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Molecular Docking and Pharmacokinetic Parameters of Moringa Chemical Compounds with Folate Receptor

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Abstract: Folic acid is a micronutrient that is needed by pregnant women in the development of the nervous system. Consumption of Moringa leaves can increase hemoglobin levels, >11 g%. Potential nutrients contained in Moringa leaves can meet the nutritional needs of pregnant women. However, the chemical compound interact with target of the molecular action of folic acid is not known. This study aims to identify the chemical compound of Moringa leaves that can interact with folate receptors in silico and predict pharmacokinetic parameters based on the SwissADME webserver. The selected molecular target is the alpha-folate receptor (PDB: 4LRH) with a molecular docking technique using Autodock 4.2 which has been previously validated to the native ligand. The results of molecular docking showed that the potential compounds of Moringa leaves were glucosinalbin, niazidin, niazinin, niazirin and rhamnetin which had an energy value of less than -8 kcal/mol. However, the potential of these compounds is not more than the energy value of folic acid as a native ligand on a folic acid receptor macromolecule. Prediction results of pharmacokinetic parameters showed that all potential compounds of Moringa leaf showed that niazinin, niazirin, and rhamnetin were highly absorbed in the gastrointestinal tract, except for niazidin and glucosine. Rhamnetin is a potential compound that can be catalyzed by CYP3A4, CYP1A2 and CYP2D6 enzymes.

Keywords: Folate, *Moringa oleifera*, Molecular Docking, Pharmacokinetic Parameters.

Introduction

Heterocyclic compounds embrace a special place among pharmaceutically significant natural products and synthetic compounds. The remarkable ability of heterocyclic nuclei to serve both as biomimetic and reactive pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs (Bara, 2006). The optimal gestation period depends on the provision of proper care, adequate antenatal preparation, and the fulfillment of nutritional needs in pregnant women. The consequences of malnutrition during pregnancy can affect the health of the mother, child, and also future generations. It is very important when entering pregnancy that the mother has a normal nutritional status and gets adequate nutrition during pregnancy for maternal health and fetal well-being. The age of the mother during pregnancy can affect the need for nutrients. The age of pregnant mothers who are relatively young or less than 20 years old requires more nutrients compared to the age of pregnant women over 20 years. Inadequate nutrients in pregnant women with the age of less than 20 years cause competition for nutrient fulfillment between mothers and babies (Chaparro *et al.*, 2014; Bara, 2006).

According to the Centers for Disease Control and Prevention [CDC] in 2015, about 2 billion people in the world are deficient in micronutrients. Based on the Food and Nutrition Technical Assistance III Project [FANTA], countries in Asia are included in the list of countries that experience a fairly high incidence of malnutrition [chronic energy deficiency and micronutrient deficiency], which is around 10-40%. Pregnant women are

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prone to micronutrient deficiencies because during pregnancy, there is rapid fetal growth, organ differentiation, and rapid cell division. Research conducted in South Asia shows that pregnant women experience folic acid deficiency of around 12-26% and zinc deficiency of about 15-74% (Chaparro *et al.*, 2014; Gernand *et al.*, 2016).

Micronutrients needed during pregnancy include folic acid and zinc which function for the development of the nervous system. The adequacy of nutrition in pregnant women has a great impact on fetal growth, development, baby birth weight, and maternal health. The impact of folic acid deficiency during pregnancy can cause anemia, abortion, low birth weight babies, anemia, and perinatal death (Lassi *et al.*, 2016). The fulfillment of folic acid needs is obtained through food consumed or through supplements. The fulfillment of folic acid nutrients is obtained from moringa leaves [Moringa oliefera]. Moringa leaves contain very high amounts of vitamin A, vitamin C, B vitamins, calcium, potassium, iron, and protein that are easily digested and assimilated by the human body. Moringa leaves contain important nutrients such as iron 28.2 mg, calcium 20.03 mg, and vitamin A 16.3 mg. Various types of antioxidant compounds are contained in moringa leaves such as ascorbic acid, flavonoids, phenolic acids, and carotenoids (Gernand *et al.*, 2016).

