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Molecular docking study of phytochemical compounds from Kustakudori ennai as a potential anti-alopecia treatment

Research Article

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Abstract

Background: Alopecia is a disorder that results in partial or complete shedding of hair from the scalp. Inhibiting the enzyme 5-α-reductase is one modern preventive strategy. By blocking the enzyme 5-α-reductase, testosterone in the circulatory system undergoes conversion into dihydrotestosterone, becoming a more powerful metabolite. Literature: *Kushtakudori Ennai*, Siddha poly herbal drug is used as an Alopecia medicine mentioned in the literature Prana Rakshamirtha Sindhu. The study aims to evaluate the anti-alopecia properties of nine recognized compounds found in *Kushtakudori Ennai*. Their interaction with the androgen receptor (PDB code 4K7a) through the use of the Auto doc software and molecular docking was the basis for their activity. Methodology: Autodock 1.5.6., preparation and optimization of three-dimensional structures of compounds, creation of androgen protein structure databases, and ADME-Tox prediction using the pk CSM tool were the stages of research that were carried out. Observation: From the various elements of the polyherbal siddha formulation, a total of nine bioactive lead compounds were solitary. From the reported data of the herbs, the phytochemicals such as Oleic acid, Chebuloside, Friedelin, Maslinic acid, Piperine, Isovitexin, Astragalin, Salsoline and Alangicine. Compounds like Astragalin, Chebuloside, Friedelin, and Piperine reveal a maximum of four to five interactions with the bioactive residues present on the target androgen receptor. Other components such as Isovitexin, Salsoline, Maslinic acid, and Alangicine reveal a maximum of three viable interactions with the target in comparison with the standard drug Finasteride. Conclusion: This suggests that these compounds may impede the binding of native ligands and restrict the process of follicle shrinkage, and these lead to hair fall. Thus, it was determined that the phyto chemical compounds mentioned above might have encouraging anti-alopecia effects.

Keywords: Molecular docking study, Alopecia, Enzyme 5-α-reductase, *Kushtakudori Ennai*, Finasteride.

Introduction

The common form of non-scarring hair loss affecting the scalp and body is called alopecia aerate, an autoimmune disease that is characterized by a sudden appearance of non-scarring hair loss ranging from small circumscribed patchy areas with no medical inflammatory indicators. The consequences are similar for men and women (1). However, some research revealed a preponderance of men (2). It impacts 0.7 percent of the country's entirety. It can happen at any age (3) ranging between 4 months and 70 years. (4) The age range between thirty and fifty-nine had the highest prevalence. (5) It affects any part of the body that bears hair, predominantly involve the scalp in many cases. (6) Alopecia is frequently reported in conjunction with other auto-immune conditions like diabetes, vitiligo, and thyroid abnormalities.(7,8) Numerous factors, such as genetics, environmental factors, and nutritional factors, can contribute to alopecia.(9) . Human scalp and body hair growth are significantly influenced by androgens. But in men with androgenic alopecia scalp hair loss is seen while beard growth is encouraged. (10,11) steroid hormones cause the balding process. (12)

Finasteride is a synthetic medication that inhibits the enzyme 5-α-reductase and prevents baldness. However, long-term use of finasteride treatment can have serious side effects (13). Mustarichie et al. (14) revealed that hair growth in male rabbit is enhanced by an Erythrina variegate ethanol extract containing polyphenol compounds, terpenoids, tannins, saponins, and steroids. Additionally, compounds isolated from Erythrina variegate were identified through chemical modeling in an in silico study these compounds bound Janus kinase 2 (JAK2) and are used for treatment of alopecia (15). Apart from synthetic medications, alternative treatments for alopecia include the use of traditional Siddha medicine known as *kushtakudori Ennai*, which is derived from the ancient Siddha text *Prana rakshamirtha Sindhu*.(16) It is used for the treatment of alopecia ,because it contains made out of

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herbs namely *Pungamia pinnata (Pungam seed), Callophyllum inophyllum (Punnai seed), Terminalia bellarica (Thandrikai seed), Psoralea corylifolia (karbogarisi), Alangium salvifolium (Alingil seed), Piper nigrum (Milagu), Terminalia chebula (Kadukkai seed)* that possess anti- alopecia properties.

