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RESEARCH ARTICLE

Molecular Docking of The Potential Compound from Cocoa Shells (*Theobroma cacao L.*) Against Androgen Receptor as Anti-Alopecia

Faruk Jayanto Kelutur[1], Resmi Mustarichie[1]*

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia 45363.

***Corresponding Author: Resmi Mustarichie**

Abstract

The determination of the components of the cocoa shells compounds that have anti-alopecia activity have not been reported, so the molecular docking approach is a very effective alternative before further testing is carried out. This study aimed to determine the potential compound components against androgen receptor targets as anti-alopecia drugs. Molecular docking used ChemDraw Ultra 12.0, Chem3D Pro 12.0, Biovia Discovery Studio 2016 Client®, and Autodock Tools 4.2, as well as to determine the pharmacokinetic properties and toxicity of drug ingredients with Pre-ADMET. It was found that that the components of the cocoa peel compound had the potential to act as anti-alopecia drugs, namely chlorogenic acid, epicatechin, and catechins with the value of the free energy binding (ΔG) and the inhibition constant (Ki) respectively (-7.87 kcal/mol; 1.70 µM)> (-6.48 kcal/mol; 17.65 µM)> (-6.36 kcal/mol; 21.91 µM) with the crucial amino acid residue formed was GLN 858. The pharmacokinetics (plasma protein binding) of epicatechin and catechin were excellent compared to chlorogenic acid and minoxidil because it could penetrate the plasma membrane when interacting. While the toxicity test, the components of chlorogenic acid, epicatechin, and catechin compounds were mutagenic, and only chlorogenic acid was carcinogenic. The study concluded chlorogenic acid, epicatechin, and catechin compounds from the cocoa shells were promising candidates for anti-alopecia drugs to be developed further targeting androgen receptors. It was consistent with the molecular docking results, which showed that ΔG and Ki's values were excellent compared to minoxidil. The pre-ADMET results also showed that the epicatechin and catechin compounds components could penetrate the plasma membrane when given topically compared to minoxidil.

Keywords: *Alopecia, Cocoa shells, Theobroma cacao, Molecular docking, Androgen receptor.*

Introduction

Alopecia is a medical term that means hair loss or baldness. It can occur in some or all areas of the body, usually on the scalp. The triggers for hair loss are various reasons, such as genetic predisposition, environment, exposure to chemicals, drugs, nutritional deficiencies, severe stress or diseases that continue to undermine the body, and other factors.

Alopecia is a dermatological disorder recognized for more than 2000 years as a common problem worldwide and is estimated to affect the world's population between 0.2% and 2% [1]. Based on the pattern and causes of hair loss, alopecia can be classified into several categories, namely alopecia areata (a highly unpredictable autoimmune skin disease that generally results in hair loss on the scalp and other parts of the body. It usually starts with one or more patches, small, round, and smooth on the scalp to result in total hair loss or alopecia totalis and hair loss throughout the body or Alopecia Universalis, the rarest alopecia) whereas androgenetic alopecia (occurs in men and women characterized by loss of hair on the scalp.

Women experience hair loss on a large part of the scalp while men begin with a reduced hairline then thinning on the head) [1]. In androgenetic alopecia, the androgen receptor pathway plays an essential role in affecting hair growth and is a transcription factor belonging to the steroid hormone receptor superfamily. Testosterone is the primary androgen in the circulation, which has converted into a more potent androgen, namely dihydrotestosterone (DHT) through

5-α reductase in the tissue. If androgen receptors have overexpressed, it can cause baldness [2]. Minoxidil is a scientifically proven synthetic drug to have a potent vasodilator for the treatment of alopecia. These drugs are available in 2% and 5% solutions, preventing hair loss on the vertex and front of the scalp but have shown very little effectiveness in stimulating regrowth of hair [3].

