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RESEARCH ARTICLE

In-silico Screening of Compounds Contained in Wera (*Malvaviscus Arboreus* Cav.) Leaves as Anti-alopecia with Androgen Receptors

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Abstract

Objective: Baldness (Alopecia) is a serious problem at this time because it is caused by stress, genetic factors, pregnancy, inadequate hair care, and less balanced nutrition. Previous studies have shown that ethanol extracts of Wera leaves (*Malvaviscus arboreus*) have anti-baldness properties. Research study was undertaken to investigate the potential of chemical compounds contained in the leaves of Wera as hair growers using androgen receptors with comparative drugs Minoxidil. Methods: This type of research is in silico where the results obtained are free energy values and hydrogen bonds that connect ligands with receptors. Results: From the 7 compounds contained in the Wera leaf, the results showed that trifolin compounds gave the lowest energy -7.08 kcal/mol and amino acids that were bound were Thr (877), Asn (705), and Phe (764) where more than the comparative drug Minoxidil namely Leu (873) and Asn (705) with free energy is -5.81 kcal/mol. In predicting pharmacokinetic properties and toxicity tests, compounds that are predicted as candidates for hair growth drugs do not meet pharmacokinetic parameters and are non-mutagenic and not carcinogenic so they cannot be administered orally. Conclusion: It is found that molecules with compounds 2- [2- (5-methyl-2-propane-2-ylphenoxy) ethoxy] naphthalene-1-carboxylate provides the lowest energy, which is -9.47 kcal/mol so that it has the potential to be a candidate for a new drug in the treatment of hair growth, namely alopecia either oral or topical used

Keywords: *Wera leaves, In-Silico, Hair growers, Androgen receptors, Malvaviscus arboreus.*

Introduction

On the scalp, there are around 100,000 strands of hair consisting of stems made from dead horn tissue and follicles where the hair grows. In the hair growth cycle there are three phases and occur in each hair at different times [1]. In early adulthood, losing 1% of the hair on the head is still normal. For most people, serious hair loss is not a problem until it reaches the age of 50 years [2].

The description of thinning hair or the appearance of bald spots is on the top of the head, in front of the head, and in the center, but generally the most obvious is the rising line hair (the forehead widens) [3]. On average, people lose 50 to 100 strands of hair every day because they fall out, but almost all grow again replaced with new hair. Hair is a crown of beauty not only in women but also in men so that everyone strives to prevent hair loss. The factors that can cause hair loss to baldness (alopecia) include stress, genetic factors, pregnancy, inadequate hair care, and less balanced nutrition. The difficulty of avoiding stress and an unbalanced diet cause's hair loss is difficult to avoid. Therefore additional nutrients are needed which are routinely given directly to his hair. One type of Indonesian plant that is traditionally used as herbal medicines in overcoming certain problems is the leaves of Wera (*Malvaviscus arboreus*) [4].

M. arboreus is reported containing astragalin, trifolin, 4-hydroxyphenyl acetic acid, beta resorcylic acid, caffeic acid [4], protocatechuic acid [5], 2-[2-(5-methyl-2-propane-2ylphenoxy)ethoxy] naphthalene-1-carboxylate [6]. This article reports in-silico

screening of these compounds as to whether they suitable for topical use for anti-alopecia treatment.

Materials and Methods

Hardware

A computer with specifications for Intel Pentium i7 CPU@3.80 GHz, RAM (Random Access Memory) 16.00 GB.

Software

Windows 10 Enterprise Pro Operating System 64-bit, x64 based processor, equipped with MGL-Tools program consisting of ADT application (Auto dock Tools), MarvinSketch 17.11.0 (Academic License), Ligand scout 4.1.4 (Universitas Padjadjaran License), Discovery Studio2016 Client® and Pre-ADMET

Test Compounds

The test compounds used were 8 compounds from wera leaves (*M. arboreus*), testosterone as natural androgen receptor ligands and minoxidil as comparative drugs.

Receptors

3D crystal receptor structure data used for molecular docking analysis was obtained from the Protein Data Bank (GDP) with the site http://www.rcsb.org/pdb/ [7,8]. Receptors were used to predict activity as hair growers, namely androgen receptors with 4K7A GDP codes.

