Current Research in Pharmaceutical Sciences
Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 16/12/2022
Revised: 29/12/2022
Accepted: 31/12/2022
Published: 08/01/2023

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DOI: 10.24092/CRPS.2022.120404

Website: www.crpsonline.com

Quick Response Code:

In Silico Study of Phytoconstituents of *Moringa Oleifera* Against Trehalose–6–Phosphate Phosphatase (TPP) Enzymes

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ABSTRACT

Numerous pathogenic microorganisms use the enzymes trehalose-6-phosphate phosphatase (TPP) to biosynthesize the sugar trehalose from trehalose-6-phosphate (T6P) as part of their infection and proliferation processes. In order to build new generation candidate medications to inhibit TPP using *in silico* approaches, the current work is being done. The majority of the 10 phytochemicals from *Moringa oleifera* that were used in docking studies with the 3D model of TPP had good binding affinities to the enzyme, with campesterol and stigmat-4-en-3-one showing the highest affinities (Binding energies of - 7.8 kcal/mole and -8.0 kcal/mole, respectively), when compared to the commonly used commercial drug isoniazid (Binding energy of –6.0 kcal/mole).The active site of TPP sequences, which coordinates Mg^{2+} and is necessary for catalysis, has been discovered to bind to phytochemical leads during docking. Binding poses and distance measurement of TPP-phytochemical complexes of 5-Hydroxymethylfurfural, Citronellol, Xylitol, 2,4-Di-tert-butylphenol, Palmitoleic acid, cis-Vaccenic acid, Phytol, Cyclopentane, 1,1-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-, Campesterol and Stigmat-4-en-3-one reveals that the lead phytochemicals were in close proximity with most of the active site amino acids (distance range from 1.88 to 3.77 Aº). This demonstrates the close affinity between the enzyme and the leads, which may open the door to the development of new generation medications to treat diseases caused by pathogenic microorganisms that produce TPP.

Key words: Trehalose–6–phosphate phosphatase, Binding affinity, *In silico*, Phytochemical, *Moringa oleifera*.

1. INTRODUCTION

Trehalose, a stress-protective molecule, is a crucial sugar in most species other than mammals.[1] Trehalose phosphate synthase (TPS) and trehalose-6-phosphate phosphatase are sequentially involved in the crucial process of trehalose production (TPP).[2] The pathogenic nature of the aforementioned pathway affects both people and animals. The TPPs in particular, which convert trehalose-6-phosphate (T6P) into sugar, demonstrate pathogenic properties in a variety of ways in various bacteria. Each year, a number of deadly diseases, including tuberculosis, aspergillosis, candidiasis, and nematode infections, are the primary cause of death globally. These diseases are typically caused by the TPPs of the relevant pathogens.[3]

A significant issue in contemporary medicine connected to drug addiction is the development of antibiotic resistance or drug resistance by microorganisms. The key to solving the aforementioned issue is to target the pathways or genes shared by infections but completely lacking in hosts.Despite the availability of specialised medications, the trehalose pathway and genes like TPP can be tested as more effective anti-pathogenic targets since they only affect infections and not humans or other animal hosts. Biomedical research is becoming increasingly active in the characterization of TPP enzymes and the development of targeted inhibitors against them in order to accomplish this goal.

TPP inhibitors have therapeutic potential for treating bacterial, fungal, and parasite disorders, including tuberculosis, but this potential has not yet been thoroughly explored. Since the beginning of recorded history, people have turned to herbal remedies to treat a wide variety of illnesses. The use of herbal medicine has increased rapidly in the past few decades. Due to their natural origin, affordability, and lack of negative side effects, they are becoming more and more popular in both developing and developed nations. Except for Allopathy, all of India's officially recognized health systems of Ayurveda, Yoga, Unani, Siddha, Homeopathy, and Naturopathy include a significant portion of herbal medications. These non-allopathic medical methods are still preferred by more than 70% of Indians.[4-5]

Moringa olifera (MO) known as drumstick tree is a member of the family Moringaceae that is typically found in South Asia, particularly in the hillock of the Himalayas in India and that can either be evergreen or deciduous and grows very quickly. It has a light and soft wood. In addition to Pakistan, Sri Lanka, Afghanistan, Arabian Peninsula, the Bangladesh, Arabian Peninsula, and East and West Africa, it has been cultivated and allowed to become naturalized in these countries. Throughout the West Indies and Southern Florida, Mexico, South America to Peru as well as in Paraguay and Brazil.[6-8] All its components, including the leaves, roots, seeds, flowers, bark, and green pods, offer a wide range of medical applications and excellent nutritional value. Anticancer, antiasthmatic, antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective, diuretic, CNS depressant, antibacterial and antifungal, anthelmintic, antibiotic, analgesic, antimalarial, antispasmodic, and heart-related problems are some of the plant's significant medicinal properties.[9-10]

