Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X
Volume 29| No. 1| April|2023

***Original article***

# Virtual prediction of fava bean (*Vicia faba* L.) active compounds to inhibit G6PD enzyme activity

Regina Shania Andyni[1,2], Regina Putri Virgirinia[1], Fatchiyah Fatchiyah[1,2]*

[1]Research Center of Smart Molecule of Natural Genetics Resource, Brawijaya University, Malang, East Java, Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

**Abstract**

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzyme defect in humans, caused by a mutation in the X-linked gene encoding G6PD. The G6PD enzyme plays an important role to produced reducing agents which maintain reduced glutathione through pentose phosphate pathway. On ingestion of Faba Bean (*Vicia faba* L.), vicine and convicine are hydrolyzed by β -glucosidase to divicine and isouramil which can cause acute hemolytic anemia in patients with G6PD deficiency. This study aims to determine the molecular mechanism of bioactive compounds from fava beans as an inhibitor of the human G6PD Canton enzyme activity through an in silico approach. Screening for toxic bioactive compounds of *V. faba* was carried out using Swiss ADME. Molecular docking was performed to identify the interaction of divicine and isouramil binding with human G6PD Canton. Dobutamine hydrochloride was used as a negative control ligand. The result of Swiss ADME screening showed that convicine and vicine were not qualified for the Lipinski rules of five. Docking analysis demonstrated similar binding interaction of divicine and isouramil compared with control ligand to bound G6PD canton allosteric sites and shift the binding position of NADP+, suggesting these compounds interrupt the NADP+ to produce NADPH. The binding affinity of divicine (-7.0 kcal/mol) was lower than dobutamine hydrochloride (-6.9 kcal/mol), while isouramil was higher (-6.5 kcal/mol). Accordingly, this study was pointed out that divicine and isouramil have the potential as inhibitor of G6PD enzyme activity. Further, in vivo and in vitro studies are needed to confirm this research.

Keywords: Divicine, G6PD deficiency, human G6PD Canton, in silico isouramil, *Vicia faba* L

Received: November 3, 2022 Revised: December 19, 2022 Accepted: December 20, 2022

## Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common hereditary enzyme defect in human erythrocytes (Howes et al., 2013). G6PD deficiency is an X-linked recessive genetic disorder caused by mutation, which 400 million people in the world suffer from (National Organization for Rare Disorders., 2019). The highest prevalence of G6PD deficiency is in Africa, the Mediterranean region and Asia (Vick., 2020). Clinical manifestations of patients with G6PD deficiency include acute hemolytic anemia, neonatal jaundice, hypovolemic shock, chronic non-sphaerocytic anemia (CNSA) neonatal hyperbilirubinemia and miscarriage (Monteiro et al., 2014; Boonyuen et al., 2017). The risk of miscarriage would be increased by 47% when the patient has a G6PD deficiency (Fadila et al., 2021). G6PD is a metabolic enzyme that has a function to catalyze the first and rate-limiting step of the Pentose Phosphate Pathway. G6PD plays an important role in catalyzing the reaction of Glucose 6 Phosphate and NADP+ into 6 phosphogluconate (6GP) and produces NADPH (Tang., 2019). The production of NADPH has an important role in red blood cells that are susceptible to damage by ROS (Cho et al., 2018). Clinical manifestation in G6PD-deficient people can develop after ingestion of fava beans which is known as favism (Luzzato & Arese., 2018).

Fava bean is a plant that belongs to the Fabaceae family. Fava beans have nutritional benefits such as lysine rich proteins, carbohydrates, minerals, and vitamins (Dhull el al., 2022). Fava bean also contains some antinutritional compounds, the substances in food that interfere the absorption of nutrients in the body, such as lectins, saponins, phytic acid vicine and convicine (Labba et al., 2021). Vicine and convicine cause hemolytic anemia in patients with G6PD deficiency (Khazaei et al., 2019). These compounds will be hydrolyzed by the β-glucosidase enzyme which then produces aglycones, divicine and isouramil. Divicine and isouramil can increase oxidative stress in the body. Oxidative stress in the form of these glucosides can cause severe damage to erythrocytes if the cells lack the G6PD enzyme (Koerim et al., 2016). However which compounds could be specifically inhibiting the human G6PD Canton is still unclear. The purpose of this research was to analyze the effect of potential compounds in fava beans (*Vicia faba* L) on activity of the enzyme Glucose-6-phosphate dehydrogenase (G6PD) with in silico approach.

*Corresponding Author:
Fatchiyah Fatchiyah
Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University. Research Center of Smart Molecule of Natural Genetics Resources, Brawijaya University
Phone: 0341-575841 Fax: 0341-575841
E-mail: fatchiya@ub.ac.id

## Methods

### Data mining

Canonical SMILES and 3D conformer bioactive compounds of *V. faba* and dobutamine hydrochloride as

http://dx.doi.org/10.23869/bphjbr.29.1.20233
Published by © PBI East Java. Open Access www.berkalahayati.org

control compound (CID:65324) were retrieved from the PubChem NCBI database (https://pubchem.ncbi.nlm.nih.gov/) set as ligands (Dhull et al., 2022) (Table 1). Dobutamine hydrochloride was chosen due to its ability as human G6PD Canton inhibitor by causing structural alterations in coenzyme and substrate binding sites (Kizilbay & Karaman, 2022). The 3D conformation of human G6PD Canton (PDB ID: 6E07) was downloaded from the RSCB PDB Website (https://www.rcsb.org/) (Hwang et al., 2018). This structure was selected because it has a co-crystallized ligand, better resolution (2.6 Å), and good percentile ranks shown by the 'blue end' of the slider plot: Rfree (0.216), clashscore (5), ramachandran outliers (0.1%), sidechain outliers (5.4%) RsRZ outliers (0.7%) (Smart et al., 2018).

