

Potential anti-alopecia constituents from *Theobroma cacao*: An *in silico* study

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ABSTRACT

Tinea capitis is local alopecia caused by a dermatophyte infection of the scalp. *Trichophyton rubrum* produces the squalene epoxidase enzyme, which has a crucial role in prolonged dermatophyte infection, as well as in synthesizing fatty acids in this dermatophyte group. This study analyzes *Trichophyton cacao* compounds as anti-alopecia by inhibiting the squalene epoxidase enzyme formation, *in silico*. The structure of *T. cacao* compounds was prepared using the MolView Web application. The compound docked to squalene epoxidase using AutoDock Vina in PyRx 0.8, followed by PyMOL for visualization, and the Proteins Plus program to analyze the complexity. The binding affinity value of catechin, epicatechin (−8.0 kcal/mol), and anthocyanin (−7.8 kcal/mol) compounds was higher than the positive control (terbinafine, −6.7 kcal/mol). Pre-ADMET demonstrated that catechin and epicatechin had moderate Human Intestinal Absorption (66.71%), but anthocyanin was very good (100%). Caco-2 parameters for catechin and epicatechin were relatively low (<4 nm s^{−1}), while anthocyanin, theobromine, and terbinafine were within 4–70 nm s^{−1}. Plasma protein binding shows catechin, epicatechin, and anthocyanin diffuse through the plasma membrane and interact with plasma proteins. The toxicity results for all compounds are mutagenic, and only terbinafine is carcinogenic. Based on the Lipinski's "Rule of Five," compounds from *T. Cacao* can be given orally. Catechin and epicatechin compounds have the potential to act as anti-alopecia. These two compounds can diffuse and interact with plasma proteins so they are directly on the target when given orally.

Key words: Alopecia, squalene epoxidase, *Trichophyton cacao* compounds, *Trichophyton rubrum*, tinea capitis

INTRODUCTION

Hair has an important role in protecting the scalp from the surrounding environment, and influences one's esthetic

appearance and self-confidence. Hence, hair loss that causes baldness has been shown to lead to a frustrating effect.^[1] Hair can show a group of people's identities, whether based on ethnicity or culture.^[2] Thus, hair loss must be diagnosed early to treat its causes more effectively.^[3]

Alopecia is a condition in which hair loss in the scalp area and other body parts is excessively abnormal. In general, a large volume occurs on the scalp.^[4] Types of alopecia consist of scarring alopecia (associated with fibrosis, inflammation, and loss of hair follicles) and nonscarring

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alopecia (associated with hair follicles present, although may miss some hair strands). External factors that cause nonscarring alopecia are tinea capitis, alopecia areata, traction alopecia, and trichotillomania.^[4-6]

Tinea capitis is local alopecia caused by a dermatophyte infection around the scalp and hair shaft, accompanied by squama. In general, this type of alopecia occurs in children.^[7] It is alopecia that is caused by *Trichophyton rubrum*, a dermatophyte that infects the skin's superficial layer by eating any keratinized areas.^[8] *T. rubrum* produces the squalene epoxidase enzyme, which plays an important role in synthesizing fatty acids in its dermatophytes and sterol biosynthesis (ergosterol),^[9] so that the enzyme becomes the primary target in the inhibition of dermatophytosis.^[10]

The drug that has been commercially used to treat dermatophytosis is terbinafine, N, 6,6-trimethyl-N-(naphthalene-1-ylmethyl)hept-2-en-4-yn-1-amine,^[11] which works by inhibiting activity of the squalene epoxidase enzyme so that ergosterol synthesis is disrupted and fungal cells die. However, the use of these drugs is inefficient in the long term.^[12] There is a risk of increasing serotonergic effects, primarily due to reduced analgesia associated with l-opioid receptors.^[13] Therefore, the design and development of a new drug are needed.

The use of natural materials as drug ingredients is essential.^[14] *Trichophyton cacao* contains polyphenol compounds (catechin, epicatechin, anthocyanin, phenolic acid, tannin, proanthocyanidin flavonoid, and others).^[15] It has been reported that the most components in this natural material, namely, catechin and epicatechin, have anti-carcinogenic, anti-inflammatory, anti-microbial, and antioxidant activities.^[16] Therefore, further research is needed to investigate promising anti-alopecia agents. This study was designed to predict anti-alopecia compounds' molecular action on *T. cacao* by inhibiting dermatophytes that can form tinea capitis. The *in silico* method was employed to investigate the ability to predict potential target proteins that bind these compounds.