Bora's research stated that pregnant women who consume moringa leaves can increase hemoglobin levels, which is >11 gr%. The potential of nutrients contained in moringa leaves is able to meet the nutritional needs of pregnant women (Bora and David, 2017). However, the identification of these active compounds against macromolecules or targets of molecular action of folic acid is not yet clearly known. Molecular action target identification is intended to optimize targeted pharmacodynamic activity based on the pattern of drug interaction with the target. The challenge faced in identifying the molecular action target of an active compound is a long and costly testing process. These challenges can be overcome through computational experiments with an in-silico approach in molecular docking techniques. The rapid development and advancement of computational techniques today allow in silico tests to accelerate the process of selecting compounds to be synthesized. Molecular docking is one of the CADD [Computer Aided Drug Design] methods that can be used to provide an overview of the interaction of a compound with the target protein by predicting its conformation and binding energy (Campbell, 2012).

Using molecular docking techniques, target proteins from several chemical contents of moringa leaves that have biological activity against folate receptors can be predicted and identified based on scores and models of ligand and protein interactions with computational techniques using the Autodock 4.0 program in Autodock Tools. Furthermore, in silico testing was carried out to predict the pharmacokinetic parameters of moringa chemical compounds against the target macromolecule of folate receptor therapy using the SwissADME web form. Based on this description, this study aims to identify the chemical content of moringa leaves that can interact with folate receptors in silico by molecular docking techniques and prediction of pharmacokinetic parameters based on the SwissADME webserver.

Materials and Methods

Macromolecular and Ligan Preparation

Folate receptor macromolecule [PDB ID: 4LRH] that was successfully identified through initial screening of SwissPred and SuperPred Prediciton was downloaded from the structure of the PDB [https://www.rcsb.org] in .pdb format. The three-dimensional structure of the test ligand that has been created with VegaZZ in .mol format is then optimized with Autodock Tools. Preparation is carried out through Autodock Tools with the separation stage of the native ligand and water molecules, as well as the addition of hydrogen and Gasteiger charges. Grid box arrangements are also performed to place test ligands similar to the native ligands that bind to the active side of the folate receptor.

Docking Method Validation

Method validation was carried out on docking of the native ligand to look for the native ligand conformation. Macromolecules that have been

prepared previously are docked with the native ligand. The conformation of the docking results obtained was then aligned with the native ligand conformation in the crystallography structure expressed in the RMSD value. The RMSD value states that the alignment of the structure conformation is still acceptable with a value of less than 2.5 Å, if it is smaller or closer to the value of 0, the alignment value is better.

Molecular Docking

The molecular docking process is carried out using the AutoDock 4.0 program with AutoDock Tools [ADT]. The docking parameters were set with a rigid macromolecule format as well as the GA Runs [200] and Population Size [150] numbers. Then select the Output submenu for Lamarckian GA [4.2]. The results of docking all test ligands produced $\Delta G_{binding}$ [kcal/mol].

Prediction of Pharmacokinetic Parameters

Pharmacokinetic profile prediction was carried out online using the SwissADME [http://www.swissadme.ch.] The initial step is to enter the SMILES code from the chemical content of Moringa oleifera as the test ligands obtained from PubChem, then click Run. Furthermore, the results of the prediction of the compounds are displayed which consist of several pharmacokinetics profile parameters. The parameters consist of radar bioavailability, physicochemical properties, lipophilicity, solubility in water, pharmacokinetic profile, and drug-likeness.

