Molecular docking of selected phytochemicals from malunggay (*Moringa oleifera*) against the chromosomal trehalose-6-phosphate phosphatase (PDB ID: 6CJ0) enzyme of *Pseudomonas aeruginosa*

ROANNE G. AZUR, RAPHAEL FRANCIS E. DEQUILLA, JOHANNA MARIE G. GAVAN, and FERNANDO CHRISTIAN G. JOLITO III

Philippine Science High School Western Visayas Campus - Department of Science and Technology (DOST-PSHSWVC), Brgy. Bito-on, Jaro, Iloilo City 5000, Philippines

Article Info

Submitted: May 13, 2021
Approved: Jun 29, 2021
Published: Aug 20, 2021

Keywords:
molecular docking
Pseudomonas aeruginosa
trehalose-6-phosphate phosphatase
phytochemicals
inhibitor

Abstract

The trehalose-6-phosphate phosphatase (TPP) enzyme, a common enzyme that is important for organism survival under stress, is utilized by some strains of *Pseudomonas aeruginosa*. Its presence in such pathogens but absence in hosts such as humans makes it a viable anti-pathogenic target. As for potential inhibitors, phytochemicals are known to possess medicinal properties and offer holistic drug action. Using molecular docking, the study screened selected phytochemicals from *Moringa oleifera* against the TPP enzyme of *P. aeruginosa*. The 13 initial phytochemicals were tested for drug-likeness using the Lipinski test, of which 11 passed and were used in the docking procedure done in AutoDock Vina. Analysis of the generated docks has shown that seven phytochemicals bind in close proximity to the active site, two bound elsewhere on the surface of the TPP enzyme, and one has both attributes. The docked phytochemicals were determined to act as either possible competitive or noncompetitive inhibitors.

Introduction. - The efficacy of antibiotics is at risk due to rapidly emerging resistant bacteria [1]. Antibiotic resistance in clinically relevant microorganisms such as *Pseudomonas aeruginosa* has been associated with an increase in hospitalization and mortality rates [2]. Antibiotic resistance is observed in microorganisms that develop the ability to survive medicine targeted against it [3]. Multidrug resistance patterns in both Gram-positive and Gram-negative bacteria are difficult to treat, if not untreatable. The increasing numbers of bacterial strains acquiring resistance to a wide range of antibiotics in recent decades is also alarming [4].

Trehalose is a disaccharide that plays an important role in the survival of some pathogens. Recent studies have highlighted its role in desiccation resistance, osmoprotection, and resistance to heat or cold [5,6,7]. One of the most studied pathways of trehalose synthesis in bacteria is the trehalose-6-phosphate phosphatase (TPP) pathway due to its conserved biosynthesis route [8]. The TPP enzyme is a member of the haloacid dehydrogenase (HAD) superfamily, a group of enzymes that facilitates the hydrolysis of a diverse range of organic phosphate substrates [9].

P. aeruginosa, a Gram-negative, opportunistic pathogen, is known to infect many organisms, including humans [10]. Surveys of genomic databases have shown that *P. aeruginosa* strains possess two different TPP coding genes which are chromosomal and extrachromosomal [11]. Trehalose can be utilized by *P. aeruginosa* as a carbon and energy source for its growth and survival [12]. An increasing number of occurrences of drug-resistant *P. aeruginosa* strains have been observed in recent years [12]; a solution to this problem must be urgently identified.

Studies have shown the potential of phytochemicals in antibiotic resistance research [13]. Phytochemicals are known to affect specific molecular targets both directly and indirectly through affecting metabolic pathways as stabilized conjugates [14]. They are also known to have a wide range of medicinal properties and offer holistic drug action against pathogens without having many side effects [15].

