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Invitro And Insilico Assessment of Anti-Cataract Activity of Ethanolic Leaf Extract of *Jasminum grandiflorum* L. Against Glucose- Induced Cataractogenesis in Goat Eye Lens

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ABSTRACT

Background of the study: The present study aimed to evaluate the anti-cataract activity of ethanolic extract of *Jasminum grandiflorum* L. plant leaf using *in silico* and *invitro* methods.

Methods:*Jasminum grandiflorum* L. plant leaf were collected, shade dried and then pulverized. The ethanolic extract of *Jasminum grandiflorum* L. were prepared by using ethanol followed by maceration method. Preliminary phytochemical screening was performed by using standard operating procedures. The anti-cataract activity of plant extract was assessed by using in silico and invitro studies. *In silico* studies are carried out using molecular docking studies and *invitro* studies were performed by photographic evaluation of goat eye lens, Determination of Total Protein Content, catalase enzyme assay.

Conclusion: The preliminary phytochemical analysis indicated the presence of flavonoids, tannins, saponins, alkaloids, proteins, carbohydrates. In *in silico* studies, ligand molecule Quercetin, Rutin, Kaempferol shows binding affinity to AKR1B1 and CRYAB receptors respectively. In this study, the results demonstrated that the ethanolic extract of *Jasminum grandiflorum* L. leaf extract possess anti-cataract activity.

Keywords: Anti-cataract activity, *Jasminum grandiflorum* L., Docking studies, *Invitro* studies.

I. INTRODUCTION

Opacification, or lens optical failure, is the main cause of cataracts, which are visual impairments that reduce lens transparency. It deteriorates vision by reducing the amount of light that reaches the eye .[1,2] Cataracts account for over 80% of cases of blindness in India. There is increasing interest in developing plant-based antioxidant nutrients that may be helpful in delaying or preventing cataract development because there is sufficient evidence that oxidative stress plays a role in the pathways behind cataract genesis .[3,4]

Clinically, cataracts can be categorized as nuclear, cortical, or posterior subcapsular cataracts according to where they are located within the lens. Blurred vision, glare, faded colours, and trouble seeing at night are some of the symptoms. Ongoing research investigates phytochemical and pharmacological interventions targeted at postponing cataract onset or progression through antioxidant and antiglycation mechanisms, even though surgical removal of the opacified lens and implantation of an intraocular lens (IOL) continues to be the most effectivetreatment.[5] According to estimates, cataracts would lead to 78.8 million cases of moderate to severe vision impairment and 15.2 million cases of blindness worldwide in 2025. Despite the fact that access to cataract surgery is improving in many areas, these numbers nonetheless reflect a persistent worldwide health concern.

TYPES OF CATARACTS

Cataracts are classified based on their location within the lens, cause, and appearance. The major types include:

