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In-Silico Analysis of Bael Leaves Phyto-Components Treatment Against Corneal Opacity of Amur Carp (*Cyprinusrubrofuscus*)

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Abstract:: India has the oldest herbal practices in the world. Many studies have investigated the use of phytoconstituents found in plants in the treatment of various diseases. However, there are currently few studies on the phytoconstituent properties and pharmacological effects of *Aegle marmelos*. This study used computer simulation methods to try to discover the therapeutic potential of plant components in the leaves of *Aegle marmelos*in treating eye opacity in Northeastern carp. Auto Dock software was used to perform in silico analysis and Bio was used to visualize the docking results via Discovery Studio.This study focuses on screening the therapeutic activity of potential pharmacophores in the gene responsible for eye opacity in *Cyprinus rubrofuscus*(i.e., Amur carp). Computer analysis. Studies such as drug probability, pharmacokinetic properties, rule of five, and bioavailability radar play a crucial role in identifying suitable pharmacophore groups for further insillico.
ico analysis. The molecular docking of seven selected compounds showed good interactions with the desired eye proteins and revealed significant binding affinities. Among the seven compounds, Cuminaldehyde (13.60 Kcal/mol) showed a higher molecular docking score, providing concrete evidence that the application of sweet-scented osmanthus plant components can be an effective strategy for treating eye opacity in carp.

Keywords: *Aegle marmelos*, corneal opacity, *Cyprinus rubrofuscus*, In-silico.

1. Introduction

The material of traditional drugs was derived from native plants since time immemorial. In our country, there are various traditional remedies involving roots of plants and other parts; thus, it is important to conduct scientific research on these roots for medical purposes (Gupta, 1994). Over the past thirty years, extracts from plants have been frequently used in treating different diseases. *Aegle marmelos*, (Bael tree), is a highly valued and complex plant with profound ecological, medicinal, and cultural significance. This deciduous tree belongs to Rutaceae family which is not only found in the Indian subcontinent but also in many other tropical regions around the world.The fruit is called "marmelos" in Latin because of its resemblance to quince while the Greek word "aiglē" means radiant or splendid (Logesh et al., 2023). The bael tree holds a prominent place in religious and cultural traditions. Known as bilva or bilwa tree, it is worshipped as Lord Shiva's sacred plant according to Hindu mythology. Ancient scriptures like Vedas and Puranas regard both leaves and flowers along with fruits from this tree as highly propitious items used during various religious rituals and ceremonies. Moreover, the extracts of bael fruits are suggested to have anti-cancer effects and are therefore being studied for their possible role in treating and preventing cancer (Sharma et al.,2016). In Ayurveda, an ancient Indian medicinal system, the therapeutic properties of bael leaves are highly valued and thus used in making various herbal drugs. These leaves have a wide range of pharmacological actions including anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, and analgesic activities. Bioactive compounds like flavonoids; alkaloids; tannins etcetera in it contribute towards its therapeutical effect. Commonly bael leaf extract is employed against many diseases such as gastrointestinal disorders like diarrhea; dysentery; IBS etcetera and also respiratory illnesses such as asthma; and bronchitis. They are also believed to possess antidiabetic nature which helps in controlling blood sugar levels among diabetic patients.

The*Cyprinus rubrofuscus*, a freshwater fish that is originally from East Asia, is widely farmed to produce food and support recreational fishing. However, they are prone to catching diseases easily and reacting badly to environmental factors such as herbicide exposure which can negatively affect their health and population dynamics. One of the principal concerns about herbicide exposure on Amur carp lies within the possible development of diseases linked with immune suppression and physiological stress. Herbicides are capable of undermining the defence mechanism of these creatures by causing hormonal imbalances associated with immunity as well as lowering the potency level displayed by immune cells thereby making them more susceptible to infections or ailments. Further still it has been found that contact with herbicides induces oxidative stress that leads to cellular injury throughout various vital organs such as the liver kidney gill etcetera in fish. Such physiological disruptions undermine overall fitness among populations of Amur carp thus rendering them less resistant to disease outbreaks besides other environmental challenges (Dhot et al., 2022). Common

carp along with amur carp species suffer from corneal opacity which gives rise to clouding or haziness over the cornea itself. The cornea is the outermost part of the eye through which light passes before getting reflected onto the lens so that vision can occur; it serves as a window through which we see the world around us. Injury infection malnutrition environment

2. Material & methods

2.1. *Protein identification and preparation*

The protein produced in the eye gene is associated with the corneal opacity disease of *Cyprinus rubrofuscus*. The protein data bank (PDB) provided the identified molecular targets linked to the eye disease of *Cyprinus rubrofuscus*that causes corneal opacity. Water molecules, heavy atoms, cofactors, and metal ions are all present in the recovered PDB. Therefore, the Discovery Studio prepared protein technique was used to generate the downloaded PDB structure.

