Original Research Article

Deciphering of Anti-Alopecia Activity of Polyphenol from *Beta vulgaris* Root against *5α-reductase*: Molecular Insight

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Abstract: *Background*: Androgenetic alopecia (AGA) is the predominant form of nonscarring alopecia, characterized by a progressive and predictable loss of hair. Androgenetic alopecia (AGA) is influenced by genetic susceptibility and heightened follicular sensitivity to androgens, predominantly in males, resulting in the gradual transformation of scalp terminal hair into vellus hair. Despite its great prevalence, it is not lethal but may exert a significant emotional influence, particularly on females and younger boys. Substantial progress has been achieved in comprehending the epidemiology and pathophysiology of AGA; nonetheless, only two medications have received FDA approval: finasteride and minoxidil. Extended administration of these medications is essential for improved therapeutic response. Nonetheless, this results in inadequate medication adherence and negative consequences from prolonged use, such as the "post-finasteride syndrome," which endures after discontinuation of the medicine. Consequently, research is required to identify more effective alternative treatments for AGA that exhibit less adverse effects. Prior study demonstrated the effectiveness of polyphenols in addressing metabolic disorders. The root of Beta vulgaris was considered for the current inquiry. *Purpose*: Current work was designed to check efficacy of root extract and their bioactive for anti-alopecic potential. *Methodology*: Scientific validation of the current investigation was done by computational based molecular docking study of lead molecules of *Beta vulgaris* root against *5α-reducatase (SRD5As)* enzyme. *Result*: The root of Beta vulgaris has been identified as an effective anti-alopecic drug, with its lead compounds, 4-picoline and Betalain, demonstrating effective binding to the target protein 5α-reductase (SRD5As) with binding energies of -4.09 and -7.93 kcal/mol, respectively. *Conclusion*: The findings indicated that each selected lead chemical for additional investigation shown significant inhibitory activity against 5'-reductase (SRD5 As), hence revealing its anti-alopecic potential.
Keywords: *Beta Vulgaris* Root, Molecular Docking, 4-Picoline and Betalain.

INTRODUCTION

Alopecia is a medical condition characterized by hair loss. The fundamental defects responsible for hair loss in alopecia patients are generally observed on the scalp, however they may impact any region of the body [1-2]. In 2014, 35 million men and 21 million women globally experienced alopecia. Genetic predisposition, environmental influences, and nutritional components are but a few of the causes of alopecia [3]. Finasteride, a synthetic pharmaceutical, is employed to inhibit the enzyme 5-reductase to mitigate hair loss; nevertheless, prolonged use of

finasteride may result in severe side effects, including diminished libido [4]. Alternative remedies for alopecia may be utilized alongside synthetic drugs, including the application of components from the B.vulgaris root to address dandruff and stimulate hair growth.

Beta is a genus under the flowering plant family Amaranthaceae. The most recognized member is the common beet, Beta vulgaris, while numerous other species are acknowledged. Nearly all possess common names that include the term "beet". Wild Beta species are distributed over the Atlantic coast of Europe, the Mediterranean shoreline, the Near East, and certain regions of Asia, including India.

Botanical classification

Kingdom	Plantae
Clade	Angiosperms
Clade	Eudicots
Order	Caryophyllales
Family	Amaranthaceae
Subfamily	Betoideae
Tribe	Beteae Moq.
Genus	*Beta*
Species	*Beta vulgaris*

Historical Background

Beets originate from the Mediterranean region. Despite the consumption of leaves predating written history, beetroot was mostly utilized for therapeutic purposes and only gained popularity as a food source until the French acknowledged its potential in the 1800s. Beet powder serves as a coloring additive in various food products. Beets were domesticated in the ancient Middle East, chiefly for its foliage, and were cultivated by the Ancient Egyptians, Greeks, and Romans. During the Roman era, it is believed that they were also cultivated for their roots. Since the middle Ages, beetroot has been utilized as a remedy for various ailments, particularly those associated with digestion and blood disorders. Bartolomeo Platina advised the consumption of beets with garlic to mitigate the effects of "garlic breath "[5].