Method

Protein Preparation

In this receptor preparation 4K7A receptors (see Fig.1) were used as hair growth receptors (alopecia) which were downloaded on the Protein Data Bank site (http://www.rscb.org/pdb). Then the receptors were visualized using the Discovery Studio 2016 Client® program. In this program, the download receptors were prepared by removing water molecules and their natural ligands. The result was a pure receptor which was then stored in the Protein Data Bank (.pdb) format.

Ligand Preparation

In the preparation of test compounds from Wera leaves and testosterone as natural androgen receptor ligands and minoxidil as comparative drugs made manually using the Marvin Sketch® program. After the construction of the structure was complete then the structure of the compound was conditioned at the body pH which was pH 7.4, then saved with the format (.pdb).

Method Validation

In the validation of the test method (ligand) used was the innate compound of receptor/protein (ligand preference) which was docking back to the receptor. The value seen was Root Mean Square Deviation (RMSD).Where the RMSD value was <2.00 Å which was commonly used as a standard molecular belay value.

Docking Test Compounds with Receptors

Docking was done using autodock4 software. (run-auto dock) by tethering between ligands and receptors, then editing cmd by deleting the directory so that in the cmd column it contains (D: / Auto dock / autodock4 -p dock.dpf -l dock.dlg &) then click launch.

Molecular Tethering Analysis and Visualization

The docking calculation results were seen in the output in notepad format. Determination of the confirmation of the test compound docking results was done by selecting the ligand configuration which had the lowest bond energy (the best pose). The position and orientation of the ligand were on macromolecules, as well as amino acids that were bound to the ligand visualized by auto dock tools.

Pre-ADMET

The ADMET parameter was calculated using the preADMET® program that was accessed through the site (https:// preadmet. bmdrc. kr/adme/). The chemical structure of the compound was drawn or uploaded in the format Mol file (.mol). The program automatically calculated predictive values from selected parameters, namely: Human colon adenocarcinoma cell permeability (Caco-2), Human Intestinal Absorption (HIA), Plasma Protein Binding, mutagenic and carcinogenic.

Results and Discussion

Computational (in silico) methods have been developed and applied to pharmacology hypothesis development and testing [9].

In this study, we used androgen receptor 4K7A Protein (see Fig. 1) whereas some researcher used JAK2 in the in-silico study of anti alopecia [10, 12].

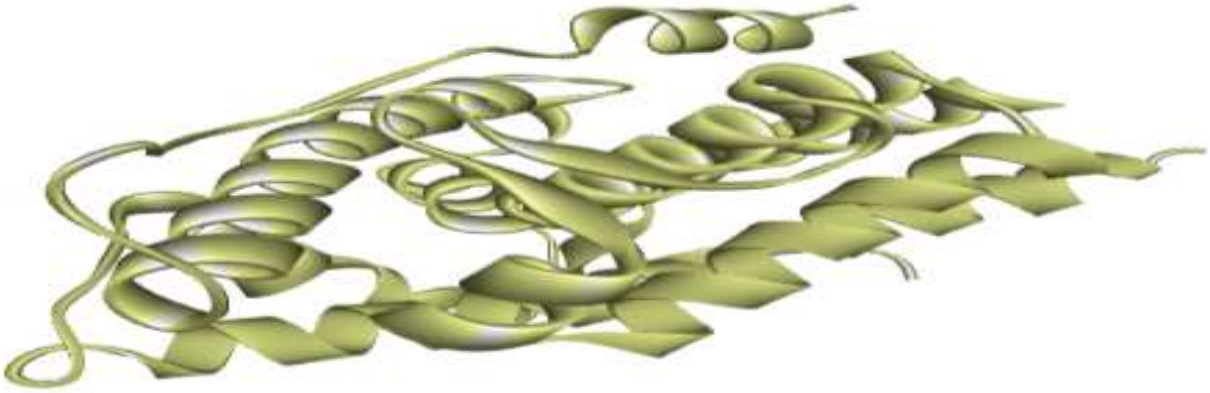


Fig. 1: 4K7A Protein (Androgen receptor)

Protein Preparation

The use of the Auto dock Tools 4.2 program was done to determine them grid box in the area known as the active side of the protein. This grid box determination included setting the location of the box parameters and determining the size of the grid box using spacing (angstrom). In 4K7A protein, the grid box results were obtained which includes: center x = -27,563, center y = 2.72 and center z = -4,945 with spacing (angstrom) of 0.375 Å. (Fig. 2).