2.2. *Selection of phytocompounds*

Important phytocompounds have been identified for the investigation of the present studyafter a thorough review of the literature. This research endeavours to clarify the mechanisms of action of these particular phytocomponents and investigate their potential applications in drug development and disease management. The primary goal of this study was to determine the mechanism of action of the phytocomponents present in *Aegle marmelos* as well as their potential for use in the development of new drugs and the treatment of disease. The review literature has helped us identify some of the phytocomponents of Aegle marmelos in our research.

2.3. *Ligand preparation and filtration*

Eight phytocomponents from *Aegle marmelos* were taken out and utilized as ligands in the docking study. The PubChem database provided the three-dimensional structures of these compounds. Then, using the prepare ligand process from Argus lab, these ligands were cleaned up, their 3D coordinators were calculated, and ligand conformations were created.when synthesis, the compounds were screened based on their molecular properties to predict their solubility and permeability in medication development. Lipinski's "rule of five,"is the most well-known physical property filter which emphasizes bioavailability. The compounds have to meet certain requirements, such as having a maximum molecular mass of 500 daltons, a maximum of five log P values for the octanol-water partition coefficient, and a maximum of 10 hydrogen bond acceptors and donors.

2.4. *Molecular docking*

A molecular docking study was carried out using AutoDockTools-1.5.6, selecting an appropriate docking strategy for each of the selected ligands with the preferred protein. To retrieve the 2D structure of each ligand, the PubChem website was used. Using the RCSB (Research Collaboratory for Structural Bioinformatics) Protein Data

Bank (https://www.rcsb.org/), the target protein's PDB code matching to the Amur carp eye has been obtained. Target protein dehydration, ligand separation, and binding site assignment are all part of the applied molecular docking approach. A visualization of the ligand-protein interactions seen in Fig. 1 was made using the BIOVIA Discovery Studio Visualizer.

2.5. *Drug likeliness*

Drug likeness is an important concept in pharmaceutical research that refers to the possibility for a compound to have qualities suitable for further development as a drug candidate. Many factors contribute to this, one of them being pharmacokinetic properties such as bioavailability, safety and efficacy among others (Lipinski & C. A., 2004). These considerations are very important since they help identify molecules with the potential to become successful drugs. For instance; Absorption Distribution Metabolism Excretion Toxicology (ADMET) assessments are necessary during drug discovery because about 60 %of all drugs fail at clinical stages due to poor ADMET profiles. Another key point is that we need good lead compounds that can be easily absorbed, transported, or eliminated from the body if we want our search for new medicines through these channels to succeed. Additionally, it is also worth mentioning that metabolism determines both the duration as well intensity of action while excretion ensures no toxicity arises through accumulation thus leading to elimination after being transformed into other forms by different organs within the system (Hughes et al., 2008).

3. Results

Various pharmacophores of *Aegle marmelos* leaves have been listed through distinctive literature surveys and their pharmacokinetic and toxicological profile (ADMET properties) was analyzed/ *viz.* Swiss-ADME software and Pro-TOX II. The canonical smiles of all the pharmacophores of bael leaves listed are copied from PubChem and pasted into the SwissADME software and Pro-TOX II software one by one to confirm their therapeutic efficacy and toxicity profile. The outcomes obtained from the studies wereobserved.

3.1. *In Silico SwissADME Analysis*

3.1.1. *Physicochemical Properties*

Ethical parameters and physicochemical properties of designed potential drug candidates play an important role in absorption, distribution, metabolism, and excretion parameters as well as in other stages of the drug development process. It is important to evaluate how medicine will interact with biological systems and take into account certain features of these specialties that should be present in a molecule and features that must be avoided in a molecule. All compound's molecular weight exhibited a value that met the required ≤500 g/mol threshold as shown in Table 1.