One important factor to consider in the invention of new drugs is the risk that such drugs would target additional or multiple receptors [16]. According to the study of Umesh et al. [13], the TPP enzyme could become a viable anti-pathogenic target due to its important role in pathogen stress tolerance while being completely absent in animal hosts. However, there has only been limited research done utilizing the chromosomal TPP enzyme of *P. aeruginosa* as an

How to cite this article:
CSE: Azur RG, Dequilla RFE, Gavan JMG, Jolito FCC III. 2021. Molecular docking of selected phytochemicals from malunggay (*Moringa oleifera*) against the chromosomal trehalose-6-phosphate phosphatase (TPP) enzyme of *Pseudomonas aeruginosa*. Publiscience. 4(1): 45–50.
APA: Azur, R.G., Dequilla, R.F.E., Gavan, J.M.G., & Jolito, F.C.C. III. 2021. Molecular docking of selected phytochemicals from malunggay (*Moringa oleifera*) against the chromosomal trehalose-6-phosphate phosphatase (TPP) enzyme of *Pseudomonas aeruginosa*. *Publiscience*, *4*(1), 45–50.

For supplementary data, contact: publiscience@wvc.pshs.edu.ph.

anti-pathogenic target.

In recent years, the incorporation of computer-based methods in medicinal chemistry has brought with it advantages in rational drug design [17]. Methods such as molecular docking are now available for the *in silico* study of biological systems and drug discovery [17].

The present study proposed to virtually screen the selected phytochemicals from *Moringa oleifera* against the chromosomal trehalose-6-phosphate phosphatase (TPP) enzyme of *P. aeruginosa*. Phytochemicals from *M. oleifera* were used as they were the most abundant types of phytochemical [18], and others have already been used in previous molecular docking studies [19]. Specifically, the study aimed to:

(i) evaluate the drug-likeness of the phytochemicals using the Lipinski rule through SwissADME;

(ii) identify the predicted binding sites of the selected phytochemicals to the TPP enzyme of *P. aeruginosa* through AutoDock Vina;

(iii) predict the interactions between the TPP enzyme and each phytochemical through LigPlot+; and

(iv) provide proof-of-concept for the mechanism of binding between each phytochemical and the TPP enzyme.

Methods. - A preliminary Lipinski test was conducted to test the drug-likeness of 13 selected phytochemicals from *M. oleifera*. The phytochemicals which passed the test were then subjected to molecular docking with the chromosomal TPP enzyme of *P. aeruginosa* using AutoDock Vina [20]. Data analysis on the predicted binding sites and interactions was done using LigPlot+ [21], PyMOL [22], and UCSF Chimera [23]. The phytochemicals' potential to be possible inhibitors of the TPP enzyme was contextualized using existing literature.

Selection of Phytochemicals. Thirteen phytochemicals involved in the studies of Lin et al. [18] and Zainab et al. [19] from *M. oleifera* were selected to be docked with the TPP enzyme. These phytochemicals and their respective PubChem Compound ID numbers are: (1) alpha-carotene (4369188), (2) anthraquinone (6780), (3) apigenin (439726), (4) excoecariatoxin (5281400), (5) flavylium (145858), (6) hemlock tannin (15559687), (7) isorhamnetin (5281654), (8) kaempferol (5280863), (9) laurifolin (102301875), (10) phenolic steroid (439726), (11) quercetin (5280343), (12) serpentine (73391), and (13) sitogluside (5742590). The three-dimensional structures of trehalose-6-phosphate (T6P)-substrate (positive control), carbon tetrachloride (negative control), and the phytochemicals in structured data format (SDF) were retrieved from the National Center for Biotechnology Information (NCBI) PubChem.

Lipinski Test for Drug-likeness. To evaluate and assess compounds during drug discovery and optimization, the Lipinski rule of five is used [24]. The phytochemicals were evaluated for their drug-likeness using the Lipinski rule by uploading their SDF files to SwissADME, a web-based application [25]. The phytochemicals that passed the rule with one or no violations were the only ligands to be tested with TPP.