This is the first research study to be done on anti-alopecia property of *Kushtakudori Ennai*. Hence we aim to examine the molecular interactions between the phytocomponds of *Kustakudori ennai* and the target proteins 4K7A to determine the anti-alopecia activity of phyto compounds included in the preparation of *Kushtakudori Ennai* using Molecular Docking.

Methodology

Hardware and software

The hardware consisted of a PC with an Intel® Core (TM) i3-4005U CPU running at 1.70GHz, an NVIDIA Ge Force GTS 710M graphics card, and four gigabytes of RAM (CPU memory). The system was running Windows 7 Home 64-bit. The following software was used for the analysis: ADME-Tox pkCSM tools, AutoDock Tools for Windows 1.5.6, and Discovery Studio Visualizer. Eighteen test ligands in total were selected from the mentioned herbal plants (Fig .1). Chem 3D Ultra 8.0 was utilized to create and optimize the three-dimensional (3D) structure of ligands through the application of the MM2 semi-empirical method.(17)

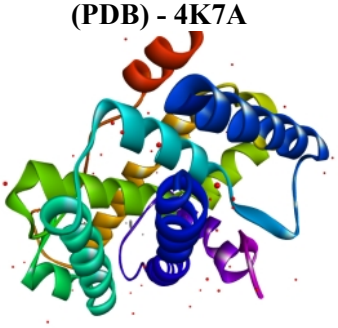
Discovery studio visualizes

The Discovery Studio program is a feature-rich software package that offers tools for basic data analysis, including the ability to view and edit data as well as analyze and model molecular structures and sequences. Using Discovery Studio, the docking result was visualized in order to calculate the nearest amino acid residue and hydrogen bond distance.(18)

Preparation of protein receptor

The androgen receptor's crystal structure (PDB code 4K7A) with a resolution of 2.44 κ was extracted from protein data bank. In addition, a grid box for figuring out spatial shape and spatial coordinates as docking materials was provided by the AutoDock Tools 1.5.6 application.(19) Based on the analysis of the interaction study, the optimal dock pose was chosen after the Autodock program assessed the lead molecules' various orientations concerning the target protein. Using AutoDock Tools 1.5.6, the ligand structure in the PDB format was transformed into the pdbqt format. To carry out the docking procedure, each ligand was tethered to an androgen receptor using the Grid Center tether coordinates of x = 40, y = 40, and z = 40 Å, as well as the Grid Box size coordinates of x = −2.592 y = 0.864 z = −6.729Å. Chemical interactions and binding energy were evaluated in the docking results. The molecular docking results were obtained in the form of a notepad with values showing the free energy bond (ΔG) and the inhibition constant (Ki).

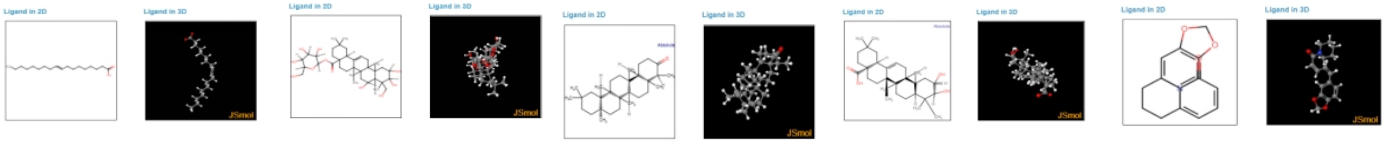
Figure 2: 3D-Structure of the androgen receptor (PDB) - 4K7A



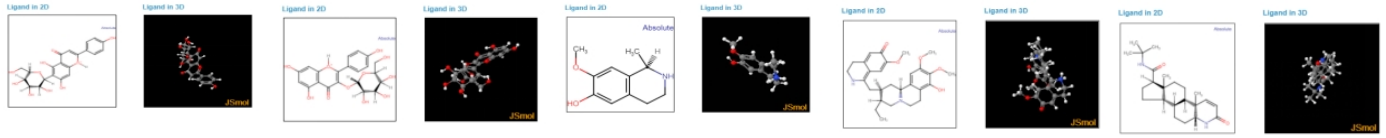
Receptor structure

Figure 1: 2D and 3D Structure of Phyto-components

Oleic acid $C_{18}H_{34}O_2$ **Chebuloside $C_{36}H_{58}O_{11}$** **Friedelin $C_{30}H_{50}O$** **Maslinic acid $C_{30}H_{48}O_4$** **Piperine $C_{17}H_{19}NO_3$**