There are less than ten rotatable bonds in each of the compounds under study. Furthermore, the compounds have molar refractivity values falling between 40 and 130 as shown in Table 1. TPSA explained another important factor that is related to the bioavailability of the drug. Highly oral bioavailable and passively absorbed have a TPSA of less than 140 Å2. (Johari et al., 2019).Table 1 shows that all of the selected compounds are polar, with cineole having the lowest TPSA value and TPSA values ranging from 9.23 Å2 to 58.56 Å2. All seven of the compounds in Table 1 have good to moderate water solubility and may effectively provide oral adsorption, with Log S values ranging from 2.03 to 2.78. The other chemicals are very soluble, but aegeline and skimmianine are just moderately soluble.

3.1.2. Pharmacokinetics Properties:

Most of the parameters related to pharmacokinetics are measured in terms of volume of distribution, half-life, clearance, bioavailability, etc. Bioavailability of a medication refers to the percentage of medication that is remaining in the bloodstream post-administration of a dose. Clearance refers to the speed at which a drug is moved out of the body; this will also have an impact on the dosage interval and duration. Other than that, the CYP superfamily, cytochrome P450, is vital. Less skin-permeant the MCU is greater the negative log Kp (cm/s) value. According to Table 2, the MCU of all previously taken medications recorded has a diverse log Kp value that varies from -6.38 to -4.52.

3.1.3. Rule-of-Five by Lipinski

The Lipinski Rule of Five is highly applicable in ADME research since it can predict the oral bioavailability of drug cells based on physicochemical features. This rule allows quick determination of "the chance of a chemical becoming a drug when taken orally" by considering four variables: molecular weight, lipophilicity (logP), hydrogen bond donors, and hydrogen bond acceptors. Since limited resources are available, the Rule of Five may be useful for the compound-selection strategy, useful for focusing resources on compounds with the best chances of succeeding. This with the aid of the Rule of Five could help drive compound selection and prioritization, which can aid in hurrying drug development. This research concludes that the series might be used in drug-like chemical expansion potential because every compound passed the ADME screening.This study illustrates how the series may be employed for drug-like compound growth potential because every compound passes the ADME screening and moves on to the molecular docking analysis (Table 1-3).

3.1.4. Medicinal Chemistry

PAINS are substances that elicit a deceptive signal and also have a strong physiological influence irrespective of the protein receptor to which they attach. Table 3 indicates that all drugs had a PAINS signal. The standard range for the SA score for candidates

in our library is between 1 and 10 1poorly organized to 10 organized. More library candidates had an SA score of less than 4, implying that the VS campaign included drugs with a moderate synthesis possibility. The synthesis feasibility of each library candidate implies that the medicinal chemist has to simplify synthesis to find compounds that may be used in follow-up checks in the biology assay. The medicinal chemist may deduce the necessary chemical for lead optimization using a rule-based approach named lead-like. Aegeline and skimminianie are valid lead optimization starting points since they do not violate lead-like as Table 3 indicates.

3.1.5. Bioavailability Radar

The bioavailability radar may be used to quickly determine how similar a chemical is to a medicine. For a molecule to be classified as drug-like, its radar plot needs to be completely contained inside this area. According to Ritchie et al. (2011), the pink area represents the physiochemical space needed for oral bioavailability. Eight compounds are deemed to have competent chemotherapeutic potential based on the Swiss ADME prediction performance since they exhibit the ideal range of all six features(Figure1).

3.2. Molecular docking analysis

The antiviral activity of the seven compounds selected by Swiss ADME was achieved by molecular docking experimental investigation. The SWISS model visualized the interaction of the selected compounds with the primary pathological factor to explore the interaction of the protein 1B8Ron the eye of the Cyprinus carpio. The first step of interaction was obtaining the coordinate crystal of the 1B8R protein from the PBD bank. The structure was taken to the auto port to dock. The Auto Dock was applied to filter out several dock complexes depending on the dock fitness value. The effective dock was defined as the dock compound that coordinates with the receptor. The results were evaluated by the binding fitness or the score at the instant a compound is chosen as a drug depending on its effectiveness toward binding with the molecule.

A 2D depiction of the protein-ligand interactions of the best postures generated by the discovery studio is presented in Table5. Every molecule assumes the binding mode, as Table-3 makes evident. These atoms' principal interactions can be determined.

