Thesis

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ABSTRACT

In molecular biology and drug discovery, the capacity to anticipate the binding conformation of ligands within receptors is of utmost relevance. The computational method known as molecular docking is essential to this procedure since it is capable of making precise predictions about ligand-receptor interactions. The objective of this thesis is to enhance molecular docking algorithms, and explore their possible applications. For lighter skin appearance, skin whitening products are available commercially - The most common disorders are melasma and post-inflammatory hyperpigmentation. All of these are addressed using whitening chemicals, which function as different degrees of melanin synthesis in the skin. The enzyme tyrosinase (the primary enzyme in melanogenesis) plays a significant role in this, as do competing inhibitors of tyrosinase. In this work, we investigated how natural whitening products may reduce skin pigmentation and assessed phytochemicals' potential tyrosinase, collagenase, and elastase inhibitory properties.

1: INTRODUCTION

Skin is an imporatant tissue covering the bodies of animals. This organ is in charge of tasks such as protection, control, and feeling (1). The human skin is classified into 3 layers: the epidermis, dermis, and hypodermis (2). The epidermis, also known as the stratum corneum that works as a shield for the internal organs and protects them from external stimulus and regulates cutaneous moisture. The dermis is mostly made up of connective tissue, sweat glands, and nerves. Its primary job is to protect the epidermis (3). Collagen fibres provide skin strength, whereas elastin fibres provide skin flexibility and resistance. Melanocytes are present in the skin's basal layer, which divides the dermis from the epidermis (4). 36 keratinocytes surround a single melanocyte (5). They join together to produce the epidermal melanin unit. Dendrites carry melanin from the melanocyte's melanosomal compartment to the overlaying keratinocytes (6).

This thesis intends to identify and assess phytochemicals with skin-lightening characteristics using computational methods, particularly molecular docking. **The main goal is to find the possible ligands who can alter target proteins involved in melanogenesis, the process of melanin formation. The study also aims to evaluate the therapeutic potential of the identified phytochemicals and clarify the molecular mechanisms underlying their skin-lightening effects.**

1.1 Research Objectives

This thesis intends to identify and assess phytochemicals with skin-lightening characteristics using computational methods, particularly molecular docking. Finding possible candidates who can alter target proteins involved in melanogenesis, the process of melanin formation, is the main goal. The research also seeks to assess how the discovered plant compounds could be used for therapeutic purposes and elucidate the molecular processes responsible for their ability to lighten the skin.

The following research questions are addressed in the thesis:

- Can molecular docking accurately anticipate the interactions and binding affinities between phytochemicals and target proteins involved in controlling skin pigmentation?
- Which phytochemicals have the most binding affinity and have the greatest potential as skin lightening treatments?
- What are the main molecular interactions and processes through which these phytochemicals influence the pathways leading to melanogenesis?

Some research articles were taken from google scholar, crossref, and pubmed. Finally some articles were selected from it in order plan this research. There is a discussion on how and which type of enzymes/proteins has to be selected in order to carry out this research. Some medicinal plants has been chosen which are reported for the treatment of skin. Lastly three enzymes have been selected and further on we will study that how the protein-ligand interaction occurs via Autodock software.

2. LITERATURE REVIEW

2.1 Mechanisms of Skin Pigmentation

Melanin, the pigment that determines skin colour, is produced, transported, and distributed as part of the intricate biological process known as skin pigmentation (7). Several proteins and enzymes, such as tyrosinase control melanogenesis, or the formation of melanin (8). To find possible targets for skin-lightening therapies, it is essential to understand the molecular processes that underlie skin pigmentation. The selection of elastase, tyrosinase, and collagenase as the three targets for a study that can be motivated by their relevance to skin health and appearance. Elastase is an enzyme responsible for breaking down elastin, a protein in the skin that helps to maintain skin's elasticity and firmness.

2.2 Skin-lightening Phytochemicals

Natural substances called phytochemicals, which are produced from plants, have drawn attention for their capacity to lighten skin (13). Numerous phytochemicals have been investigated for their capacity to prevent melanogenesis, including flavonoids, polyphenols, and terpenoids (14). By specifically targeting important enzymes involved in the melanogenesis pathway, such as Tyrosinase and Tyrosinase-related proteins, these substances can influence the formation of melanin (15).