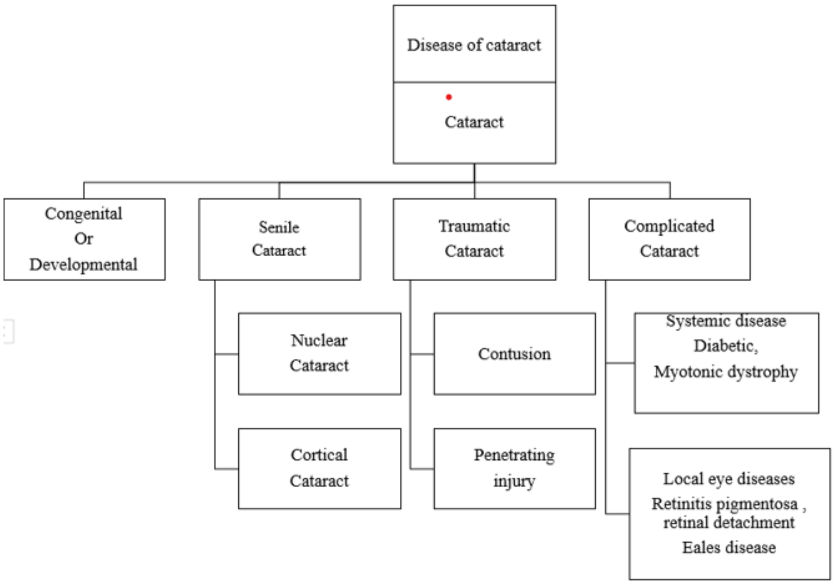

Fig no:1.1 Types of cataract

PATHOPHYSIOLOGY

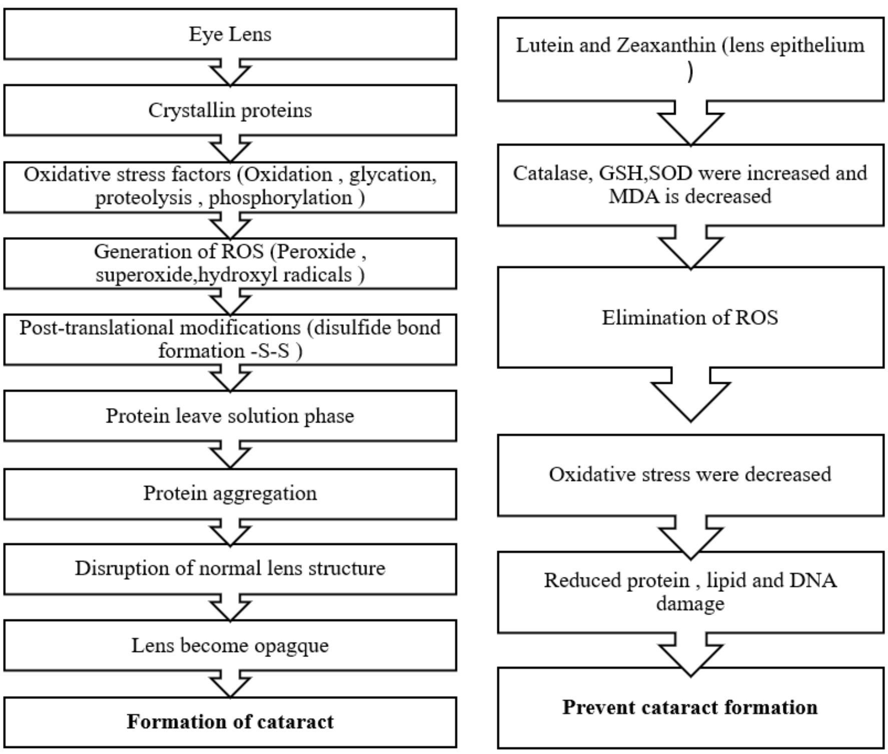

Fig no :1.2 pathophysiology of cataract

COMPLICATIONS OF CATARACT

1. Visual Impairment and Blindness
2. Lens-Induced Glaucoma
3. Lens Dislocation
4. Inflammation
5. Secondary Complications after Surgery[6,7]

DIAGNOSIS

Cataract is primarily diagnosed through clinical examination and ophthalmic investigations to assess the type, severity, and visual impact of lens opacity.

1. Patient History and Symptom Evaluation
2. Visual Acuity Test
3. Slit-Lamp Examination
4. Retinal Examination (Ophthalmoscopy)
5. Tonometry
6. Additional Tests[8]

PLANT PROFILE

One species of jasmine, Jasminum grandiflorum Linn (Oleaceae), is indigenous to Asia, including China, Afghanistan, Persia, India, the Philippines, Myanmar, and Sri Lanka. Its many parts, including the leaf, stem, bark, flower, and root, are highly valuable and significant in the pharmaceutical industries. They have been shown to have positive effects as an antiseptic, anthelmintic, aromatherapy, depurative, diuretic, emollient, suppurative, and to treat amenorrhea, constipation, dysmenorrhea, giddiness, loose teeth, leprosy, ringworm infection, sterility, stomatitis, skin conditions, ulcers, and wounds. Our extensive literature search has uncovered an intriguing fact: although this plant is widely used as a treatment for a number of illnesses, very little research has been done to confirm its effectiveness by scientific screens in animal models and clinical trials. [9-10]

