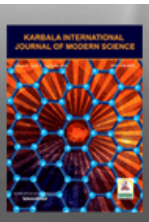

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Molecular Docking Reveals Phytoconstituents of the Methanol Extract from Muntingia calabura as Promising α-Glucosidase Inhibitors

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Keywords

Diabetes; Fisetin; Methanol; Muntingia calabura; Systematic review

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RESEARCH PAPER

Molecular Docking Reveals Phytoconstituents of the Methanol Extract From *Muntingia calabura* as Promising α-glucosidase Inhibitors

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Abstract

Inhibition of α-glucosidase has been used as a management of type 2 diabetes mellitus, where studies are focused on finding more efficacious and safe drugs. Herein, the research aimed to unveil the potential of phytoconstituents contained in methanol extract of *Muntingia calabura* leaves in inhibiting α-glucosidase through molecular docking simulation. From a systematic search (Scopus), we found 5 eligible articles and identified 28 phytocompounds. Fisetin, pinostrobin, and rhamnetin identified in *M. calabura* extract were predicted to have good bioavailability. Finally, fisetin was revealed as the most potential α-glucosidase inhibitor candidate with a binding affinity of −7.5 kcal/mol.

Keywords: Diabetes, Fisetin, Methanol, *Muntingia calabura*, Systematic review

1. Introduction

To date, type 2 diabetes mellitus (T2DM) persists as a leading cause of mortality and morbidity worldwide, especially in developing countries [1]. A recent report revealed that there are 451 million adults living with diabetes around the world, predicted for an increment up to 693 million in 2045 with currently available preventive and curative treatments [2]. With the presence of the coronavirus disease (COVID-19) pandemic, a persistent case of T2DM could worsen the burden on global health [3,4]. T2DM itself occupies at least 90% of the entire diabetes cases (another 10% are type 1 diabetes mellitus) [2]. Patients with T2DM might have a normal or increased level of insulin, in which undergoing non-insulin management is the best option [5]. Of which, an α-glucosidase inhibitor is a potent non-insulin anti-diabetic therapy, particularly for patients with a high risk of hypoglycemia or lactic acidosis [6].

Acarbose, voglibose, and miglitol are commonly prescribed α-glucosidase inhibitors, but their continuous usage could cause uncomfortable abdominal conditions and flatulence [7]. Furthermore, increased drug resistance is expected due to the wide and frequent usage of these drugs. Hence, the discovery of α-glucosidase inhibitors has become the aim of many researche projects, including those employing natural products [8,9]. Anti-inflammation and antioxidant properties are the primary mechanism of phytoconstituents in ameliorating insulin dysregulation [8]. *Muntingia calabura* has been reported as an emerging source of antioxidants that has a high abundance in nature [10]. Moreover, a water extract from *M. calabura* leaves has been studied for its anti-diabetic properties in animal models [11]. The study revealed that *M. calabura* leaves extract had anti-diabetic

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properties by lowering plasma glucose levels [11]. Unfortunately, the aforementioned study is the only one that investigated the anti-diabetic properties of *M. calabura* extract.

Our research group intends on investigating the methanolic extract of *M. calabura* leaves in treating T2DM. Indeed, water extract could contain rich flavonoids that are effective in inhibiting α-glucosidase [12]. However, methanol could also draw rich content of flavonoids, but with fewer impurities [13]. So far, methanolic extract of *M. calabura* leaves has only been studied for its ability to ameliorate hepatoxicity [14,15], reduce gastric ulceration [16], prevent carcinogenesis [17], relieve pain [18], and protect liver from CCl_4-induced injury [19]. By considering its antioxidant profile reported previously [14–19], it is very potential that the extract might perform α-glucosidase inhibition. Herein, we systematically acquired the already identified phytocompounds from methanol extract of *M. calabura*, and subsequently performed in-silico screening through molecular docking simulation. A systematic review on phytocompounds deriving from methanolic extract of *M. calabura* has never been reported. Moreover, we conducted the in-silico analysis adding the innovation to this article. This research acts as a guide to explore the potential of *M. calabura* in the treatment of T2DM. Moreover, data on *M. calabura* antioxidant potentials could be used for preparing nanoparticles with a green method as reported previously [20–24].