Chemical Profile

Beetroot is recognized for its health-enhancing properties attributed to distinctive bioactive components, including betalains, phenolics, glycosides, carotenoids, vitamins, nitrates, and minerals. Betalains are present in two forms, betaxanthin and betacyanin, and are marketed as food dyes owing to their non-toxic properties. The distinctive chemical composition of beetroot facilitates the extraction of physiologically active chemicals, highlighting its significance in the food and pharmaceutical sectors. Bioactive chemicals in beetroot are known to demonstrate substantial antioxidant, antibacterial, and anti-inflammatory properties. [6]. These chemicals confer upon beetroot a wide array of biological actions, encompassing antioxidant, antihypertensive, anti-inflammatory, antimicrobial, hypoglycemic, hepatoprotective, hypolipidemic, and antidepressant effects.

The objective of the present study was to evaluate the efficacy of *Beta vulgaris* root extract in stimulating hair growth and to empirically substantiate this effect by molecular docking analyses targeting the enzyme *5α-reductase (SRD5As)*.

EXPERIMENT WORK
Scientific Validation of Hair growth Potential by Molecular Docking

As per literature survey *B.vulgaris root* contained volatile constituents of which the major ones were, pyridine (5.6 %) and 4-picolene (54.4 %). It is a rich source of a group of red and yellow pigments known as betalains comprising the red violet betacyanins and yellow betaxanthins. Betanin is the major constituent (75-95%) of the red pigment and vulgaxanthine I, the principal pigment of the yellow betaxanthin group. It also contains isobetanin, isobetanidine, prebetanin, isoprebetanin, and vulgaxanthin II. Anthocyanins and betalains have been reported in rich amount [7-8]. So, 4-picolene and betalain compound were selected as lead molecule for computational based docking studies against 5α-reductase (SRD5 As) enzyme to explored hair growth efficacy of *root* extract of *B.vulgaris*.

Ligand Preparation:

The 2D structures of 4-picoline and betalain were created using ChemSketch, and the two-dimensional representation of the synthesized ligand was transformed into optimum 3D geometries. The optimized structure was preserved in PDB format for compatibility with Auto Dock. The fundamental structures of the synthesized ligand are presented below:
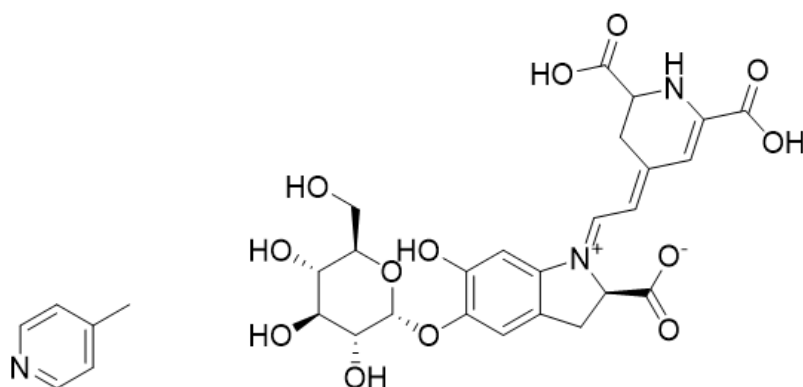
**Figure 1: 2D structure of 4-picoline and betalain**

Preparation of the Grid File

The regions of interest utilized by Autodock were delineated by establishing a grid box encompassing the operational sites. The grid box is crucial in the docking process as it encompasses all amino acids in the active sites required for binding, excluding those found in the receptor. The grid box contains three thumbwheel widgets that allow for the adjustment of the number of points in the x, y, and z dimensions. The spacing and grid points for all receptors examined in this investigation are presented in Table 1 [10, 11].