The literature offers details on the structure-activity connections, modes of action, and prospective uses of phytochemicals in skin-lightening remedies.

2.3 Techniques for Molecular Docking

A popular computer method for analysing and predicting the interactions between ligands (phytochemicals) and target proteins is molecular docking (16). To calculate the binding affinity and locate advantageous binding conformations, docking methods use scoring functions. A scoring function is a mathematical model that calculates a score or energy value for each ligand pose generated during the docking process. This score represents the predicted binding affinity or energy of the interaction. Scoring functions typically consist of several components: Van der Waals Interactions that account for the attractive and repulsive forces between atoms and molecules (17). Electrostatic Interactions that consider the charges and distribution of charges within the molecules. Hydrogen Bonding in which scoring functions assess the potential for hydrogen bonding between the ligand and the protein (18). Solvation Energy is the component accounts for the change in energy when the ligand enters the solvent (water) environment. Entropy in which some scoring functions include terms related to entropy or conformational changes upon binding (19). The scoring function computes a score or energy value for each ligand pose based on the components mentioned above. The poses are ranked based on their computed scores. Lower scores generally indicate better binding affinity or stronger interactions. The top-ranked ligand poses are often selected as potential binding modes, and these poses can then be further analyzed or refined. Scoring functions are developed through the parameterization of their components. These parameters are often optimized using experimental data to improve the accuracy of the scoring function (20).

Recent advancements include the use of machine learning algorithms to develop scoring functions that can better predict binding affinities based on training data (21). AutoDock,

GOLD, and Vina are frequently used molecular docking software that provide a variety of

search methods and score options. These methods have been useful in lead optimisation and virtual screening, offering insightful information on ligand-receptor interactions (22).

2.4 Benefits of Molecular Docking in Research on Skin Lightening

Molecular docking has various benefits for skin-lightening studies, including:
It makes it possible to search through enormous chemical databases to find prospective skin-lightening chemicals.
The computational method helps with both the rational design of skin lightening agents and the study of structure-activity correlations (23).

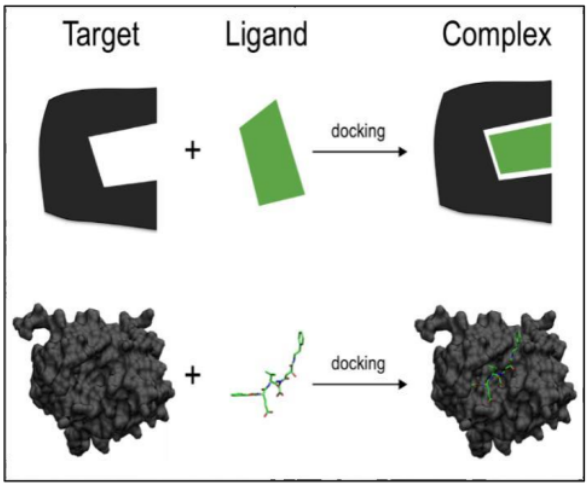


Figure 1: Schematic diagram showing the theory behind molecular docking

2.5 Molecular Docking

Molecular docking predicts the orientation of each molecule within a complex. While one molecule is docked per second, others form stable, permanent connections. This explains that molecular docking predicts the orientation of individual molecules within a complex (24). Information can be mounted in any direction of rotation expect a strong commitment or bond. Two molecules bind with each other by affinity, Molecules such as proteins, peptides and nucleic acids, carbohydrates and lipids play a central role in signal transduction (25). Besides Relative orientation of two interacting partners It can affect the type of signal formed (e.g., Agonist vs. antagonist). Agonists and antagonists are two types of molecules or compounds

that interact with receptors in the body, particularly in the context of cell signaling. They have opposite effects on these receptors and play critical roles in various physiological processes and drug actions. Agonists are molecules or compounds that bind to specific receptors and activate them. When an agonist binds to its target receptor, it triggers a cellular response or signaling cascade. Agonists mimic the natural ligand (substance) that typically binds to the receptor, leading to the same biological response. They promote receptor activation and the downstream physiological or pharmacological effect (26). Antagonists are molecules or compounds that bind to specific receptors but do not activate them. Instead, they block or inhibit the receptor's activation by preventing other molecules, including agonists, from binding.