Fig no:1.3 *Jasminum grandiflorum* .L

CULTIVATION AND COLLECTION[11]

The plant is cultivated in well drained loamy soil (pH 6.5–7.5) and also on a variety of soils such as black, lateritic and clay loam with good drainage system as the plant is highly susceptible to water logging. It can be propagated by shoot tip culture method. Flowering of jasmine plants starts in the first year itself. The harvesting of the flower is done in the month of May to December. The leaves of *Jasminum grandiflorum* L. are used for medicinal purposes. Leaves were collected manually without damage. Collected plant material is cleaned and dried for further research.

CHEMICAL CONSTITUENTS[12]

Jasminum grandiflorum L the plant contains alkaloids such as jasminine and nuciferine, flavonoids like quercetin, kaempferol, and rutin, and terpenoids like linalool and methyl jasmonate.

MEDICINAL USES

- Anti-inflammatory
- Anti-microbial
- Wound healing
- Anti-diabetic activity

IN-SILICO STUDY

MOLECULAR DOCKING OF LIGANDS WITH SELCTED TARGET[13,14]

Procedure

1. Selection of Target Protein

- Open the Protein Data Bank (PDB) website.
- Search for the desired target protein (Aldose Reductase, α - crystalline using its name or PDB ID).
- Select the required protein entry.
- Open the detailed page of the selected protein.
- Download the protein file in PDB format (Legacy PDB).
- Repeat the same procedure if multiple target proteins are required.

2. Selection of Ligand

- Open the PubChem database.
- In the search bar, type the name of the required drug/ligand.
- A page containing information about the drug will open.
- Select the required compound and go to the Compound Summary page.
- From the download section, select the 3D conformer in SDF format.

- Repeat the procedure for all selected ligand

3. Docking Procedure

- Open the docking software (CB – Dock 2)
- Choose the available server/software and click on the Docking option.
- Upload the protein (PDB file) and ligand (SDF file) into the designated fields.
- Select the option for binding site prediction or auto-docking.
- Run the docking process.
- Wait until the docking result is generated.

4. Analysis of Docking Results

- The output page displays predicted binding cavities and docked poses of ligands.
- Binding affinity values (e.g., docking score or binding energy) are provided.
- Visualize the protein-ligand complex using molecular visualization tools (e.g., PyMOL, Chimera).
- Change receptor style to cartoon/ball-and-stick for better visualization.
- Interpret the binding interactions (hydrogen bonds, hydrophobic interactions, etc

IN VITRO STUDY [15-17]

***In vitro* Anti-Cataract Activity by Glucose-Induced Cataract in Incubated Goat Lens Models**

1. Materials

Goat lens, sodium chloride, potassium chloride, magnesium chloride, Sodium bicarbonate, Sodium phosphate, Calcium chloride, Glucose, Penicillin-G, Streptomycin, Ascorbic acid, Ethanolic extract of *Jasminum grandiflorum* Lleaf.

2. Collection of Eye Balls

Fresh Goat eye balls were collected from slaughterhouse in Parassala. The eyeballs were removed when the animal was died and kept them between 0-4°C.

3. Preparation of Lens Culture

Extracapsular lenses were removed, then grown in synthetic aqueous humour were incubated for 72hrs with maintaining pH 7.8. The solution including 140Mm NaCl, 5mM,KCl, MgCl2 2mM, NaHCO3 0.5mM, NaHPO4 0.5mM, CaCl2 0.4mM,Glucose 5.5mM,Glucose 55mM, Pencillin-G 32mg and streptomycin 250mg were added to the culture media to prevent bacterial contamination .

