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Investigating the anti-cataract potential of *Spirulina platensis*: Metabolite screening and molecular docking studies

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ABSTRACT

Diabetes is a chronic metabolic condition that can lead to various complications, including cataracts, a chief cause of visual impairment. There is growing promise in the use of natural compounds as preventive and therapeutic agents for cataracts. Oxidative stress has been implicated as a significant factor in cataract development. Natural compounds with antioxidant properties show potential in combating oxidative stress and preventing the formation of cataracts. Among these compounds, *Spirulina platensis* has been chosen for investigation in this study due to its well-established antioxidant properties. Additionally, this study aims to explore *Spirulina platensis* as a potential inhibitor of human aldose reductase (hAR), which is proven to show a fundamental role in cataract development. In the course of the work, *Spirulina platensis* underwent Soxhlet extraction with methanol, yielding four-column fractions (eluotropic solvent series). Each extract was subjected to HR-LCMS metabolite screening. Molecular docking studies were conducted to identify potential hAR inhibitors, and *in vitro*, anti-cataract activity was assessed using a goat lens model. The methanol fraction (500 µg/ml) showed the most significant anti-cataract activity, with a lens morphology grade 1 and improvements in superoxide dismutase (SOD) activity (1.565±0.10 U/mg of tissue, $p<0.001$), catalase (CAT) levels (6.59±0.25 U/mg of tissue, $p<0.001$), glutathione (GSH) concentrations (21.19±21.19 nmoles/100 mg, $p<0.001$) and protein content (255.5±5 mg/dl, $p<0.001$). Molecular docking identified Dubamine (methanol fraction, docking score: -11.0 kcal/mol) as a potential hAR inhibitor with the highest binding affinity. These results demonstrate the molecular pathways underlying *Spirulina platensis* therapeutic potential against cataracts.

INTRODUCTION

The worldwide incidence of diabetes exceeds 285 million individuals, and it is predicted to arise to 439 million by 2030, according to the International Diabetes Federation. Among the various complications associated with diabetes, there are several eye-related conditions, such as diabetic macular edema, cataracts, tear film dysfunction & retinopathy. The incidence of diabetic cataracts has been steadily rising with the increasing number of individuals affected by type 1 and 2 diabetes [1,2].

Cataracts is illustrated by clouding of eye lens, leading to a progressive decline in visual acuity, contrast sensitivity, and colour perception. This age-related condition is a significant everyone's health worry because it represents the primary cause of visual impairment in older adults, with the global prevalence expected to rise due to the ageing population. Cataracts can notably influence a person's capacity to execute regular activities, ultimately affecting their overall quality of life and independence [3,4].

The formation of cataracts is a multifactorial process involving various physiological, environmental and genetic factors. Key contributors to cataract development include oxidative stress, protein aggregation and post-translational modifications of lens proteins, which result in the opacification of the lens. Accumulation of reactive oxygen species in the lens is one of the primary factors leading to oxidative damage, initiating a flow of events that conclude in the disruption of lens transparency [5-7].

Cataract treatment options are predominantly surgical, with phacoemulsification being the most performed procedure. Although cataract surgery is highly effective in restoring visual function, it is not without risks and potential complications. Additionally, access to cataract surgery can be limited in certain regions, particularly in developing

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countries, where the burden of cataract-induced blindness is the greatest. Consequently, there is a pressing need for alternative therapeutic strategies to prevent or delay cataract progression [8-10]. Spirulina platensis, a cyanobacterium popularly known as a "superfood," has gained attention for its rich nutrient report and promised health advantages. This microalga is a rich source of proteins, minerals, vitamins and bioactive compounds which exhibit various pharmacological properties, including antimicrobial, anti-inflammatory and antioxidant activities. Such properties make *Spirulina platensis* a hopeful candidate for developing novel therapeutic agents against various diseases, including cataracts [11-13].

In our previous research, we investigated one complication of diabetes mellitus, focusing on the nephroprotective effects of Spirulina platensis. This study aims to perform a comprehensive virtual screening of phytochemicals identified in our previous research on *Spirulina platensis* and to assess their anticataract potential, a complication of diabetes mellitus, through in-vitro evaluations using a goat lens model [14]. This research is expected to contribute to the existing knowledge on the therapeutic potential of *Spirulina platensis* and pave the way for further investigation into its potential applications in cataract prevention and treatment. By identifying the bioactive compounds responsible for the observed anticataract effects, this research will provide valuable understandings into the possible mechanisms of action of *Spirulina platensis* for cataract prevention and management [15].