Table 1: Grid parameters used in current docking analysis of GSK3β, Aldosereductase, α-amylase and α-glucosidase

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	5α-reductase	58	40	44	0.375	-29.547	15.112	37.14
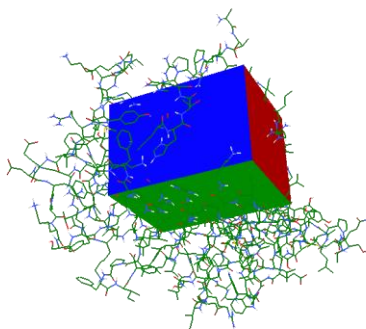
**Figure 2: Grid box covering all active sites in 5α-reductase receptor.**

Preparation of the Docking File

All computations were conducted with Autodock 4.2 as the docking tool. The visualization and other programs required for docking investigations were conducted using Pymol, Chimera, DS Visualizer, and MMP Plus [12-14].

Docking Study
Crystal Structure

The crystal structure of the protein consisting of 5α-reductase receptor is downloaded from the Protein Data Bank portal. All the primary information regarding all the receptor's structure was registered in the Protein data bank [15, 16]. The complex ligand was separated by using Chimera software for all the target receptors.
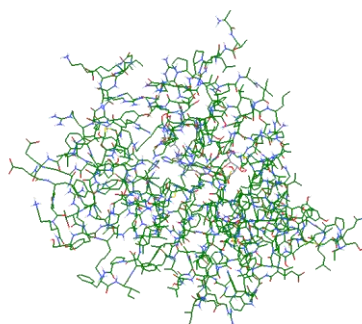
Figure 3: Crystal structure of 5α-reductase receptor (PDB ID-7bw1)

Processing of Protein

All the downloaded receptor proteins are having only one chains, i.e. chain A, which has been selected for experimental purpose and complicated ligand was removed from it. The bound ligand was isolated from the macromolecular complex by using software Chimera [17-20].

Molecular Docking Simulation Studies

Ligands such as 4-picoline and betalain were docked against the 5α-reductase receptor using Autodock. All bonds of each ligand were maintained in a flexible state, however no residues in the receptor were rendered flexible [21-23].
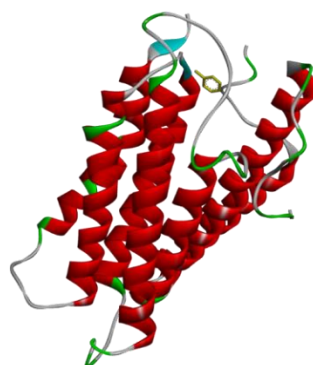
Figure 4: Binding mode of 4-picoline within the active site of 5α-reductase receptor
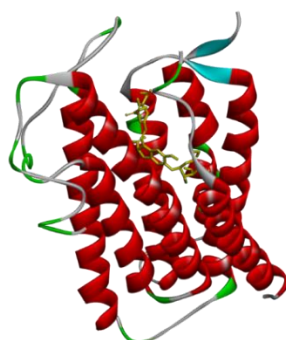
Figure 5: Binding mode of betalain within the active site of 5α-reductase receptor

Toxicity & ADME-T Studies

The ligand compounds, specifically 4-picoline and betalain, were analyzed using the online tool OSIRIS to anticipate the existence of any hazardous groups and to assess ADME-T characteristics [24].

RESULT AND DISCUSSION

Androgenetic alopecia, also known as male pattern baldness, is the primary type of progressive hair loss in males. Anti-alopecia medications must be developed to stimulate hair follicles into the anagen growth phase. The development and advancement of androgenetic alopecia depend on the interaction of hormonal factors and genetic susceptibility. Androgenetic alopecia is characterized by the progressive atrophy of hair follicles due to androgenic effects on the epithelial cells of genetically susceptible hair follicles in androgen-sensitive areas. The exact pathophysiology of androgenetic alopecia has to be clarified; nonetheless, research suggests it is a polygenic condition.