Isovitexin $C_{21}H_{20}O_{10}$ **Astragalin $C_{21}H_{20}O_{11}$** **Salsoline $C_{11}H_{15}NO_2$** **Alangicine $C_{28}H_{36}N_2O_5$** **Finasteride**



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Results

Fig 3: Protein ligand docking pose

Fig 3.a Visualization of interactions between the Oleic acid and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 865 SER, 869 ILE

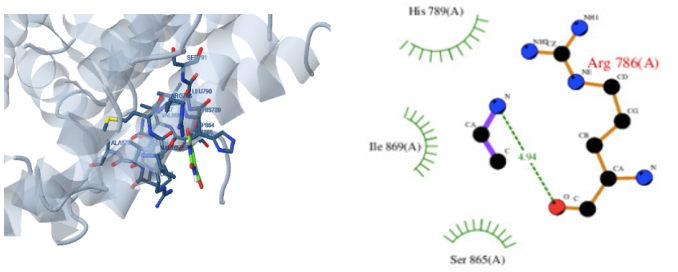

Fig 3.b Visualization of interactions between the Chebuloside and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU,861 LYS,865 SER, 869 ILE

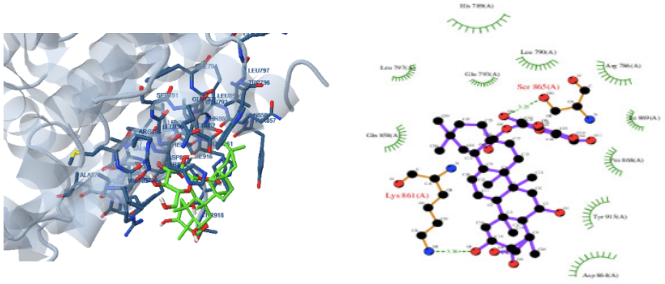

Fig 3.c Visualization of interactions between the Friedelin and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU,861 LYS,865 SER, 869 ILE

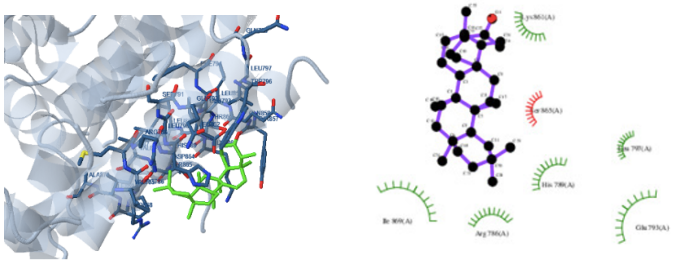

Fig 3.d Visualization of interactions between the Maslinic acid and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU,861 LYS, 865 SER

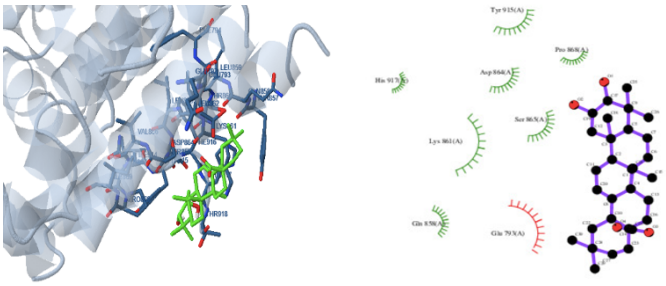

Fig 3.e Visualization of interactions between the Piperine and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, 793 GLU,861 LYS, 862 LEU, 865 SER, 869 ILE