4.Generating the Glucose Induced Cataract Model

Using 55Mm Glucose we induced cataract. The high amount of Glucose inside the lens cause oxidative stress and it lead to cataractogenesis.

5. Group design

Goat lenses were divided into 3 groups containing one lens in each and incubated.
Group I : A normal control glucose level is 5.5 mM
Group II : A disease control group is 55mM Glucose
Group III: 55mM Glucose + Ascorbic acid 40µg/ml
Group IV: 55mM Glucose + 100µg/ml leaf extract
Group V : 55mM Glucose + 200 µg/ml leaf extract

6.Studying an images lens opacity

To determine the lens opacity, the posterior side of the lens were set on a sheet of graph paper and left incubated for a whole day. The following standard help to evaluate the opacity degree.
0 indicates no opacity
+ indicates a small amount of opacity
++ indicates diffuse opacity
++++ indicates a significant amount of thick opacity

7.Creating the lens homogenate

10 % of every lens was homogenised using a pH 7.4, 0.1 M sodium phosphate buffer after 72hour incubation. The homogenate was then refrigerated and centrifuged for 30 minutes at 10,000 g and -4^0 C. The supernatant was then gathered and refrigerated at – 20^{0}C to enable the next experiment.

8.Biological parameter

Among the biochemical measurements for the supernatant were total protein and catalase activity.
The total protein content of the lens was determined using Bradford's technique.
The degree of catalase activity was assessed using ultra violet spectrophotometry assay.

ASSAY METHOD [18-19]

1. Estimation of Protein: (Bradford's method)

Principle

The Bradford protein assay is based on the principle that the binding of a dye molecule, Coomassie Brilliant Blue, to proteins results in a shift in the dye's absorption spectrum. The dye binds to protein and its absorption maximum shifts from 465 nm to 595 nm resulting in change of colour from brown to blue. The extracted protein from sample interacts with a dye, Coomassie brilliant blue, which turned blue on binding, will have maximum absorbance at 595nm.

Procedure Homogenate preparation

The enzyme extract was prepared by homogenizing 0.1g of tissue in 1ml 0.1M Tris-HCl buffer (pH 7.4) using liquid Nitrogen and was then centrifuged at 10000rpm for 10 minutes at 4°C. The supernatant was used for further assay.

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2. Catalase enzyme Assay

Catalase is an enzyme found broadly in animals, plants microorganisms and cultured cells it is the main enzyme of clearing H_2O_2 which plays an important role in the active oxygen scavenging system H2O2 has characteristic absorption peak at 240nm it can be decomposed in to water and oxygen by catalase which makes the absorbance at 240 nm to decrease the activity of catalase can be calculated according to the rate of change of absorbance.

Procedure

Homogenate preparation

The enzyme extract was prepared by crushing the tissue 0.1g in 1 ml of 0.1M Tris-HCl buffer using liquid nitrogen and was then centrifuged at 10000rpm for 10 min at 5^0C.

Assay

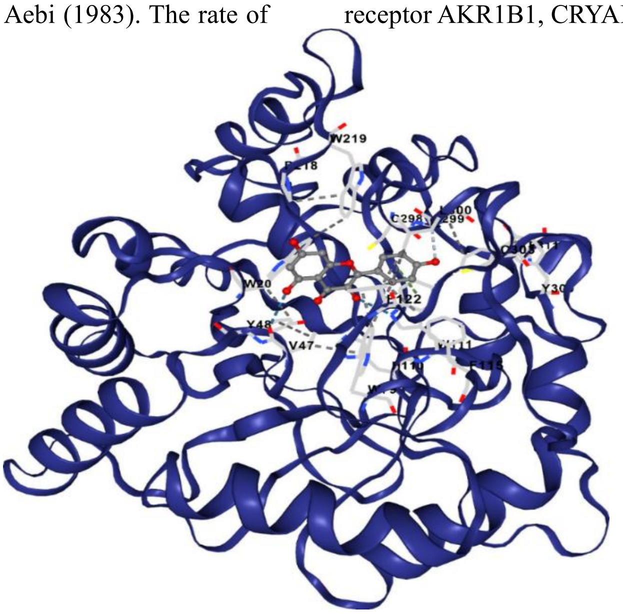
The catalase enzyme activity was determined according to the method of Aebi (1983). The rate of decomposition of H_2O_2 was determined by the decrease in absorbance at 240nm in a reaction mixture containing 1.5ml phosphate buffer, 1.2ml of hydrogen peroxide and 300 µl of enzyme extract.