Also, it has side effects, not safe, and not effective for treating alopecia, do drugs of natural origin are needed to replace these synthetic drugs. Empirically, the Cocoa shells has been used by the people of Dinggaa Linggarjati at the foot of Mount Galunggung, West Java, Indonesia, to treat corroded and bald children's heads by boiling then extracting the water and giving it to the head to be treated.

Mustarichie and Hasanah [4] conducted in-vivo research on the cocoa shell using test animal rabbits. The results showed that the ethanol extract of the cocoa shell at a 15% concentration had activity in stimulating hair growth. Whereas in testing the activity of fractions of the cocoa shell, the n-hexane fraction was perfect compared to other fractions and positive control (2% minoxidil), and the results of phytochemical screening obtained secondary metabolites of flavonoids, saponins, polyphenols, tannins, monoterpenes, sesquiterpenes, steroids, and triterpenoids.

It has also has reported that the cocoa shell contains theobromine, theophylline, caffeine, epicatechin, catechin, paraxanthine, 3-methylxanthine, uric acid, gallic acid, and procyanidin compounds [5]. It has not been reported the determination of compound components that have potential as anti-alopecia, so molecular docking using the in-silico method is very appropriate for screening potential compounds against androgen receptors before other tests have been carried out.

Material and Method

Material

The software used the windows ten home single language 64-bit operating system (10.0, build 18362) intel (R) Core (TM) i5-10210u CPU @ 1.60 GHz (8 CPUs), ~2.1 GHz. The crystalline structure of the androgen receptor binding domain with the minoxidil complex was downloaded on https://www.rcsb.org/ in PDB format, Protein Data Bank (PDB) code 4K7A which had a Root Mean Square Deviation (RMSD) value of 2.44 Å. Meanwhile, the test ligand was the cocoa shell compound (3- methylxanthine, caffeine, catechin, chlorogenic acid, epicatechin, gallic acid, paraxanthine, theobromine, theophylline, dan uric acid) downloaded from https://pubchem.ncbi.nlm.nih.gov/ with the 2D structure in SDF format.

This research had conducted using the application ChemDraw Ultra 12.0, Chem3D Pro 12.0, Biovia Discovery Studio 2016 Client®, and Autodock Tools 4.2. To find out the pharmacokinetic properties and toxicity of the test ligand as a candidate for drug ingredients using Pre-ADMET at https://preadmet.bmdrc.kr/.

Method

The complex between androgen receptors and minoxidil (native ligand) had prepared to remove water molecules and separated between the receptors and ligands using the Biovia Discovery Studio 2016 Client®, then saved in PDB format. Meanwhile, the test ligands obtained from PubChem were converted to PDB format first using Chem3D Pro 12.0. The test ligands had prepared using Autodock Tools 4.2 by adding a Gasteiger charge, hydrogen, and merge non-polar, then stored in the pdbqt format.

Before using androgen receptors for the molecular docking process, the method had validated first. The main requirement was that the RMSD value ≥ 2 Å from the re-docking results compared with the crystallographic results. The docking process used Autodock Tools 4.2, and the grid box parameters obtained had used for the test ligand. Besides, it should also note that the Genetic Algorithm parameter, which had set was only the number of GA runs (100×) while the others had left as default. It aimed to determine the best pose or conformations when the receptors and ligands bind [6].

The molecular docking results obtained in the form of a notepad value the free energy bond (ΔG) and the inhibition constant (Ki). Visualization of pose and orientation of ligands in both 2D and 3D bound

macromolecules and amino acids using the Biovia Discovery Studio 2016 Client® [7]. The pharmacokinetics and toxicity were also carried out.

Result and Discussion

This research was conducted to know and obtain the cocoa shell potential compound components (Fig. 1) by molecular docking as a candidate for anti-alopecia drug ingredients. Targeted treatment was the androgen receptor because it had an essential role in influencing hair growth and transcription factors. The androgen receptors used for the docking process with the ligands were obtained from PDB (Fig. 2).

Fig.1: Cocoa Shell (*T. cacao* L.)