2. Materials and methods

2.1. Study design

Phytocompounds were collected through a systematic review of previously published reports. Molecular docking was performed on VivoBook S13 X330UA using Intel(R) Core(TM) i3-8130U CPU (2.20 GHz; 2.21 GH) processor and Windows 10 operating system. The protocol of molecular docking in general followed the previous report [25]. A potential α-glucosidase inhibitor was selected based on the binding affinity and properties of the phytochemical molecules and their interaction with the receptor's pocket region.

2.2. Search strategy

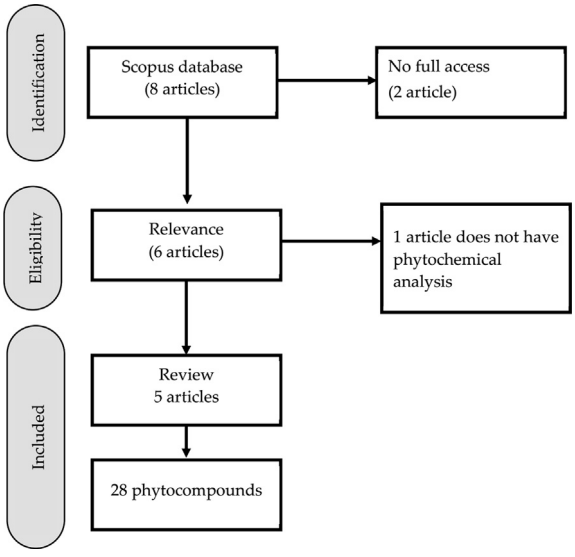
A literature search was conducted on the Scopus database on 20th October 2021 using the following terms: "*Muntingia calabura*" AND "methanol" AND "extract". Only papers reporting phytochemical analysis on the methanol extract of *Muntingia calabura* were included. Data extracted from the references were phytocompound isolation methods, identified phytocompounds, and results of bioactivity tests. The preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow-chart of the search method performed herein could be seen in Scheme 1.

2.3. Protein and ligand preparation

The protein or receptor used in this research was downloaded from Protein Data Bank (https://www.rcsb.org/). α-glucosidase (5NN6) was downloaded in PDB format, in the presence of an inhibitor ((2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-trio) along with solvent molecules (triethylene glycol; sulfate ions; glycerol; 1,2-ethanediol; and chloride ions). To prepare the receptor, the molecule file was uploaded to modrefiner (https://zhanglab.dcmb.med.umich.edu/ModRefiner/). The receptor was obtained with repaired missing residue, removed solvent molecules, and minimized energy. As for the ligand, after being downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), the molecule was added with hydrogen on Chimera 1.15. The receptor was added with hydrogens and then merged on AutoDockTools 1.5.6.

2.4. Molecular docking

Docking was performed on Autodock Vina 1.1.2 [26], where the exhaustiveness was set at 8. The docking grid box was centered on the inhibitor (x: -13.968; y: -32.728; and z: 96.336) with a size of



Scheme 1. PRISMA flow-chart of literature search and selection.

34 Å × 26 Å x 26 Å. Docking results with the lowest binding affinity were then observed and visualized on Biovia Discovery Studio Visualizer 21.1. Prior to the execution of molecular docking simulation on the targeted compounds, we validated the grid box position and its size through redocking protocol with its inhibitor ((2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-trio). The results showed that only one amino acid residue that was not in accordance with that of the experimental version (Figure S1 (https://kijoms.uokerbala.edu.iq/cgi/editor.cgi?article=3236&window=additional_files&context=home)).

2.5. Molinspiration

A simplified molecular-input line-entry system (SMILE) of each ligand published on PubChem was inserted onto molinspiration (https://www.molinspiration.com/) to obtain the structure. The calculation was carried out by the system to acquire information pertaining to molecule properties including the molecular weight, statistically calculated LogP, number of H donors, and number of H acceptors.