4. Conclusion

The above In-Silico ADMET predictions and docking study can offer important insights, but they cannot entirely substitute thein-vitro or in-vivo experiments. The present in-silico investigation on *Aegle marmelos* leaf extract highlights its applicability in Ocular opacity in *Cyprinus rubrofuscus* owing to its free radical scavenging activity, anti-inflammatory activities, and wound healing activities. Selected 7 compounds from the leaf extract have proven their eligibility as potential drug candidate owing to their pharmacokinetic and drug likeliness evaluation. Molecular docking analysis of the selected 7 components revealed significant inhibition of the target protein and higher

binding affinity with the host ocular cell of *Cyprinus rubrofuscus* confirmingits potentialapplicability. Compounds like Aegeline,Citronellal,Cinole, and Cumaldehyde have shown higher binding affinity revealing their effective pharmacological ability. Although no comprehensive studies have been published, it is important to highlight that various in-Silico methodologies that are pertinent for ADMET prediction can be categorized as promising because they appear to have a probable future. The pharmacological and molecular analyses provided by these preliminary outcomes can serve as a helpful framework for the development of novel therapies for improving the life span and growth of *Cyprinus rubrofuscus*.

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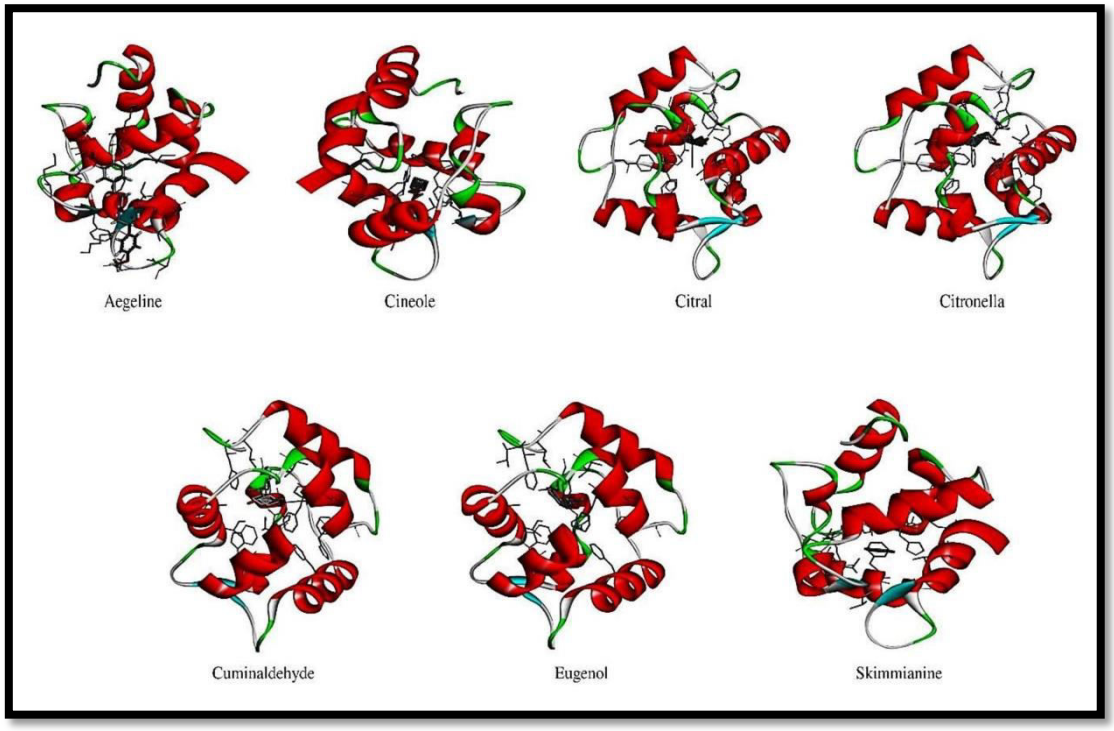


Figure 1: Protein-ligand interaction; a- 1B8R-Aegeline interaction, B- 1B8R-Cineloe interaction, C-1B8R-Citral interaction, D- 1B8R-Citronellal interaction, E- 1B8R-Cuminaldehyde interaction, F- 1B8R-Eugenol interaction, G- 1B8R-Skimmianine interaction.