Fig.2: Androgen Receptor (PDB code: 4K7A)

Method validation was performed by re-docking the native ligand to the androgen receptor. It aimed to see the RMSD value and the location of the binding. The method parameter was reliable/valid if the RMSD value is ≥ 2 Å. The smaller the RMSD value, the closer the position between the native ligand results from re-docking and crystallography [8]. The docking system was used on ligands under flexible conditions. These conditions allowed the ligands to adjust when conforming and stabilize when they bind to the receptor's active sites [9].

The receptor was optimized by adding Kollman to charged amino acid residues in electrostatic potential energy based on quantum mechanical calculations [10]. Besides, a polar only hydrogen atom was added, which meant that the receptor was water-soluble. Meanwhile, the ligand was added with a Gasteiger charge to adjust to the molecular docking environment so that the calculation process had been correct [11]. Then also added a hydrogen atom (protonation process), which aimed to adjust to the pH conditions in the cell cytoplasm (~7) [12] as well as completes the missing amino acid residues of hydrogen atoms due to structural damage due to x-ray radiation during the crystallography process.

The hydrogen atom that had been considered was polar because it was imperative in hydrogen bonding. Meanwhile, non-polar hydrogen atoms had not been considered in the ligand-receptor interactions on molecular docking, so they needed to combine with the binding atom [13]. It was necessary to choose a non-polar merge [10].

Besides, a grid box set had also carried outnaiming to determine the ligand coordinate space at the receptor. Determining the grid box included setting the parameter location of the box and the size of the grid box using the distance (angstrom, Å) [14]. At the androgen receptors, a grid box docking with coordinates x = -2.592; y = 0.864; and z = -6,729 with a grid point of 40×40×40 and a grid point distance of 0.375 Å. Lamarckian Genetic Algorithm was an algorithm method used with 100× docking simulations. The validation of the method obtained was an

RMSD value of 1.732 Å (Table 1), which was smaller than the crystallography results (2.44 Å). It meant that the positions of the atoms in the ligands resulting from re-docking were almost close to the location of the ligands on crystallography (Fig. 3) [15-17]. Thus, it could be stated that the androgen receptor could be used for molecular docking processes.

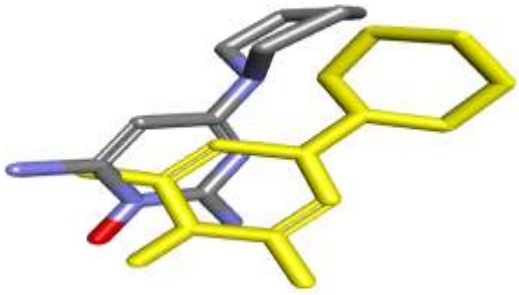


Fig.3: Visualization of overlap between crystallographic (yellow) and re-docking (gray) native ligand

Table 1: Results of native ligand validation with androgen receptor

Compounds	Amino Acid Residue Interactions		RMSD (Å)	ΔG (Kcal/mol)	Ki (µM)
	Hydrogen Bonds	Van Der Waals Bonds (Hydrophobic)			
Minoxidil	TRP 796	GLN 858, TYR 857, GLU 793, HIS 789, LEU 862	1.732	-4.83	286.80