Results and Discussion

Validation of Molecular Docking Methods

Validation of the molecular docking method is a preliminary test and it is important to do it before the molecular docking process on the test ligand. At this stage, the process of re-tethering or re-docking the native ligand to the target protein is carried out. Determining the center of the grid box is the first step in the validation stage. Gridbox is an analogy to the space for the native ligand or active compound to form a conformation when tethered to the target protein. The determination of the grid box is carried out to find out the coordinate point at the binding site or the active side of a protein. The grid box settings carried out are grid center coordinates and grid size settings (Drie, 2005).

The redocking process is carried out by the rigid method, which is to arrange macromolecules to be rigid so that there is no change in the shape of the binding site during the redocking process while the ligand to be docked is flexible. The validation parameter in molecular docking is in the form of the Root Mean Square Deviation (RMSD) value. RMSD shows a comparison of the native ligand conformation from re-docking with the native ligand conformation from crystallographic measurements. The acceptable RMSD value limit is ≤ 3Å (Rachmania, 2016; Tjahjono, 2013).

The folate macromolecule (folate alpha receptor) has an RMSD value of 2.80 Å consisting of 8 protein chains. The validation of the docked ligand showed good results with an RMSD value of <2Å for each chain. This shows that the conformation of the native ligand resulting from re-binding to the target macromolecule is similar to the conformation resulting from crystallography. Table 1 shows the results of the validation of the re-tethering of the native ligand with the binding energy value or Gibbs energy that qualifies. Thus, the macromolecule selected as the target protein can be used in molecular docking.

Table 1. Molecular docking result of potential compounds on *Moringa oleifera*.

Chemical Compounds	$\Delta G_{binding}$ (kcal/mol)							
	Chain A	Chain B	Chain C	Chain D	Chain E	Chain F	Chain G	Chain H
Native Ligand	**-12,11**	**-12,36**	**-14,00**	**-13,53**	**-14,22**	**-14,08**	**-14,40**	**-13,67**
Glucosinalbin	-9,09	-8,85	-9,14	-8,89	-8,61	-8,58	-8,63	-9,49
Niazidin	-9,21	-9,31	-9,14	-8,93	-8,92	-9,10	-9,32	-9,04
Niazinin	-9,10	-9,17	-8,91	-8,70	-8,61	-9,10	-8,71	-9,09
Niazirin	-8,61	-8,42	-8,31	-8,25	-8,25	-8,12	-8,14	-8,32
Rhamnetin	-8,37	-8,81	-8,75	-8,81	-8,81	-8,25	-8,08	-8,78

Analysis of Molecular Docking

The selection of macromolecules as target proteins is based on the potential interaction of various chemical contents of moringa described in Table 1 to the target protein. Molecular docking is performed using the Autodock 4.2 program in AutoDock Tools with similarly customized grid box settings when validating methods. Molecular docking provides results in the form of the lowest bonding energy value or free energy ($\Delta G_{binding}$) and ligament conformation pose. $\Delta G_{binding}$ is useful in the calculation of the value of the reaction velocity constant, which is a parameter of the strength of the ligand's binding affinity to the receptor. The lower the value, the more stable the bonds between the receptor and the ligand will be because the attraction between atoms is greater and the repulsion-repulsion force becomes minimal so that the conformation becomes more stable (Ideaconsult, 2011).

Based on the results of molecular docking of 8 macromolecule chains, it was found that the test ligand or chemical content of moringa could potentially interact with folate receptors. There are five best test ligands as well as potential compounds with the lowest binding energy values that are smaller than the native ligands and less than -8 kcal/mol. However, the bond energy value of the chemical content of moringa is not lower than the native ligand, namely the folic acid complex.

Table 2. Physicochemical properties of potential compounds on *Moringa oleifera*.