Antagonists essentially "compete" with agonists for receptor binding sites. When an antagonist occupies the receptor, it prevents the natural ligand or agonist from binding, thereby inhibiting the receptor's signaling pathway (27). Therefore, docking helps to predict both potency and type of interaction between a molecule (typically a ligand) and its target receptor. Molecular docking calculates a score or energy value for each potential binding pose or conformation of a ligand within the receptor's binding site. This score reflects the predicted binding affinity or binding energy. Molecular docking also provides insights into the type of interaction between a ligand and its target receptor. This includes information about the binding mode, such as the orientation of the ligand within the binding site (28).

Basics steps of docking:

- STEP1: Getting the complex PDB.
- STEP2: Cleaning the complex.
- STEP3: Adding the missing hydrogels/side chain atoms and minimize the complex.
- STEP4: Preparing the docking suitable files.
- STEP5: Cleaning of the minimized complex.
- STEP6: Preparing all the required files for docking.
- STEP7: Run the docking.
- STEP8: Analyze the results of docking.

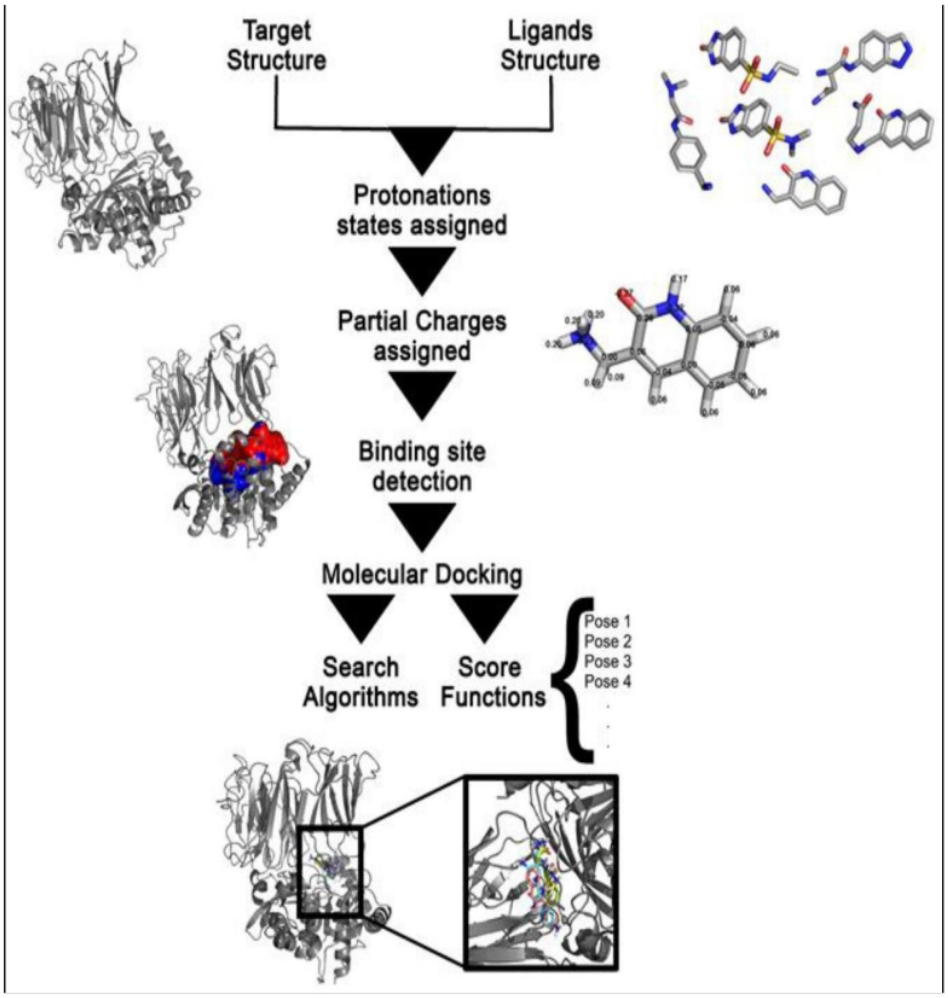


Figure 2: Steps involved in molecular docking

2.6 Mechanism of docking with its importance

The atomic level interactions between tiny chemicals and proteins may be modelled using molecular binding techniques. Small molecule behavior at the binding site can be explained by this. Two fundamental phases make up the joining procedure: i) determination of binding affinity as well as the shape of the ligand, its orientation inside these sites (often referred to as postpositions). Utilizing these two actions scoring should be performed. The effectiveness of docking is considerably increased by knowing the binding site's position prior to docking (29). Comparing the target protein to a protein that crystallizes with a protein family or another ligand with a related function can also reveal information about the location. Blind docking