Preparation of Molecular Models. The three-dimensional structure of TPP (PDB ID: 6CJ0) in PDB format was retrieved from Protein Data Bank (PDB). The natural ligands (CO_3 and Mg^{+2}) of the TPP enzyme were deleted using UCSF Chimera before the file was saved in PDB format. This file was then opened in AutoDock Tools to remove water molecules to avoid distortion in the search for possible binding sites [26]. Afterward, polar hydrogen atoms were added to establish the hydrogen bonds that may be involved in the binding of the protein and ligand. The whole macromolecule was enclosed by the grid box. The offset numbers and the number of points in the x, y, and z dimensions were noted down to define the search space for ligand binding in AutoDock Vina . The TPP was saved as a PDBQT file. Moreover, the SDF files of each phytochemical were converted in PDB format using UCSF Chimera. The substrate and each phytochemical were then opened in AutoDock Tools to detect its root to assign rotatable torsion angles of the ligand. After that, the controls and each phytochemical were saved as a PDBQT file.

Molecular Docking Proper. The config file was written in Python programming language. The input placed were the receptor (TPP enzyme) and the ligand (phytochemicals and controls). The filenames of the output of AutoDock Vina in PDBQT and TXT format were then stated. After, the offset values and grid box size were also stated. The exhaustiveness was then set to 24. The MS/DOS command prompt was opened to run AutoDock Vina. The directory was changed to the file path of the folder where the PDBQT and config files were saved. To dock the ligand and the receptor, the file path of the .exe file of AutoDock Vina was pasted on the command prompt. This was then followed by two dashes and the word 'config' and its TXT file extension.

Data Analysis. AutoDock Vina automatically generates the top nine conformations per ligand. Each conformation of the docked ligand and receptor was individually saved as a PDB file using PyMOL The PDB files of the conformations were analyzed using UCSF Chimera and LigPlot+. LigPlot+ was utilized to analyze the two-dimensional (2D) structure of the conformations and to generate schematic 2D diagrams of ligand-protein interactions. The amino acids involved in hydrogen bonding and hydrophobic interactions were noted down and verified using UCSF Chimera, which was also used to generate three-dimensional (3D) structures of the conformations.

Safety Procedure. Since the study was done *in silico*, the researchers took frequent breaks and practiced the 20-20-20 rule; every 20 minutes, watch an object 20 feet away for 20 seconds. This was done to ensure that the researcher's eyes were not strained from long exposure to digital screens during the data gathering procedure.

Results and Discussion. - The study aimed to virtually screen the selected phytochemicals from *M. oleifera* against the chromosomal trehalose-6-phosphate phosphatase (TPP) enzyme of *P. aeruginosa*. The 13 selected phytochemicals were subjected to the Lipinski rule. Those that passed were docked with the TPP enzyme using AutoDock Vina. Each generated conformation was then analyzed using UCSF Chimera and LigPlot+.

Lipinski Drug-likeness Test. According to the Lipinski rule, the requirements for a compound to be considered drug-like state that an orally active drug must not violate more than one of the following criteria: the molecular weight should not exceed 500 grams/mole, the MlogP [27] should not exceed 4.15, there must not be more than five hydrogen bond donors, and there must not be more than ten hydrogen bond acceptors [28]. The 13 phytochemicals were subjected to Lipinski drug-likeness test using SwissADME. Among the 13 phytochemicals, 11 passed the Lipinski drug-likeness test. These can be referred to in Table 1.

Table 1. Drug-likeness of selected *M. oleifera* phytochemicals based on Lipinski rule.

a	*b*	*c*	*d*	*e*	*f*
Alpha-carotene*	536.87	12.46	0	0	2
Anthraquinone	208.21	1.86	0	2	0
Apigenin	270.24	0.52	5	3	0
Excoecariatoxin	528.63	1.47	3	8	1
Flavylium	207.25	3.28	0	1	0
Hemlock tannin*	578.52	-0.26	10	12	3
Isorhamnetin	316.26	-0.31	4	7	0
Kaempferol	286.24	-0.03	4	6	0
Laurifolin	356.37	1.09	3	6	0
Phenolic steroid	256.38	4.46	1	1	1
Quercetin	302.24	-0.56	5	7	0
Serpentine	349.40	2.21	0	4	0
Sitogluside	576.85	3.96	4	6	1

a Phytochemical; *b* Molecular weight (g/mol); *c* MlogP; *d* No. of hydrogen bond donors; *e* No. of hydrogen bond acceptors; *f* No. of violations; *Phytochemicals that did not pass the Lipinski test.