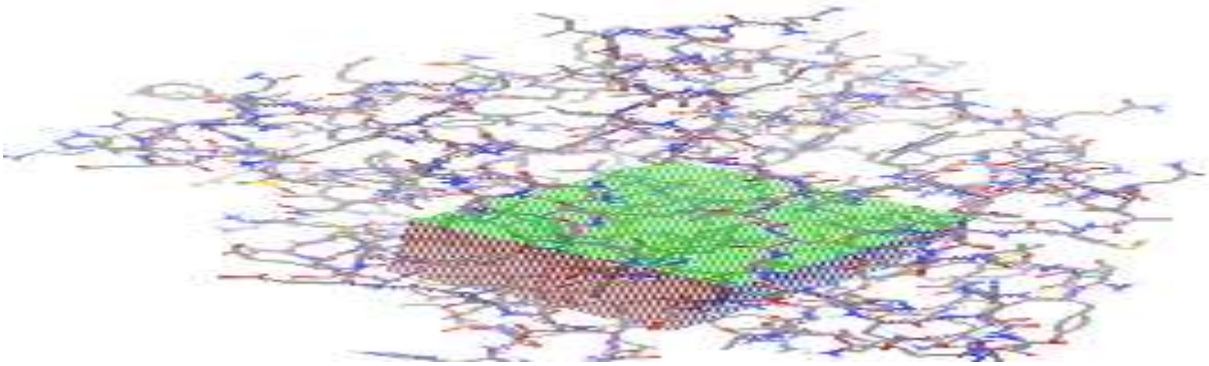
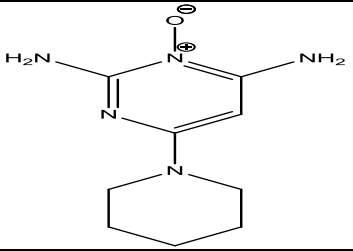
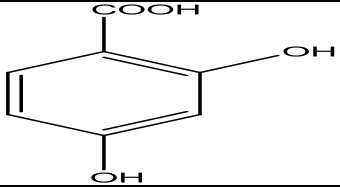
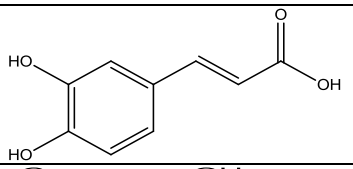
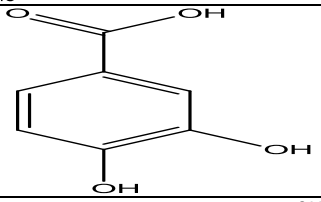
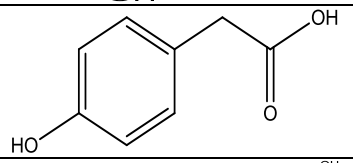
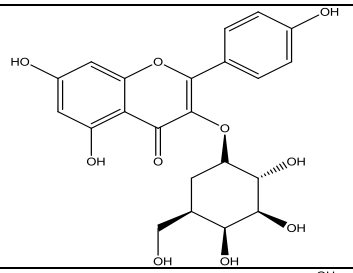
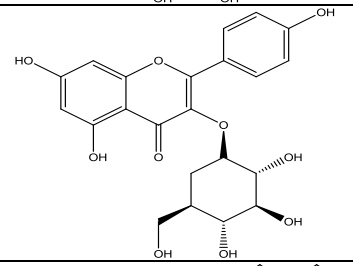
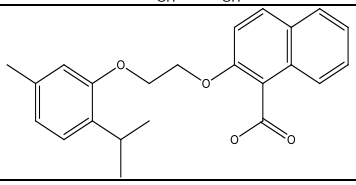


Fig. 2: Protein preparation with Auto dock 4.2

Ligand preparation: In the preparation of ligands, the compounds contained in the leaves of Wera had 6 test compounds, namely 4 phenolic acid groups (cataflytic acid, caffeic acid, protocatechuic acid, 4-hydroxyphenyl acetic acid) and 2 flavonoids (trifolin and astragalin) and used Minoxidil comparison. Then it is optimized by conditioning the structure of the compound at body pH which is pH 7.4. (See Table 1).

