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Original Article

# *IN SILICO* STUDY OF 12 PHYTOSTEROL COMPOUNDS IN MORINGA (*MORINGA OLEIFERA* LAMK.) SEED OIL ON 5A-REDUCTASE ENZYME INHIBITION ACTIVITY AS ANTI-ALOPECIA

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**ABSTRACT**

**Objective:** This study aimed to determine the interaction of 12 phytosterol compounds in moringa seed oil to the 5α-reductase enzyme (PDB code: 7BW1) as anti-alopecia by *in silico*.

**Methods:** The research was conducted using a molecular docking approach using autodock Tools 1.5.6. Data analysis was carried out by looked at the binding affinity values and inhibition constants (Ki) of 12 phytosterol compounds, as well as visualization of amino acid interactions using Biovia Discovery Studio 2021.

**Results:** 12 Phytosterol compounds had the potential to be a candidate for anti-alopecia medicines based on *in silico* test simulations using auto dock with high binding affinity values in the range of-11.47 to-12.76 kcal/mol and stable inhibition constants in the range of 1.87. nM–4.30 nM involving hydrogen bonds with Arg179, Tyr178, Arg105, Arg114, Ser177, Tyr98, Glu57, and Tyr91 amino acids.

**Conclusion:** Ergostadienol compound in moringa seed oil was predicted to be a better anti-alopecia on the inhibition of 5α-reductase enzyme with binding energy value was-11.60 kcal/mol, inhibition constant was 3.17 nM and interaction of amino acid residues on the inhibition of 5α-reductase enzyme was similar with native finasteride ligands namely Glu57 and Tyr91.

**Keywords**: *Moringa oleifera* Lamk, Ergostadienol, 5α-reductase, Anti-alopecia


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## INTRODUCTION

Alopecia is a condition of hair loss or head hair is not growing which can occur as a result of severe stress, heredity, hormonal or certain diseases, such as diabetes mellitus [1, 2]. Alopecia is classified into three types, namely non cicatricial, which is reversible, cicatricial which is irreversible, and alopecia due to abnormalities in the hair cavity [3]. Noncicatricial alopecia is divided into four types, namely hair loss (telogen effluvium), general baldness (androgenic alopecia), isolated repeated baldness (alopecia areata), and hair loss due to physical contact (traction alopecia) [4].

Androgenic alopecia is caused by the presence of 5α-reductase enzyme which is known as the cause of adrogenic alopecia [5]. This enzyme is present in the body that can convert the testosterone hormone into the form of dihydrotestosterone (DHT) [6, 7]. 5α-reductase enzyme is divided into two types, namely type 1 and type 2 [8]. 5α-reductase enzyme type 1 is found in the newborn scalp, skin and liver, while 5α-reductase enzyme type 2 is found in genital skin, liver, and prostate [9]. Dihydrotestosterone (DHT) will then bind to androgen receptors in hair follicles so that it will reduce blood flow to hair follicles and cause hair growth inhibition, hair damage, and hair follicle reduction [10].

Alopecia treatment using synthetic drugs, such as minoxidil and finasteride [11, 12] but can give side effects, especially in long-term use [13]. The use of finasteride can cause sexual disorders such as impotence, while minoxidil can cause irritation or allergic [14]. Therefore, it is necessary to find new compounds that can be used as antialopecia agents that are safer for the body, one of which is research related to plants that have 5α-reductase inhibitor activity [15, 16].

Moringa (*Moringa oleifera* Lamk.) seed oil contains phytosterol compounds, namely brassicasterol, ergostadienol, 24-methylenecholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β-sitosterol, stigmastanol, 7-avenasterol, stigmastadienol, 28-isoavenasterol and stigmastenol [17, 18]. β-sitosterol, stigmasterol and campasterol compounds can block the formation of DHT, which causes alopecia by inhibiting the 5α-reductase enzyme found in hair follicles [19]. The content of phytosterol compounds at concentrations of 0.01% to 0.5% in plants had been shown to have anti-alopecia effect [20]. Phytosterols are insoluble in water but soluble in alcohol [21].

Computational and mutagenesis studies showed an interaction in 5α-reductase enzyme inhibited by finasteride as therapeutic for benign prostatic hyperplasia [22, 23] and pharmacokinetic-pharmacodynamic studies as anti-alopecia [24]. Finasteride and phytosterols had mechanism of inhibiting 5α-reductase enzyme, which tested based on *in vivo* approach [25-27]. The existence of this interaction allowed a study about the activity of phytosterol compounds in moringa seed oil in inhibiting 5α-reductase enzyme by *in silico* as anti-alopecia needed to be done testing by molecular docking used The AutoDock Tools 1.5.6 with PDB code: 7BW1. Visualization of ligand interaction with target molecule using the Biovia Discovery Studio 2021 program to provide an overview of amino acids types that played role in inhibiting 5α-reductase enzyme to prevented DHT formation that caused alopecia.

