibitory potential of *P. niruri* extract reported previously could be attributed to geraniin, corilagin, gallic acid, and quercetin. Our study showed that the docking score of the corilagin tested showed a consistent trend with the *in vitro* α-glucosidase inhibition data. Corilagin showed a stronger binding affinity with a MolDock score of −152, suggesting it plays an important role in inhibiting α-glucosidase. Fractions containing Corilagin, such as PNF-1 to PNF-5, appear to have inhibitory activity. Meanwhile, fractions PNF-6 to PNF-8, which do not show the presence of Corilagin, exhibit weak inhibitory activity as shown in Tables 3 and 4.

The interaction between compounds and α-glucosidase involves various binding forces, including hydrogen bonding and steric interactions, which are essential in molecular recognition and stability. In Table 4, corilagin provides hydrogen bonds with amino acids with glucosidase, namely Asn241, Ser244, His245, His279, Glu304, Pro309, and Gln350. Amino acid Gln350 is found only in corilagin, geraniin, and quercetin. In addition, Gln350 is found in isomaltose, which is a native ligand in α-glucosidase. Hydrogen bonds are crucial for the protein stability of ligand-protein complexes, and the number of hydrogen bonds is positively correlated with the binding energy and interaction specificity [42,70]. In addition, steric interactions with amino acids observed in corilagin, namely Phe157, Thr215, Leu218, His279, Phe300, and Asp349, contribute to the inhibition of activity, which isomaltose has steric interactions with amino acids Asp214 and Asp349. Furthermore, the corosolic acid exhibited steric interactions with specific amino acids such as Phe157, Thr215, Leu218, His279, Phe300, Glu304, and Asp349. Steric interactions are interactions between atoms or groups of the ligand that occur due to physical size (volume space), where the shape and size fit with the amino acids in the binding pocket of the protein [71].

The MolDock score represented the binding energy between the target receptor, α-glucosidase, and ligands. Corilagin had the most negative Moldock score of −152, which was lower than that of isomaltose, corosolic acid, and other ligands. Negative values imply a higher degree of bond stability, suggesting that the contact between the receptor and the ligand may impede the enzyme's performance. Figure 3 illustrates the interaction between ligands and amino acid residues of the

Table 4. MolDock score and hydrogen bonding with amino acid after docked target α-glucosidase and ligands.

Ligand	MolDock score	Hydrogen bonding with amino acid	Steric interactions with amino acid
Isomaltase	−131	**Asp 68**, **His 111**, **Gln 181**, **Arg 212**, **Asp 214**, **Glu 276**, His 348, **Asp 349**, **Gln 350**, **Arg 439**	Asp214, **Asp 349**
Mucic acid	−87	**Asp 68**, **Gln 181**, **Arg 212**, **Asp 214**, **Glu 276**, **His 348**, **Asp 349**, **Arg 439**	Asp 68, **Asp 349**
Gallic acid hexoside	−120	Lys 155, Phe 157, Gly 217, His 239, **Glu 276**	Phe 157, Leu 218
Gallic acid	−83	**Asp 68**, **His 111**, **Gln 181**, **Asp 214**, **Glu 276**, Asp 314, **Arg 439**	Asp 68, Tyr 71, Thr 215, Glu 276
Gallic acid methyl ester	−82	Lys 155, Phe 157, Arg 312, Asp 408, Asn 412	Phe 157, Phe 311, Arg 312
Geraniin	−43	Lys 155, Asn 241, His 279, Tyr 313, Phe 310, Arg 312, **Asp 349**, **Gln 350**, Asp 408, Asn 412	Phe 157, Phe 158, Thr 215, His 239, Asn 241, Glu 276, Ala 278, His 279, Phe 300, Phe 310, Arg 312, Arg 439
Corilagin	−152	Asn 241, Ser 244, His 245, His 279, Glu 304, Pro 309, **Gln 350**	Phe 157, Thr 215, Leu 218, His 279, Phe 300, **Asp 349**
Gallic dimethyl ester	−81	His 245, **Glu 276**	Ser 156, Leu 218, Asn 241
Quercetin	−115	**Asp 68**, **Asp 214**, **Glu 276**, Tyr 313, **Gln 350**, Asp 408, **Arg 439**	Phe 300, **Asp 349**, Arg 439
3-hydroxyflavone	−93	**Glu 276**, **Asp 349**, **Arg 439**	Phe 157, Phe 177, Phe 300, **Asp 349,** Arg 439
7-hydroxyflavone	−104	Phe 157, Leu 176, Asn 241	Phe 157, Asn 241, Glu 276
Galangin-8-sulfonate	−103	Lys 155, Phe 157, Glu 304, Arg 312, Asp 408, Asn 412	Phe 157, Arg 312, Asp 408
Corosolic acid	−87	His 279, Pro 309, **Arg 439**	Phe 157, Thr 215, Leu 218, His 279, Phe 300, Glu 304, **Asp 349**

Bold residues indicate same interaction with the native ligand.