Table 1: Compounds content in *M.Arboreus* leaf

No	Compounds	Molecular Formula	Structure
1	Minoxidil	$C_9H_{15}N_4$	
2.	Betha Resorcylic Acid	$C_6H_6O_4$	

3.	Caffeic Acid	$C_9H_8O_4$	
4.	Protocatechuic Acid	$C_7H_6O_4$	
5.	4-hydroxyphenyl Acetic Acid	$C_8H_8O_3$	
6.	Trifoline	$C_{22}H_{22}O_{10}$	
7.	Astragalin	$C_{22}H_{22}O_{10}$	
8.	2-[2-(5-methyl-2-propan-2-ylphenoxy)ethoxy]naphthalene-1-carboxylate	$C_{23}H_{23}O_4$	

Method Validation

Analysis used to evaluate the results of validation, namely the RMSD value, binding site and parameters used is considered valid if the RMSD results obtained are ≤ 2 Å. Based on the results of the validation obtained RMSD value of 1.41 Å, the RMSD value has met the validation requirements which is less than 2.00 Å [13] so that the parameters can be used for the simulation of the test compound docking (Fig.3).

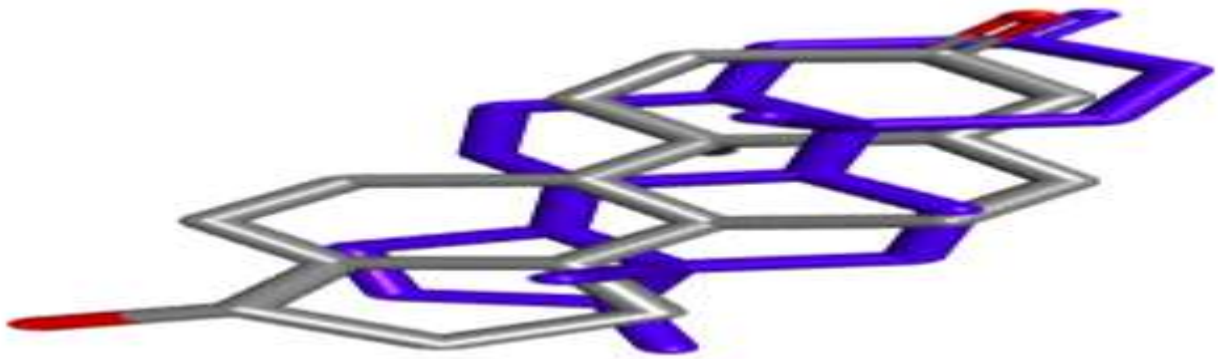


Fig. 3: Overlapping visualization of 4K7A (gray) natural ligands with re-docking ligands (blue)

Inhibition of ligand compounds to receptors: In this study, the six compounds contained in the leaves of Wera were applied to the androgen receptor. This tethering used Auto dock tools. This belaying process was carried out using a grid box dimension of 40x40x40.

Table 2 shows that the value of the free energy of the sixth bond of the test compounds showed the lowest bond free energy (trifolin) with a value of -7.08 kcal/ mol but not lower with the androgen receptor natural ligand 5-alpha-dihydrotestosterone -11.26 kcal/mol. This result was higher than the results of the minoxidil value of -5.81 kcal/ mol.