2.6. Molecular dynamic

The molecular dynamic simulation was performed on NAMD 2.14 (Urbana, IL, USA) following the input preparation using CHARMM-GUI (https://www.charmm-gui.org/) with 0.15 M KCl and rectangular water box type (edge distance = 10.0). The trajectory simulation was set to run for 100 ps and recording frequency of 10 ps using NAMD. As for the production, it was set to run for 500 ps and run on NAMD. All files were then loaded into VMD 1.9.3 (Urbana, IL, USA) to calculate the root mean square deviation (RMSD) of the docked α-glucosidase and its inhibitor candidate.

3. Results and discussion

3.1. Phytocompounds from methanolic extract of Muntingia calabura

Out of 8 reported studies, only 5 articles were selected for the review. So far, there is a total of 28 phytocompounds have been collaboratively identified. Maceration was commonly used in the reported studies, where only one study performed sequential fractionation using petroleum ether, ethyl acetate, and distilled water. All of the identified compounds are phenols with the majority coming from the flavonoid group. Of which, quercetin is the most reported phytocompound found in the *M. calabura* methanolic extract, followed by quercitrin and gallic acid. Those three compounds are often associated with antioxidant properties of the extract contributing to multiple bioactivities (anti-inflammation, anticancer, and so on) [16,17]. It is worth noting that rutin, kaempferol, and myricetin are among the identified compounds.

In the case of α-glucosidase inhibition, quercetin has been reported to play a significant role in reducing diabetes-related enzymes, including α-glucosidase [12]. Even further, glycosylated quercetin could possess a better inhibitory effect against α-glucosidase [12]. It is in accordance with the fact that quercetin derivative, quercetin O-glycoside or quercitrin, could cause α-glucosidase inhibition via hydrogen bonding and van der Waals forces [27]. Rutin and kaempferol isolated from *Morus atropurpurea* leaves using water solvent could inhibit α-glucosidase, where rutin was revealed to have stronger activity. Comparatively, quercetin has been proven to be more capable of inhibiting α-glucosidase than rutin [28]. Quercetin alongside rutin could have higher inhibition via synergistic interaction than that of single flavonoids [12].

Gallic acid is not a new natural compound that has been studied for its anti-diabetic properties [29]. A recent study suggested that gallic acid in combination with metformin could improve the dysregulated system and glucose metabolic system in diabetic rats [30]. Optimum inhibition against α-glucosidase in-vivo could be achieved by combining gallic acid and acarbose with a ratio of 1:1 [31]. Similarly, myricetin had been noticed for its potential in T2DM treatment. In-vitro [32] and in-vivo [33] studies revealed the effectiveness of myricetin in inhibiting α-glucosidase.

From this point, we have understood that many of the identified phytocompounds in methanol extract of *M. calabura* leaves are active against α-glucosidase. To further select the best α-glucosidase inhibitor, we performed a molecular docking simulation on the specific active site of the enzyme. Nonetheless, compounds with molecular weight exceeding 500 g/mol were excluded from the study to optimize the drug's bioavailability [34].

3.2. Potential α-glucosidase inhibitors

The results of molecular docking have been provided in the supplementary file (Table S1 (https://kijoms.uokerbala.edu.iq/cgi/editor.cgi?article=3236&window=additional_files&context=home)). Quercetin which is the most frequent compound identified in the methanol extract of *M. calabura* leaves, has a

Table 1. Phytocompounds reported in published literatures regarding methanolic extract from M. calabura leaves.