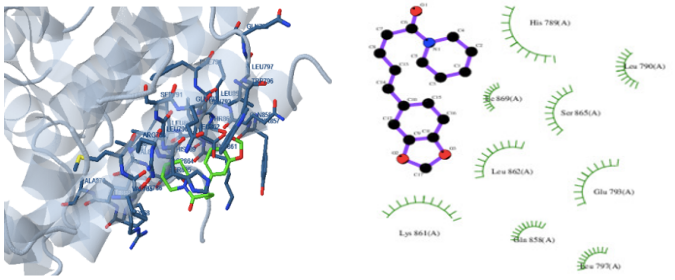

Fig 3.f Visualization of interactions between the Isovitexin and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU, 861 LYS, 865 SER

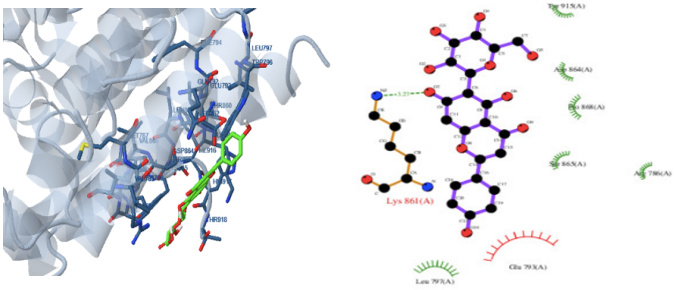

Fig 3.g Visualization of interactions between the Astragalin and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU, 861 LYS, 862 LEU, 865 SER, 869 ILE

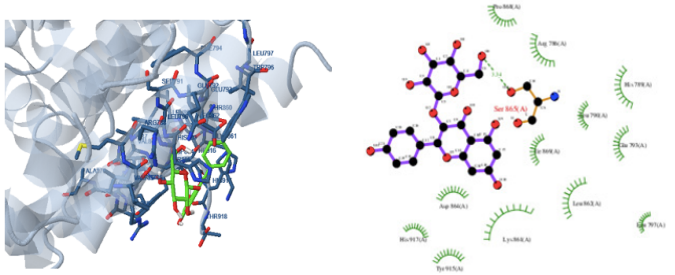

Fig 3.h Visualization of interactions between the Salsoline and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU, 861 LYS, 862 LEU, 865 SER

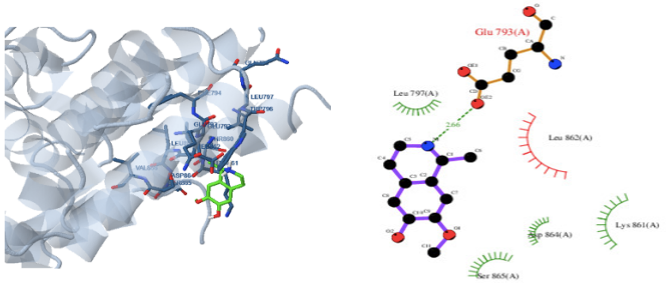

Fig 3.i Visualization of interactions between the Alangicine and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU, 862 LEU, 865 SER

Fig 3.j Visualization of interactions between the Finasteride and androgen receptors (4K7A). Hydrogen bonds are represented by green bonds, Amino acid residues forming hydrogen bonds are 793 GLU, 861 LYS, 862 LEU, 865 SER