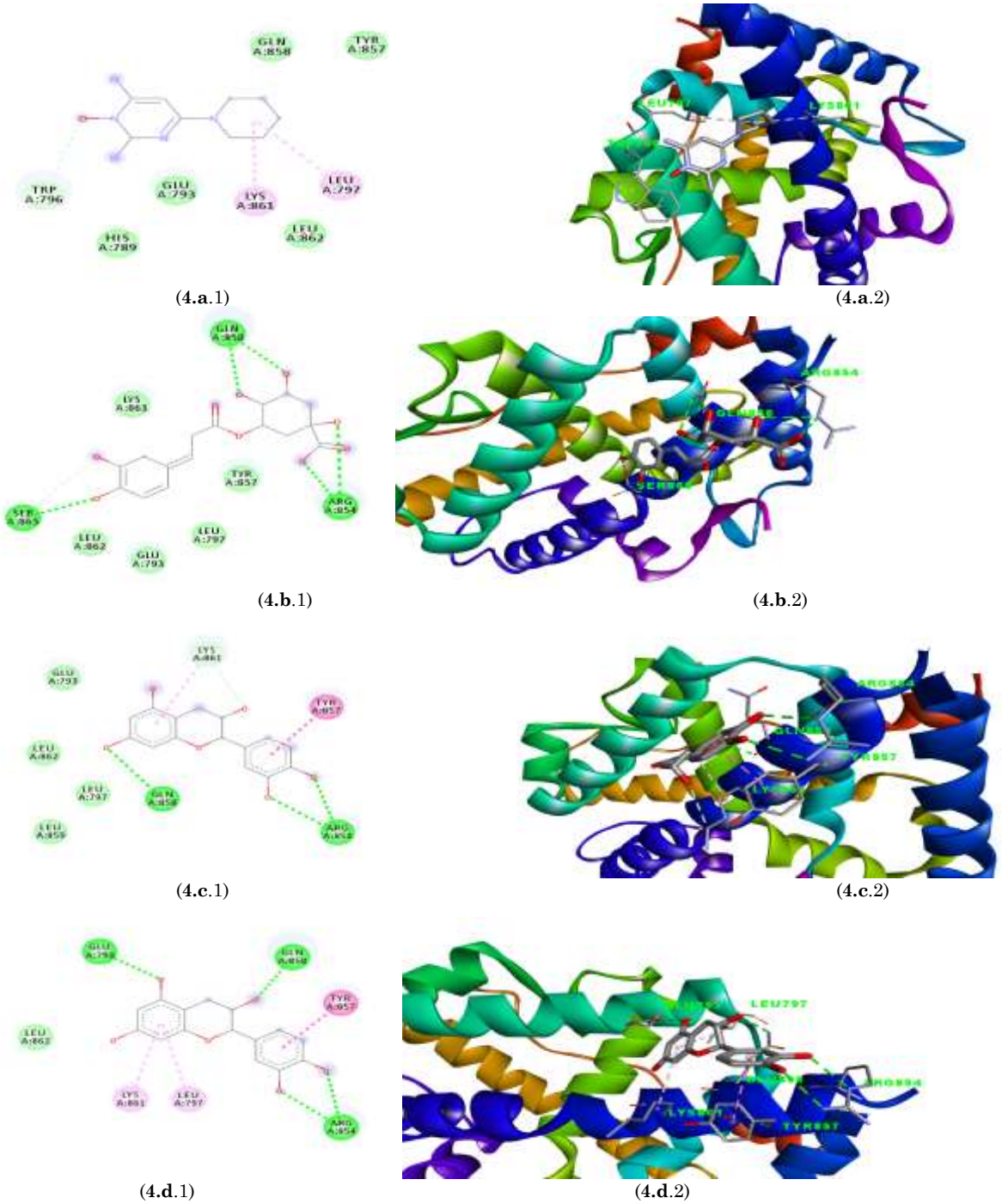
Binding affinity was a crucial factor that needed to be considered when the ligand-receptor interaction, namely the value of the free energy bond (ΔG) and the inhibition constant (Ki). If the binding affinity was lower, it meant that a compound requires less energy when it bound or interacted with the receptor. In other words, the lower the binding affinity value meant that it had a more significant potential to interact with the target protein [18]. The value of ΔG showed the amount of energy released from a compound when it interacted or formed bonds with receptors. So, the smaller the number or, the more extensive the minus, the stronger the energy used to create the bond [19]. Based on the results of molecular docking, the ones with strong bonds were chlorogenic acid (-7.87 kcal/mol) > epicatechin (-6.48 kcal/mol) > catechin (-6.36 kcal/mol), respectively (Table. 1). If we compare the value of ΔG with minoxidil (-4.83 kcal/mol) (Table 2) as a widely used medicinal substance, the three components of the cocoa shell had potential as anti-alopecia drug ingredients.