**Table 1**. The identity of bioactive compounds of fava bean

| Bioactive Compounds | Pubchem CID |
|---|---|
| Protocatechuic acid | 72 |
| Ferulic acid | 445858 |
| Vanilic acid | 8468 |
| Caffeic acid | 689043 |
| Sinapic acid | 637775 |
| Salvianolic acid | 5281793 |
| Eucomic acid | 23757219 |
| Caffeoylquinic acid | 1794427 |
| Dicaffeoylquinic acid | 12358846 |
| Raffinose | 439242 |
| Stachyose | 439531 |
| Verbascose | 441434 |
| Vicine | 135413566 |
| Convicine | 88000 |
| Catechin | 9064 |
| Epicatechin | 72276 |
| Folic acid | 135398658 |
| Genistein | 5280961 |
| Daidzein | 5281708 |
| Phytic acid | 890 |

**Virtual screening**

Virtual Screening is carried out using the Swiss ADME (http://www.swissadme.ch/) (Daina & Zoete, 2019). Swiss ADME is a web tool that provides access to a free collection of fast yet powerful predictive models for determining the physicochemical properties, pharmacokinetics, drug-likeness, and chemical assays of drugs (Daina et al., 2017). The parameters used are the number of hydrogen bond acceptors, the number of hydrogen bond donors, GI absorption, lipophilicity (MLOGP Po/w) and Lipinski Drug Likeness. Hydrogen bond donor is ≤ 5. Hydrogen bond acceptor is ≤ 10. Molecular < 500 Daltons. Octanol-water partition coefficient (log *P*) < 5 and > 1 (Lipinski et al., 1997).

**Ligand and protein preparation**

The 3D structure of aglycones Divicine (CID: 135413566) and Isouramil (CID: 77518) were obtained from PubChem NCBI database (https://pubchem.ncbi.nlm.nih.gov/). Pyrx autodock Vina software (https://pyrx.sourceforge.io/) was used to minimize the energy and convert the .sdf format into .pdb format (Dallakyan & Olson, 2015). The 3D conformation of Human G6PD Canton (PDB ID: 6E07) was downloaded from RSCB Protein Data Bank (https://www.rcsb.org/) and was retrieved from water molecules and ligand using Discovery Studio 4.1 Software (http://3dsbiovia.com/products/) (Hwang et al., 2018).

**Molecular docking and visualization**

Molecular docking was simulated using Pyrx autodock Vina software with a specific docking. The grid setting for allosteric site: Center (X: 13.0211 Y: 23.0626 Z: 6.0114) and dimension (X: 27.5148 Y: 21.9603 Z: 21.5730). The grid setting for NADP+ binding site: Cite (X:15.1746 Y: 22.3661 Z: 4.6612) and dimension (X: 34.3113 Y: 22.6962 Z: 21.6119) (Kizilbay & Karaman, 2022) (Hwang et al., 2018). Interactions and energy binding formed between ligands (dobutamine hydrochloride, divicine, and isouramil) were calculated. Docking complex combined with Pymol Software (https://pymol.org/2/). Visualization and analysis of docking results were done using the Discovery Studio software.

## Result and Discussion

**Virtual screening of bioactive compounds in Fava Beans (*Vicia faba.* L)**

Virtual screening of bioactive compounds in fava beans based on Swiss ADME showed that there were 8 active compounds such as verbascose, phytic acid, stachyose, dicaffeoylquinic acid, raffinose, folic acid vicine and convicine were not qualified to Lipinski rule of five (Table 2). These bioactive compounds are categorized as toxic compounds to the human body. Lipinski drug likeness is a rule for determining whether a compound with a particular pharmacological or biological activity has similar chemical and physical properties to drugs that are orally active in humans (Tian et al., 2015). These rules describe molecular properties that are important for the pharmacokinetics of drugs in the human body, including absorption, distribution, metabolism, and excretion (ADME) (Feher & Schmidt, 2003). Total violations to Lipinski rule of five for vicine, convicine and follic acid are 2 violations. Meanwhile verbascose, phytic acid, stachyose, dicaffeoylquinic acid, and raffinose have 3 violations. A drug that can be taken orally has no more than 1 violation (Ivanovic et al., 2020). The 8 bioactive compounds of fava beans have low GI absorption values and TPSA >140(Å²). Low GI absorption indicates a low absorption process for the compound in the GI. Oral drug absorption involves transport across the membrane of the epithelial cells of the gastrointestinal tract. Absorption is influenced by differences in luminal pH along the GI, surface area per luminal volume, blood perfusion, presence of bile and mucus, and epithelial properties. The TPSA value for polarity must be 20-130(Å²) (Vertzoni et al., 2019).

Fava beans mainly contain galacto-oligosaccharides such as raffinose, stachyose, and verbascose, which are not digested due to the lack of galactosidase in the small intestine (Sharan et al.,2021). Therefore, these

oligosaccharides are fermented in the colon and release the gas (Pico et al., 2021). Dicaffeoylquinic acid plays a role in anti-inflammatory and have acute effects on a rat with the dose >800mg/kg (Humphreys & Busath, 2019). Folic acid is not toxic in healthy people, but it can harm the nervous system in people with undetected pernicious anemia (Berry, 2019). According to a recent study, acute hemolytic anemia is caused by vicine and convicine aglycones (divicine and isouramil) in patients with a G6PD deficiency in their red blood cells (Labba et al., 2021). Based on the results of screening using Swiss ADME and literature studies, divicine and isouramil were selected as candidate compounds that are harmful to patients with G6PD deficiency and will be continued in molecular docking analysis.