MATERIALS AND METHODS

Materials

The test ligands from *T. cacao* compounds (catechin, anthocyanin, theobromine, and epicatechin) were prepared using the MolView program (<http://molview.org/>) and positive control; terbinafine was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) with CID, 1549008. The squalene epoxidase target enzyme used in this study was obtained from UniProt (<http://www.uniprot.org/>) with the gene name Q4JEX9.^[17]

Methods

Preparation of test ligands and target enzyme

The test ligands for the *T. cacao* compounds (catechin, anthocyanin, theobromine, and epicatechin) were prepared using the MolView program and then stored in the MOL file format. The OPEN BABEL 2.4.1 program was used to change the positive control (terbinafine) to the PDB file format. The target enzyme (squalene epoxidase) was prepared using the SWISS-MODEL (<https://swissmodel.expasy.org/>), and converted to the PDB file format.

Molecular docking of *Theobroma cacao* compounds against squalene epoxidase

The docking process was carried out using AutoDock Vina software, which is connected to the PyRx 0.8 tools.^[18,19] The target enzyme, squalene epoxidase, was selected for the macromolecule. The test ligands from *T. cacao* compounds and positive control were docked at the active sites of squalene epoxidase using PyRx. The result obtained is binding affinity (kcal/mol) to compare each protein-ligand complex value. The conformation was chosen based on the lowest binding affinity, which has a value of Root Mean Square Deviation (RMSD) ≤ 1.0 Å.^[20,21]

Visualization and analysis of molecular docking results

PyMOL was used to visualize poses best when docking and protein-ligand molecular interactions.^[20] Meanwhile, Protein Plus (<https://proteins.plus/>) used to analyze the amino acid residues formed in the form of two dimensional (2D) and 3D structure images. The similarity can be seen of ligand poses to one another on the target protein's active sites when bound.

Pre-ADMET and lipinski rule of five

The pre-ADMET program was accessed through <http://preadmet.bmdrc.kr/adme>, with the chemical structures of the compounds, described or uploaded in Mol file format (*.mol). The program automatically calculates the value of the selected parameter, including human intestinal absorption (HIA), cell permeability of human colon adenocarcinoma (Caco-2), plasma protein binding (PPB), mutagenic, and carcinogenic.^[22] Lipinski's "Rule of Five" was accessed through scfbio-iitd.res.in/software/drugdesign/lipinski.jsp with the Protein Data Bank file format (*.pdb). The parameters observed were molecular weight, log P, hydrogen bond donor and acceptor, and molar refractivity.^[23]

RESULTS

Results of molecular docking of *Theobroma cacao* compounds against squalene epoxidase enzyme

Table 1 shows the binding affinity and interaction between the ligands and the squalene epoxidase.

Table 2 shows that the compound structure from *T. cacao* is not much different from the positive control (terbinafine).

Visualization and analysis of the molecular docking results between the ligand and squalene epoxidase in Figures 1 and 2 demonstrated the active sites' binding.

Pre-ADMET

Predictions of absorption, distribution, metabolism, excretion, and toxicity are shown in Table 3.

Lipinski rule of five

The predictive route of oral drug administration is shown in Table 4.

DISCUSSION

Prediction of bioavailability and anti-alopecia activity from *Theobroma cacao* compounds through molecular interaction with squalene epoxidase

Binding affinity shows the amount of energy released by a compound (ligand) when it interacts or binds to a target (receptor). In general, a high-affinity ligands' binding result was obtained from a greater intermolecular force between the ligands, which shows good activity.^[24,25] The smaller the value, the stronger the energy used to form a bond.^[26] Based on the molecular docking results in Table 1, the compounds with strong bonds are catechin = epicatechin > anthocyanin > theobromine, with binding affinity values of -8.0, -8.0, -7.8, and -6.1 kcal/mol, respectively. Compared to the positive control (terbinafine, -6.7 kcal/mol), the catechin, epicatechin, and anthocyanin compound better bond to the squalene epoxidase enzyme, so that it can be predicted that the three compounds contained in *T. Cacao* have potent