Chemical Compounds	Physicochemical properti						
	Formula	MW	HBA	HBD	TPSA	Log P	Solubility
Glucosinalbin	C14H19NO10S2	425,43	11	6	220,02	0,49	Soluble
Niazidin	C15H18N2O6S	354,38	7	4	156,29	1,66	Soluble
Niazinin	C15H21NO6S	343,40	6	4	132,50	2,03	Soluble
Niazirin	C14H17NO5	279,29	6	3	102,94	1,68	Soluble
Rhamnetin	C16H12O7	316,26	7	4	120,36	2,23	Soluble

Pharmacokinetics Prediction

Prediction of physicochemical properties and pharmacokinetic profiles of a chemical compound is intended to be more effective in modifying the structure of drug compounds before synthesis after obtaining the best results from molecular docking. Prediction of pharmacokinetic profiles can be done in silico with an online database approach to the best compounds that are suspected of having pharmacological activity with certain target proteins. Thus, pharmacokinetic profile information of several compounds is needed that can interact with folic acid therapy target proteins. The following is a description of the pharmacokinetic profile of the best compounds carried out online through the SwissADME webform.

Table 3. Pharmacokinetic parameters of potential compounds on *Moringa oleifera*.

Chemical Compounds	Pharmacokinetic Parameters							
	Abs. GI	BBB Perm.	Subs. Pgp	Inh. 1A2	Inh. 2C19	Inh. 2C9	Inh. 2D6	Inh. 3A4
Glucosinalbin Niazidin	Low	No	No	No	No	No	No	No
Niazinin	Low	No	No	No	No	No	No	No
Niazirin	High	No	Yes	No	No	No	No	No
Rhamnetin	High	No	No	No	No	No	No	No
	High	No	No	Yes	No	No	Yes	Yes

Determination of the physicochemical properties of a test ligand when crossing the cell membrane can be carried out by identification of Lipinski's rule of five. The conditions that must be met by potential compounds based on Lipinski's rule start from molecular weight (BM) <500 Da, log value P <5, number of donor hydrogen bonds (HBD) <5, and number of hydrogen acceptor bonds <10, as well as molar reactivity in the range of 40 – 130. Ligands with BM <500 Da can easily penetrate cell membranes. The P log value indicates the polarity of the ligand in the fat solvent or non-polar if the P >5 log value because it can interact more slowly through the lipid bilayer and is widely distributed in the tissue. A low log P value indicates that the ligand tends to be soluble in water. The number of hydrogen bonds of donors and acceptors is related to the chemical activity of drug molecules in the body. If the ligand meets the criteria of Lipinski's rule without any deviation in value, the ligand is drug-likeness or a potential compound as a drug candidate. Drug-likeness refers to the similarity of a compound to an oral drug based on the evaluation in Lipinski's rule (Jain and Nicholls, 2008; Lipinski, 2011).

The pharmacokinetic phase consists of the process of absorption by the gastrointestinal tract, distribution in the blood towards the therapeutic target, and metabolism in the liver into the form of active metabolites until it is excreted out of the body through certain organs. Pharmacokinetic profile predictions showed that the compounds niazinin, niazirin, and rhamnetin were the test ligands that were highly absorbed in the gastrointestinal tract, while the other three compounds showed high absorption rates.

Discussion

The preparation of macromolecule is the first step in carrying out the process of molecular docking. Macromolecular structure used can be downloaded from the webform: Protein data bank (http://www.rscb.org/). The selected macromolecule was a folate receptor (PDB ID: 4LRH) that was successfully identified through initial screening of SwissPred and SuperPred Prediciton. Determination method of macromolecular structure as well as crystallography method used is X-ray diffraction because it can be applied to large and more precise macromolecular structures. The conformation resolution value must also be considered with the RMSD criterion of <2.5Å. The organism used is human (*Homo sapiens*) (Hypercube, 2002).