Ref.	Extraction	Analytical method	Phytocompound	Bioactivity
[16]	Maceration using methanol and followed by fractionation using petroleum ether, ethyl acetate, and distilled water.	HPLC	Fisetin, quercetin, rutin, quercitrin, and gallic acid	- Superoxide and DPPH scavenging - Nitric oxide, LOX, and XO inhibition
[14]	Maceration using methanol.	HPLC	Rutin, quercetin, and fasetin	- Hepatoprotection via alanine aminotransferase and aspartate aminotransferase downregulation
[17]	Maceration using methanol.	HPLC	Rutin, gallic acid, ferulic acid, quercitrin, kaempferol, quercetin, afzelin, pinobaksin, pinocembrin, kaemferide, and ermanin	- Anti-carcinogenesis activity against colon cancer - Upregulation of SOD, catalase, and glutathione and downregulation of malonaldehyde
[15]	Maceration using methanol.	UHPLC-ESI-MS/MS	Gallic acid, citric acid, loganin acid, coumaryl hexoside, elenolic acid, protocatechuic acid, myricetin, quercetin, pentagalloyl-hexoside, kaempferol, 6′-O-trans-Cinnamoyl-8-epikingisdic acid, quercitrin, afzelin, pinostrobin, pinocembrin, ermanin, pinobaksin, chysrin, and kaempferide	- DPPH, SOD, and ORAC scavenging - anti-inflammatory activity via LOX - Hepatoprotective activity
[19]	Maceration using methanol.	UHPLC-MS	Gallic acid, protocatechuic acid, ferulic acid, quercetin, quercitrin, pentagalloyl-hexoside, kaempferol, myricetin, isoferulic acid, afzelin, pinocembrin, rhamnetin, pinobaksin, chyrsin, kaempferide, genistein, ermanin, and pinostrobin	- Hepatotoxicity amelioration via alanine transaminase, aspartate transaminase, and nitric oxide downregulation. - Reducing TNF-α, IL-1β, and IL-6 - Increasing liver catalase and SOD

HPLC: High-performance liquid chromatography; UHPLC: Ultra high-performance liquid chromatography; ESI: Electrospray ionization; MS: Mass spectroscopy; DPPH: 2,2-diphenyl-1-picrylhydrazyl; SOD: Superoxide dismutase; LOX: lipoxygenase; XO: Xanthine oxidase; ORAC: Oxygen radical absorbance capacity; TNF-α: tumor necrosis factor α; IL: interleukin.

binding affinity of −7.3 kcal/mol. The value is close to that obtained from myricetin interaction with the pocket region of α-glucosidase (−7.4 kcal/mol). Phenolic acids, such as gallic acid, citric acid, ferulic acid, and elenolic acid achieve binding affinity as much as −5.6, −5.5, −5.5, and −5.9 kcal/mol, respectively. The molecular docking values obtained herein only indicate the likeliness of a compound to inhibit the enzyme by disabling the catalytic side (similar to that of acarbose). Hence, compounds with poor binding affinity may perform the inhibition through other pathways, like altering the enzyme conformation [27]. Furthermore, quercitrin in this study has stable hydrogen bond interaction with Asp518 and Asp616 which are the catalytic sites of α-glucosidase [35] (see Table 1).

To focus our study on finding alternative drugs that could replace acarbose, we then selected several compounds with binding affinity scores of −7.5 kcal/mol onward, where the results have been presented in Table 2. Among the phytocompounds, pinostrobin has the most likeliness to form a stable interaction with α-glucosidase (affinity = −7.6 kcal/mol). Others (afzelin, fisetin, quercitrin, and rhamnetin) have a binding affinity as much as −7.5 kcal/mol, where their 2D representations of the molecular docking simulation have been presented in Fig. 1. As a kaempferol derivative, afzeline could

Table 2. Molecular docking and molecular properties of some phytocompounds from M. calabura leaves methanolic extract.

Compound	MW (g/mol)	LogP	nON	nOHNH	Affinity (kcal/mol)	Interaction	Amino acid
Afzelin	432.38	1.13	10	6	−7.5	H bond	Asp518, Arg600, Asp282
						Hydrophobic	Phe649, Leu405
Fisetin	286.24	1.97	6	4	−7.5	H bond	Arg600, Asp518, Leu678, Ser676
						Hydrophobic	Leu678, Phe649, Trp367
Pinostrobin	270.28	3.13	4	1	−7.6	Hydrophobic	Leu405, Leu650, Leu677, Leu678, Trp376
						Electrostatic	Asp404
Quercitrin	448.38	0.64	11	7	−7.5	H bond	Asp404, Asp518, Asp616, Leu677, Ser676
						Hydrophobic	Leu678
Rhamnetin	316.26	2.22	7	4	−7.5	H bond	Arg600, Leu678
						Hydrophobic	Phe649, Trp376
						Donor clash	Arg600

nON: Number of hydrogen bond acceptors; nOHNH: Number of hydrogen bond donors. MW, LogP, nON, and nOHNH should be ≤ 500, ≤5, ≤10, and ≤5, respectively, for optimum bioavailability and absorption.