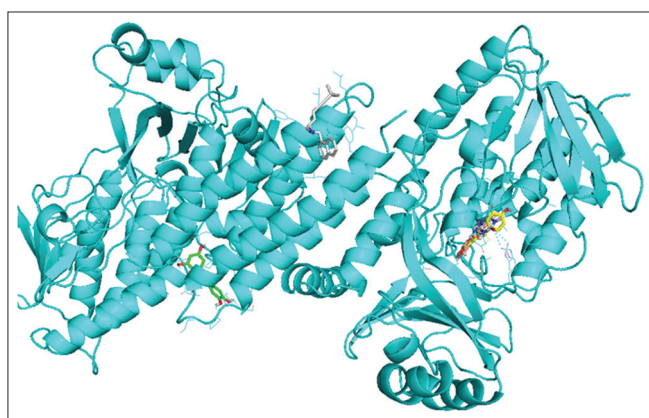


Figure 1: Squalene epoxidase enzyme

activities as anti-alopecia. In addition, the RMSD value of the three potent compounds is 0, which indicates a stable position when interacting, and is strongly bound to the substrate (squalene epoxidase enzyme). The interaction's stability is influenced by the distance and hydrogen bonds formed between the ligand and protein, so that the smaller the distance, the stronger the bond energy.^[27]

Pre-ADMET

In the development of a new drug substance, absorption, distribution, metabolism, excretion, and toxicity must be thoroughly investigated before clinical testing is carried out. Predictable parameters include pharmacokinetic properties and toxicity predictions. Pharmacokinetic properties consist of absorption (HIA and permeability of human colon adenocarcinoma/Caco-2 cells) and distribution (PPB). Meanwhile, toxicity was done through the prediction of mutagenic and carcinogenic properties.^[22]

HIA shows the percentage of bioavailability and absorption evaluated by excretion ratio through urine, bile, and feces. Meanwhile, Caco-2 has been widely used *in vitro* modeling to predict drug transport through human colon adenocarcinoma's intestinal epithelium, which has multiple transport routes.^[28] Distribution parameters pay attention to PPB and are closely related to drug disposition ability when giving effect.^[29] In general, only drugs in the unbound form can diffuse through the membrane and interact with pharmacological targets, so plasma protein bonds are crucial in drug efficiency. PPB is a fraction of the available drug-free of charge for distribution to various tissues.^[30]

The catechin and epicatechin compounds have a moderate HIA pharmacokinetics value (66.71%) during absorption in the intestine, in contrast to the very good anthocyanins (100%). The Caco-2 parameters showed very low catechin and epicatechin (<4 nm s⁻¹) *in vitro* cell permeability. Meanwhile, anthocyanins, theobromine, and terbinafine compounds have moderate permeability (4–70 nm s⁻¹). In terms of PPB, the catechin, epicatechin, and anthocyanin compounds can diffuse through the plasma membrane and interact with plasma proteins. However, the toxicity results demonstrate that all compounds were mutagenic, and only terbinafine was carcinogenic.

Table 1: Prediction of anti-alopecia activity from *Theobroma cacao* compounds

Ligands	Binding Affinity (kcal/mol)	rmsd/ub	rmsd/lb	Amino acids
Catechin	-8.0	0	0	Lys380B, Lys456A, Thr454A
Anthocyanin	-7.8	6.3	1.5	Phe115B, Arg409B, Gln408B, Leu239B, Tyr114B
Theobromine	-6.1	0	0	Tyr200A, Arg76A
Epicatechin	-8.0	0	0	Asp327A, Pro308A
Terbinafine	-6.7	37.0	33.6	Phe484A