II. RESULT AND DISCUSSION

In silico* docking study *of Jasminum grandiflorum L

In silico docking studies facilitate interaction among the components in a system and mathematical and computed models are established and predict the interaction between ligand and target molecules.

From various literature reviews founded that *Jasminum grandiflorum L* plant leaf posses flavonoids that is Quercetin, Rutin, Kaempferol which has the ability to produce anti- cataract activities. Quercetin, Rutin, Kaempferol shows binding affinity with AKR1B1, CRYAB receptors. Fig no 1.4,1.5,1.6,1.7,1.8,1.9 shows the docking images of Quercetin, Rutin, Kaempferol with receptor AKR1B1, CRYAB.



FigureNo :1.4 Docking image of Kaempferol with receptor AKR1B1

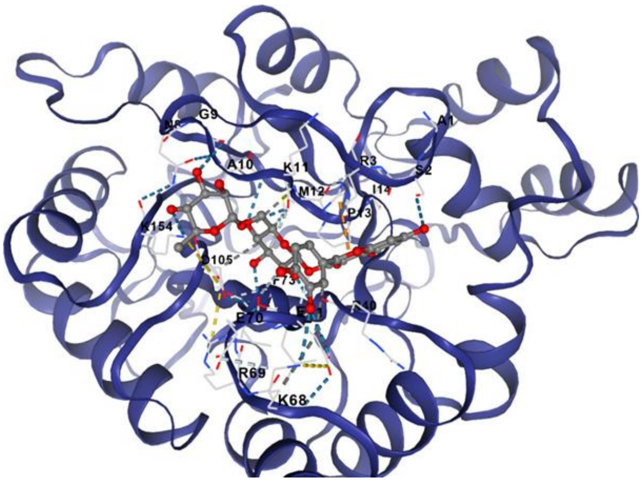


Figure No :1.5 Docking image of Rutin with receptor AKR1B1

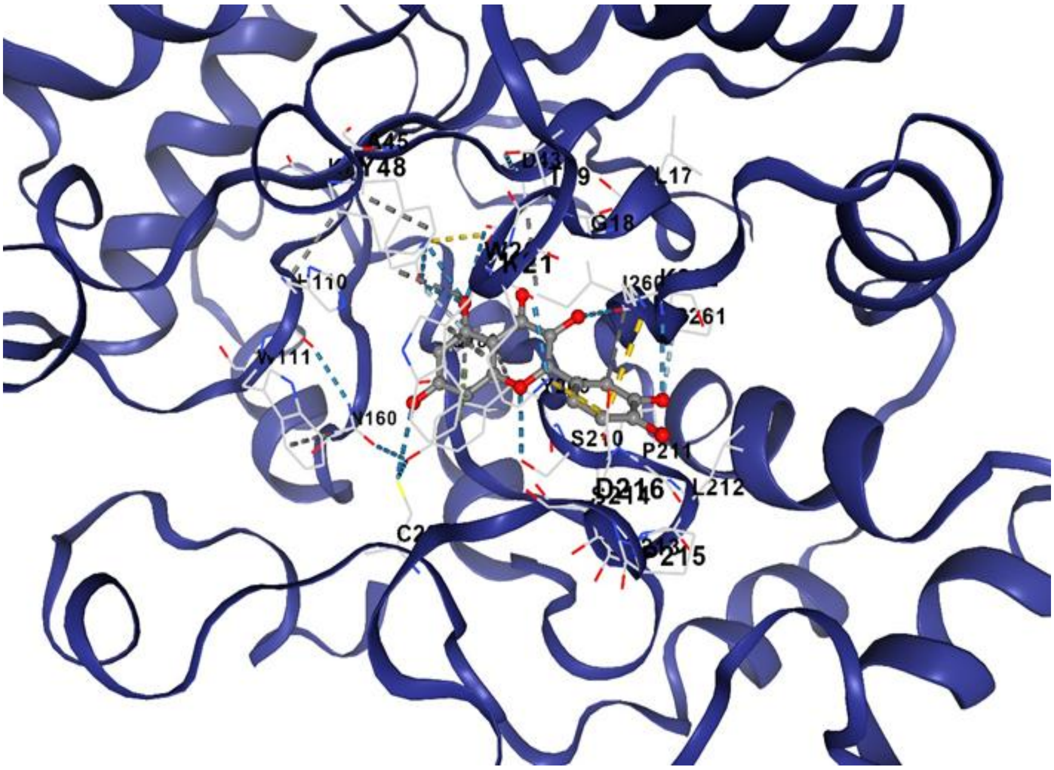


Fig no :1.6Docking image of Quercetin with receptor AKR1B1

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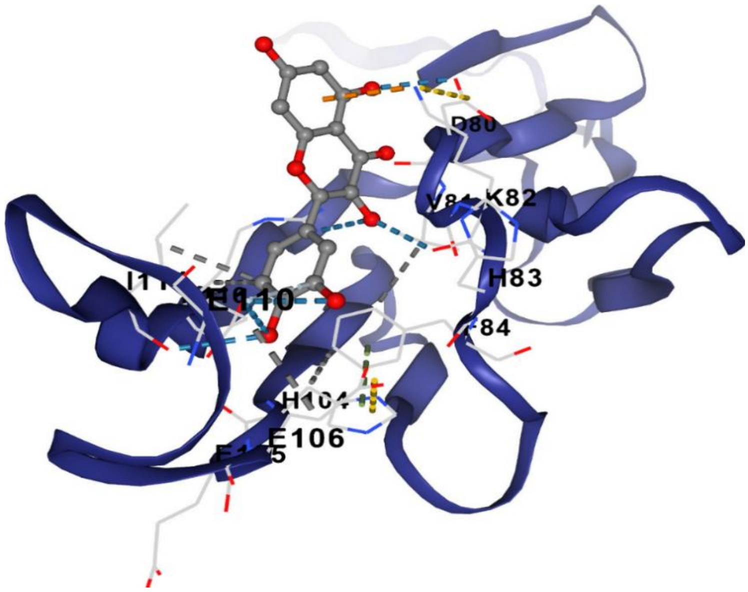