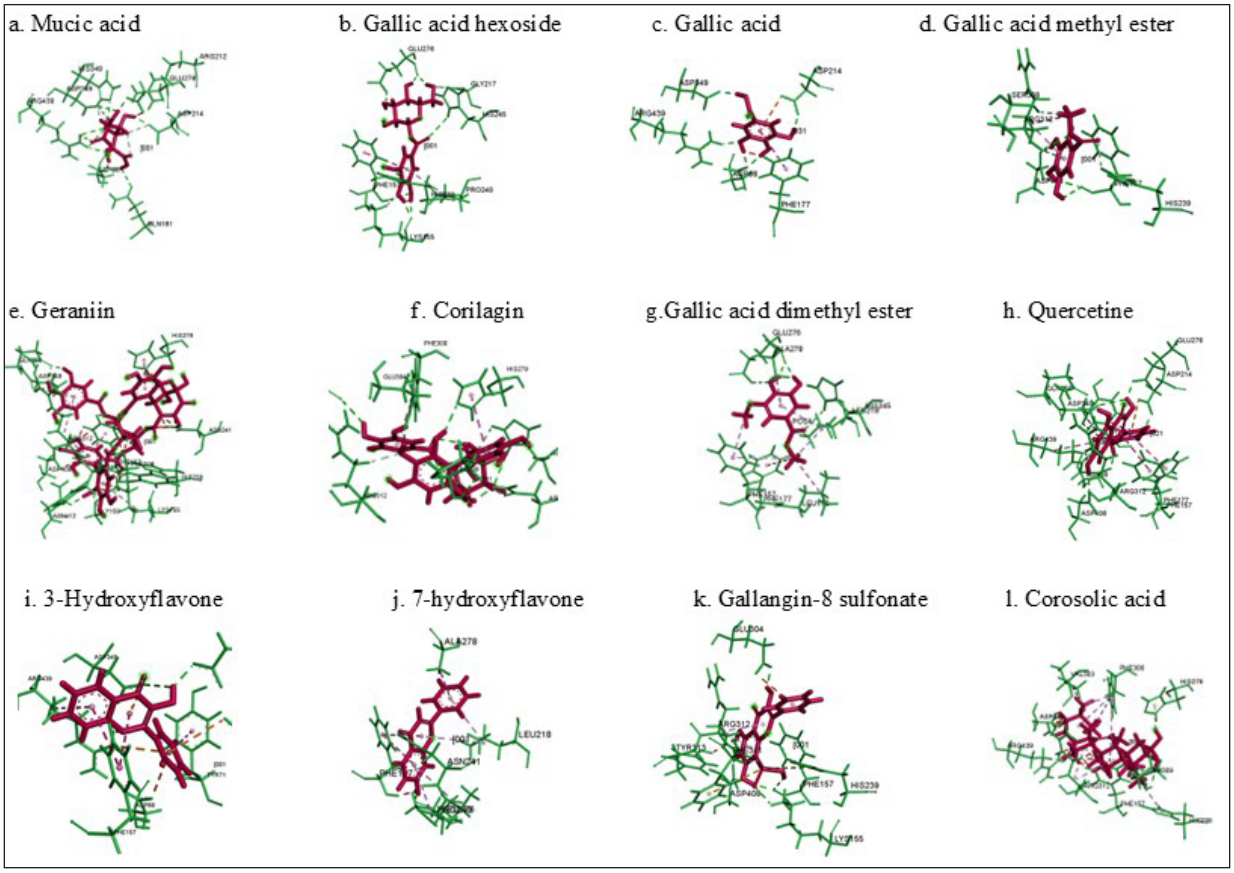

Fig. 3. Predicted binding modes, H-bond interactions of *α*-glucosidase with mucic acid (a), gallic acid hexoside (b), gallic acid (c), gallic acid methyl ester (d), geraniin (e), corilagin (f), gallic dimethyl ester (g), quercetin (h), 3-hydroxiflavone (i), 7-hydroxyflavone (j), galangin-8-sulphonate (k), and corosolic acid (l). Green is *α*-glucosidase and red is target of ligand.

enzyme. Corilagin established hydrogen bonds, electrostatic contacts, and steric interactions with various amino acids present in the enzyme residues, including those located in the active sites.

CONCLUSIONS

The bio-guided fractionation of *P. niruri* extract showed that fractions PNF-1 to PNF-6 had higher α-glucosidase inhibition activity compared to PNF-7 and PNF-8. This analysis revealed that the major compounds of consistently in PNF-1 and PNF-5 were tannin (corilagin) and phenolic acid (gallic acid dimethyl ester) class compounds that can be used as chemical marker candidates to control the quality. According to the results of the docking simulation, it is projected that corilagin, which contains several phenol groups, plays a significant role in inhibiting the enzyme. Further research is required to validate this, which should involve the isolation of the active chemical, led by bioassay.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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