Table 2: Results of Molecular Tethering Analysis

No	Compounds	Interaction with Amino Acids		Ki (nM)	(ΔG) (kcal/mol)
		Hydrogen bond	Van Der Waals (Hydrophobic)		
1.	5-alpha-dihydrotestosterone	LEU(873), ARG(752), GLN(711)	GLY(708), TRP(741), MET(895), THR(877), PHE(876), LEU(701), VAL(746), MET(749)	5.60	-11.26
2	Minoksidil	LEU(873), ASN(705)	PHE(876), THR(877), LEU(880), PHE(891), LEU(701), TRP(741), MET(895), GLY(708), MET(742), MET(780)	54.92	-5.81
3	Betha Resorcylic Acid	MET(745), GLN(711), ARG(752)	ALA(748), LEU(707), LEU(704), MET(742), TRP(741), LEU(873), VAL(746)	218.99	-4.99
4	Caffeic Acid	PHE(764), ARG(752), GLN(704), LEU(704), ASN(705)	MET(895), TRP(741), LEU(707), MET(749), MET(745), GLY(708)	83.30	-5.57
5	Protocatechuic Acid	MET(742), GLN(711), MET(745)	TRP(741), LEU(873), PHE(764), ALA(748), ARG(752), LEU(707)	200.52	-5.04
6	4-hydroxyphenyl Acetic Acid	LEU(704), MET(745), GLN(711)	MET(749), VAL(746), TRP(741), GLY(708), ASN(705), MET(895), PHE(764), ARG(752), ALA(748)	68.95	-5.68
7	Trifolin	THR(877), ASN(705), PHE(764)	LEU(880), LEU(701), PHE(876), LEU(873), TRP(741), ILE(899), PHE(891), GLY(708), ALA(765), ARG(752), GLN(711), VAL(756), LEU(704), MET(787), MET(780)	6.49	-7.08
8	Astragalin	MET(742), GLN(711), LEU(707), ASN(705)	MET(749), ARG(752), PHE(764), GLY(708), LEU(712), TRP(741), MET(895), ILE(899), PHE(891), LEU(880), PHE(876), LEU(873), MET(787), VAL(746)	13.67	-6.64
9.	2-[2-(5-methyl-2-propan-2-ylphenoxy)ethoxy]naphthalene-1-carboxylate	THR(877)	ILE(699), MET(895), ASN(705), LEU(704), GLY(708), VAL(746), GLN(711), ARG(752), LEU(701), PHE(876), TRP(741)	114.71	-9.47

Molecular Tethering Analysis and Visualization

Analysis of the results of the docking test included the value of free energy, inhibition constants, interactions that occur between ligands and amino acids from protein receptors, types of amino acids that interact with ligands and results based on the rules of

Lipinski's Rule of Five. The results of the analysis based on the best energy values, namely compounds 2- [2- (5-methyl-2-propane-2-ylphenoxy) ethoxy] naphthalene-1-carboxylate have strong potential to be candidates for hair growth drugs because they have free energy values that smaller than the comparative drug, Minoxidil (see Fig. 4 and Fig. 5)