and targeted docking are two approaches in molecular docking studies, and they differ in their methods and objectives. Here's an elaboration of the key differences between blind and targeted docking. Blind docking is an approach where molecular docking simulations are performed without prior knowledge of the ligand's binding site on the target receptor. The goal is to explore potential binding sites and orientations across the entire receptor surface. Blind docking is typically used in cases where the binding site is unknown or poorly characterized, or when researchers want to investigate the possibility of alternative binding sites on the receptor (30). Targeted docking, on the other hand, involves performing docking simulations with prior knowledge of the binding site on the target receptor. The goal is to predict the binding mode and affinity of a ligand within a specific, predefined binding site. Targeted docking is employed when the binding site is well-defined or when researchers want to explore the interaction of a ligand with a known binding site on the receptor. Blind docking is particularly useful when the binding site is unknown or when researchers want to explore potential off-target interactions. Whereas, targeted docking is applied when the binding site is well-characterized and researchers are interested in studying ligand-receptor interactions within a specific site (31).

Theory of docking: Utilizing computational methods molecular docking aims to anticipate the structure of the ligand receptor complex. Docking can be accomplished by two linked stages. Then use a score system to order these conformations. The scoring function used in molecular docking should assign the highest score or lowest energy to the ligand conformation that closely matches the experimentally observed binding mode. This indicates that the docking method has successfully reproduced the known or expected binding geometry. Placing the experimental binding mode first among all produced conformations is crucial as it indicates the accuracy and reliability of the docking results (32).

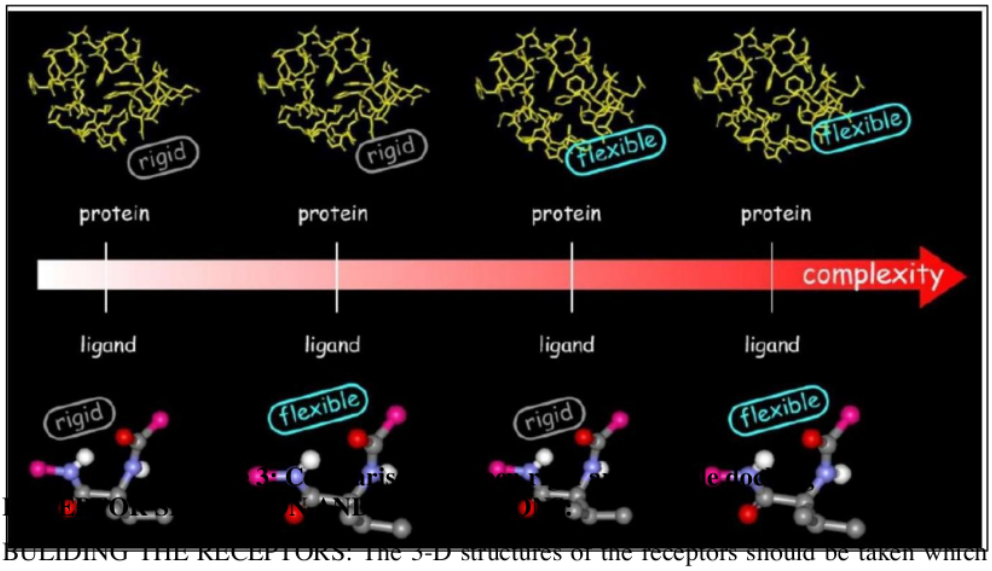
Importance of molecular docking are as follows:

1. Predicting the binding affinity (scoring function)
2. Identifying the ligands in binding sites.
3. Designing of drugs rationally

2.7 Types of Docking

RIGID DOCKING: The inner geometry of both ligands and receptors is assumed to be stiff. These are also referred to as lock and key.