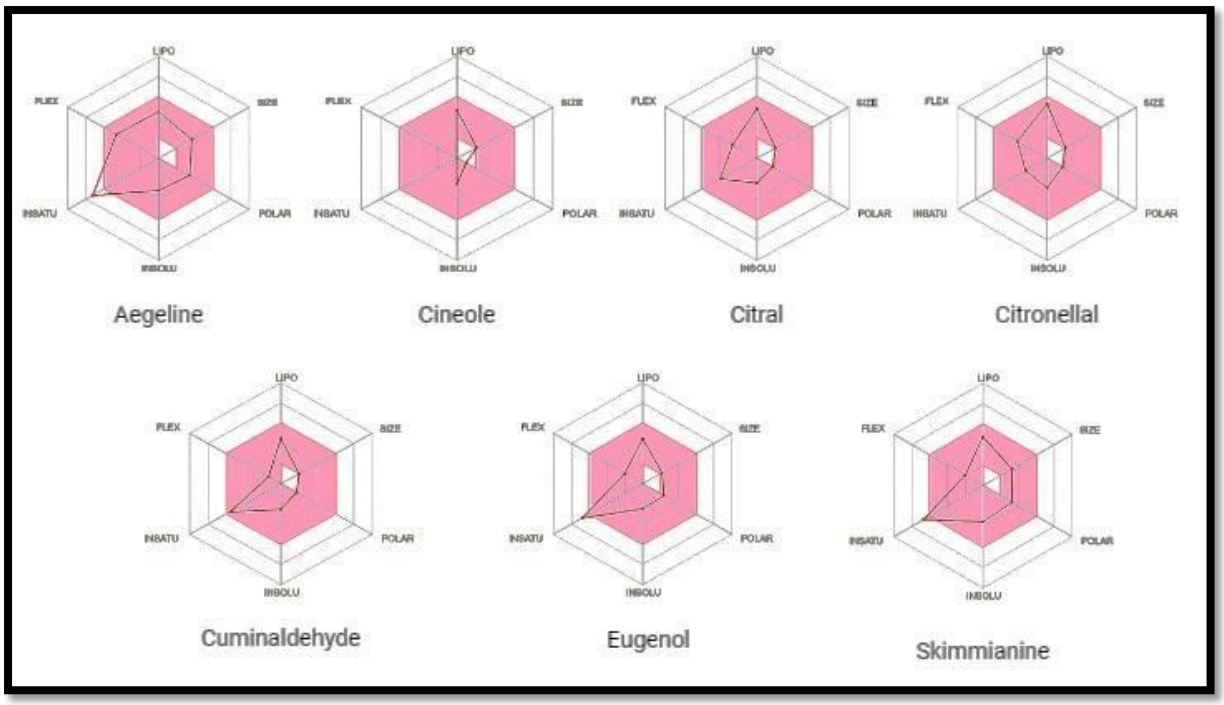


Figure 2:For the compounds under investigation, the bioavailability radar (pink area) shows the ideal range for each particular property: LIPO (lipophilicity as XLOGP3), SIZE (size as molecular weight), POLAR (polarity as TPSA (topological polar surface area), INSOLU (insolubility in water by log S scale), INSATU (instauration as per fraction of carbons in the sp3 hybridization), and FLEX (flexibility as per rotatable bonds).

Table 1: Prediction of the physiochemistry of a few chemicals found in *Aegle marmelos* leaves MW: molecular weight, fraction Csp3 ≤ 0.25, permissible range: <500 g/mol Acceptable ranges for HBA and HBD are ≤10 and ≤5, respectively, for hydrogen bond acceptors and donors. ESOL stands for water solubility, Log p for high lipophilicity (recommended range: ≤5), Molar refractivity (MR): 40 to 130 is the acceptable range. Topological polar surface area, or TPSA.

Sl no.	**Compound**	**Formula**	**Molecular weight**	**Fraction Csp3**	**RB**	**HBA**	**HBD**	**MR**	**TPSA**	**ESOL**	**Lipophilicity Conseco Log p**
1.	Aegeline	C18H19NO3	297.35 g/mol	0.17	7	3	2	86.10	58.56 A^2	M. SOL	2.54
2.	Cineole	C10H18O	154.25 g/mol	1.00	0	1	0	47.12	9.23 A^2	SOL	2.58
3.	Citral	C10H16O	152.23 g/mol	0.50	4	1	0	49.44	17.07 A^2	SOL	2.47
5.	Citronellal	C10H18O	154.25 g/mol	0.70	5	1	0	49.91	17.07 A^2	SOL	2.49
6.	Cuminaldehyde	C10H12O	148.20 g/mol	0.30	2	1	0	46.41	17.07 A^2	SOL	2.03
7.	Eugenol	C10H12O2	164.20 g/mol	0.20	3	2	1	49.06	29.46 A^2	SOL	2.37
8.	Skimmianine	C14H13NO4	259.26 g/mol	0.21	3	5	0	70.99	53.72 A^2	M.SOL	2.78

Table 2: Prediction of the pharmacokinetics of pharmacophores obtained from of *Aegle marmelos*.