Fig no :1.7Docking image of Quercetin with receptor CRYAB

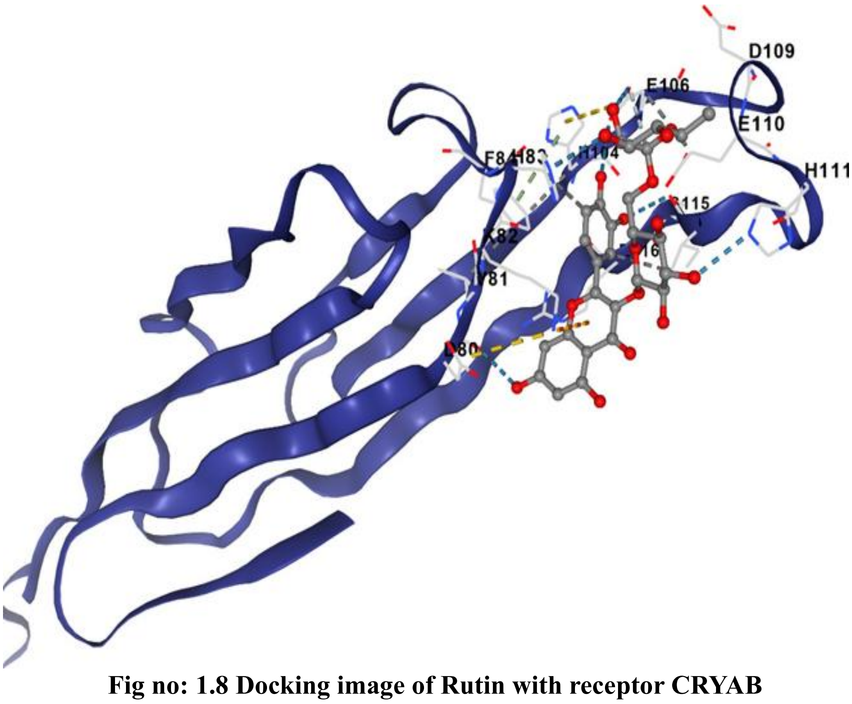

Fig no: 1.8 Docking image of Rutin with receptor CRYAB

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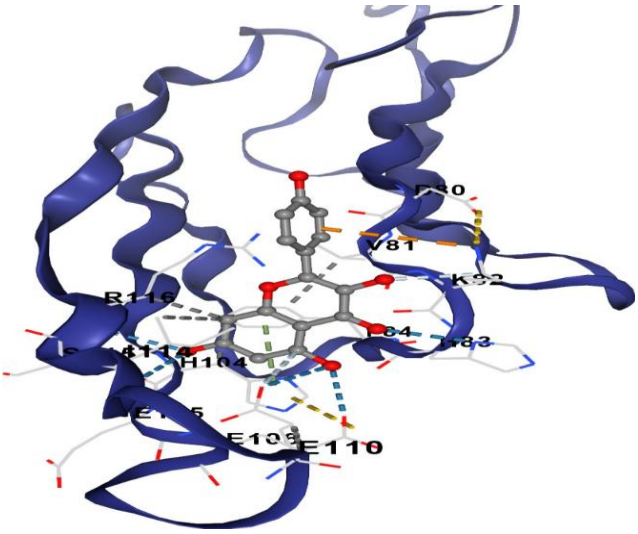
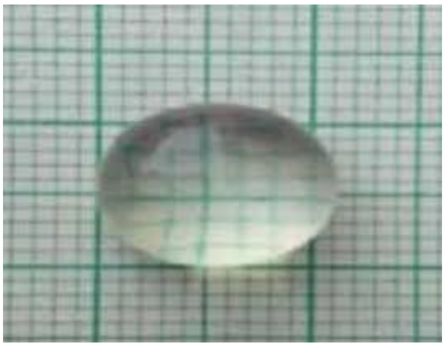


Figure No :1.9 Docking image of Kaempferol with receptor CRYAB

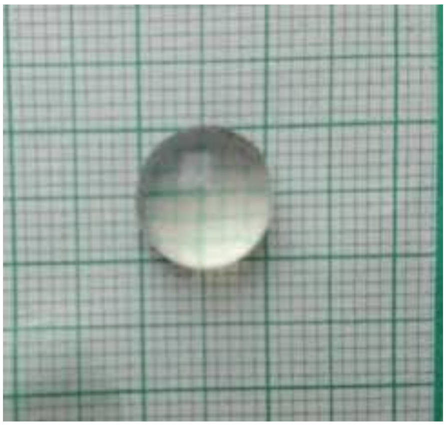
In vitro study of *Jasminum grandiflorum L.*

5.1.4.1 Photographic Evaluation



GROUP-I (Normal control)

GROUP-II (Disease control)



GROUP-III (Standard control)