The results of the macromolecule download in .pdb format still contain the native ligand complex and the crystallized water molecule. The native ligands and water molecules must be removed from the macromolecules through optimization so as not to interfere with the molecular docking process. The native ligand is removed to obtain individual macromolecules to be docked with the test ligand. Water molecules are eliminated because they can mediate the interaction between ligands and receptors and the molecular docking results obtained are not good due to the complexity of mathematical calculations in docking, causing the required docking time to be longer. Preparation or optimization is carried out through the AutoDock Tools program. In addition to the removal of native ligand molecules and water molecules, preparation is also carried out by removing non-amino acid residue molecules and adding charges for chemical environment

adjustment. Residues other than amino acids are removed because they can interfere with the interaction between the ligand and amino acid residues on the active side of the macromolecule. The removal of residues other than amino acids needs to be reviewed by reviewing selected macromolecule journals attached by protein data bank (Ideaconsult, 2011).

The three-dimensional structure of the test ligand was obtained through Canonical SMILES line notation conversion using the VegaZZ program. The conversion of SMILES Canonical line notation to three-dimensional representation is done with an energy minimization approach. The preparation of the test ligand is carried out by removing water molecules so as not to interfere with the docking process by simplifying mathematical calculations in molecular docking. Furthermore, it is necessary to add hydrogen atoms with the aim of adjusting the docking process to be close to the pH atmosphere in the body and can re-emerge hydrogen atoms in the molecule so that hydrogen bonds can be observed in the interaction of the ligand with the target receptor (Tjahjono, 2013).

The structure of the test ligand is then studied in obtaining a more convergent structure or able to be centered on the binding pocket receptor. This is based on the amount of active torque that each test ligand has. The large amount of active torque that is possessed makes the search time for the best conformation and molecular docking results longer and more difficult to obtain. The determination of the amount of active torque is intended to determine the active bonds that can rotate during the docking process (Drie, 2015).

Based on the physicochemical properties of the test ligands in Table 2, it shows that all potential compounds because do not have a large enough molecular weight (>500), so all the best test ligands are predicted to penetrate the cell membrane. The blood-brain barrier is part of the brain barrier with characteristics in the form of an essential diffusion layer as a physiological regulator of passive transfer or diffusion of a drug molecule. Passive diffusion of BBB through paracellular hydrophilic diffusion or paracellular lipophilic. Lipophilic drug molecules or less than 400-600 Daltons can pass through the endothelium freely, as well as molecules with less than 10 hydrogen bonds can enter the brain via the transcellular route (Fiori *et al.*, 2020). The test ligand must be lipophilic to be able to penetrate the BBB. The P-glycoprotein (Pgp) substrate is an efflux transporter system that can restrict drug molecules to brain tissue. Table 3 shows that niazinin as a test ligand is a Pgp substrate so that the penetration of its active molecule becomes inhibited by the blood-brain barrier. Cytochrome P450 enzyme (CYP) is an oxidase enzyme involved in the metabolism of various endogenous or exogenous compounds (drugs) in the liver. All best test ligands are not inhibitors of CYP1A2, CYP2C19, CYP2C9, CYP2D6 enzymes, and CYP3A4. Rhamnetin test ligan can catalyze or inhibit CYP1A2, CYP2D6 and CYP3A4. The CYP2D6 enzyme can catalyze alkaline compounds with 4-7 A protonated atoms such as some types of flavonoids and alkaloids. The CYP3A4 enzyme is a type of P450 that can catalyze most active molecules that are lipophilic (Zanger and Schwab, 2013). The prediction of pharmacokinetic parameters in Table 3 shows that only rhamnetin as an inhibitor of CYP3A4, CYP1A2, and CYP2D6 can be catalyzed by these enzymes.

Conclusions

Based on the results of the study, it can be concluded that glucosinalbin, niazinin, niazidine, niazirin, and rhamnetin are predicted to have binding affinity and interact with alpha folate receptor target proteins based on bioinformatics analysis and molecular docking. Pharmacokinetic predictions of potential compounds of moringa leaf show that niazine, niazirin, and rhamnetin are highly absorbed in the gastrointestinal tract, as well as rhamnetin can be catalyzed by the enzymes CYP3A4, CYP1A2, and CYP2D6.

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