Phytochemicals derived from plants provide pathogens with appealing, efficient, and all-encompassing therapeutic action with little adverse effects. The goal of the current work was to virtually test possible *Moringa oleifera* phytochemicals as trehalose-6-phosphate phosphatase inhibitory medication candidates (TPPs) [11] M. oleifera's phytochemicals were utilised since they were the most prevalent kinds of phytochemicals,[12-13] and others were previously employed in molecular docking research.[14-15] In order to compare the binding affinities of various phytochemicals to a model of the TPP protein and known antimicrobial medicines, the current study focuses on *in silico* docking studies on a few selected phytochemicals as lead compounds. This may aid in identifying potential next-generation therapeutic targets for virulent TPPs that are implicated in the infection and growth of numerous diseases. Table 1 illustrates the three-dimensional structure, PubChem Database IDs of Standard commercial drugs, and phytoconstituents of *Moringa oleifera*.

2. MATERIAL AND METHODS

2.1 Selection of Phytochemicals

Ten phytochemicals from M. oleifera that were used in the investigations by Lin *et al.*,[11] Bolade *et al.*,[12] and Zainab *et al.*,[13] was chosen to be docked with the TPP enzyme. (Table 1) lists these phytochemicals along with their corresponding PubChem Compound ID numbers. The phytochemicals in structured data format (SDF) and the three-dimensional structures of isoniazid (a positive control) were downloaded from the National Center for Biotechnology Information (NCBI) PubChem.

2.2 Ligand Preparation

The three-dimensional structures of Isoniazid (positive control), and the phytochemicals in structured data format (SDF) were then opened in pyrx software and their energy minimized. They were then converted to pdbqt file via open babel in pyrx software for further docking process.

2.3 Preparation of Protein

Protein Data Bank provided the three-dimensional structure of TPP in PDB format (PDB ID: 1U02) (PDB). The TPP enzyme's natural ligands (CO_3 and Mg^{+2}) were then removed from this file in BIOVIA Discovery studio visualizer, along with the water molecules, to prevent distortion when looking for potential binding sites.[16] The file was then saved in PDB format after polar hydrogen atoms were added to create any hydrogen bonds that could be necessary for the protein and ligand to bind. Figure 1 displays the crystal structure of a protein related to trehalose-6-phosphate phosphatase (PDB ID: 1U02) with the water molecules removed and polar hydrogens and kollman charges added.

2.4 Molecular Docking

Protein 1U02's 3D structure was opened in Pyrx and then transformed to a macromolecule. The grid box encircled the entire macromolecule. For the purpose of defining the search space for ligand binding in the pyrx software, the offset values and the quantity of points in the x, y, and z dimensions were recorded. As a PDBQT file, the TPP was stored. Additionally, the vina wizard in Pyrx was used to choose the pdbqt files of each phytochemical for docking with proteins. The best-scoring ligands from a blind docking of ligands with proteins were chosen to produce 3D and 2D figures of them. Using empirical-free energy functions and the Lamarckian genetic algorithm, the docking energies of all the ligand molecules and the standard drug, substrate-T6P (trehalose-6-phosphate), were determined. Based on distinct electrostatic, Vander Waal, hydrogen bonding, and desolvation effects, these tools determine binding-free energy (G).

For each docking run, "flexible" ligand-docking mode and "regular precision" docking settings were used. Using Pyrx energy calculations, the stability of every docked posture was assessed.[17]

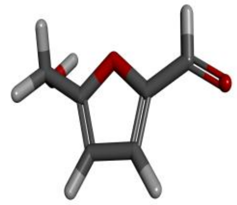
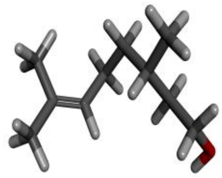
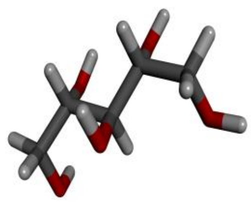
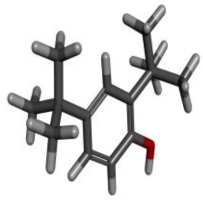
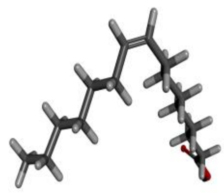
3. RESULT AND DISCUSSION