Research related to activity of moringa seed oil as an anti-alopecia is still rarely done so it is necessary to conduct an assessment through sustainable research to support data that can confirm information related to the development of moringa seed oil as an anti-alopecia. This study aimed to determine the interaction between 12 phytosterol compounds on inhibition of 5α-reductase enzyme, which can be developed in predicting its activity as anti-alopecia.

## MATERIALS AND METHODS

### Sample preparation

Hardware used in this research was Windows 10 (Processor 1 GHz and RAM 1 GB for 32-bit). Software used in this research were

Autodock 4, MGL Tools, analysis and visualization using Biovia Discovery Studio 2021 software.

Samples used in this study were 12 phytosterol compounds identified from moringa seed oil, namely β-sitosterol, campasterol, stigmasterol, brassicasterol, campestanol, ergostadienol, clerosterol, stigmastanol, 7-avenasterol, stigmastadienol, 28-isoavenasterol and 24-methylenecholesterol. Structure of the compound was obtained by downloading from https://pubchem.ncbi.nlm.nih.gov/. Protein used in this study was 5α-reductase enzyme with PDB code: 7BW1 which was obtained from https://www.rcsb.org.

**Preparation of native ligands and proteins**

Protein native ligands preparation and test ligands were carried out using Biovia Discovery Studio 2021 software. The preparation was done by separating enzymes, native ligands and test ligan from unused residues such as hydrogen or water molecules [28].

**Molecular docking method validation**

Validation of molecular docking method was carried out by re-docking the native ligand (finasteride) to the prepared target protein (5α-reductase enzyme). Validation using the Autodock Tools 1.5.6 program. The method was said valid if the value of Root Mean Square Deviation (RMSD) obtained was<2Å [29].

**Optimization of test compounds**

Three-dimensional structure of phytosterol compound was created and geometrically optimized using Chemdraw 19.0 and Chem3D 19.0 programs. Optimization results were saved in pdb format.

**Docking phytosterols on target proteins**

The optimized phytosterol compounds were docked with the target protein using Autodock Tools 1.5.6 program with a validated grid box. Molecular docking would produce a compound with a certain conformation that had the lowest binding energy to the target protein. Dimensions were determined based on the size of each ligand and the grid box coordinates were determined based on the ligand coordinates of the protein file used.

**Data analysis**

Data were analyzed based on the bond energy obtained from molecular docking. The binding energy value indicated the bond strength (affinity) between the test compound and the target protein. Mechanism of activity of the test ligands were determined from the type of interaction (hydrogen bond) formed between phytosterols and the target protein. Determination of docking ligand (best pose) conformation was done by selecting the conformation of ligand that had the lowest binding energy. Analysis was also carried out by observed the inhibition constant (KI) and the interaction of the test ligand with amino acids [30].

**RESULTS**

Initial stage of molecular docking in this research was the preparation of protein macromolecular structures. Function of structural preparation was to separate the desired macromolecule from other residues. Preparation of ligand structure was carried out by downloading the macromolecular pdb structure from the website at http://www.rcsb.ord/pbd/with PDB code: 7BW1 (fig. 1).

Preparation process using Biovia Discovery Studio 2021 software to removed residues in the form of non-polar hydrogen atoms and water molecules and separated receptor and ligand into two different folders. This preparation step aimed to ensured that residues contained in ligands and enzymes did not interfere when the docking simulation was carried out, beside that also to ensured that the interactions that occurred were only between the ligands and enzymes. Addition of hydrogen atoms during preparation was also carried out to adjusted the docking atmosphere to approach pH 7 which was neutral [31].

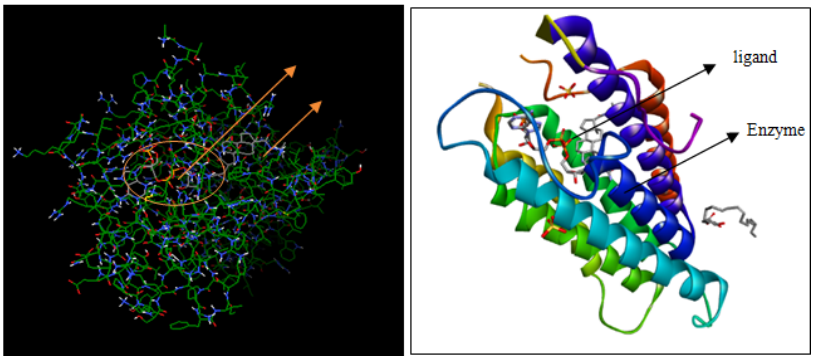



**Fig. 1: Enzyme and ligand structure in PDB code: 7BW1 [23]**

Enzyme and ligand that had been separated and cleaned then stored in PDB format. Separation of proteins from native ligands aimed to provided pocket as place for the test compound to bind to the 5α-reductase enzyme. Result of the preparation of 5α-reductase enzyme separation from native ligand finasteride could be seen in fig. 2.