FLEXIBLE DOCKING: Generally, smaller molecules are counted as they rotate, and after each revolution, energy is computed to determine the best position. Protein-ligand, protein-protein, and protein-nucleotide interactions may all be docked. Electrostatic, electrodynamic, steric, and other forms of forces all come into play when two objects dock (33).



BUILDING THE RECEPTORS: The 3-D structures of the receptors should be taken which can be downloaded from RCSB official site in .pdb file format. the available structures should be processed. The receptors should be biologically active and stable (34).

SELECTION OF LIGAND: It can be taken from various databases like zinc, pubchem etc. the ligands are docked onto a receptors are the interactions are checked. After that the scoring function generates the scores depending upon the best fit ligand is selected (35).

THE USE OF AUTODOCK: AutoDock Vina is a computer-assisted docking program based

on a simple scoring function and a fast gradient-optimized conformational tool. The docking of drug-like ligands to proteins is very simple and efficient. This is a free open source molecular docking program. It was originally designed and implemented at the Molecular Graphics Lab. The main reasons for its popularity include:

1. Accuracy: AutoDock Vina significantly improves the typical accuracy of the result predictions.

2. Easy to use: All that's needed is that the structures of the molecules being docked and the specification of the search area including the bindingsite (36).

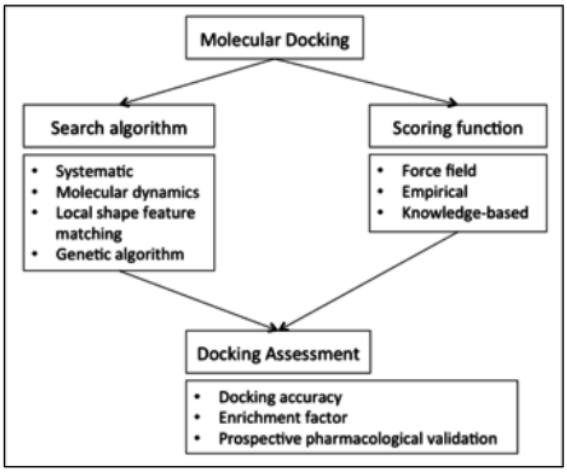


Figure 4: Components of molecular docking

3: METHODOLOGY

3.1 Phytochemical Database Selection

A broad and varied database of phytochemicals is necessary to undertake the screening of skin-lightening phytochemicals. Compounds with proven skin-lightening effects and the availability of structural data are among the selection criteria for the phytochemical database. For gathering the required molecules, well-known databases like PubChem, ChemSpider, or specialised phytochemical databases might be an invaluable resource.

3.2 ADMET properties:

Swiss ADME (http://www.swissadme.ch/) was used to predict the ADME properties of the phytochemicals. The simplified molecular-input line-entry system (SMILES) of the ligands were given as the input and corresponding results were noted down. Swiss ADME also predicts the solubility of the molecules and its qualitative solubility class.

3.3 Selection of Protein Targets

For the screening procedure, it is essential to identify relevant protein targets implicated in melanogenesis.

Target proteins should include important enzymes that are involved in regulating the production of melanin. Hence in this research we have taken 3 different proteins that play a pivotal role in skin pigmentation and health:

1.Tyrosinase (PDB CID: 2Y9X)

2. Collagenase (PDB CID: 1CGL)

3. Elastase (PDB CID: 1BRU)

These proteins are potential locations for the control of skin pigmentation and play crucial functions in the melanogenesis process.

3.4 Protocol for Molecular Docking

STEP1: Getting the complex PDB.

STEP2: Cleaning the complex.