Table 2: Results of the validation of test ligands with androgen receptor

Compound	Amino Acid Residue Interactions		ΔG (Kcal/mol)	Ki (µM)
	Hydrogen Bonds	Van Der Waals Bonds (Hydrophobic)		
3-methylxanthine	GLN 858, ARG 854	-	-3.72	1870
Caffeine	SER 865, LEU 862, GLU 793, GLN 858	LEU 797	-3.70	1950
Catechin	GLU 793, GLN 858, ARG 854	LEU 862	-6.36	21.91
Chlorogenic acid	SER 865, GLN 858, ARG 854	LYS 861, LEU 862, GLU 793, LEU 797, TYR 857	-7.87	1.70
Epicatechin	GLN 858, ARG 854, LYS 861	GLU 793, LEU 862, LEU 797, LEU 859	-6.48	17.65
Gallic acid	GLN 858, ARG 854	TYR 857	-4.73	341.39
Paraxanthine	LYS 861	SER 865, GLN 858, TYR 857	-3.66	2090
Theobromine	SER 865, LEU 862	GLU 793, LEU 797, GLN 858, TYR 857	-3.55	2510
Theophylline	SER 865, GLN 858	LEU 790	-3.87	1460
Uric acid	ARG 854	TYR 857, LYS 861, GLN 858	-4.02	1140

The value of Ki showed the ability of the resistance of a compound to the receptor. If the smaller the value of Ki, the stronger the resistance strength [19]. Table 2 showed that

there were 3 test ligands for the compound component of the cocoa shell which was potent as anti-alopecia with the order of the best potential was chlorogenic acid (1.70 μM) > epicatechin (17.65 μM) > catechin (21.91 μM), which has a much Ki value smaller than minoxidil (286.80 μM) (Table 1). Thus, based on the ΔG and Ki values, chlorogenic acid, epicatechin, and catechin compounds were potential candidates for the components of the cocoa shell compound as an anti-alopecia drug. Visualization of the interaction of amino acid residues with the bonds formed, namely hydrogen and Van der Waals on minoxidil, chlorogenic acid, epicatechin, and catechin, was shown in Fig.4.



(**4.a**.1) (**4.a**.2)

(**4.b**.1) (**4.b**.2)

(**4.c**.1) (**4.c**.2)

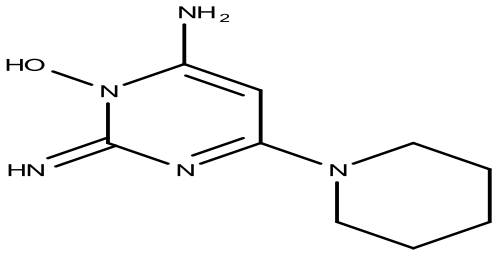
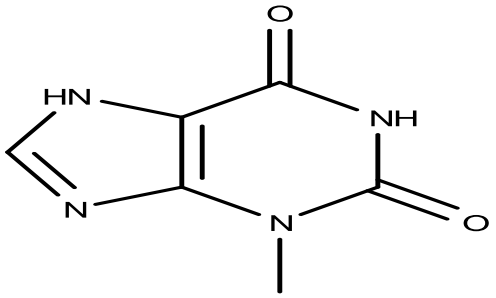
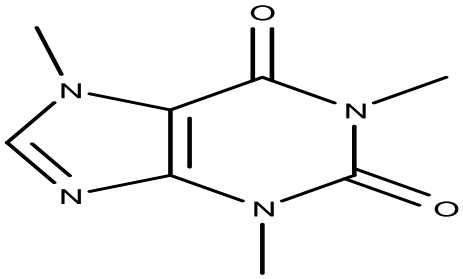
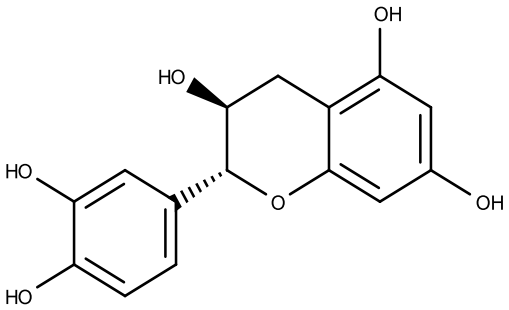
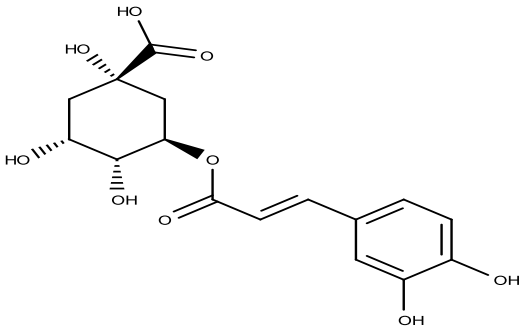
(**4.d**.1) (**4.d**.2)