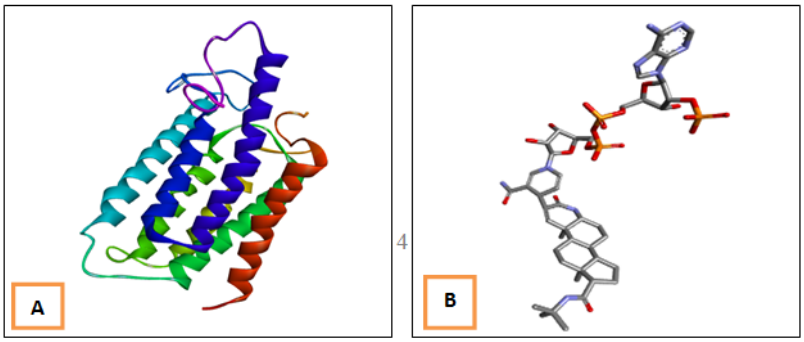



**Fig. 2: Results of 5α-reductase enzyme and finasteride preparation (A) 5α-reductase enzyme and (B) native ligand finasteride**

Validation of molecular docking method was carried out using the redocking method, namely re-docking the ligand with the protein on the binding site used for the molecular docking process. Molecular docking method validation was performed using Autodock Tools 1.5.6 software. Validation parameter for docking was expressed by the RMSD value, which was the value that indicated level of deviation of docking ligand results against test ligands at the same binding site. RMSD was deviation distance from the native ligand binding position with the protein after docking to true native ligand binding position [32]. The receptor used was declared valid if RMSD value was less than or equal to 2Å [33]. Validation results obtained RMSD value of 1.37, which indicated that the docking protocol was valid for used in the docking process of the test compound. The native ligand overlapping of finasteride and redocking with 5α-reductase enzyme at PDB code 7BW1 with the help of Pyrex application can be seen in fig. 3.

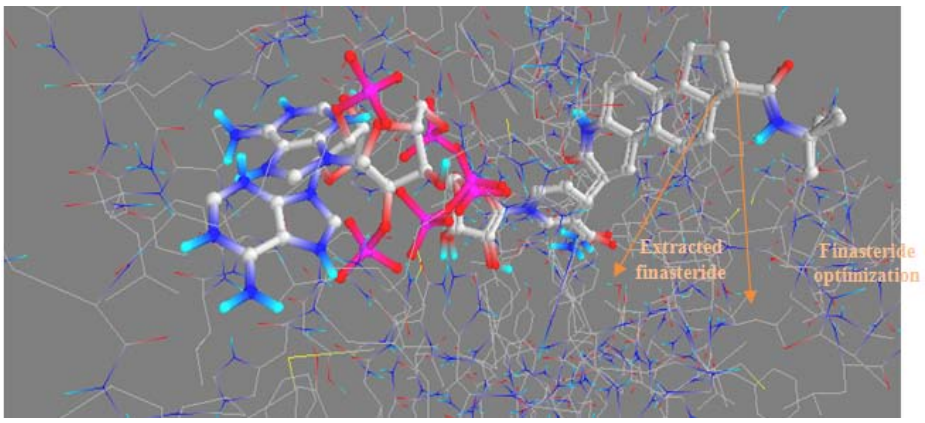
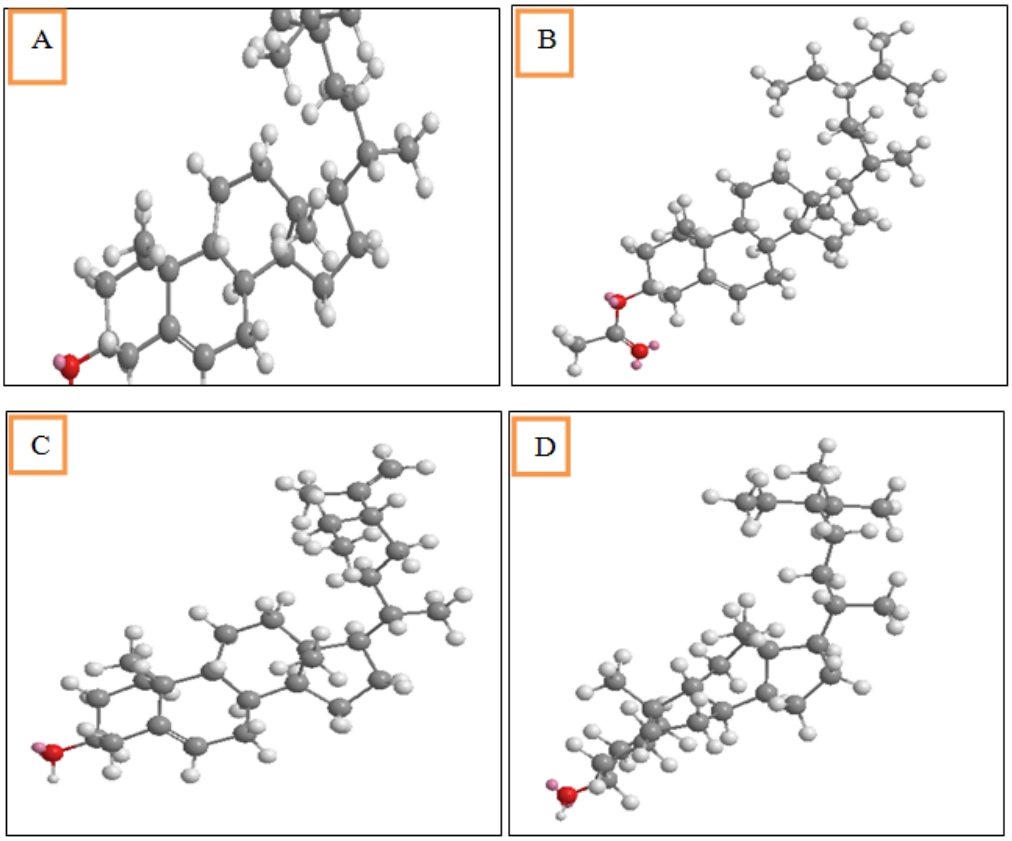