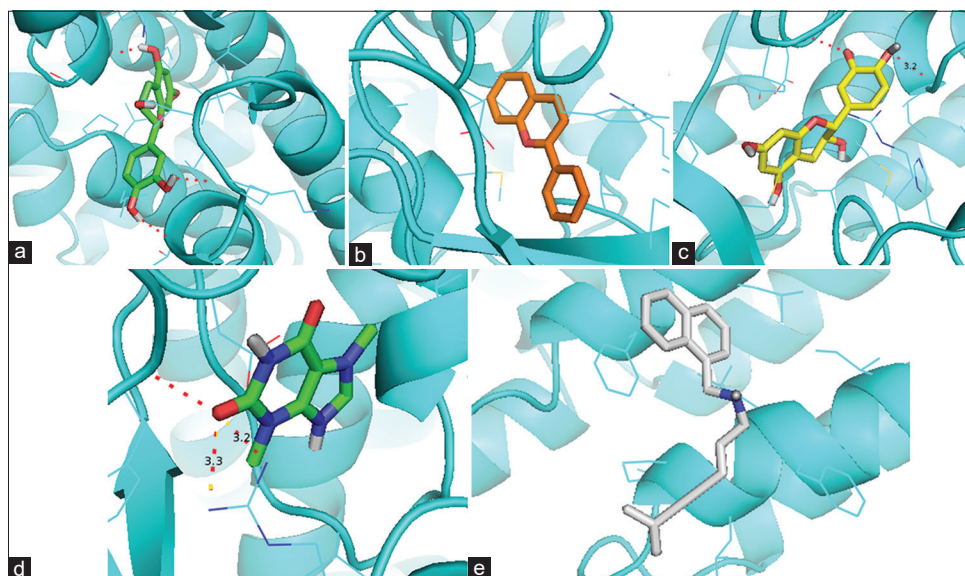


Figure 2: Visualization of catechin (a), anthocyanin (b), epicatechin (c), theobromine (d), and terbinafine (e) bound to the active sites of squalene epoxidase

Table 2: Structure and a molecular formula of the *Theobroma cacao* compounds and positive control

Compounds	Structure	Formula	Molecular formula
Catechin			$C_{15}H_{14}O_6$
Anthocyanin			$C_{15}H_{11}O$
Theobromine			$C_7H_8N_4O_2$
Epicatechin			$C_{22}H_{18}O_{10}$
Terbinafine			$C_{21}H_{25}N$

Solubility and permeability predictions based on the Lipinski rule of five

The solubility and permeability of a compound play a crucial role in considering drug development to the next stage. This was done to prevent the drug's failure by low absorption or permeation.^[20] Based on the rules of Lipinski on the discovery and development of a candidate drug substance used orally, it must satisfy the five conditions known as the "Rule of Five," including having a molecular weight value of not more than 500 daltons, having high lipophilicity, which is indicated by a log *P* of not more than 5, a hydrogen bond donor not more than 5, a hydrogen bond acceptor not more than 10, and a molar refractivity value between 40 and 130.^[23] Based on these rules [Table 4], all *T. Cacao* compounds can be administered orally.

CONCLUSIONS

The results of molecular docking using the *in silico* method of *T. cacao* compounds as anti-alopecia in inhibiting the formation of the squalene epoxidase enzyme have concluded to have a binding affinity value, namely, catechin=epicatechin(-8.0kcal/mol)>anthocyanin(-7.8kcal/mol), compared to terbinafine (-6.7 kcal/mol), as a drug that has been used commercially. The Pre-ADMET (pharmacokinetics and toxicity) showed that the catechin and epicatechin compounds had moderate HIA (66.71%) when there was absorption in the intestine, but it was perfect anthocyanin (100%). Caco-2 parameters showed low catechin and epicatechin (<4 nm s⁻¹) *in-vitro* cell permeability, but anthocyanin, theobromine, and terbinafine had moderate permeability (4–70 nm s⁻¹). The PPB for catechin, epicatechin, and anthocyanin compounds can diffuse through the plasma membrane and interact with plasma proteins. However, the toxicity results indicate that all

Table 3: Preabsorption, distribution, metabolism, excretion, and toxicity prediction results

Compounds	Absorption		Distribution	Toxicity	
	HIA (%)	Caco-2 (nm/sec)	PPB (%)	Mutagenic	Carcinogenic
Catechin	66.71	0.66	100	Yes	No
Anthocyanin	100	28.09	100	Yes	No
Theobromine	87.96	20.99	17.28	Yes	No
Epicatechin	66.71	0.66	100	Yes	No
Terbinafine	100	58.16	88.38	Yes	Yes

HIA: Human intestinal absorption, Caco-2: Human colon adenocarcinoma, PPB: Plasma protein binding

Table 4: Prediction results of compounds based on the Lipinski's rule of five

Compounds	MW	Log P	Hydrogen donor	Hydrogen acceptor	Molar refractivity	Information
Catechin	290.26 g/mol	1.55	5	6	72.62	Qualification
Anthocyanin	207.25 g/mol	4.19	0	1	65.40	Qualification
Theobromine	180.16 g/mol	-0.28	1	5	44.47	Qualification
Epicatechin	290.26 g/mol	1.55	5	6	72.62	Qualification
Terbinafine	290.26 g/mol	4.12	0	0	95.14	Qualification

MW: Molecular Weight

compounds were mutagenic, and only terbinafine was carcinogenic. The Lipinski Rule of five indicates that the *T. Cacao* compounds can be given orally.

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Conflicts of interest

There are no conflicts of interest.

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