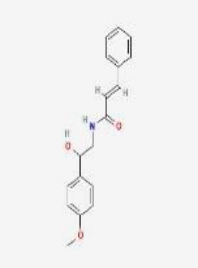
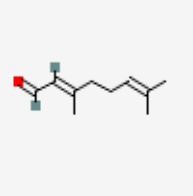
Sl no.	Compound	GIA	BBBP	P-gpS	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log Kp
1.	Aegeline	High	Yes	No	Yes	No	No	Yes	Yes	-6.38cm/s
2.	Cineole	High	Yes	No	No	No	No	No	No	-5.30cm/s
3.	Citral	High	Yes	No	No	No	No	No	No	-5.08cm/s
4.	Citronellal	High	Yes	No	No	No	No	No	No	-4.52cm/s
5.	Cuminaldehyde	High	Yes	No	Yes	No	No	No	No	-5.52cm/s
6.	Eugenol	High	Yes	No	Yes	No	No	No	No	-5.69cm/s
7.	Skimmianine	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-5.87cm/s

Table 3: Prediction of the drug likeness and medicinal chemistry of a few chemicals found in *Aegle marmelos* leaves

		Drug Likeness						Medicinal Chemistry		
Sl no.	Compound	Lipinski	Ghose	Veber	Egen	Muegge	BAS	PAINS	Lead Likeness	Synthetic accessibility
1.	Aegeline	Yes	Yes	Yes	Yes	Yes	0.55	0 alert	Yes	2.85
2.	Cineole	Yes	No	Yes	Yes	No	0.55	0 alert	No;1 violation	3.65
3.	Citral	Yes	No	Yes	Yes	No	0.55	0 alert	No;1 violation	2.49
4.	Citronellal	Yes	No	Yes	Yes	No	0.55	0 alert	No;2	2.57

									violation	
5.	Cuminaldehyde	Yes	No	Yes	Yes	No	0.55	0 alert	No;1 violation	1.00
6.	Eugenol	Yes	Yes	Yes	Yes	No	0.55	0 alert	No;1 violation	1.58
7.	Skimmianine	Yes	Yes	Yes	Yes	Yes	0.55	0 alert	Yes	2.93

Table- 4: Docking interactions between a few of *A. marmelos*'s bioactive substances.

Sl. No.	**Compound**	**Pub Chem ID**	**2D Structure**	**Binding score**	**Residue**	**Hydrophobicity**	**Pka**
1.	Aegeline	15558419		-9.276	ASP8	-3.5	3.9
					ASP10	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					ASP22	-3.5	3.9
					HIS26	-3.2	6
					LYS27	-3.9	10.4
2.	Cineole	10106		-12.98	ASP8	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					ASP22	-3.5	3.9
3.	Citral	638011		-11.56	ASP8	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					HIS26	-3.2	6
4.	Citronellal	7794		-10.43	ASP8	-3.5	3.9