**Fig. 3: Native ligand overlapping of finasteride and redocking with 5α-reductase enzyme at PDB code 7BW1 with the help of Pyrex application**

Downloaded 3D structure of phytosterols, then optimized by geometrical molecular methods. The parameter observed was total energy value (kcal/mol) using ChemDraw software version 19.0 and Chem3D version 19.0 which aimed to obtained the most stable test ligand structure of 12 phytosterol compounds. The success of test compound optimization was indicated by the acquisition of total energy value that was smaller than total energy value from single point calculation [34]. In the geometry optimization process, total energy of molecule was minimized so that the most stable compound structure was obtained [35]. The results of phytosterol compounds optimization and the total value of optimization energy were shown in fig. 4.



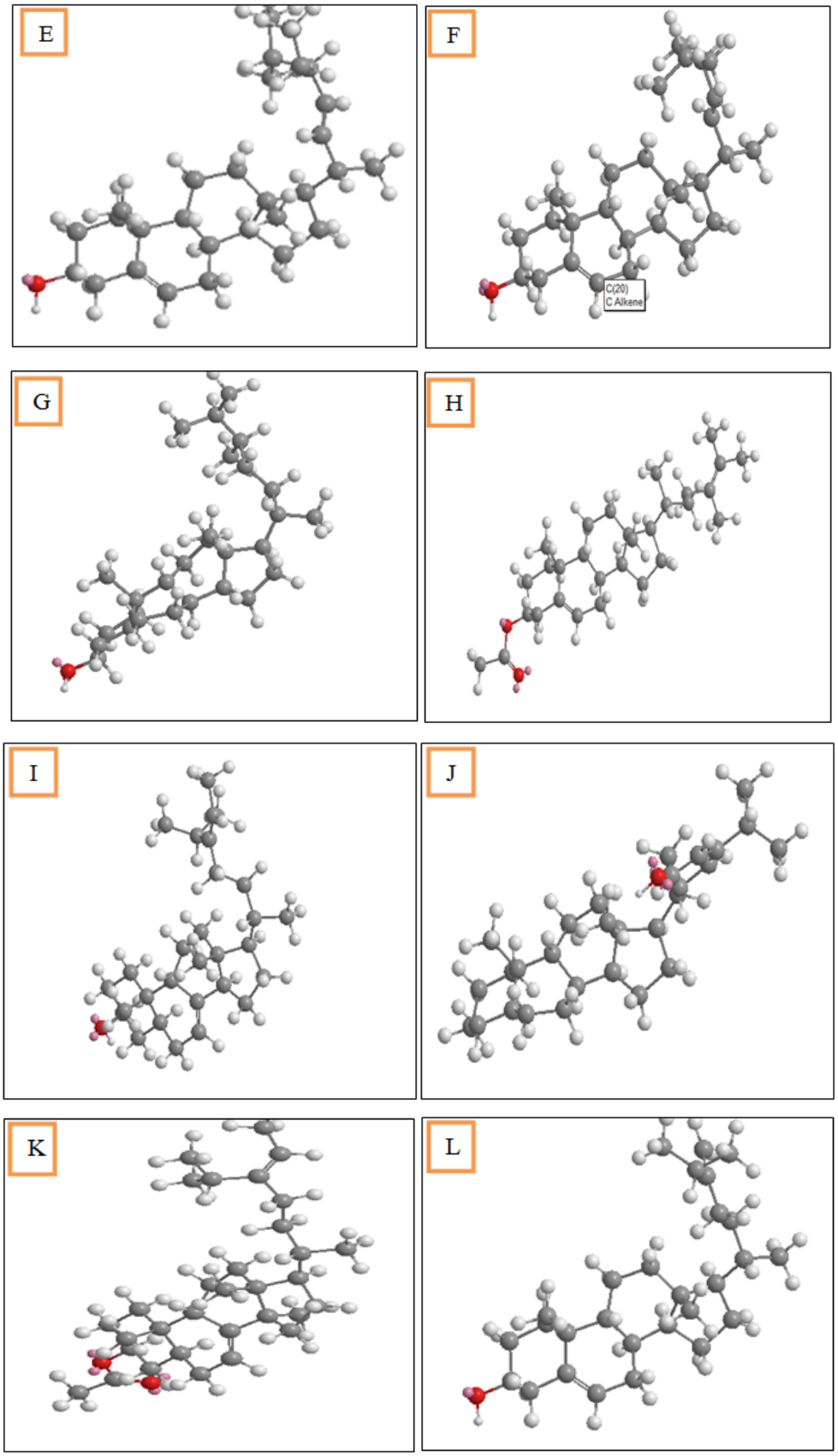



**Fig. 4: The 3D structure and optimization results of phytosterol compounds using Chemdraw 19.0 and Chem3D 19.0. (A) β-sitosterol (66.37 kcal/mol), (B) campasterol (60.2 kcal/mol), (C) stigmasterol (58.86 kcal/mol), (D) brassicasterol (53.23 kcal/mol), (E) campestanol (69.65 kcal/mol), (F) ergostadienol (59.15 kcal/mol), (G) clerosterol (57.48 kcal/mol), (H) stigmastanol (68.80 kcal/mol), (I) Δ7-avenasterol (62.86 kcal/mol), (J) stigmastadienol (75.32 kcal/mol), (K) 28-isoavenasterol (70.91 kcal/mol), (L) 24-methylenecholesterol (59.30 kcal/mol). Values in the brackets were tested ligand optimization of geometry structure**

Geometry structure optimization results based on fig. 4 showed that 12 phytosterol compounds had reached convergence condition because they had a low total energy value. A compound was said to be stable if it had a low total energy value so that it was considered to had a good final conformation [36]. Optimization results of 12 phytosterol compounds obtained were expected to be easier to form molecular interactions with the target protein, namely 5*α*-reductase enzyme.