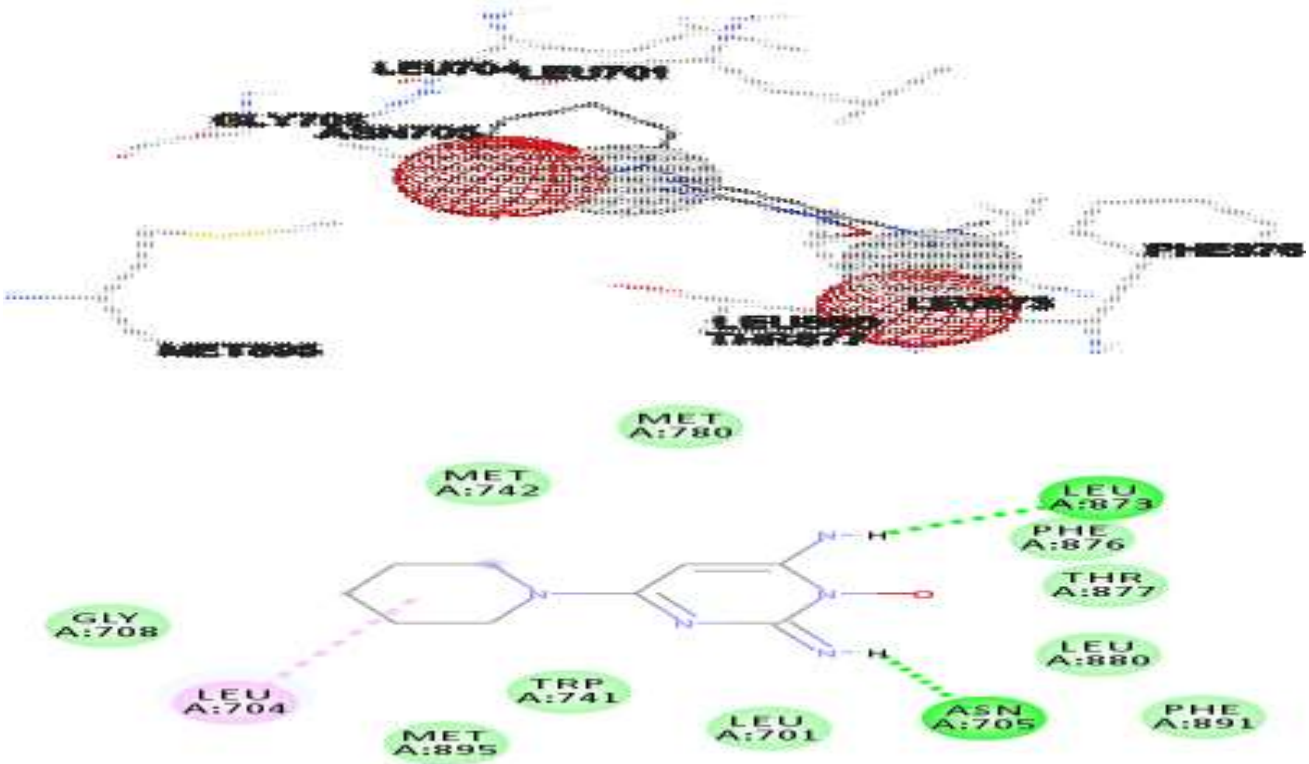
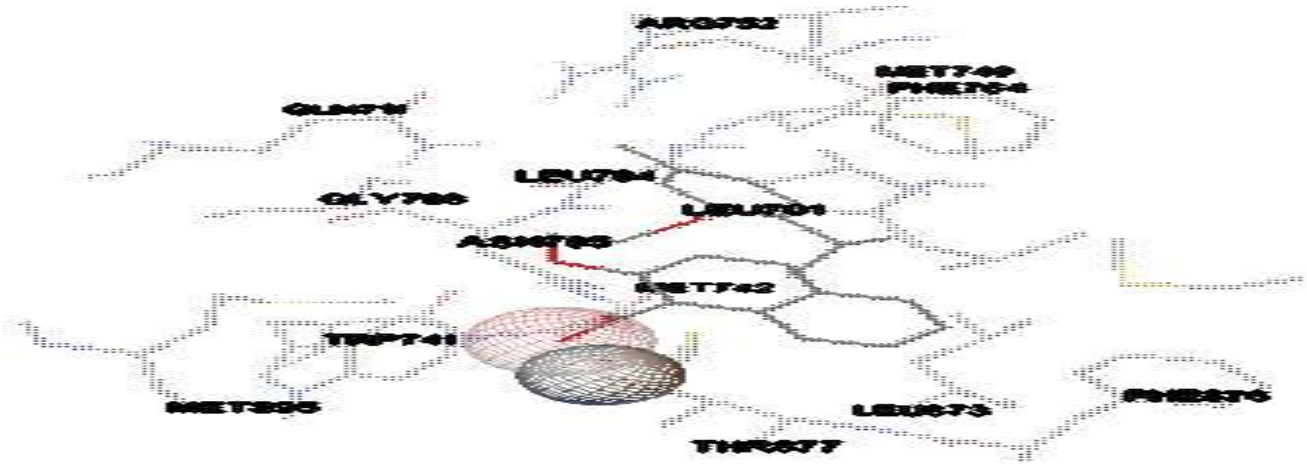
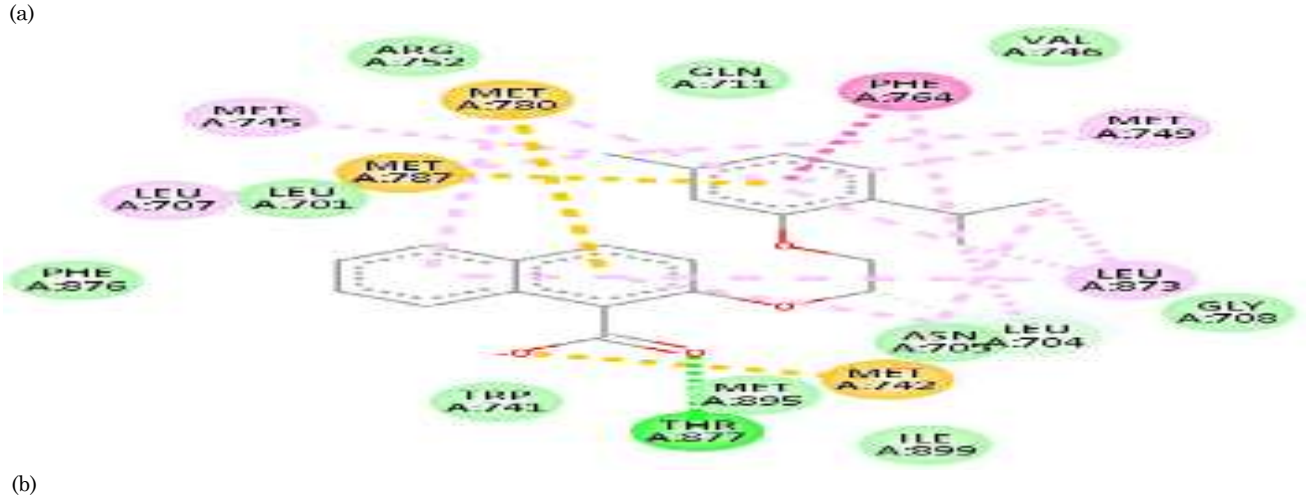