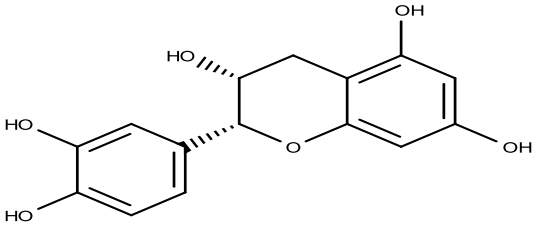
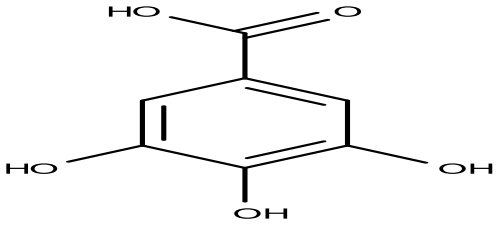
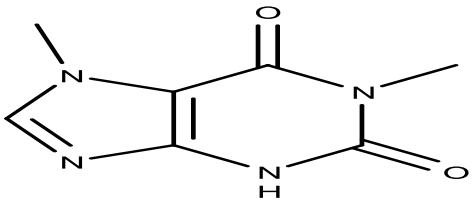
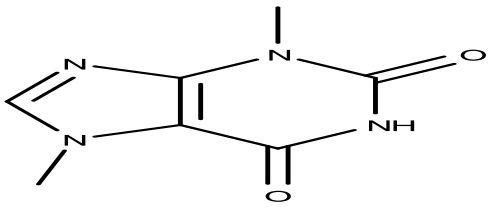
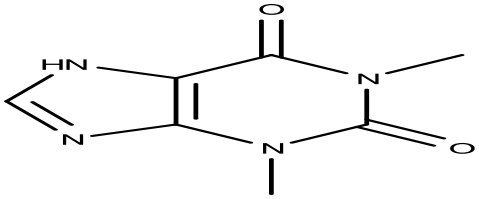
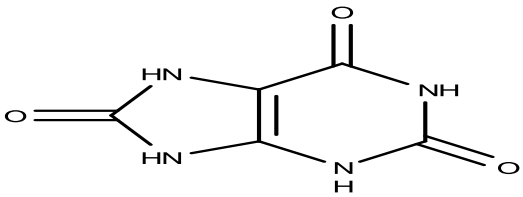
Fig. 4: Visualization 2D and 3D of interactions of amino acid residues with hydrogen and Van der Waals bonds of minoxidil (a), chlorogenic acid (b), epicatechin (c), dan catechin (d)

Table 3 showed that the structure of the test ligand compound from the cocoa shell was not much different from the native ligand (minoxidil). It could be seen from the presence of amine and hydroxyl groups. The interaction of amino acid residues between the ligand and the receptor was in hydrogen and Van der Waals bonds. The compound components of chlorogenic acid, epicatechin, and catechins form hydrogen bonds with the amino acid residues of GLN 858 and ARG 854. Whereas in minoxidil, the amino acid

residues of GLN 858 form Van der Waals bonds (Table 2). It had also been reported that some of the mobile residues of 2 helices (α8 and α11) on androgen receptors when binding to minoxidil were GLU 793, TRP 796, LEU 797, TYR 857, GLN 858, and LYS 861 [2]. Among the amino acid residues, GLN 858 was a crucial amino acid against anti-alopecia activity at androgen receptors.