Docking of phytosterol test compound that had been optimized against 5α-reductase enzyme was carried out with the same procedure and coordinate as in the validation method, which had been validated with the specified grid points x: 64; y: 46 and z: 60 values, and the center grid box values were x: -29.212; y: 13.225 and z: 35.629. Process of determined the best pose in the docking ligand conformation was done by selecting ligand conformation that had the lowest bond energy where the smaller (negative) score of a docking result, the more stable the protein-binding complex with the ligand, so that the observed test ligand was more potent as anti-alopecia [37, 38].

**Table 1: Results of docking 12 phytosterols to the 5α-reductase enzyme**

| No | Compound | (ΔG) (kcal/mol) | Estimated inhibition constant (nM) | Interaction with amino acid residues | | | |
|---|---|---|---|---|---|---|---|
| | | | | Hydrogen bonds | Hydrophobic Interaction | | |
| | | | | | Alkyl and phi-alkyl | Donor | Phi-sigma |
| 1 | Finasteride | -17.77 | 94.17 | Arg114, Glu57, Tyr91 | Leu23, Cys119, Phe223 | Tyr235 | Phe118 |
| 2 | β-Sitosterol | -11.56 | 3.35 | Glu197, Ser220 | Leu20, Leu111, Phe223, Tyr33, Phe216, Arg114, Phe118, Cys119 | | |
| 3 | Campasterol | -11.49 | 3.80 | Glu197, Ser220 | Leu111, Arg114, Phe219, Phe223, Tyr33, Cys119, Phe118, Phe216 | | |
| 4 | Stigmasterol | -11.86 | 2.04 | Glu197, Ser220 | Leu20, Leu111, Phe223, Tyr33, Phe216, Phe219, Phe118, Cys119 | | |
| 5 | Brassicasterol | -11.72 | 2.56 | Glu197, Tyr91 | Leu20, Leu111, Tyr33, Trp53, Phe219, Leu224, Arg114, Phe216, Phe118 | | |
| 6 | Campestanol | -11.63 | 2.98 | Leu111 | Leu224, Phe216, Phe223, Phe219, Tyr33, Phe118, His90, Tyr91, Arg94 | Gly115 | |
| 7 | Ergostadienol | -11.60 | 3.17 | Glu57, Tyr91 | Leu224, Phe223, Phe216, Trp201, Phe219, Cys119, Trp53, Phe118, Leu111, ARG114 | | |
| 8 | Clerosterol | -11.47 | 3.94 | Glu197, Ser220 | Arg114, Leu111, Phe216, Cys119, Phe118, Phe219, Leu20, | | |
| 9 | Stigmastanol | -11.69 | 2.71 | Glu197, Ser220 | Leu111, Trp53, Tyr33, Leu224, Phe219, Phe216, Phe118, | Trp201 | |
| 10 | Δ7-avenasterol | -11.74 | 2.47 | Glu197, Ser220 | Leu111, Phe223, Phe216, Phe219, Phe218, Cys119, Leu20 | | |
| 11 | Stigmastadienol | -12.76 | 4.30 | Arg114 | Leu111, Tyr33, Trp53, Leu224, Phe216, Phe118, Phe219, | | |
| 12 | 28-isoavenasterol | -11.91 | 1.87 | Tyr91, Arg94, Phe118 | Leu111, Tyr33, Phe223, Cys119, Ala24 | | Phe118 |
| 13 | 24-methylenecholesterol | -11.50 | 3.71 | Glu197, Ser220 | Leu111, Tyr33, Phe223, Phe118, Phe216, Arg114 | | |