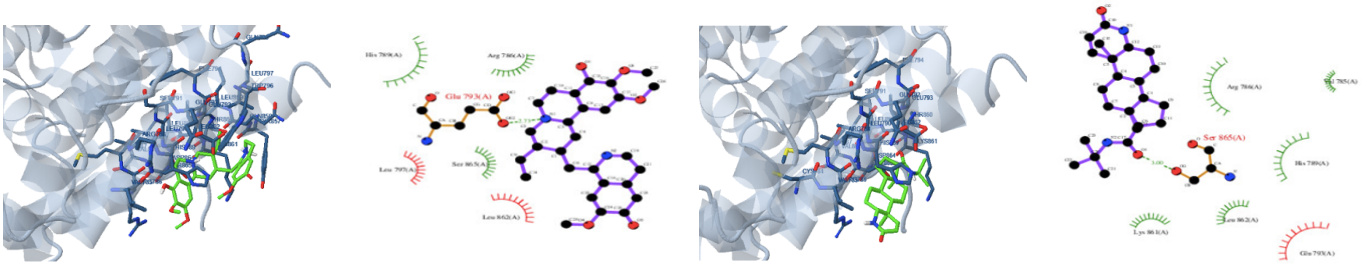

Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/ mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Oleic acid	282.5 g/mol	C18H34O2	1	2	15
Chebuloside	666.8 g/mol	C36H58O11	8	11	5
Friedelin	426.7 g/mol	C30H50O	0	1	0
Maslinic acid	472.7 g/mol	C30H48O4	3	4	1
Piperine	285.34 g/mol	C17H19NO3	0	3	3
Isovitexin	432.4 g/mol	C21H20O10	7	10	3
Astragalin	448.4 g/mol	C21H20O11	7	11	4
Salsoline	413.6 g/mol	C27H43NO2	2	3	0
Alangicine	480.6g/mol	C28H36N2O5	2	7	6
Finasteride	372.5 g/mol	C23H36N2O2	2	2	2

Table 1 shows Chebuloside and Astragalin consists of highest number of Hydrogen acceptor bonds - 11 bonds followed by Isovitexin having 10 acceptor bonds, Alangicine having 7 acceptor bonds, Maslinic acid having 4 acceptor bonds, Piperine and Salsoline having 3 acceptor bonds each, Oleic acid and Finasteride having 2 acceptor bonds each, Friedelin being the one to possess the least i.e. 1.

Table 2: Summary of the molecular docking studies of compounds against Androgen receptor (PDB) - 4K7A

Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	No .of Interactions
Oleic acid	-3.76 kcal/mol	1.77 mM	-3.49 kcal/mol	-0.56 kcal/mol	2
Chebuloside	-9.94 kcal/mol	51.40 nM	-10.16 kcal/mol	-0.48 kcal/mol	4
Friedelin	-7.01 kcal/mol	7.29 uM	-7.03 kcal/mol	-0.03 kcal/mol	4
Maslinic acid	-7.65 kcal/mol	2.47 uM	-7.65 kcal/mol	-0.24 kcal/mol	3
Piperine	-6.36 kcal/mol	21.96 uM	-6.67 kcal/mol	-0.07 kcal/mol	5
Isovitexin	-7.76 kcal/mol	2.04 uM	-5.45 kcal/mol	-1.01 kcal/mol	3
Astragalin	-8.63 kcal/mol	474.48 nM	-8.39 kcal/mol	-0.52 kcal/mol	5
Salsoline	-5.40 kcal/mol	110.97 uM	-3.79 kcal/mol	-1.45 kcal/mol	4
Alangicine	-7.23 kcal/mol	5.05 uM	-6.91 kcal/mol	-1.12 kcal/mol	3
Finasteride	-6.66 kcal/mol	13.13 uM	-7.21 kcal/mol	-0.12 kcal/mol	4

Table 2 shows the Values of Estimated Free Energy of Binding were Chebuloside (-9.94 kcal/mol) > Astragalin (-8.63 kcal/mol) > Isovitexin (-7.76 kcal/mol) > Maslinic acid(-7.65 kcal/mol) > Alangicine(-7.23 kcal/mol) > Friedelin (-7.01 kcal/mol) > Piperine (-6.36 kcal/mol) > Oleic acid(-3.76 kcal/mol) > Salsoline(-5.40 kcal/mol).

Table 3: Amino acid Residue Interaction of Lead against Androgen Receptor (PDB) - 4K7A

Compound	Interactions	Amino acid residues												
Astragalin	5	786 ARG	789 HIS	790 LEU	793 GLU	797 LEU	861 LYS	862 LEU	864 ASP	865 SER	868 PRO	869 ILE	915 TYR	917 HIS
Chebuloside	4	786 ARG	789 HIS	790 LEU	793 GLU	797 LEU	858 GLN	861 LYS	864 ASP	865 SER	868 PRO	869 ILE	915 TYR	
Friedelin	4	786 ARG	789 HIS	793 GLU	797 LEU	861 LYS	865 SER	869 ILE						
Isovitexin	3	786 ARG	793 GLU	797 LEU	861 LYS	864 ASP	865 SER	868 PRO	915 TYR					
Salsoline	3	793 GLU	797 LEU	861 LYS	862 LEU	864 ASP	865 SER							