**Table 2.** Swiss ADME Prediction Results for Bioactive Compounds of Fava Bean

| Bioactive Compounds | TPSA (Å²) | MW (g/mol) | No. of H Bond acceptors | No. Of H Bond donors | GI absorption | Lipophilicity (MLOGP Po/w) | Lipinski Drug Likeliness |
|---|---|---|---|---|---|---|---|
| **Verbascose** | **426.98** | **828.72** | **26** | **17** | **Low** | **-9.93** | **No; 3 violations** |
| **Phytic acid** | **459.42** | **660.04** | **24** | **12** | **Low** | **-7.36** | **No; 3 violations** |
| **Stachyose** | **347.83** | **666.58** | **21** | **14** | **Low** | **-8.02** | **No; 3 violations** |
| **Dicaffeoylquinic acid** | **211.28** | **516.45** | **12** | **7** | **Low** | **-0.35** | **No; 3 violations** |
| **Raffinose** | **268.68** | **504.44** | **16** | **11** | **Low** | **-6.15** | **No; 3 violations** |
| **Vicine** | **197.17** | **304.26** | **8** | **7** | **Low** | **-3.76** | **No; 2 violations** |
| **Convicine** | **191.12** | **305.24** | **8** | **7** | **Low** | **-3.30** | **No; 2 violations** |
| **Folic acid** | **213.28** | **441.40** | **9** | **6** | **Low** | **-0.62** | **No; 2 violations** |
| Protocatechuic acid | 77.76 | 154.12 | 4 | 3 | High | 0.40 | Yes; 0 violation |
| Ferulic acid | 66.76 | 194.18 | 4 | 2 | High | 1.00 | Yes; 0 violation |
| Vanilic acid | 66.76 | 168.15 | 4 | 2 | High | 0.74 | Yes; 0 violation |
| Caffeic acid | 77.76 | 180.16 | 4 | 3 | High | 0.70 | Yes; 0 violation |
| Sinapic acid | 75.99 | 224.21 | 5 | 2 | High | 0.73 | Yes; 0 violation |
| Salvianolic acid | 184.98 | 494.45 | 10 | 7 | Low | 1.34 | Yes; 1 violation |
| Eucomic acid | 115.06 | 240.21 | 6 | 4 | High | 0.20 | Yes; 0 violation |
| Caffeoylquinic acid | 164.75 | 354.31 | 9 | 6 | Low | -1.05 | Yes; 1 violation |
| Catechin | 110.38 | 290.27 | 6 | 5 | High | 0.24 | Yes; 0 violation |
| Epicatechin | 110.38 | 290.27 | 6 | 5 | High | 0.24 | Yes; 0 violation |
| Genistein | 90.9 | 270.24 | 5 | 3 | High | 0.52 | Yes; 0 violation |
| Daidzein | 70.67 | 254.24 | 4 | 2 | High | 1.08 | Yes; 0 violation |
| Divicine | 118.02 | 142.12 | 3 | 4 | High | -2.36 | Yes; 0 violation |
| Isouramil | 111.97 | 143.10 | 3 | 4 | High | -1.91 | Yes; 0 violation |

**Molecular docking native ligand (NADP+) dobutamine hydrochloride, divicine and isouramil to human G6PD canton**

The interaction between dobutamine hydrochloride, divicine, and isouramil to human G6PD Canton has been carried out through molecular docking. Ligand-protein interaction was shown as the type of chemistry bond formed and the binding site of amino acid residue (Table 3). Dobutamine hydrochloride formed conventional hydrogen bond to human G6PD Canton at amino acid residues HIS 374, ASN 229, LYS 403, and GLU 389. There was also Pi- Donor hydrogen bond interaction between dobutamine hydrochloride and ASN 388 residue. THR 402 was the amino acid bound to dobutamine hydrochloride mediating by Pi-Sigma through hydrophobic interaction. ASP 375, VAL 376, ILE 220, PHE 221, LEU 390, MET 404, ILE 224, and PHE 373 were amino acid residues that interact with dobutamine hydrochloride through van der Waals force (Figure 1B).

Divicine formed conventional hydrogen bonds to human G6PD Canton at amino acid residues ASN 229, ASN 388, and ASP 375. VAL 376 was the amino acid bound to divicine mediating by Pi-Alkyl through hydrophobic interaction. There were also van der Waals forces between divicine and ALA 377, ILE 224, PHE 373, and HIS 374 amino acid residues (Figure 1C). Isouramil formed conventional hydrogen bonds and van der Waals forces with amino acid residues ARG 215, ARG 348, PHE 221, PHE 216, ASN 218, LYS 275, and TRP 225 (Figure 1D). Amino acid residues on the allosteric side of human G6PD Canton are ASN 229, PHE 373, ILE 220, LEU 420, LEU 422, TYR 401, VAL 400 ASN 388, ASP 375, ASP 421, ARG 215, and ARG348 (Kizilbay & Karaman, 2022).