reach IC_{50} as little as 0.94 nM against α-glucosidase in a pre-clinical trial study [36]. Quercitrin also has a good inhibition activity against α-glucosidase [27].

According to the rule of 5 for drug permeation, Log P should be < 5, the number of hydrogen bond acceptors (nON) — < 10, and the number of hydrogen bond donors (nOHNH) — < 5 [34]. Hence, the α-glucosidase inhibitor candidates selected were fisetin, pinostrobin, and rhamnetin. Fisetin is a good inhibitor candidate since it has stable hydrogen bound with Asp518. Molecular interaction with a specific substrate binding site, Arg600, was also shown in 4' hydroxyl group of fisetin. As in the case of pinostrobin, its inhibitor potential was shown by an electrostatic interaction formed between the aromatic ring of pinostrobin and Asp404. Nonetheless, in comparison with hydrogen bond interaction, electrostatic interaction could be affected by the surrounding environment suggesting its less stability [37]. Meanwhile, rhamnetin has shown stronger inhibition against α-amylase than acarbose, but not in the case of α-glucosidase [38]. It is possibly due to the donor-donor clash interaction with Arg600 which is a common pocket region target of α-glucosidase inhibitors (such as 1-deoxynojirimycin, N-hydroxyethyl-deoxynojirimycin, and acarbose) [35]. Taken together, fisetin appears as the most promising α-glucosidase inhibitor candidate, where the 3D representation of fisetin—α-glucosidase interaction has been presented in Fig. 2.

In previously published works, fisetin has been acknowledged to play a significant role in T2DM treatment [39–41]. In an ex-vivo study using human monocytic (THP-1) cells, fisetin could ameliorate hyperglycemia, associated with its ability to inhibit the nuclear factor of kappa B (NF-κB) [40]. The same study also observed the reduction of proinflammatory cytokines; interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [40]. In line with a study using the rats model, renal TNF-α, IL-1β, and IL-6 were attenuated significantly [41]. However, its role in improving plasma insulin is still debatable among researchers [41,42]. Taken together, it corroborates the fact that, as a candidate for anti-diabetic drug, fisetin works primarily by reducing oxidative stress and inflammation as well as through carbohydrate metabolism.

In comparison with acarbose (6.7 kcal/mol), fisetin has a better molecular docking score (−7.5 kcal/mol). The simulation of molecular interaction between acarbose and α-glucosidase could be seen in Figure S2 (https://kijoms.uokerbala.edu.iq/cgi/editor.cgi?article=3236&window=additional_files&context=home). This is in accordance with the fact that fisetin has superior ability in inhibiting α-glucosidase in-vitro, in comparison with that of acarbose [43,44]. Moreover, the interaction between docked fisetin and α-glucosidase was found stable with RMSD <3 nm (Fig. 3). A previous in-silico study has revealed that fisetin inhibits α-glucosidase through a non-competitive binding pathway [43]. Their argument was supported by the fact that fisetin has only one binding site with α-glucosidase [43]. However, having one binding site is not mutually exclusive to proving that fisetin does not form interaction on the substrate-binding site. Secondly, their study used α-glucosidase molecules from *Saccharomyces cerevisiae* [43]. Therefore, our study suggests a novel finding of fisetin interaction on the substrate and catalytic site of α-glucosidase. It further implies fisetin mode of action in the inhibition includes competitive binding.