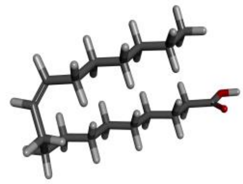
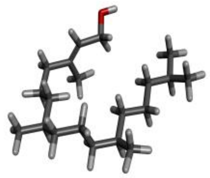
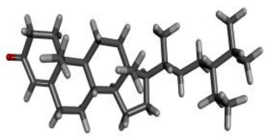
The results of a molecular docking investigation that demonstrated strong interactions between 10 possible ligands and trehalose–6–phosphate phosphatase in various forms of ligand-protein interactions with specific binding affinities are summarized in (Table 2). The most stable binding between the ligand and the target protein was indicated by the docked model with the lowest binding energy and highest binding affinity. We chose ligands with sizable binding affinities. Specific amino acids important in ligand-protein binding were discovered through visualization of structures with high dock scores in PyMOL and Discovery Studio 4.0. Crystal structure of trehalose-6-phosphate phosphatase related protein 1U02 Through interactions with Histidine via H-bonds and Lysine via Hydrogen bond interactions, Isoniazid interacted -6.0 kcal/mol (Figure 2). Campesterol, Stigmast-4-en-3-one with a binding affinity of less than -8.2, -7.8 kcal/mol, the docking of campesterol bind with glutamine with two hydrogen bond Stigmast-4-en-3-one having -7.8 kcal/mol but no hydrogen bond formed Figure 3.

4. CONCLUSION

The enzymes involved in the metabolism of trehalose, particularly the TPP enzyme, have emerged as promising candidates for use in medicinal and other prospective applications. TPP has received little attention thus far, and there is still more work to be done, despite the fact that the structures and catalytic processes of a number of other phosphatases are well established. Selecting TPPs as a therapeutic target has certain distinct advantages. TPP avoids difficulties brought on by secondary recognition sites, unlike other phosphatases, by binding only the short molecule ligand, which makes it easier for the inhibitor to imitate the substrate. Inhibitors with high affinities can be created because structural investigations of a small number of TPPs indicated the existence of an active site within a crevice that appears to bind substrate with great specificity. The aforementioned claim is supported by the current *in silico* molecular modelling investigation we conducted employing phytochemicals as TPP inhibitors.

Table 1: Compound name with molecular weight, Pubchem database ID and 3-dimensional structure of Standard marketed drug and phytoconstituents of *Moringa oleifera*

S. No	**Compound Name with Molecular Formula**	**PubChem Database ID**	**3D Structure**
1.	Isoniazid ($C_6H_7N_3O$)	3767	
2.	5-Hydroxymethylfurfural ($C_6H_6O_3$)	237332	
3.	Citronellol ($C_{10}H_{20}O$)	8842	
4.	Xylitol ($C_5H_{12}O_5$)	6912	
5.	2,4-Di-tert-butylphenol ($C_{14}H_{22}O$)	7311	
6.	Palmitoleic acid ($C_{15}H_{14}O_5$)	445638	

7.	Cis-Vaccenic acid (C18H34O2)	5282761	
8.	Phytol (C20H40O)	5280435	
9.	Cyclopentane, 1,1′-[3-(2-cyclopentylethyl)-1,5-pentanediyl] bis- (C22H40)	281840	
10.	Campesterol (C28H48O)	173183	
11.	Stigmast-4-en-3-one (C29H48O)	5484202	

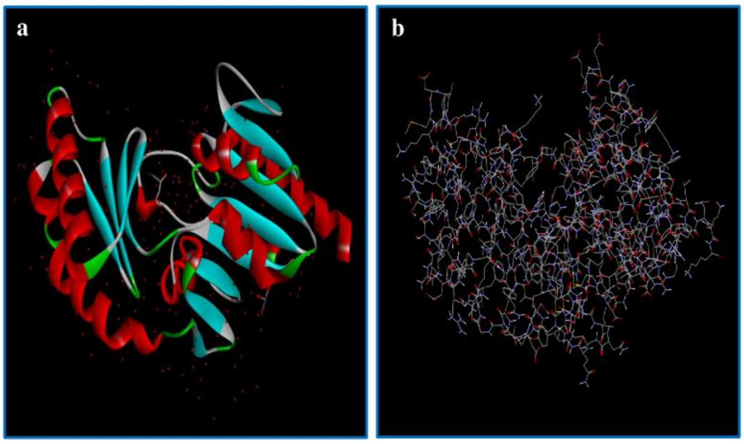


Figure 1: (a) Crystal structure of trehalose-6-phosphate phosphatase related protein (PDB ID: 1U02), (b) Target protein: trehalose-6-phosphate phosphatase (PDB ID: 1U02) with removed water molecules and with added polar hydrogens and kollman charges.