Table 3: Structure and a molecular formula of native ligand and test ligands

Compounds	Structure Formula	Molecular Formula
Minoxidil		$C_9H_{15}N_5O$
3-methylxanthine		$C_6H_6N_4O_2$
Caffeine		$C_8H_{10}N_4O_2$
Catechin		$C_{15}H_{14}O_6$
Chlorogenic acid		$C_{16}H_{18}O_9$

Epicatechin		$C_{15}H_{14}O_6$
Gallic acid		$C_7H_6O_5$ or $C_6H_2(OH)_3COOH$
Paraxanthine		$C_7H_8N_4O_2$
Theobromine		$C_7H_8N_4O_2$
Theophylline		$C_7H_8N_4O_2$
Uric acid		$C_5H_4N_4O_3$

To develop a new drug substance, it was necessary to pay attention to the parameters before clinical testing, including pharmacokinetic properties and prediction of toxicity. Parameters of pharmacokinetic properties were absorption (human intestinal absorption / HIA and Human colon adenocarcinoma / Caco-2) and distribution (plasma protein binding / PPB). Meanwhile, toxicity predicted mutagenic and carcinogenic properties [20]. In this study, the administration of medicinal ingredients was topical because it was aimed at hair growth. What was concerned was the distribution parameters (PPB) and toxicity (mutagenic and carcinogenic). The PPB parameter was closely related to the ability of drug disposition to give effect [21]. Pre-ADMET results can be seen in the table. 4, which indicateed that the PPB value of the test ligands of epicatechin and catechin compounds was excellent, namely 100% compared to chlorogenic acid (41.96%) and minoxidil (56.86%) compounds. It explained that epicatechin and catechin compounds were potential anti-alopecia drugs because they could diffuse through the plasma membrane and interact with plasma proteins [22].Meanwhile, epicatechin, catechin, chlorogenic acid, and minoxidil compounds were mutagenic and carcinogenic only in chlorogenic acid compounds.

Table 4: Pre-ADMET prediction results

Compounds	Distribution	Toxicity	
	PPB (%)	Mutagenic	Carcinogenic
Minoxidil	56.86	Yes	No
3-methylxanthine	34.21	Yes	No
Caffeine	14.08	Yes	No
Catechin	100	Yes	No
Chlorogenic acid	41.96	Yes	Yes
Epicatechin	100	Yes	No
Gallic acid	65.39	Yes	No
Paraxanthine	13.20	Yes	No
Theobromine	17.28	Yes	No
Theophylline	22.15	Yes	No
Uric acid	3.40	Yes	No

Conclusion

The purpose of this study was to screen the components of the compounds contained in the cocoa shell as anti-alopecia drugs targeting androgen receptors. The method used was the in-silico method with molecular docking. Based on the results of molecular docking, the potential components of the compound are chlorogenic acid > epicatechin > catechin with the free energy bond value and inhibition constant (-7.87 kcal/mol; 1.70 μM) > (-6.48 kcal/mol; 17.65 μM) > (-6.36 kcal/mol; 21.91 μM) was much better than minoxidil (-4.83 kcal/mol; 286.80 μM). The crucial amino acid residue produced when the ligand with the receptor binds was GLN 858. The results of plasma protein binding indicated that epicatechin and catechin compounds ccoul penetrate the plasma membrane because they were 100% worth. Meanwhile, the toxicity of epicatechin, catechin, and chlorogenic acid compounds were mutagenic, and only chlorogenic acid was carcinogenic.

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