Visualization observations showed that area of the binding protein to the ligand (binding site) would affect the conformation and function of protein which showed amino acid residues that play an important role in forming interactions between macromolecules and ligands in the form of hydrogen and hydrophobic bonds [39].

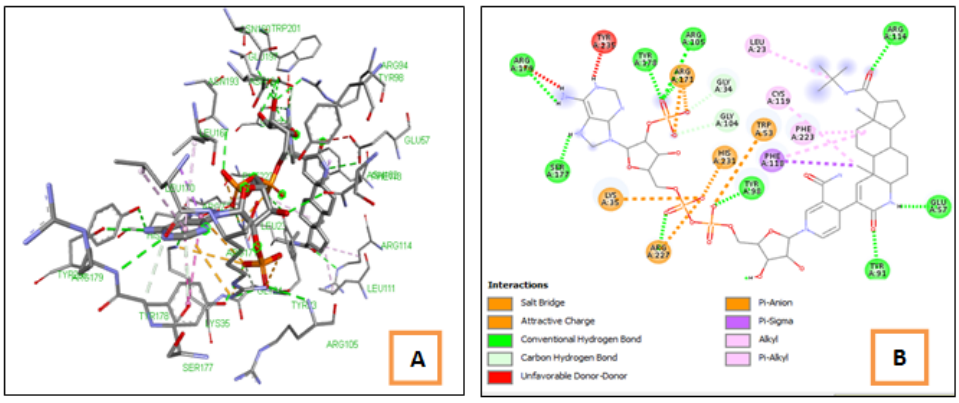



**Fig. 5: Interaction of finasteride with 5α-reductase enzyme (A) 3D interaction form, (B) 2D interaction form**

2D visualization of the native ligand docking of finasteride as an anti-alopecia agent with 5α-reductase enzyme in fig. 5 showed the presence of hydrogen bonds involved the amino acid residues Glu57 and Tyr91. Hydrogen bonds had big impact in the stability of interactions between molecules, so the more hydrogen bonds formed, the more energy bonds between proteins and ligands were formed [39].