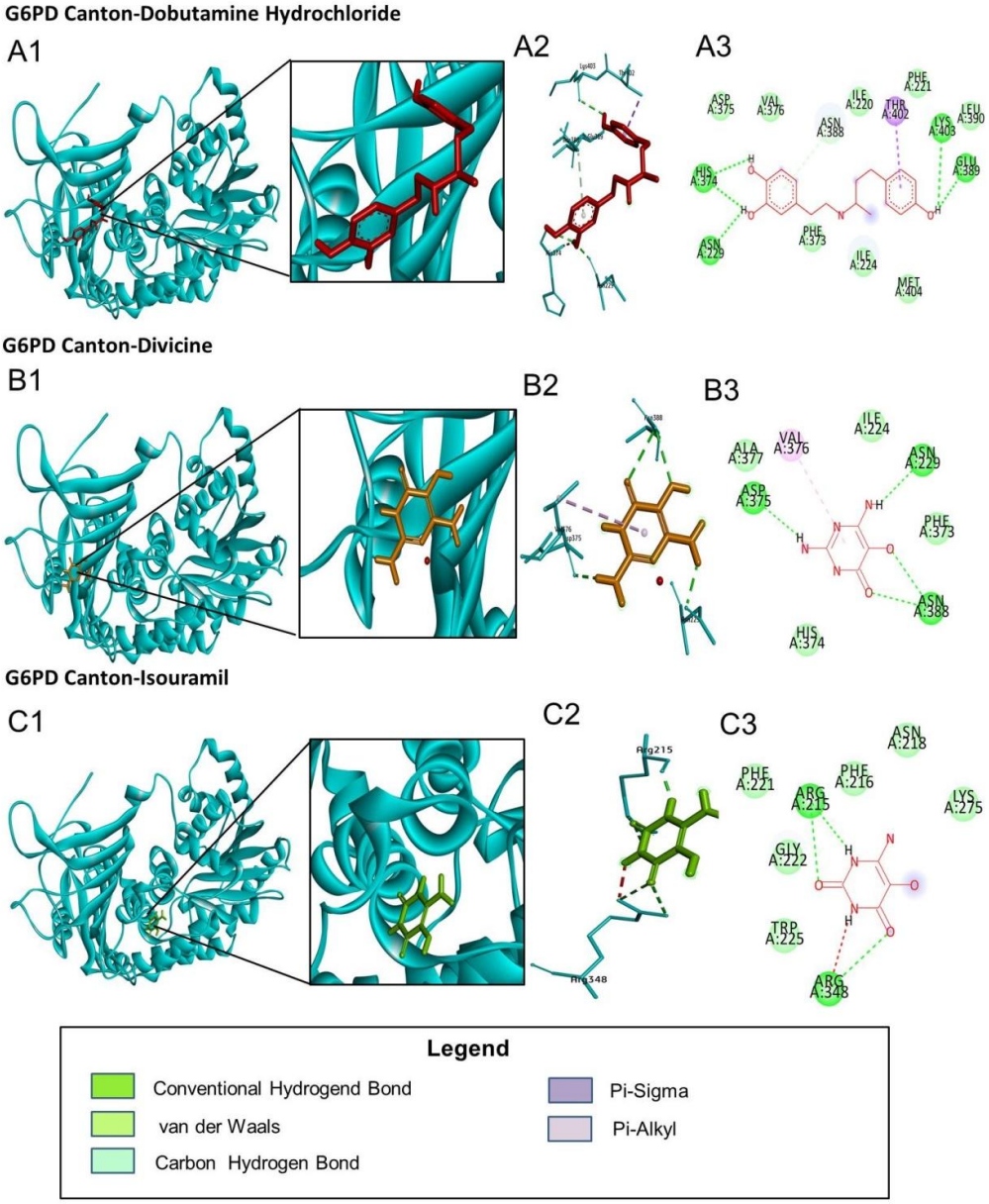


**Figure 1.** Interaction between Human G6PD Canton with (A) Dobutamine Hydrochloride (B) Divicine (C) Isouramil. Number 1 show complex docking. Number 2 show 3D Structure. Number 3 show 2D Structure.

NADP+ formed hydrogen bonds at amino acid residues ASP 421, THR 423, ARG 393, and TYR 401. Hydrophobic bonds with the Pi-Alkyl type are on the amino acid residues TYR 401 and TRP 509. The electrostatic hydrogen bond with the salt bridge type is on the amino acid residues 366 and 403. The electrostatic bond with the attractive charge type is on the amino acid residue LYS 238, LYS 366, ARG 370, and ARG 487 (Table 4).

The aromatic rings of NADP+ are embedded between delocalized π-electron clouds. Adenine is between TYR 503 and ARG 487 and nicotinamide is between TRP 509 and TYR 401. The 2′-phosphate forms hydrogen bonds to ARG 487, ARG 357, LYS 238, and LYS 366. The bisphosphate interacts with ARG 370. The amide function of the nicotinamide interacts with ARG 393 and ASP 421. The role of ARG 487 is typical of NADP+-binding sites in several enzymes (Au et al., 2000).

The presence of hydrogen bonds plays a role in the ability of drug molecules to maintain molecular complexes, which leads to the formation of biological responses to target proteins (Williams & Ladbury, 2003). The formation of hydrogen bonds contributes significantly to ligand-receptor interactions. Hydrogen bonds are crucial to obtain the specificity of the ligand to the protein target (Chen et al., 2018).

Binding affinities of Dobutamine hydrochloride, divicine isouramil, and NADP+ to human G6PD Canton are -6.9, 7.0, -6.5, -6.2 (kcal/mol) (Table 2 &3). Binding affinity is a parameter of the ability of a compound or drug to bind to the receptor (Williams & Ladbury, 2003). Binding affinity is influenced by hydrogen bonding, electrostatic, hydrophobic, and Van der Waals (Gholami

& Bordbar,2014). The lower binding affinity value indicating as stronger ligand-protein binding (Stepniewska et al., 2018).

Dobutamine hydrochloride, divicine, and isouramil that bind to human G6PD Canton, shift the position of the binding site native ligand (NADP+). There were 22 amino acid residues in the interaction between NADP+ and human G6PD Canton. Meanwhile, there were 20 amino acid residues involved in the complex docking between NADP+, dobutamine hydrochloride, divicine, isouramil, and human G6PD Canton. Interestingly, there was no interaction on ASN 363 amino acid residue on human G6PD Canton-dobutanine hydrochloride - NADP+ and human G6PD Canton - divicine - isouramil - NADP+ complex dockings. Moreover, the presence of ARG 357 and the absence of ASN 397, ASP 493, and VAL 391 indicate a shifting residue mechanism in the native ligand (NADP+) binding site (Figure 2).