In recent years, beetroot (Beta vulgaris L.) has emerged as a promising "functional food." These bioactive phytonutrients are vital in managing several chronic diseases, including cardiovascular and cerebrovascular disorders, cancer, diabetes, and chronic respiratory ailments. Betalains, chiefly betanin, are powerful antioxidants obtained from beetroot. Numerous lines of evidence suggest that betalains may reduce the incidence of some cancers, cardiovascular and cerebrovascular diseases, as well as liver and kidney damage. Betalains exhibit antibacterial and antimalarial effects. The present

study employed a computer molecular docking analysis to scientifically evaluate lead compounds from B. vulgaris against the 5α-reductase (SRD5As) enzyme. Chiranan Khantha *et al.*, (2021) assert that SRD5As are NADPH-dependent enzymes that play a crucial role in steroidogenesis by accelerating the transformation of 4-ene-3-keto steroids into more active 5α-reduced derivatives, such as the reduction of testosterone (T) to dihydrotestosterone (DHT). Dihydrotestosterone (DHT), the most potent androgen hormone, is a crucial causative element in androgenetic alopecia (AGA), or hair loss. Steroid 5-alpha reductases (SRD5As) facilitate the production of dihydrotestosterone (DHT) in scalp hair follicles, resulting in hair thinning and loss. Synthetic SRD5A inhibitors, such finasteride and dutasteride, effectively treat androgenetic alopecia but exhibit adverse effects. This has led to increased interest in alternative medicines originating from natural sources [1]. The grid parameter employed in the docking analysis of 5-alpha reductases (SRD5As) is detailed in Table 10. The research revealed that the selected chemicals, specifically 4-picolene and betalain, serve as effective inhibitors of 5α-reductase (SRD5As) in the following manner: Betalain to 4-picoline.

B.vulgaris root found to be effective anti-alopecic agent and their lead molecules effectively binds to be target protein *5α-reducatase (SRD5As)* with binding energy -4.09 and -7.93 kcal/mol for 4-picoline and betalain respectively. The result was tabulated in table 2. The binding mode of selected lead molecules showed in fig.4-5. The 2D and 3D interaction of selected compound displayed in fig.6-9. The interaction of 4-picoline and betalain with active site at *5α-reducatase(SRD5As)* showed as follows:
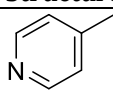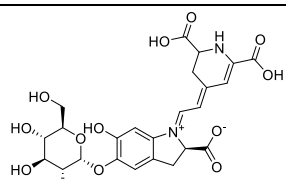
| Compound	Conventional Hydrogen bounding	Pi-alkyl	Pi-Pi	Alkyl	Week Vander's interaction	Pi-Sigma	Pi-Anion
4-picoline	Lys35, Tyr235	Leu170, Leu 167	Tyr178	Arg171	Pro181, Arg179, Ser177	----	-----
betalain	Cys119 Glu57 Asn102 His231		Try53	------	Phe216 Tyr91 Phe118 Gly115 Gln56 Ala49 Gly104 Arg103 Arg105 Tyr178 Lys35 Asp164 Asn1160 Glu197 Tyr33 Phe194 Phe223	Leu224	Arg94 Tyr98

The pharmacokinetic profile reveals that it is having good pharmacokinetic profile but with the presence of any major toxic effects including mutagenicity, tumorogenicity and reproductive effects. The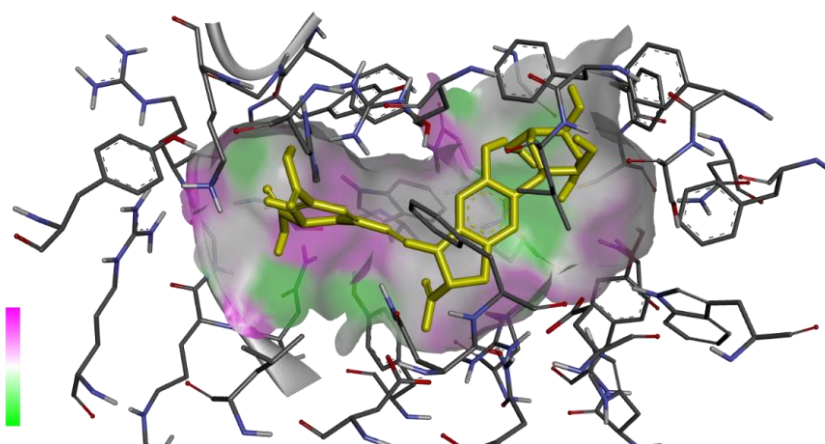 pharmacokinetic and toxicity profiling results of ligands like 4-picolin e and betalain were shown in figure 10-11 & table 3-5. Theoretically, all the ligand molecules have shown encouraging docking score.