Our research provides an insight pertaining to the role of natural compounds contained in the methanolic extract of *M. calabura* in inhibiting

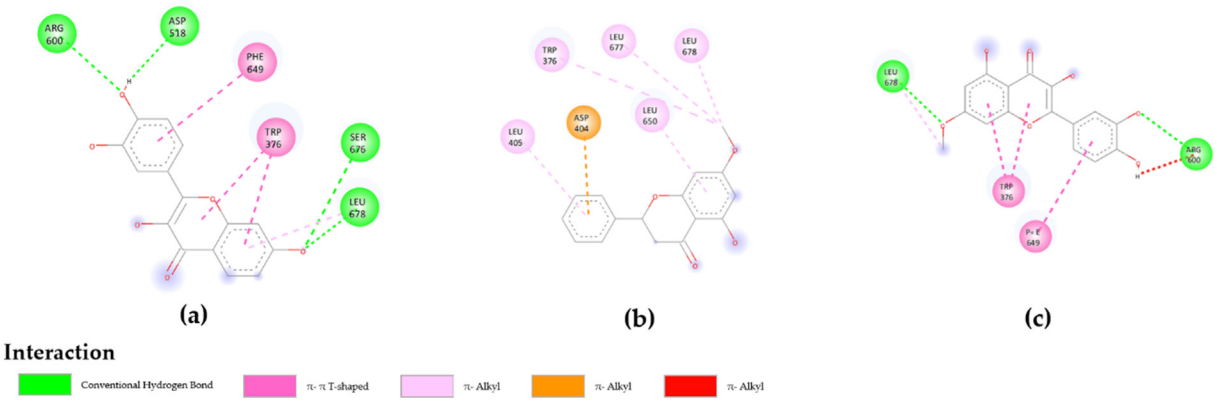


Fig. 1. 2D representation of (a) fisetin, (b) pinostrobin, and (c) rhamnetin interacting with the receptor's residues in the pocket region.