**Table 3.** Interaction of Human G6PD Canton with Dobutamine hydrochloride, Divicine and Isouramil.

| Point Interaction | Chesmitry Bond | Type | Donor atom | Acceptor atom | Binding Affinity (kcal/mol) |
|---|---|---|---|---|---|
| ***Human G6PD Canton-Dobutamine Hydhrochloride*** | | | | | |
| :LIG1:H-A:GLU389:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:GLU389:O | |
| :LIG1:H-A:LYS403:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:LYS403:O | |
| :LIG1:H-A:ASN229:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:ASN229:O | |
| :LIG1:H-A:HIS374:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:HIS374:O | -6.9 |
| :LIG1:H-A:HIS374:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:HIS374:O | |
| A:ASN388:ND2-:LIG1 | Hydrogen Bond | Pi-Donor Hydrogen Bond | A:ASN388:ND2 | :LIG1 | |
| A:THR402:CG2-:LIG1 | Hydrophobic | Pi-Sigma | A:THR402:CG2 | :LIG1 | |
| ***Human G6PD Canton – Divicine*** | | | | | |
| A:ASN388:ND2 - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ASN388:ND2 | :LIG1:O | |
| A:ASN388:ND2 - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ASN388:ND2 | :LIG1:O | |
| :LIG1:H - A:ASP375:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:ASP375:O | -7.0 |
| :LIG1:H - A:ASN229:OD1 | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:ASN229:OD1 | |
| :LIG1 - A:VAL376 | Hydrophobic | Pi-Alkyl | :LIG1 | A:VAL376 | |
| ***Human G6PD Canton - Isouramil*** | | | | | |
| A:ARG215:NE - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG215:NE | :LIG1:O | |
| A:ARG215:NH2 - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG215:NH2 | :LIG1:O | |
| A:ARG348:NH1 - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG348:NH1 | :LIG1:O | -6.5 |
| A:ARG348:NH2 - :LIG1:O | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG348:NH2 | :LIG1:O | |
| :LIG1:H - A:ARG215:O | Hydrogen Bond | Conventional Hydrogen Bond | :LIG1:H | A:ARG215:O | |

The complex docking indicated that there were several types of binding changes when NADP+ binds to Human G6PD Canton. ASP 421 amino acid residue changed from conventional hydrogen bonds to van der Waals forces. Van der Waals force, a relatively weak electric force that attracts neutral molecules (Israelachvili, 2011). The complex (dobutamine hydrochloride - NADP+ - human G6PD Canton) showed a change from conventional hydrogen bond to unfavorable donors on ARG 393. The complex (divicine isouramil - NADP+ - human G6PD Canton) showed a change from hydrophobic pi alkyl to carbon hydrogen bond hydrogen bond on TRP 509. Unfavorable donor-donor bond indicates the repulsion between 2 molecules or atoms which reduces the stability of the complex (Figure 2) (Dhorajiwala et al., 2019).

Dobutamine hydrochloride, divicine, and isouramil affected the binding positions of NADP+ by interacting with the allosteric site residues. Allosteric site allow molecules to inhibit enzymatic activity. The role of NADP+ and the stability of Human G6PD activity depends on the structural NADP+ site (Agarwal & Mehrotra, 2016). Compounds that bind to allosteric inhibitors can reduce enzyme activity. Low G6PD enzyme causes NADP+ unable to produce NADPH and lead to the destruction of red blood cells or hemolysis (Mason et al., 2007).

The sequence alignment of Human G6PD reveals 3 conserved regions such as 9 residue peptide (RIDHYLGKE, residues 198–206 of the G6PD enzyme), a nucleotide-binding fingerprint (GxxGGDLA, residues 38–44 of the G6PD enzyme), and the sequence EKPxG (residues 170–174 of G6PD enzyme). Amino acid

aspartate, histidine, and lysine are important in NADP+ binding and catalysis in G6PD enzyme in 9 residue peptide (Sirdah et al., 2021). The catalytic binding domain of G6PD enzyme located on residue 1-206 (da rocha et al., 2022). Although the interactions formed are far from the catalytic site, dobutamine hydrochloride, divicine, and isouramil are potential to inhibit activity of the G6PD enzyme.

Divicine and isouramil as aglycones of vicine and convincine bound specifically to the allosteric site and inhibit human G6PD Canton activity. Vicine and convicine exist almost in *Vicia faba.* However, vicine and convicine have been also found in other species of *Vicia* genus, such as *Vicia narbonsensis* and *Vicia bithynica* but in low amounts (<0.1mg/g) (Griffiths & Ramsay,1992). Therefore, this study can be a recommendation for patients with G6PD deficiency to avoid foodstuffs from the genus *Vicia* as prevention.

In conclusion, dobutamine hydrochloride as control compound, divicine, and isouramil as bioactive compounds of fava beans has similar interactions to bind with human G6PD Canton. The bond formed at the allosteric site can shift the position of the native ligand (NADP+) and inhibits the activity of human G6PD Canton. Inhibited G6PD enzyme activity can trigger clinical manifestations in patients with G6PD deficiency. Further, in vivo and in vitro studies are needed to confirm this research.