Alpha-carotene and hemlock tannin did not meet two and three out of four criteria, respectively; they were not included in the list of phytochemicals that were used in the molecular docking process.

Analysis of Predicted Binding Sites and Interactions. After generating the top nine conformations in AutoDock Vina, the top two poses of each docked ligand (phytochemical or control) were selected to be analyzed further. For ligands which are bound restrictively to one chain, the two poses correspond to the top-ranked conformations for Chain A and Chain B, respectively. For ligands that interacted with both chains, the first and second-ranked conformations were selected.

The chromosomal TPP enzyme of *P. aeruginosa* is a member of the haloacid dehydrogenase (HAD) superfamily [11], which comprises enzymes such as phosphatases, ATPases, phosphomutases, phosphonatases, and dehalogenases [29]. TPP possesses a core phosphatase domain with α/β-hydrolase fold, which is common among the hydrolase family, as well as a cap domain [30]. While the fold of the core domain, which functions as base and side walls of the active site, is well conserved among the HAD superfamily, the cap domain, which functions as the cover, can vary in size and structure [30]. Through comparisons to other bacterial TPPs, the structure of the TPP enzyme from *P. aeruginosa* is also revealed to have four HAD conserved motifs located in the core domain [11].

The study found that all of the top predicted conformations of the docked phytochemicals interacted with amino acid residues located in the core domain. The top predicted conformations (for both chain A and B) of seven phytochemicals were in close proximity to the active site of the TPP enzyme; this is because they bound to one or more motifs within the core domain [11]. The exception is the top predicted conformation of sitogluside in chain A since it is bound near the β12 sheet. The other two phytochemicals (flavylium and serpentine) that did not bind near the active site were also bound to amino acid residue/s near the β12 sheet, the hydrophobic interface that links the two monomers.

The active site of an enzyme is defined as the region that binds the substrate (a ligand that becomes the starting material of an enzymatic reaction) and converts it into a product [31]. It is formed by amino acid residues; the properties and spatial arrangement of these determine which molecules can bind to and become substrates for the enzyme. The forces which bind the substrate are multiple weak forces such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, and van der Waals bonds [31]. This study only observed the hydrogen and hydrophobic bonds that each phytochemical had with the TPP enzyme.

The phytochemicals anthraquinone, apigenin, isorhamnetin, kaempferol, laurifolin, phenolic steroid, and quercetin may be possible competitive inhibitors since these phytochemicals bind in close proximity to the active site of the TPP enzyme – all of these phytochemicals bound to one or more motifs – through steric hindrance. Steric hindrance prevents the further interaction of the natural substrate to the receptor when a competitive inhibitor is bound to the active site [32]. This effect of steric hindrance implies that if the phytochemicals bound to the active site of the TPP enzyme, T6P (the natural substrate) could not be catalyzed by the TPP enzyme into trehalose.

As for flavylium and serpentine, they could be considered as allosteric modulators or possible noncompetitive inhibitors due to their binding site being quite different from the active site. Allosteric

modulators bind elsewhere on the protein surface other than the active site and induce an allosteric conformational change of the active site of the receptor by shifting the free energy landscape [33,34] However, while noncompetitive inhibitors may or may not affect the structure of the protein, it is less certain what effect they may have on the binding affinity of the natural substrate since noncompetitive inhibitors do not compete with the substrate for active site binding [35].

As for sitogluside, its different conformation in each chain may suggest that it may possibly act as an allosteric modulator (in the case of Chain A) or a competitive inhibitor (in the case of Chain B). This is illustrated in Figures 1 and 2.

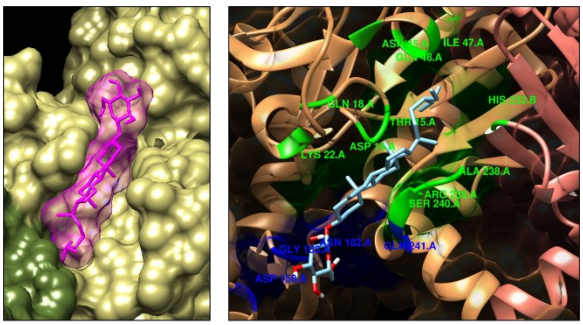

Figure 1. *(a)* Third-ranked predicted binding site of sitogluside in the TPP enzyme; *(b)* Top predicted binding site of sitogluside in chain A of the TPP enzyme with corresponding amino acid residues with *(green)* hydrogen bonds or *(blue)* hydrophobic bonds.