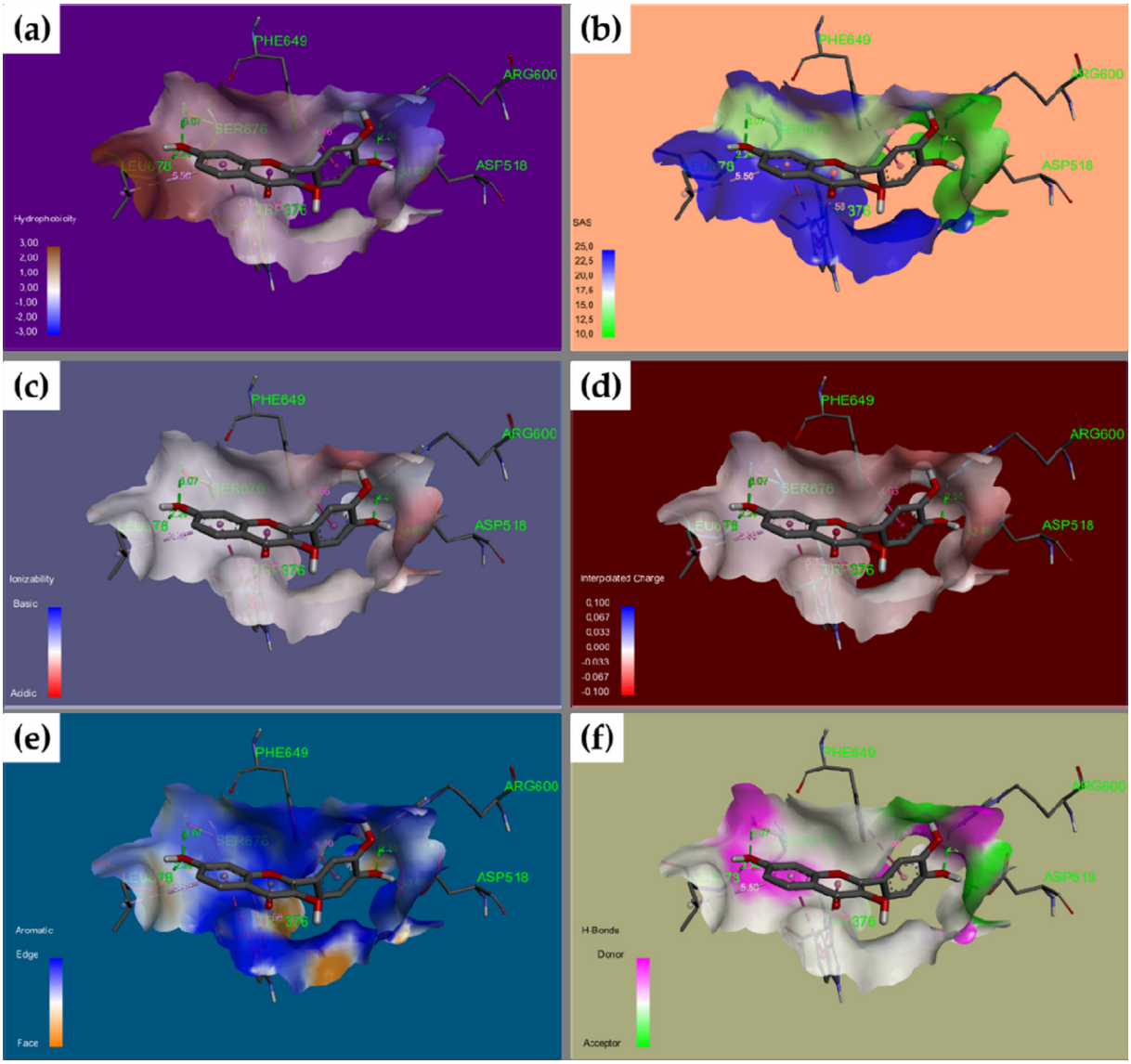


Fig. 2. 3D representation of (a) hydrophobicity, (b) solvent accessibility, (c) ionizability, (d) charge, (e) aromatic, and (f) H-bonds surface of α-glucosidase surrounding pocket regions interacted with fisetin.

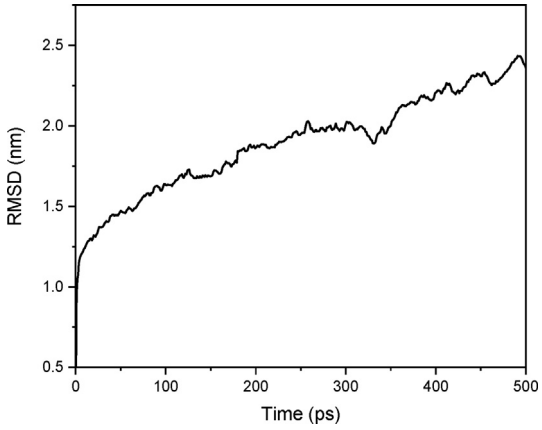


Fig. 3. RMSD plot obtained from a molecular dynamic simulation of fisetin and α-glucosidase.

α-glucosidase through molecular docking simulation, where drug bioavailability-related molecular properties were also taken into consideration. The limitation of our study includes our inability to present other inhibition mechanisms that could be possessed by the phytocompounds. Moreover, this study did not consider the synergistic interaction between phytocompounds which could reveal another superiority of natural compounds [45]. This study could act as a starting point for investigating methanolic extract from *Muntingia calabura* leaves in the treatment of T2DM.

4. Conclusion

This systematic review combined with molecular docking simulation revealed three potent glucosidase inhibitor candidates, namely fisetin, pinostrobin, and rhamnetin. Fisetin, one of flavonoids, was prevailed as *Muntingia calabura* leaves-derived phytocompound with the highest potential as an α-glucosidase inhibitor candidate, based on molecular docking simulation and its molecular properties. There are three recommended trajectories for future research: (1) Investigating fisetin as an anti-diabetic therapy in a clinical trial; (2) Revealing other potential candidates of glucosidase inhibitor from the *Muntingia calabura* leaves extract; And (3) investigating the synergistic mechanism of phytoconstituents contained in the extract.

Data availability

The data presented in this study are openly available as Supplementary File in Harvard Dataverse at 10.7910/DVN/TSZAUJ, reference number [46].

Ethical clearance

Not applicable.

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