STEP3: Adding the missing hydrogels/side chain atoms and minimize the complex.

STEP4: Preparing the docking suitable files.

STEP5: Cleaning of the minimized complex.

STEP6: Preparing all the required files for docking.

STEP7: Run the docking.

STEP8: Analyze the results of docking.

3.5 Preparation of Ligand

The phytochemicals from the chosen database need to be prepared as ligands prior to docking simulations. In order to make a ligand, hydrogen atoms must be added, molecule geometry must be optimised, and the proper charges must be applied. For ligand preparation chores, software tools like Open Babel or DISCOVERY STUDIO can be employed.

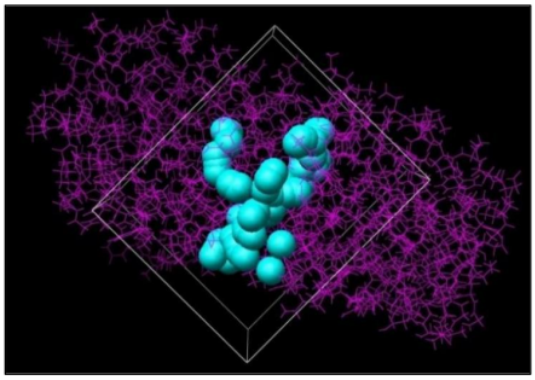
3.6 Preparation of Protein

Prior to running docking simulations, the chosen protein structures must be ready (for example, from the Protein Data Bank). In order to prepare proteins, water molecules must be removed,

hydrogen atoms must be added, partial charges must be assigned, and the protein structure must be optimised. For the preparation of proteins, one can utilize programmes like AutoDock Tools,

3.7 Grid Generation

To specify the area where ligand binding should take place, a docking grid is created around the target protein.The active site or pertinent binding pockets, where ligands are anticipated to



interact with the protein, are covered by the grid.To guarantee thorough sampling while retaining computational efficiency, the grid size and spacing parameters should be properly specified.

Figure 5: Grid box preparation for AutoDock Vina

3.8 Scoring and Docking Simulation

By putting the ready-made ligands inside the created docking grid, docking simulations are carried out. While looking for the ideal binding pose, the docking programme investigates various ligand conformations and orientations. Ranking algorithms assess the ligands' anticipated binding energies and rank them according to their binding affinities. To improve sampling and capture ligand flexibility, we used several docking runs (run number set to 9).

3.9 Evaluation and Analysis

The results of the docking simulations are examined to find top-ranked ligands that may have skin-lightening potential. Focusing on important residues and binding motifs, the molecular interactions between the ligands and target proteins are investigated.

4: RESULTS AND ANALYSIS

4.1 Analysis of ADMET properties

The ADMET properties of all the phytochemicals that are present in the parent database of 112 compounds are presented in Table S2. Initial screening revealed that 37 phytoconstituents do not pass the Lipinski's filter for drug-likeliness of molecules. Hence those compounds are not considered in our subsequent molecular docking. The phytochemicals that do not violate the Lipinski's rule of five are selected as our potential target ligands and the screening of hit molecules is done using the subset of parent database (i.e. containing 175 compounds).

4.2 Analysis of binding affinity and molecular interactions

A set of docking findings is produced by screening the phytochemical database against the chosen protein targets using molecular docking. The entire table of binding affinity of all the chosen ligands with the three receptor molecules is presented in Table S1. The docking outcomes include details on the projected binding mechanisms and binding affinities of the ligands with the target proteins. The intensity of the interaction between the ligand and the protein is indicated by the binding affinities, which are frequently reported as docking scores or binding energies. The outcomes are examined to determine the ligands with the highest binding affinities, which may have skin-lightening potential.