Maslinic acid	3	793 GLU	858 GLN	861 LYS	864 ASP	865 SER	868 PRO	915 TYR	917 HIS					
Oleic acid	2	786 ARG	789 HIS	790 LEU	865 SER	869 ILE								
Piperine	5	789 HIS	790 LEU	793 GLU	797 LEU	858 GLN	861 LYS	862 LEU	865 SER	869 ILE				
Alangicine	3	786 ARG	789 HIS	793 GLU	797 LEU	862 LEU	865 SER							
Finasteride	4	785VAL	786 ARG	789 HIS	793 GLU	861 LYS	862 LEU	865 SER						

Table 3 shows the conserved residues that formed hydrogen bond interactions

Discussion

The nuclear hormone receptor known as the androgen receptor can be made more active by bonding interactions with androgen hormones (15). This androgen receptor acts as a transcription factor and regulates expression of gene in developing young adolescent males (20). From the components of the polyherbal siddha formulation *KushtaKudori Ennai*, a total of nine bioactive lead compounds were isolated namely astragalin, oleic acid, cheloside, Friedelin, maslinic acid, piperine, isovitexin, salsoline, and alangicine . Since finasteride is a medication that is known to prevent baldness we have chosen finasteride as a standard. In nature, hydrogen (H)-bonds are ubiquitous and are crucial for the folding of proteins, (21) interaction of protein-ligand (22), and catalysis (23). Enhancing ligand molecular weight, rotatable bonds, and lipophilicity is frequently necessary for lead optimization since these factors impact the ligands' pharmacokinetics and ADMET characteristics (24). Chebuloside and Astragalin consist of the highest number of Hydrogen acceptor bonds and hence it may be said to possess the highest interaction with the receptor. Drug absorption and enzyme catalysis depend heavily on H-bond pairing. Binding affinity was a crucial factor that needed to be considered when the ligand receptor interaction occurs, which depends on the number of free energy bond and the inhibition constant (Ki). Negative binding energy is the favorable energy of binding that is skewed in favor of the bound complex A lower binding affinity indicated that a substance needed less energy to attach or interact with the receptor. Stated otherwise, a lower binding affinity value indicated a greater potential for interaction with the target protein(25) The quantity of energy released from a chemical during interactions or bond formation with receptors was indicated by the value of free energy. Therefore, the greater the energy required to form the bond, the smaller the number or the more extensive the minus (26). Chebuloside (-9.94 kcal/mol), Astragalin (-8.63 kcal/mol), Isovitexin (-7.76 kcal/mol), Maslinic acid(-7.65 kcal/mol), Alangicine(-7.23 kcal/mol), Friedelin (-7.01 kcal/mol) possess more negative binding energy than Finasteride(-6.66 kcal/mol) so the bond created by Chebuloside, Astragalin, Isovitexin, Maslinic acid, Alangicine, Friedelin seems to be stronger with less energy utilized compared to Finasteride.

Astragalin and Piperine forms hydrogen bonding at 793 GLU,861 LYS,862 LEU,865 SER,869 ILE positions, whereas Chebuloside, Friedelin, forms hydrogen bonding at 793 GLU, 861 LYS, 865 SER, 869 ILE, Salsoline forms hydrogen bonding at 793 GLU,861 LYS,862 LEU,865 SER which is more similar to Finasteride that has bonding at 793 GLU, 861 LYS, 862 LEU, 865 SER.

Isovitexin, soline, mastinicine, and alangicine reveal a maximum of three feasible interactions with the target. This interaction may prevent native ligand binding and limit the process of follicle shrinkage, which is the main cause of hair fall.

Conclusion

The computational analysis revealed that bioactive compounds such as astragalin, chebuloside, Friedelin, piperine, isovitexin, salsoline, maslinic acid, and alinagicine exhibit significant interactions with the receptor's active site. This suggests that these compounds may restrict the process of follicle shrinkage, and these lead to hair fall. Thus it can be concluded that the aforementioned drug *Kushtakudori Ennai* with the phyto compounds Astragalin, Chebuloside, Friedelin, Piperine, Isovitexin, Salsoline, Maslinic acid, and Algicine may therefore have potential anti-alopecia effects.

Conflict of Interest: Nil
Sources of Funding: Nil

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