Table 2: Results of docking of ligands like 4-picoline and betalain against 5α-reductase receptor

S. No.	Compound Name	Structure	B.E
1	**4-Picoline**		-4.09
2	**Betalain**		-7.93
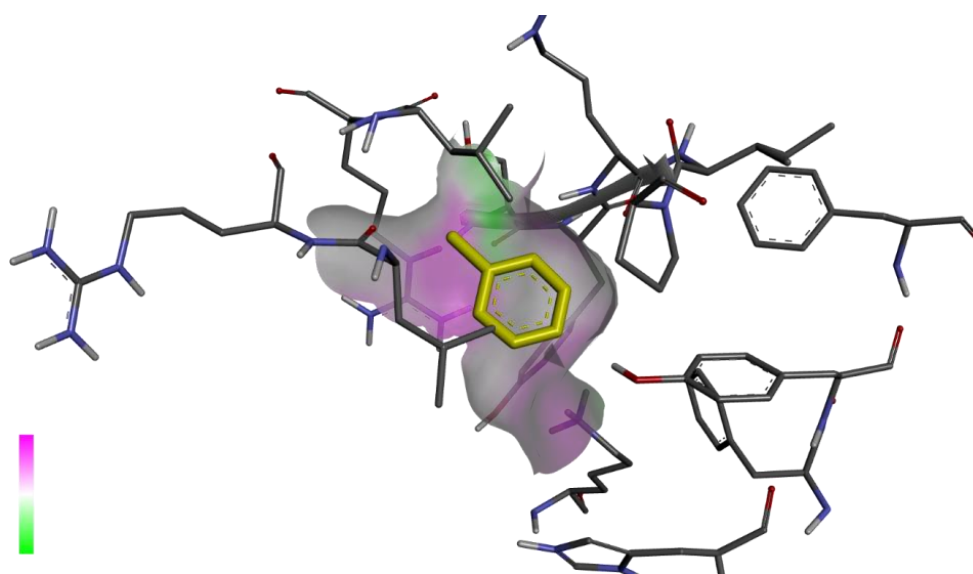
Figure 6: Three-dimensional binding mode of 4-picoline within the active site of 5α-reductase receptor

Figure 7: Three-dimensional binding mode of betalain within the active site of 5α-reductase receptor

Figure 8: Two-dimensional binding mode of 4-picoline within the active site of 5α-reductase receptor