Fig. 4: Visualization of the results of docking of Minoxidil 3D (a) Minoxidil 2D (b) compounds to 4K7A proteins



(a)



(b)

Fig. 5: Visualization of the results of compound docking 2- [2- (5-methyl-2-propan-2-ylphenoxy) ethoxy] naphthalene-1-carboxylate 3D (a) and trifolin 2D (b) against 4K7A protein

The Lipinski's Rule

The Lipinski's rule of five is a rule of thumb for evaluating the physicochemical properties of a compound for oral administration. This rule describes the physicochemical properties in the phases of the pharmacokinetics of drugs in the human body, including absorption, distribution, metabolism, and excretion. Therefore, in designing a drug that will be given orally it is expected to meet Lipinski's Rule Of five, namely: [14]

- No more than 5 hydrogen donors
- No more than 10 hydrogen bond acceptors.
- The molecular mass of fewer than 500 daltons.
- LogP value is not more than 5.

Based on these rules, from the results of all compounds, there are several compounds that meet the rules of Lipinski's Rule of Five (see Table 3). When ranked from all compounds, compound number 9, namely 2-[2- (5-methyl-2-propane-2-ylphenoxy) ethoxy] naphthalene-1-carboxylate was the best compound that meets the rules of Lipinski's Rule of Five.

So that it was orally activated to become a drug candidate whereas trifolin compounds did not meet the rules of Lipinski's Rule of Five so that it was inactive or could not be given orally to be used as an oral drug. The route that could be chosen was another route which was topical.

Table 3: Compound Screening Results Based on Lipinski's Rule Of Five rules.

No.	Compounds	Molecular weight	Log P	Hydrogen Donor	Hydrogen Acceptor
1.	5-alpha-dihydrotestosterone	290.447	3.959	2	1
2.	Minoxidil	211.226	0.756	3	2
3.	Betha Resorcylic Acid	153.113	0.98	2	4
4.	Caffeic Acid	181.167	1.53	2	4
5.	Protocatechuic-Acid	153.113	1.02	2	4
6.	4-hydroxyphenyl Acetic Acid	151.141	1.31	1	3
7.	Trifolin	450.396	0.344	7	10
8.	Astragalin	446.408	0.12	8	10
9.	2-[2-(5-methyl-2-propan-2-ylphenoxy)ethoxy]naphthalene-1-carboxylate	361.462	2.34	0	4

Pre-ADMET

In designing a new drug, predictions of pharmacokinetic properties and predictions of toxicity from new compounds need to be done to obtain candidate drug compounds that have good pharmacokinetic and toxicity to the body [15]. Predicted parameters of pharmacokinetic properties include absorption (Human Intestinal Absorption (HIA) and Caco-2) and distribution (Plasma Protein Binding).