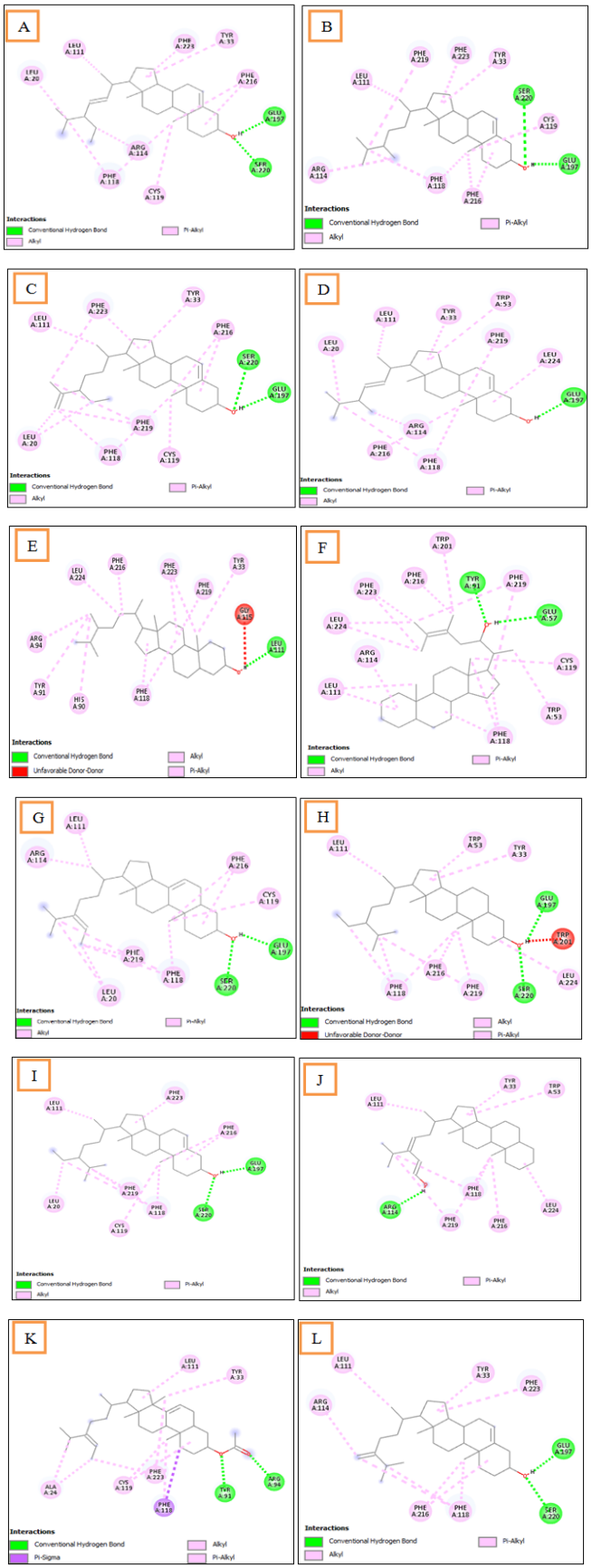



**Fig. 6: The 2D visualization of phytosterol compounds interaction with amino acids on the inhibition of 5α-reductase enzyme with PDB code: 7BW1 by Biovia Discovery Studio 2021. (A) β-sitosterol (B) campasterol, (C) stigmasterol, (D) brassicasterol, (E) campestanol, (F) ergostadienol, (G) clerosterol, (H) stigmastanol, (I) Δ7-avenasterol, (J) stigmastadienol, (K) 28-isoavenasterol, (L) 24-methylenecholesterol**

Visualization results in fig. 6 showed that Glu197 was amino acid that had the most interactions with test ligands, namely 8 test ligands and Ser220 which had interactions with 7 test ligands. Ergostadienol compounds had similar interactions to finasteride in binding amino acid residues, namely Glu57 and Tyr91. The more similar and close to the type of amino acid indicated similarity in interaction [40].

Amino acid residues that interacted with test ligand through hydrogen bonding include glutamate (Glu197; Glu57), tyrosine (Tyr91), arginine (Arg114; Arg94), serine (Ser220), and leucine (Leu111). In addition to hydrogen bonds, there were also hydrophobic interaction such as alkyl, phi alkyl, phi sigma bonds and donor bonds involved the interaction between Trp201 with stigmastanol and interaction of Tyr91 with campestanol.

## DISCUSSION

Molecular docking provided information related to molecular interactions prediction between test ligands and enzymes through binding energy value obtained from docking results [41]. Parameters observed in molecular docking of 12 phytosterol compounds included binding energy values (ΔG), inhibition constants (Ki), and hydrogen bond interactions between ligands and enzymes [42].

*In silico* study of 12 phytosterol compounds in moringa seed oil on 5α-reductase enzyme inhibition activity as anti-alopecia using PDB code: 7BW1. Selection of this code was based on the target protein complex with appropriate ligand compound, in this case protein target of 5α-reductase enzyme was obtained, which formed complex bond with finasteride so that it could prevent alopecia. Another reason of this PDB code selection was because its targets to *Homo sapiens* organism which provided an overview of anti-alopecia activity in humans, besides that it was not mutated and its resolution qualified the standard, namely less than 2Å [33].

Docking results in table 1 showed that finasteride as native ligand has more stable binding to 5α-reductase enzyme compared to 12 phytosterol test compounds because it had smaller (negative) ΔG of-17.77 kcal/mol, but in 12 phytosterols compounds can also inhibited 5α-reductase enzyme, because it had ΔG in the range of-11.47 to-12.76 kcal/mol. Inhibition interaction of phytosterol test compound against 5α-reductase enzyme caused the formation of DHT so that it would prevent alopecia. Comparison of docking results between 12 phytosterol compounds showed that stigmastadienol had a lower ΔG than other phytosterol compounds, namely-12.76 kcal/mol, which means that its interaction with 5α-reductase enzyme was more stable.

The Ki gave a description of how potent the activity of a compound or the range of concentrations required for 50% inhibition. The smaller the Ki value, the stronger the affinity of ligand for macromolecules [43, 44]. Binding affinity was said to be high if the value of Ki had a value was 250 nM and ΔG was<-9 kcal/mol respectively [45, 46]. Value of Ki of finasteride as native ligand against 5α-reductase enzyme showed a higher value, namely 94.17 nM compared to phytosterol as test ligand, so it could be said that the phytosterol compound was much more stable than native ligand. Stigmastadienol compound gave a higher inhibition value when compared to other phytosterol compounds, namely 4.30 nM while the lowest inhibition constant was indicated by 28-isoavenasterol compound, namely 1.87 nM which showed stronger affinity compared to finasteride and other compounds.

Analysis of good docking results was seen by compared the value of ΔG, Ki, and also the hydrogen bond interaction between the test ligand and the enzyme. Bond interactions were said to be more spontaneous and stable when the bond energy value (ΔG) was lower [47]. Hydrogen bond was bond that occured because of attractive force between the hydrogen atom and other atoms, where one atom of the molecule acted as donor and acceptor. In fig. 6, presence of hydrogen bonds between the test ligand and protein 5α-reductase enzyme was indicated by the green dotted line. Hydrogen bonds are quite strong bonds, because hydrogen bonds could be formed even though distance between the ligand and the receptor was quite far, but it was still weaker than covalent bonds or ionic bonds [48].