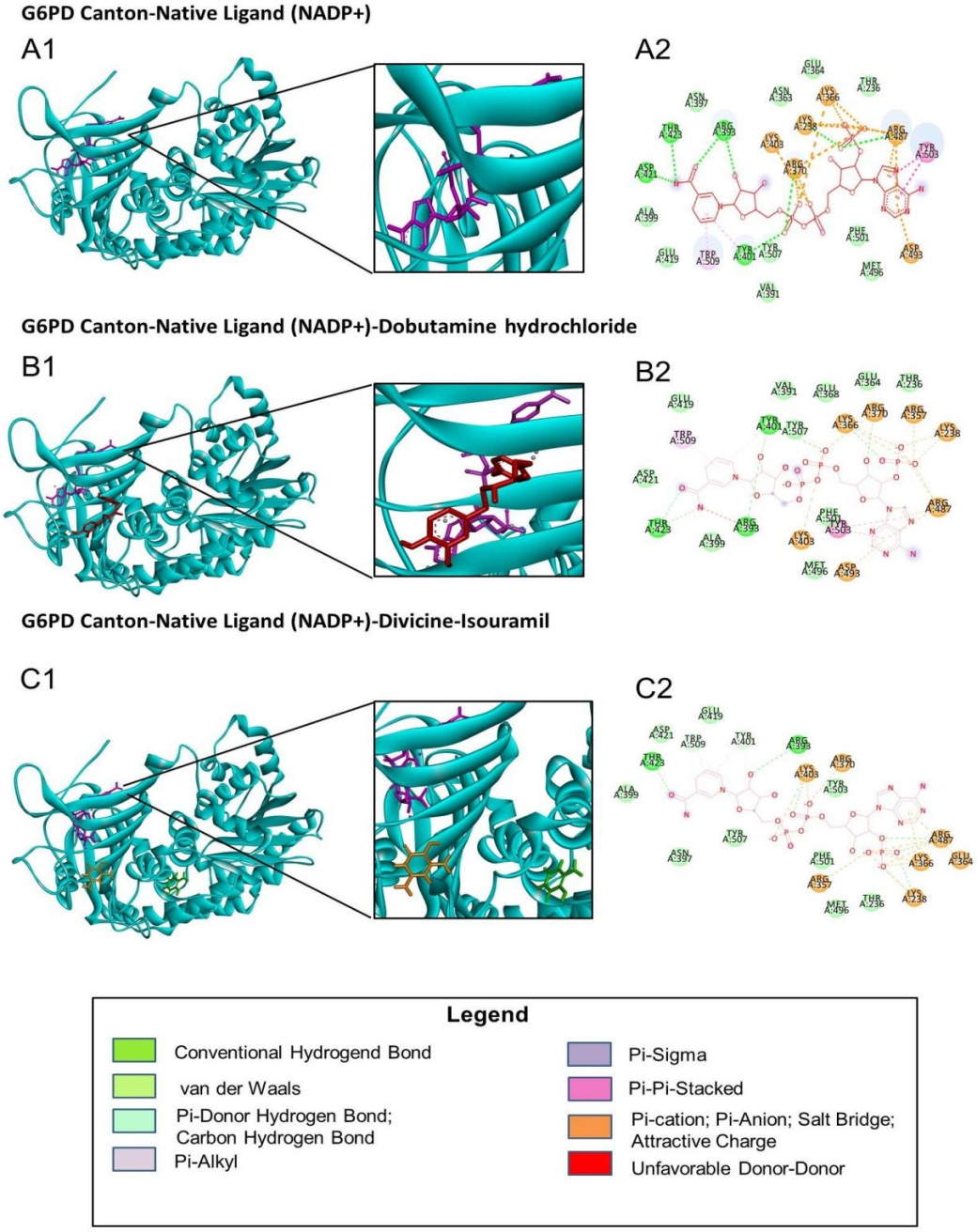


**Figure 2.** Interaction between Human G6PD Canton with (A) Native Ligand (NADP+) (B) Native Ligand (NADP+) – Dobutamine hydrochloride (C) Native Ligand (NADP+)- Divicine-Isouramil. Number 1 show complex docking. Number 2 show 2D Structure.

**Table 4.** Interaction of Human G6PD Canton and Native ligand (NADP+)

| Point Interaction | Chesmitry Bond | Type | Donor atom | Acceptor atom | Binding Affinity (kcal/mol) |
|---|---|---|---|---|---|
| | | ***Human G6PD Canton - Native Ligand (NADP+)*** | | | |
| A:LYS366:NZ - A:NAP601:O2X | Hydrogen Bond;Electrostatic | Salt Bridge | A:LYS366:NZ | A:NAP601:O2X | |
| A:LYS403:NZ - A:NAP601:O2A | Hydrogen Bond;Electrostatic | Salt Bridge | A:LYS403:NZ | A:NAP601:O2A | |
| A:LYS238:NZ - A:NAP601:O1X | Electrostatic | Attractive Charge | A:LYS238:NZ | A:NAP601:O1X | |
| A:LYS238:NZ - A:NAP601:O2X | Electrostatic | Attractive Charge | A:LYS238:NZ | A:NAP601:O2X | |
| A:LYS366:NZ - A:NAP601:O1N | Electrostatic | Attractive Charge | A:LYS366:NZ | A:NAP601:O1N | |
| A:LYS366:NZ - A:NAP601:O1X | Electrostatic | Attractive Charge | A:LYS366:NZ | A:NAP601:O1X | |
| A:ARG370:NH1 - A:NAP601:O2A | Electrostatic | Attractive Charge | A:ARG370:NH1 | A:NAP601:O2A | |
| A:ARG370:NH2 - A:NAP601:O1N | Electrostatic | Attractive Charge | A:ARG370:NH2 | A:NAP601:O1N | |
| A:ARG370:NH2 - A:NAP601:O2X | Electrostatic | Attractive Charge | A:ARG370:NH2 | A:NAP601:O2X | |
| A:ARG487:NH2 - A:NAP601:O1X | Electrostatic | Attractive Charge | A:ARG487:NH2 | A:NAP601:O1X | |
| A:ARG487:NH2 - A:NAP601:O2X | Electrostatic | Attractive Charge | A:ARG487:NH2 | A:NAP601:O2X | |
| A:LYS238:NZ - A:NAP601:O3X | Hydrogen Bond | Conventional Hydrogen Bond | A:LYS238:NZ | A:NAP601:O3X | |
| A:ARG370:NH1 - A:NAP601:O2N | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG370:NH1 | A:NAP601:O2N | |
| A:ARG370:NH2 - A:NAP601:O2N | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG370:NH2 | A:NAP601:O2N | -6.2 |
| A:ARG393:NH1 - A:NAP601:O2D | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG393:NH1 | A:NAP601:O2D | |
| A:ARG393:NH2 - A:NAP601:O7N | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG393:NH2 | A:NAP601:O7N | |
| A:TYR401:OH - A:NAP601:O2N | Hydrogen Bond | Conventional Hydrogen Bond | A:TYR401:OH | A:NAP601:O2N | |
| A:ARG487:NE - A:NAP601:O3X | Hydrogen Bond | Conventional Hydrogen Bond | A:ARG487:NE | A:NAP601:O3X | |
| A:NAP601:N7N - A:ASP421:OD1 | Hydrogen Bond | Conventional Hydrogen Bond | A:NAP601:N7N | A:ASP421:OD1 | |
| A:NAP601:N7N - A:THR423:OG1 | Hydrogen Bond | Conventional Hydrogen Bond | A:NAP601:N7N | A:THR423:OG1 | |
| A:NAP601:C5D - A:NAP601:O1A | Hydrogen Bond | Carbon Hydrogen Bond | A:NAP601:C5D | A:NAP601:O1A | |
| A:NAP601:C6N - A:NAP601:O5D | Hydrogen Bond | Carbon Hydrogen Bond | A:NAP601:C6N | A:NAP601:O5D | |
| A:ARG487:NH2 - A:NAP601 | Electrostatic | Pi-Cation | A:ARG487:NH2 | A:NAP601 | |
| A:ARG487:NH2 - A:NAP601 | Electrostatic | Pi-Cation | A:ARG487:NH2 | A:NAP601 | |
| A:ASP493:OD1 - A:NAP601 | Electrostatic | Pi-Anion | A:ASP493:OD1 | A:NAP601 | |
| A:TYR503 - A:NAP601 | Hydrophobic | Pi-Pi Stacked | A:TYR503 | A:NAP601 | |
| A:NAP601 - A:TYR503 | Hydrophobic | Pi-Pi Stacked | A:NAP601 | A:TYR503 | |
| A:TYR401 - A:NAP601 | Hydrophobic | Pi-Alkyl | A:TYR401 | A:NAP601 | |
| A:TRP509 - A:NAP601 | Hydrophobic | Pi-Alkyl | A:TRP509 | A:NAP601 | |
| A:TRP509 - A:NAP601 | Hydrophobic | Pi-Alkyl | A:TRP509 | A:NAP601 | |