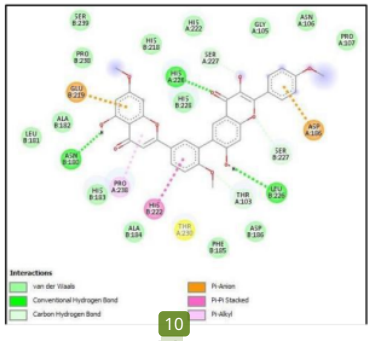
The investigation of the precise molecular interactions between the top-ranked ligands and the target proteins is the main goal of the analysis. Hydrogen bonds, hydrophobic contacts, electrostatic interactions, and - stacking interactions are among the interactions. The binding motifs and potential hotspots are highlighted by the identification of key residues involved in ligand-protein interactions. The molecular processes by which the ligands influence the target proteins and prevent melanogenesis are better understood

4.3 Prioritisation of Potential Candidates for Skin Lightening

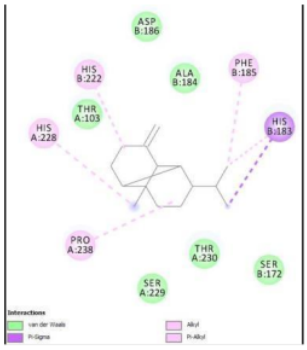
Potential candidates for skin lightening are ranked according to the, binding affinities i.e, molecular interactions. Promising choices are ligands with the highest binding affinities, robust molecular interactions, and advantageous structural characteristics. The potential for skin lightening of the prioritised candidates can be confirmed using further computational techniques or experimental testing.

Table 1: Phytochemicals exhibiting strong interaction with all the chosen 3 receptors

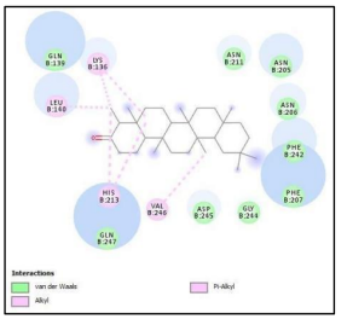
Compound	Binding affinity (in kcal/mol)		
	Tyrosinase	**Elastase**	**Collagenase**
1.beta-Copaene	-10	-9.1	-8.6
2.Kaempferol-7-rhamnoside	-10.1	-8.8	-10.6
3.Abiesin	-10.2	-8.7	-11.5
4.Flavanomarein	-9.2	-8.2	-10.1
5.Quercitrin	-8.5	-8.2	-9.5
6.Taraxerol	-10.9	-8	-10.8
7.Friedelin	-9	-8	-8.6
8.Hesperidin (standard skin-lightening agent)	-9.9	-8.4	-11.2


Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Anion
Pi-Pi Stacked
Pi-Alkyl

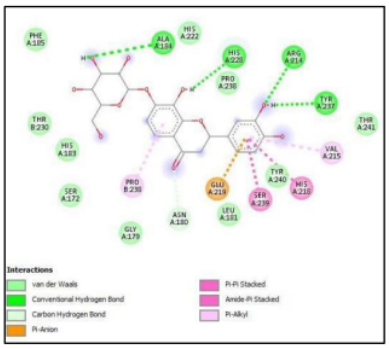
(a)


ASP B:186
HIS B:222
ALA B:184
PHE B:185
THR A:103
HIS A:228
HIS B:183
PRO A:238
THR A:230
SER B:172
SER A:229
Interactions
van der Waals
Pi-Sigma
Alkyl
Pi-Alkyl

(b)


Interactions
van der Waals
Alkyl
Pi-Alkyl

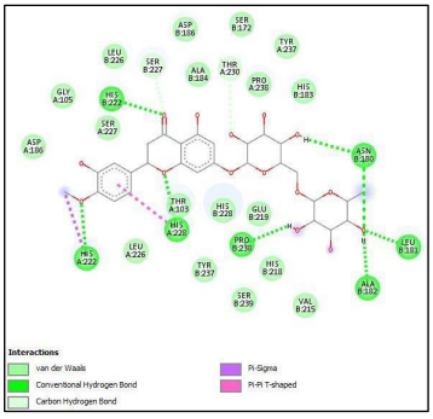
(c)

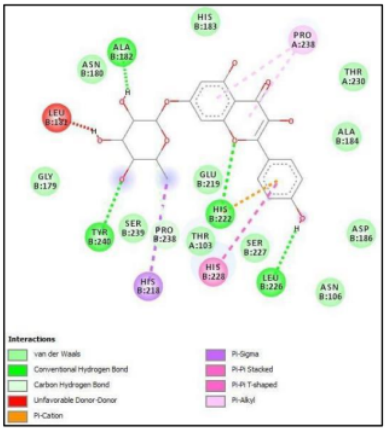

Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Anion
Pi-Pi Stacked
Amide-Pi Stacked
Pi-Alkyl