Table 2: Binding affinities and Hydrogen Bond interactions of Standard drug Isoniazid and 10 different ligands with protein 1U02

Compound	**Binding Affinity (kcal/mol) with protein 1U02**	**Hydrogen Bond interactions (Distance in A) [Type of bond]**	**Total number of Hydrogen -bonds**
Isoniazid	-6.0	HIS A:66 (3.49) [C-H Bond], LYS A:161 (5.79), ASP A:9 (2.67) [Conventional H-Bond]	3
5-Hydroxymethylfurfural	-5.1	ILE A:60 (3.55), ASN A:61 (3.77) [C-H Bond], SER A:53 (1.88), LYS A:71 (2.18), ASN A:61 (2.81) [Conventional H-Bond]	5
Citronellol	-4.9	NIL	-
Xylitol	-5.1	ASP A:7 (1.96, 2.42, 2.71), ASP A:180 (1.93), LYS A:161 (2.12) [Conventional H-Bond]	5
2,4-Di-tert-butylphenol	-6.4	NIL	-
Palmitoleic acid	-5.6	HIS A:66 (3.65) [C-H Bond], LYS A:161 (2.74), ASP A;179 (2.51), ASP A:7 (2.85) [Conventional H-Bond]	4
Cis-Vaccenic acid	-5.6	HIS A:66 (3.57) [C-H Bond], GLY A:46 (2.59), LYS A:161 (2.39, 3.01) [Conventional H-Bond]	4
Phytol	-5.7	LYS A:161 (2.75), ASP A:179 (2.89) [Conventional H-Bond]	2
Cyclopentane, 1,1′-[3-(2-cyclopentylethyl)-1,5-pentanediyl] bis- (C22H40)	-6.8	NIL	-
Campesterol	-8.2	GLU A:200 (2.28, 2.83) [Conventional H-Bond]	2
Stigmast-4-en-3-one	-7.8	Nil	-

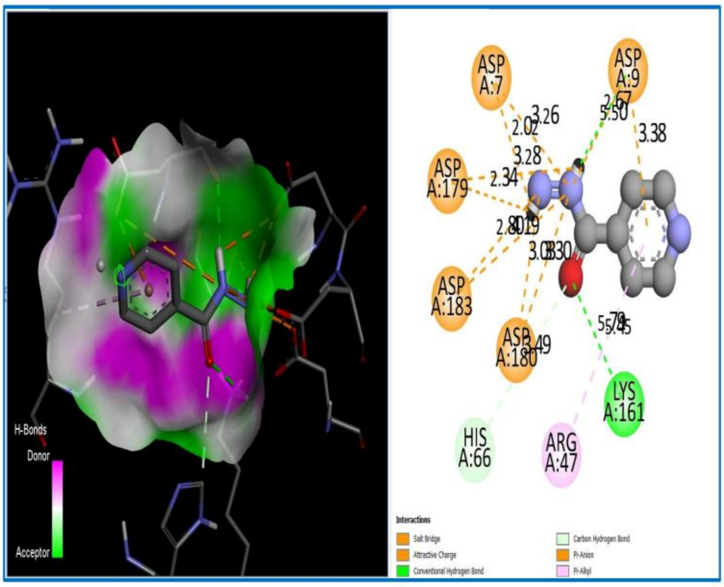
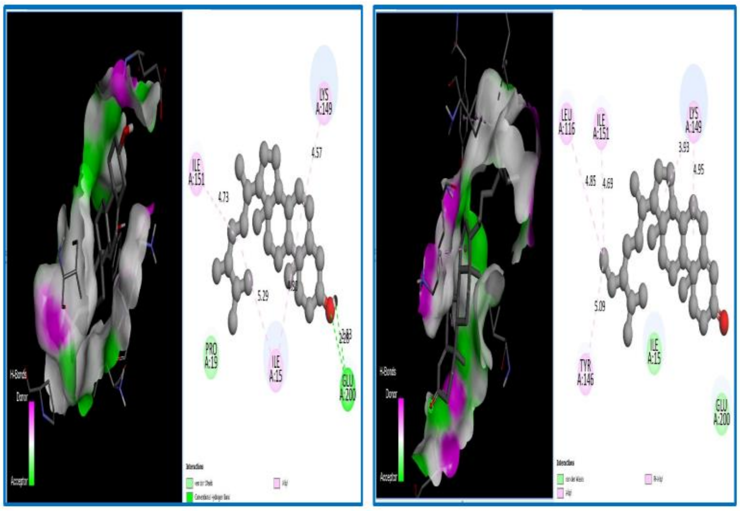


Figure 2: 3D and 2D structures of Donor-acceptor interactions obtained by docking of Standard drug Isoniazid and receptor 1U02 trehalose-6-phosphate phosphatase



(a) 3D and 2D structure of protein-ligand interaction of first pose of Campesterol with Crystal structure of trehalose-6-phosphate phosphatase related protein (PDB ID: 1U02).

(b) 3D and 2D structure of protein-ligand interaction of first pose of Stigmast-4-en-3-one with Crystal structure of trehalose-6-phosphate phosphatase related protein (PDB ID: 1U02).

Figure 3: 3D and 2D structures of Donor-acceptor interactions obtained by docking of ligands having best dock score i.e., (a) Campesterol and (b) Stigmat-4-en-3-one and receptor 1U02 trehalose-6-phosphate phosphatase

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