## Acknowledgement

We send our regards to the members of research center of SMONAGENES for discussion and support

## References

Agarwal S. & Mehrotra R.J.J.C., 2016. An Overview of Molecular Docking. JSM chem, 4(2): 1024-1028.

Au S.W., Gover S., Lam V.M., Adams M.J., 2000. Human Glucose-6-Phosphate Dehydrogenase: The Crystal Structure Reveals a Structural NADP+ Molecule and Provides Insights into Enzyme Deficiency. Structure, 8(3):293-303.

Berry R.J., 2019. Lack of Historical Evidence to Support Folic Acid Exacerbation of The Neuropathy caused by Vitamin B12 Deficiency. The American Journal of Clinical Nutrition, 110(3):554-561.

Boonyuen U., Chamchoy K., Swangsri T., Junkree T., Day N.P., White N.J., Imwong M., 2017. A Trade Off Between Catalytic Activity and Protein Stability Determines The Clinical Manifestations of Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency. International journal of biological macromolecules, 104:145-156.

Chen D Li., Zhao Y., Tan M., Li W., Savidge X., T. Guo W. Fan X. 2018. Effective Lead Optimization Targeting The Displacement of Bridging Receptor-Ligand Water Molecules. Phys Chem Chem Phys, 20: 24399-24407.

Cho E.S., Cha Y.H., Kim H.S., Kim N.H., Yook J.I., 2018. The Pentose Phosphate Pathway as A Potential Target for Cancer Therapy. Biomolecules & Therapeutics, 26(1):29.

Daina A., Michielin O., Zoete V., 2017. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. Scientific Reports, 7(1):1-13.

Daina A., & Zoete V., 2019. Application of The Swiss Drug Design Online Resources in Virtual Screening. International Journal of Molecular Sciences, 20(18):4612.

Dallakyan S.,Olson AJ (2015) Small-Molecule Library Screening by Docking with PyRx. In: Methods Mol. Biol, 243–250.

da Rocha, J.B., Othman, H. and Hazelhurst, S., 2022. Molecular Dynamics of G6PD Variants from sub-Saharan Africa. Biochemistry and Biophysics Reports. 30:101-236.

Dhorajiwala T.M., Halder S.T., Samant L., 2019. Comparative in Silico Molecular Docking Analysis of l-Threonine-3-Dehydrogenase, A Protein Target Against African Trypanosomiasis Using Selected Phytochemicals. Journal of Applied Biotechnology Reports, 6(3):101-108.

Dhull S.B., Kidwai M.K., Noor R., Chawla P., Rose P.K., 2022. A Review of Nutritional Profile and Processing of Faba Bean (Vicia faba L.). Legume Science, 4(3):129-135.

Fadila F., Ekawardhani S., Fauziah N., Hutagalung J., 2021. Risk Factors of Miscarriage in Malaria-Endemic Region: A Case-Control Study in Eastern Indonesia. European Journal of Molecular & Clinical Medicine, 8(3):1281-1292.

Gholami S., Bordbar A.K., 2014. Exploring Binding Properties of Naringenin with Bovine β-Lactoglobulin: A Fluorescence, Molecular Docking and Molecular Dynamics Simulation Study. Biophysical Chemistry, 187:33-42.

Feher M., & Schmidt J.M., 2003. Property Distributions: Differences Between Drugs, Natural Products, and Molecules from Combinatorial Chemistry, J. Chem. Inf. Comput. Sci, 43: 218-227.