Figure 9: Two-dimensional binding mode of betalain within the active site of 5α-reductase receptor
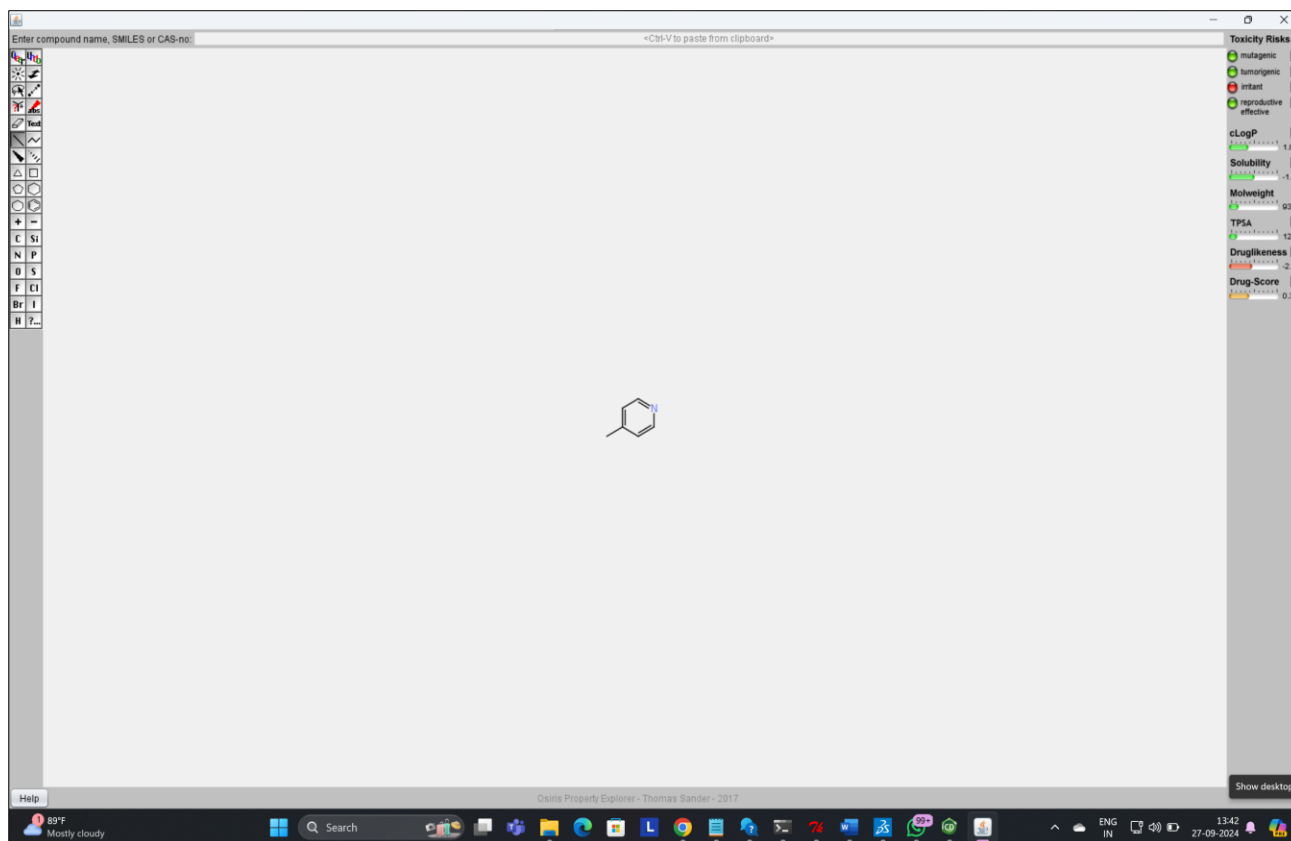
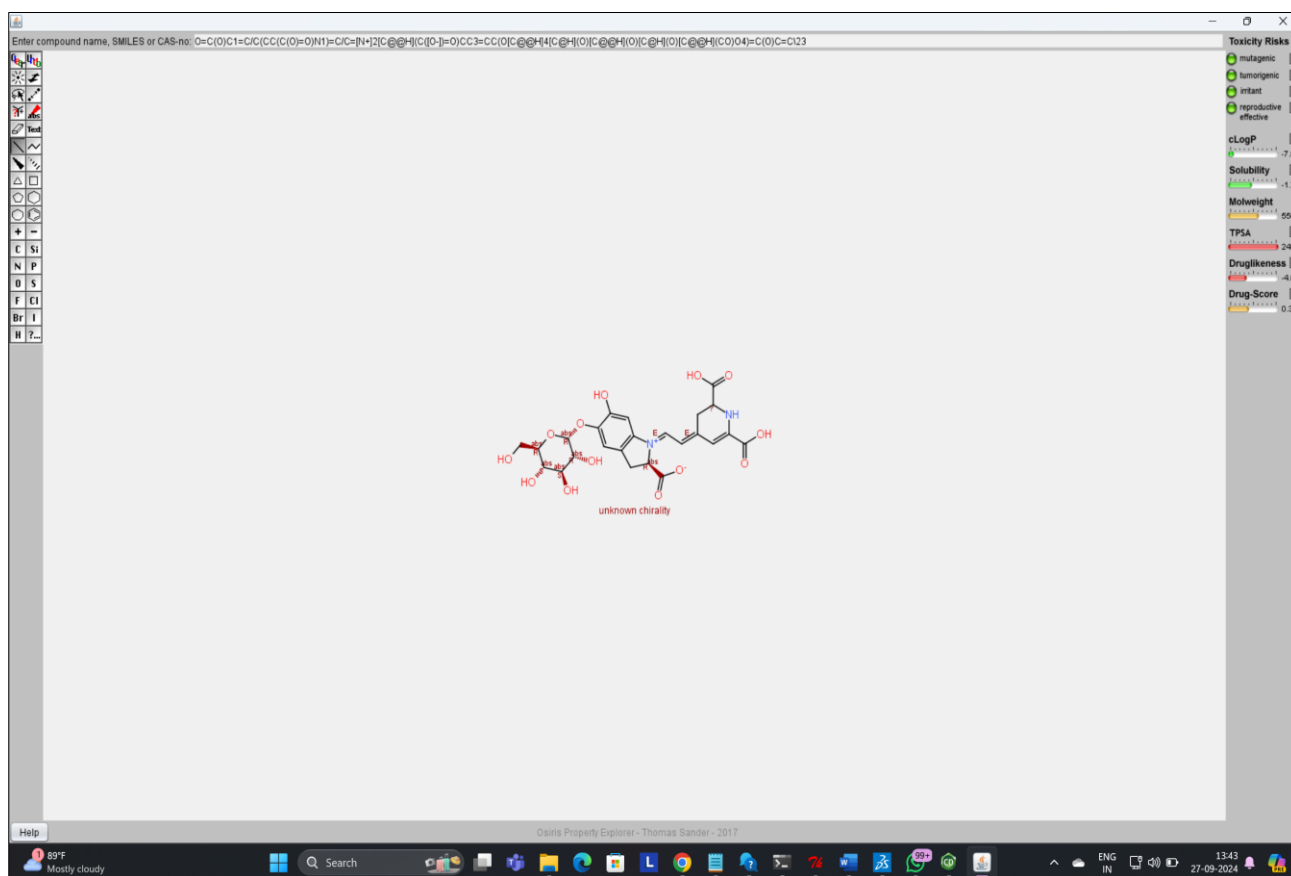**Figure 10: Pharmacokinetic and toxicity profiling of 4-picoline**

Figure 11: Pharmacokinetic and toxicity profiling of betalain

Table 3: Pharmacokinetic Profiling of lead molecules

Compound	ADMET			
	Mutagenic	Tumorigenic	Irritant	Reproductive effectivity
4-picoline	NO	NO	Yes	NO
Betalain	NO	NO	NO	No

Table 4: Lipinski Properties of lead molecules

Compound	*cLogP*	Solubility	Mol.wt.	TPSA	Drug likeness	Drug score
4-picoline	1	-1.15	93	12.06	-2.12	0.32
betalain	-7	-1.70	550	24.01	-4.04	0.34

Table 5: Drug likeness of lead molecules

Compound	Lipinski rule of five	H bond donar(<5)	H bond acceptor (<10)
4-picoline	Mild	8	14
Betalain	Yes	0	1

CONCLUSION

The results demonstrated that each selected lead compound from *B. vulgaris* examined had considerable inhibitory activity against 5'-reductase (SRD5A), hence highlighting its anti-alopecic potential.

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