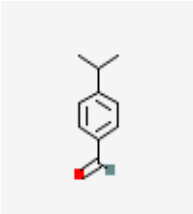
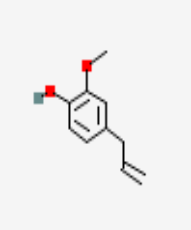
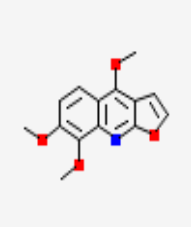
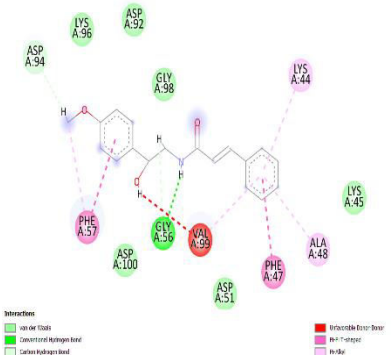
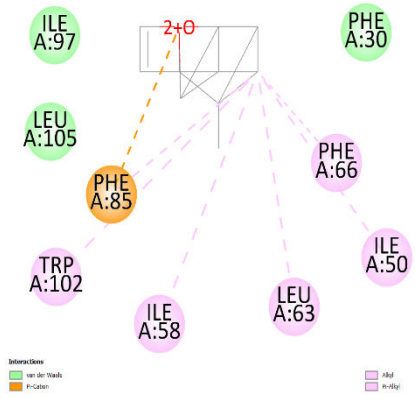
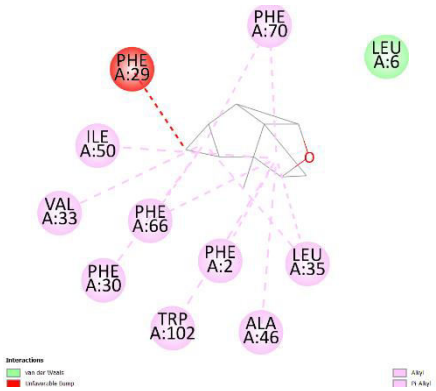
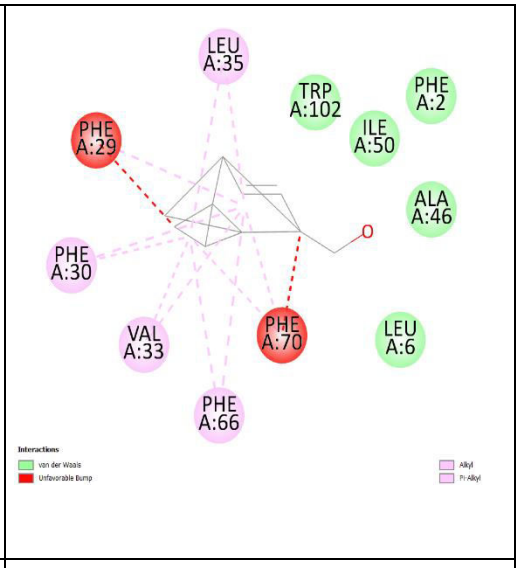
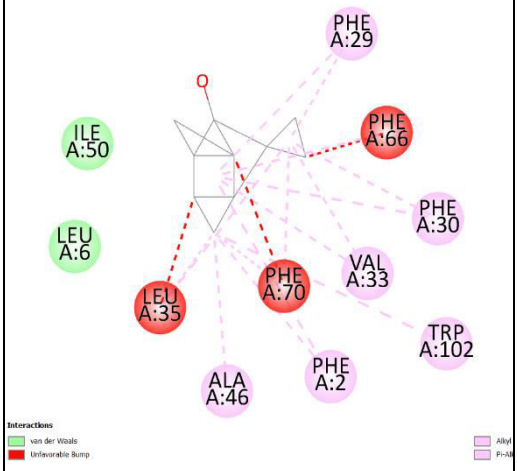
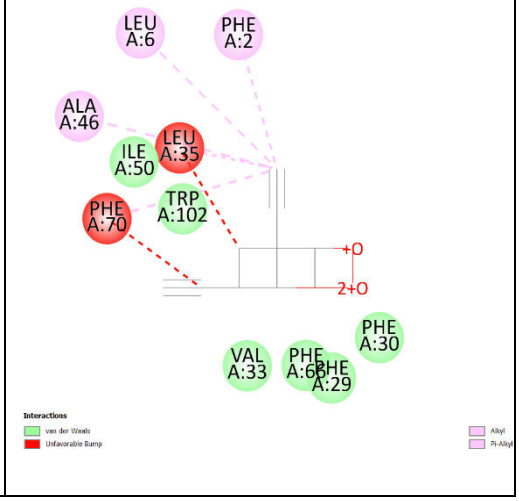
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					HIS26	-3.2	6
5.	Cuminaldehyde	326		-13.60	ASP8	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					HIS26	-3.2	6
6.	Eugenol	3314		-9.47	ASP8	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.9	10.4
					HIS26	-3.2	6
7.	Skimmianine	6760		-3.38	ASP8	-3.5	3.9
					GLU16	-3.5	4.3
					CYS18	2.5	9
					LYS19	-3.5	10.4
					HIS26	-3.2	6

Table5: Screened chemicals as ligands in silico molecular docking interactions of 2Dstructures involving ocular proteins.

SL No.	Compound	Protein	2D Structure
1.	Aegeline	1B8R	
2.	Cineole	1B8R	
3.	Citral	1B8R	

4.	Citronellal	1B8R	
5.	Cuminaldehyde	1B8R	
6.	Eugenol	1B8R	

7.	Skimmianine	1B8R