Griffiths DW & Ramsay G. 1992. The Concentration of Vicine and Convicine in Vicia faba and Some Related Species and Their Distribution within Mature Seeds. J Sci Food Agric, 59:463-468.

Humphreys B., Busath D.D., 2019. Anti-Influenza Nutraceuticals: Antiviral and Anti-Inflammatory Effects. Advances in in Complementary and Alternative Medicine, 4(3):358-373.

Howes R.E., Battle K.E., Satyagraha A.W., Baird J.K., Hay S.I., 2013. G6PD Deficiency: Global Distribution, Genetic Variants and Primaquine Therapy. Advances in Parasitology, 81:133-201.

Hwang S., Mruk K., Rahighi S., Raub A.G., Chen C.H., Dorn L.E., Horikoshi N., Wakatsuki S., Chen J.K., Mochly-Rosen D., 2018. Correcting Glucose-6-Phosphate Dehydrogenase Deficiency with A Small-Molecule Activator. Nature Communications, 9(1):1-12.

Israelachvili J.N., 2011. Intermolecular and surface forces. Academic press

Ivanović V., Rančić M., Arsić, B., Pavlović A., 2020. Lipinski's Rule of Five, Famous Extensions and Famous Exceptions. Popular Scientific Article, 3(1):171-177.

Khazaei H., Purves R.W., Hughes J., Link W., O'Sullivan D.M., Schulman A.H., Björnsdotter E., Geu-Flores F., Nadzieja M., Andersen S.U., Stougaard, J., 2019. Eliminating Vicine and Convicine, The Main Anti-Nutritional Factors Restricting Faba Bean Usage. Trends in Food Science & Technology, 91:549-556.

Kizilbay G., & Karaman M., 2022. Possible Inhibition Mechanism of Dobutamine Hydrochloride as Potent Inhibitor for Human Glucose-6-Phosphate Dehydrogenase Enzyme. Journal of Biomolecular Structure and Dynamics, 40(1):204-212.

Koriem K.M., Arbid M.S., El-Gendy N.F., 2016. The Protective Role of Anise Oil in Oxidative Stress and Genotoxicity Produced in Favism. Journal of Dietary Supplements, 13(5):505-521.

Labba I.C.M., Frøkiær H., Sandberg, A.S., 2021. Nutritional and Antinutritional Composition of Fava Bean (Vicia faba L., var. minor) Cultivars. Food Research International,140:11-38.

Lipinski CA., Lombardo F., Dominy B.W., Feeney, P.J. 1997. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings, Adv Drug Deliver Rev, 23:3-25.

Luzzatto L., Arese P., 2018. Favism and Glucose-6-Phosphate Dehydrogenase Deficiency. New England Journal of Medicine, 378(1): 60-71.

Mason P.J., Bautista J.M., Gilsanz, F., 2007. G6PD Deficiency The Genotype-Phenotype Association. Blood reviews, 21(5):267-283.

Monteiro W.M., Franca G.P., Melo G.C., Queiroz A.L., Brito M., Peixoto H.M., National Organization for Rare Disorders. 2019. https://rarediseases. org/rare-diseases/glucose-6-phosphate-dehydrogenase-deficiency.

Pico J., Vidal N.P., Widjaja L., Falardeau L., Albino, L., Martinez M.M., 2021. Development and Assessment of GC/MS and HPAEC/PAD Methodologies for The Quantification of α-Galacto-Oligosaccharides (GOS) in Dry beans (Phaseolus vulgaris). Food Chemistry, 349:129-151.

Sharan, S., Zanghelini G., Zotzel J., Bonerz D., Aschoff J., Saint-Eve A., Maillard M.N., 2021. Fava bean (Vicia faba L.) for Food Applications: From Seed to Ingredient Processing and its Effect on Functional Properties, Antinutritional Factors, Flavor, and Color. Comprehensive Reviews in Food Science and Food Safety, 20(1):401-428.

Sirdah M., Reading N.S., Vankayalapati H., Prchal, J.T., 2021. A Computational Study of Structural Differences of Binding of NADP+ and G6P Substrates to G6PD Mediterraneanc. 563T, G6PD A− c. 202A/c. 376G, G6PD Cairoc. 404C and G6PD Gazac. 536A mutations. Blood Cells, Molecules, and Diseases, 89: 102-572.

Smart OS., Horský V, Gore S., Svobodová Vařeková R., Bendová V., Kleywegt GJ., Velankar S., 2018. Worldwide Protein Data Bank Validation Information: Usage and Trends. Acta Crystallographica Section D: Structural Biology. 174(3):237-44.

Stepniewska-Dziubinska M.M., Zielenkiewicz P., Siedlecki P., 2018. Development and Evaluation of A Deep Learning Model for Protein–Ligand Binding Affinity Prediction. Bioinformatics, 34(21):3666-3674.

Tang B.L., 2019. Neuroprotection by Glucose-6-Phosphate Dehydrogenase and The Pentose Phosphate Pathway. Journal of Cellular Biochemistry, 120(9):14285-14295.

Tian S., Wang J., Li Y., Li D., Xu L., Hou T., 2015. The Application of in Silico Drug-Likeness Predictions in Pharmaceutical Research. Advanced drug delivery reviews, 86:2-10.

Vertzoni M., Augustijns P., Grimm M.,. 2019 Impact of Regional Differences along The Gastrointestinal Tract of Healthy Adults on Oral Drug Absorption: An UNGAP review. Eur J Pharm Sci, 134:153-175.

Vick D.J., 2020. Glucose-6-Phosphate Dehydrogenase Deficiency and COVID-19 Infection. In Mayo Clinic Proceedings, 95(8):1803-1804.

Williams M.A., Ladbury J.E., 2003. Hydrogen Bonds in Protein-Ligand Complexes. Protein-Ligand Interactions: from Molecular Recognition to Drug Design, 20:137-161.