Human intestinal absorption (HIA) is an important roadblock in the formulation of new drug sub-stances [16]. Caco-2 (immortalizedhuman colon adenocarcinoma cell line) monolayers are now used because they exhibit remarkable morphological and functional similarity to the small intestinal columnar epithelium [17, 18]. The toxicity parameters are seen as mutagenic and carcinogenic. Human Intestinal Absorption (HIA) has a percentage of intestinal absorption in humans that is if more than 80%, the absorption is better and if it is less than 30% the absorption is poor. Based on the prediction of all compounds having the value of Human Intestinal Absorption (HIA) above 80% which indicates that all these compounds can be absorbed through the intestine and have a good absorption rate in the intestine (well absorption) (see Table 4). While all compounds exhibit moderate permeability in Caco-2 cells, 4 to 70. Caco-2 cells are derivatives of human adenocarcinoma colon and have various drug transport pathways through the intestinal epithelium. For toxicity tests by looking at their toxicity based on simple methods to test the mutagenicity of compounds and carcinogens as mutagen frame shift [19].

Based on the predictions of its pharmacokinetic properties, trifoline compounds had plasma protein bonds of 59.30% which meant that they were not strongly bound to plasma proteins (strong bounds chemicals) and exhibit moderate permeability to Caco-2 cells and had a poor absorption rate in the intestine.

Based on the prediction of toxicity all compounds had mutagenic properties except for trifolin and astragalin compounds and were negatively carcinogenic.

So that for all compounds there was no problem with carcinogenic properties but it had a problem with mutagenic properties because if mutagenic compounds had a direct impact on health. Based on the best results for trifolin compounds based on the pre-admet test results some did not meet the parameters so that when given orally it would have an impact body and the best route choices could be given topically. Compounds 2- [2- (5-methyl-2-propane-2-ylphenoxy)ethoxy] naphthalene-1carboxylate, on the other hand, had plasma protein bonds above 90% which meant that the compound was strongly bound to plasma proteins (chemicals strong bonds) and showed moderate permeability in Caco-2 cells and had a good absorption rate in the intestine. So the 2- [2- (5-methyl-2-propane-2 ylphenoxy)ethoxy]naphthalene-1-carboxylate compound might be used for both oral and topical.

Table 4: Pre-ADMET Prediction Results

No	Compounds	Absorbtion		Distribution	Mutagenic	Carcinogenic
		HIA (%)	CACO-2	PPB (%)		
1	Minoxidil	82.11	0.17	70.66	+	-
2	Betha Resorcylic Acid	74.15	12.45	59.33	+	-
3	Caffeic Acid	82.30	21.10	40.29	+	-
4	Protocatechuic-Acid	74.74	18.30	27.11	+	-
5	4-hydroxyphenyl Acetic Acid	89.47	20.52	0.00	+	-
6	Trifolin	31.37	6.13	59.30	Non-Mutagenic	-
7	Astragalin	25.17	11.14	57.57	Non-Mutagenic	-
8.	2-[2-(5-methyl-2-propane-2-ylphenoxy)ethoxy]naphthalene-1-carboxylate	97.39	46.78	92.37	+	-

Conclusion

Of the 7 molecular compounds contained in the leaves of *W. arboreus*, ranked according to their free energy values, it is found that molecules with compounds 2- [2- (5-methyl-2-propane-2-ylphenoxy) ethoxy] naphthalene-1-carboxylate provides the lowest energy, which is -9.47 kcal/mol so that it has the potential to be a candidate for a new drug in the treatment of hair growth, namely alopecia either oral or topical used. For long-term used, its mutagenic characteristic, an impact on health problems should be considered.

Acknowledgment

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