Docking results in table 1 showed the ΔG and the Ki of finasteride and ergostadienol compounds which had the lowest values but had high inhibition constants, as well as 28-isoavenasterol compounds which had lower inhibitory constant values but interact with amino acids were still different from finasteride. Ergostadienol compounds had binding energy values and inhibition constants in the low range of-11.60 kcal/mol and 3.17 nM as well as interactions with amino acid residues in inhibiting 5α-reductase enzyme which similar to finasteride, namely Glu57 and Tyr91 so that it could be predicted to be a compound that could be developed as the candidate of anti-alopecia.

Observation of interaction between amino acid residues and test ligand aimed to identified the interactions that occurred between 12 phytosterol compounds and 5α-reductase enzyme where the presence of hydrogen bonds could stabilize the interaction between ligand and enzyme, beside that the electrostatic interaction could increase stability in conformation [49]. The interaction through hydrogen bond between test ligands with the same amino acid residues as native ligands could show similar types of interactions which also illustrated the similarity of activity [50]. Ergostadienol compound had similarities with native ligand finasteride in interacting with the important amino acids in 5α-reductase enzyme inhibited, namely the amino acids Glu57 and Tyr91 through hydrogen bond, which means they had the same activity and interaction. Amino acid residues Glu57 and Tyr91 had a very important role in the interaction of 5α-reductase enzyme with finasteride as antiandrogen with mechanism described in fig. 7.

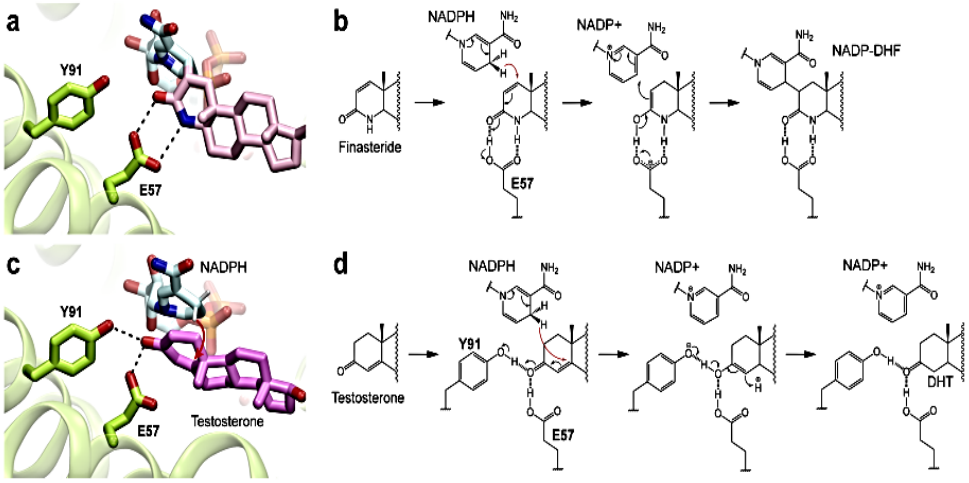



**Fig. 7: Mechanisms for 5α-reductase catalysis and inhibition [23]**

Fig. 7 showed that the presence of DHF binding pose, where the amino acid Tyr9 is not directly hydrogen bonded to DHF (fig. 7a). Potential mechanism of finasteride inhibition against 5α-reductase enzyme is through the formation of covalent bond with NADPH facilitated by Glut57 which transfers the hydride to the Δ1, 2 bond of finasteride (fig. 7b). Testosterone-binding pose based on the docking results showed an interaction between Glut57 and Tyr91 which forms hydrogen bond 5α-reductase enzyme and facilitates the transfer of hydride to $\Delta^{4,5}$ bonds of testosterone, leading to the formation of DHT (fig. 7c and 7d). Hydrogen bonds are shown as dashed lines and hydride transfer is shown as red curved arrows [23].

In addition to formed interactions with amino acid residues, phytosterol compunds also formed other interactions with amino acid residues on enzyme's active site. Phytosterol compounds had a planar shaped aromatic ring that allowed the interaction of π (*phi)*. Aromatic structure was rich in electrons and in electron-deficient anions could form π anion interactions. While it could play an important role in protein stability, ligand binding and this interaction was energetically beneficial [51, 52].

## CONCLUSION

Phytosterol compounds have the potential to be candidates for anti-alopecia medicine based on *in silico* test simulations using autodock. Ergostadienol in moringa seed oil is predicted to have potential as anti-alopecia by inhibiting 5α-reductase enzyme with ΔG of-11.60 kcal/mol and Ki of 3.17 nM. Interaction of ergostadienol with amino acid residues in inhibiting 5α-reductase enzyme is similar to finasteride, namely Glu57 and Tyr91.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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