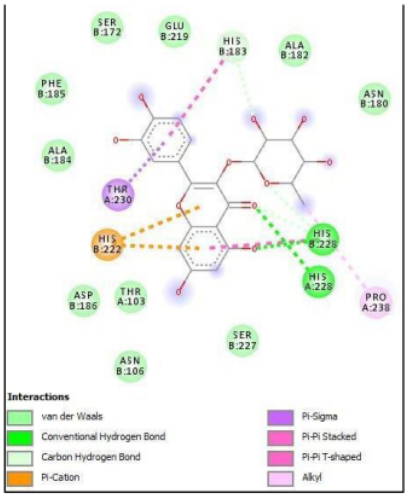
(d)

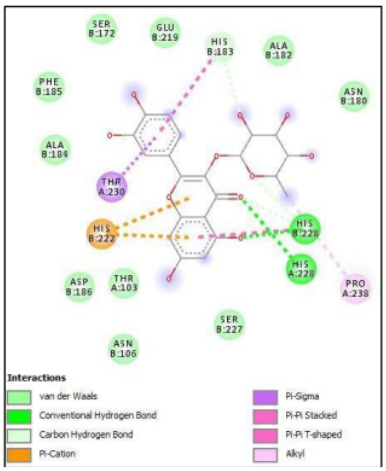
(e)

(f)


Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sigma
Pi-Pi T-shaped


Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Cation
Pi-Sigma
Pi-Pi Stacked
Pi-Pi T-shaped
Pi-Alkyl





(g) **(h)**

Figure 6: 2D interaction diagram showing the interacting residues of the hit molecules with the receptor collagenase: (a) abiesin, b) beta-Copaene, (c) friedelin, (d) flavanomarein, (e) Hesperidin, (f) kaempferol-7-rhamnoside, (g) quercitrin, (h) taraxerol

Figure 6 reveals the binding pockets of the hit molecules with the receptor collagenase. It is observed that the phytochemicals abiesin, beta-Copaene, friedelin, flavanomarein, kaempferol- 7-rhamnoside, quercitrin, and

taraxerol share some identical interacting residues with the standard skin lightening agent hesperidin. This elucidates similar mechanism of action of the highlighted phytochemicals on the three enzymes tyrosinase, elastase and collagenase when compared with the standard compound hesperidin.

4.4 Evaluation in Relation to Common Skin Lightening Agents

The outcomes of molecular docking can be contrasted with those of well-known skin- lightening products, both natural and artificial. By considering the ligands' binding affinities to those of known skin-lightening agents, the comparison of their values aids in determining the effectiveness and potential of the compounds. It sheds information on the uniqueness, superiority, and possibility for use as substitutes for traditional skin lightening agents.

The intrinsic limitations of molecular docking, such as its reliance on structural data and simulation assumptions, may be one source of restrictions. Future efforts might include experimental confirmation of the prioritised ligands, more optimisation using computational methods, or in vivo tests to assess safety and effectiveness.

In molecular biology and drug discovery, the capacity to anticipate the binding conformation of ligands within receptors is of utmost relevance. The computational method known as molecular docking is essential to this procedure since it is capable of making precise predictions about ligand-receptor interactions. The objective of this thesis is to enhance molecular docking algorithms, and explore their possible applications. For lighter skin appearance, skin whitening products are available commercially - Melasma and Post- inflammatory hyperpigmentation are the major disorder of this. These all are treated by whitening agents which acts as various levels of melanin production in the skin. The enzyme tyrosinase plays a major role in this known as competitive inhibitors of tyrosinase (the key enzyme in melanogenesis). In this study we elucidated how natural skin lightening agents may decrease the pigmentation in skin and highlighted the potential phytochemicals that can act as multi-targeted inhibitors of tyrosinase, collagenase and elastase.

Thesis

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