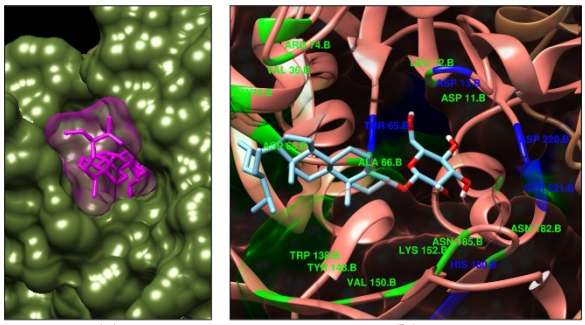

Figure 2. *(a)* Top-ranked predicted binding site of sitogluside in the TPP enzyme; *(b)* Top predicted binding site of sitogluside in chain B of the TPP enzyme with corresponding amino acid residues with *(green)* hydrogen bonds or *(blue)* hydrophobic bonds.

Inhibiting the T6P-substrate from binding to the active site would eventually lead to its accumulation. The intracellular accumulation of T6P is toxic to host organisms [36]. Previous studies determined that T6P accumulation can be lethal to *Caenorhabditis elegans* and *Mycobacterium tuberculosis* [36,37]. This adverse effect is due to the inhibition of metabolic enzymes such as phosphotransferases caused by sugar-phosphatases acting as antimetabolites [38]. The effects of the accumulation of T6P in *P. aeruginosa* are still unknown. However, the widespread toxicity of T6P and the presence of a glycolytic enzyme - known to be inhibited by T6P in other organisms - in *P. aeruginosa* all suggest that T6P accumulation may have adverse effects on the bacteria [11].

Antibacterial Properties of Phytochemicals. Several of the phytochemicals are already known to exhibit antibacterial properties against bacteria. Likewise, *M. oleifera* is also known to exhibit antibacterial properties against both Gram-positive and Gram-negative bacteria [39]. As for the phytochemicals, four of the seven possible competitive inhibitors are known to exhibit antibacterial activity against *P. aeruginosa* [40,41,42,43]. There is a lack of studies on the antibacterial activity of isorhamnetin, and the noncompetitive inhibitors flavylium and serpentine, against *P. aeruginosa*. Sitogluside exhibited low activity against *P. aeruginosa* in a study testing the antibacterial activity of daucosterol isolated from the roots of *Cissus populnea* [44].

Limitations. The study was not able to analyze the top rankings of excoecariatoxin since the generated conformation files could not be opened in LigPlot+ for further data analysis. This affected the third objective because the study was not able to predict the interactions between the TPP enzyme and the excoecariatoxin.

Conclusion. - The study concluded that after virtually screening the selected phytochemicals *of M. oleifera* against the chromosomal trehalose-6-phosphate phosphatase (TPP) enzyme of *P. aeruginosa*, all of the 11 docked phytochemicals that were analyzed are either possible competitive inhibitors or allosteric modulators of the enzyme.

Recommendations. - The researchers would recommend the utilization of software programs which could process the docked excoecariatoxin conformations. *In situ* analysis via nuclear magnetic resonance spectroscopy is also encouraged to examine the possible allosteric sites. Isothermal titration calorimetry to confirm the intended binding target of the TPP enzyme and each phytochemical is also recommended.

Acknowledgement. - The authors would like to thank the SIDHI Mentorship community for providing the opportunity to hold consultations and provide insights regarding the study. The authors would also like to thank Mr. Jose Sandino Bandonil of Academia Sinica and of the SIDHI Mentorship community, and Mr. Romie Azur, Science Research Specialist II at the National Institute of Molecular Biology and Biotechnology of the University of the Philippines-Diliman, for their guidance in the data gathering and data analysis procedures.

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