GROUP-IV (Test-1) 100µg/ml

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GROUP-V (Test-2) 200µg/ml

Figure No 5.1.4.1.1 Result for Photographic Evaluation

5.1.4.2. Evaluation of Lens Opacity :

Sl.No	Treatment with *Jasminum grandiflorum* L. leaf extract	Degree of opacity
1	Group I :A normal control glucose level is 5.5 mM .	0
2	Group II :A disease control glucose level is 55 Mm.	++++
3	Group III : 55mM glucose + Ascorbic acid 40µg/ml .	+
4	Group IV : 55mM glucose + 100µg/ml leaf extract	+++
5	Group V : 55mM glucose + 200µg/ml leaf extract	++

Table no: 5.1.4.2 Evaluation of Lens opacity

5.1.4.3 Estimation of Total protein content in Homogenate Lens :

Sl.No	Group	Total protein content
1.	Group I : Normal control	215.3±0.8
2.	Group II : Negative control	163±0.5
3.	Group III: Standard	192.6±0.6
4.	Group IV : Test-1	181±0.3
5.	Group V : Test-2	189±0.6

Table No :5.1.4.3 Estimation of total protein content

5.1.4.4 Catalase Activity

Sl.No	Group	Catalase Activity
1.	Group I : Normal control	High
2.	Group II : Negative control	Low
3.	Group III: Standard	High
4.	Group IV : Test-1	Moderate
5.	Group V : Test -2	High

Table no :5.1.4.4 Catalase activity

III. DISCUSSION

Cataracts, the leading cause of blindness in India, result from lens opacification that reduces light entry and impairs vision. They usually develop slowly with age but can also arise from injury, diabetes, prolonged UV exposure, or certain medications. Oxidative stress plays a central role in cataractogenesis by causing protein denaturation and aggregation in lens fibres, disrupting transparency. The lens is highly vulnerable due to its polyunsaturated fatty acids and constant light exposure, with reactive oxygen species (ROS) oxidizing proteins and lipids into insoluble aggregates that scatter light. Reduced activity of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase further accelerates damage. Because oxidative stress is pivotal, plant-based antioxidants are being explored to delay or prevent cataract development.

Jasminum grandiflorum is a medicinal plant belonging to family Oleaceae growing in various parts of the world. It is traditionally used to treat various disease condition such as anti-inflammatory, anti-oxidant, skin disorders, wound healing pain relief and oxidative stress .in this study the extract of *Jasminum grandiflorum* was selected for evaluating the anti-cataract activity. The plant is collected and authenticated, washed, dried and powdered. The powdered part is extracted with ethanol using maceration process, Then the extract is screened through phytochemical analysis which indicate the presence of flavonoid, tannins, alkaloids, saponins , carbohydrates and proteins.

The flavonoids quercetin, kaempferol, Rutin were present in the plant *Jasminum grandiflorum* L which is responsible for the anti-cataract activity it is determined through *In silico* and *In vitro* analysis method

In silico method is a computerised tool for drug discovery, by this tool can find out the ligand binding with macromolecule. From various literature reviews founded that *Jasminum grandiflorum* possess flavonoids i.e, Quercetin, Kaempferol, and Rutin which has the ability to produce anti-cataract activity. Quercetin, Kaempferol and Rutin show binding affinity with AKR1B1, CRYABreceptors the docking study indicates quercetin has anti-cataract activity.These result indicate that flavonoid is active constituent present in extract of *Jasminum grandiflorum* responsible for anti-cataract activity

In-vitro assay method suggests Estimation of protein by, Catalase enzyme assay it show that extract of *Jasminum grandiflorum* produce anti-cataract activity and which is compared to standard anti-cataract drugs .These result obtained from *In vitro* studies show that active constituent present in plants show reduction in the intensity in the cloudy formation in the lens and finally we conclude that plant *Jasminum grandiflorum* has anti-cataract activity.

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