MATERIAL AND METHODS

Collection and authentication

The algae was collected from the PIRENS, located in Bhableshwar, Rahata, Ahmednagar 413714. Authentication of sample was undertaken by BSI, Pune 411001, with Ref No-BSI/WRC/100-1/DEN.CER/2018/101, Specimen No. 204830, as *Spirulina platensis* (Nordstedt) Geitler 1925, a member of the Phormidiaceae family.

Soxhlet extraction and fractionation

The collected algae, Spirulina platensis, was dried and powdered. The algae powdered sample underwent Soxhlet extraction by methanol for a day. Whatman filter paper no.42 was used for the filtration of the methanolic extract. The solvent was then evaporated via rotary evaporator. Employing a gradient elution technique, the crude extract was eluted into (n-hexane, dichloromethane, ethyl acetate and methanol) fractions by column chromatography according to their polarity. The elution was continued until each solvent showed no spots on thin-layer chromatography (TLC), indicating that the compounds within the extract had been successfully separated into their respective fractions [16-18].

Phytochemical investigation

In an earlier study, we reported on a phytochemical investigation by HR-LCMS to identify the phytochemicals in the fractions of *Spirulina platensis* extract by metabolite screening from the METLIN Metabolite Database [14,19-21].

Selection of Target receptor

In studying the anticataract potential of Spirulina platensis, selecting an appropriate target receptor is crucial for understanding the molecular interactions and mechanism of action. hAR has been chosen as the target receptor for this research due to its significant role in the development of cataracts. hAR, with the Protein Data Bank (PDB) ID: 1US0, is an enzyme implicated in the polyol pathway, in which sorbitol is obtained from glucose under hyperglycemic conditions [22]. Sorbitol accumulation in the eye lens leads to osmotic stress and, eventually, cataract formation. By investigating the interactions between the phytochemicals present in *Spirulina platensis* and the hAR enzyme, the study aims to unveil the potential of this microalgae in preventing or treating cataracts [23,24].

Docking studies

Molecular docking studies were carried out to assess the binding relationships between the target protein and the identified phytochemical. The target protein was obtained from the PDB and ChemDraw and Chem3D (v.16) tools were applied to create and optimize the phytochemical structures. Discovery Studio Visualizer (v.4.5), AutoDock Vina (Auto Dock v.1.2.0) and LigPlot++ program (v.2.2) software were used for docking studies [25].

In vitro anticataract activity

Collection of eyeballs

Goat eyeballs were brought from the slaughterhouse and stored at freezing temperature.

Lens culture

Artificial humour was prepared with composition of Magnesium Chloride at 2 mM, Sodium Chloride 140 mM, Sodium Bicarbonate at 0.5 mM, Calcium Chloride at 0.4 mM, Potassium Chloride at 5 mM, Sodium Hydrogen Phosphate 0.5 mM and Glucose at 5.5 mM. Lenses were incubated for 72 hours in artificial aqueous humour at ambient temperature and pH 7.8. Streptomycin and Penicillin G were added to stop the growth of microorganisms.

Cataract formation

A 55 mM glucose solution was used to induce cataract formation.

Experimental design

A total of 11 groups were designed, with 3 lenses in each group. Various solutions were administered to the groups: standard drug, negative control, normal control, and varied high (500 µg/mL) and low (250 µg/mL) concentration of *Spirulina platensis* extract fractions. Low and high concentration of glucose (5 mM and 55 mM) were also employed (Table 1) [26-30].

Table 1. Experimental design for the *in vitro* anticataract study

Group No.	Treatment
I	Normal control aqueous humour + low concentration of Glucose
II	Toxic control aqueous humour + high concentration of Glucose
III	100 µg/mL Ascorbic acid) Standard drug-treated aqueous humour + 55 mM glucose
IV	Methanolic Extract (low concentration) aqueous humour + high concentration of Glucose
V	Methanolic Extract (high concentration) aqueous humour + high concentration of Glucose
VI	Ethyl Acetate Extract (low concentration) aqueous humour + high concentration of Glucose
VII	Ethyl Acetate Extract (high concentration) aqueous humour + high concentration of Glucose
VIII	Dichloromethane Extract (low concentration) aqueous humour + high concentration of Glucose
IX	Dichloromethane Extract (high concentration) aqueous humour + high concentration of Glucose
X	n-Hexane Extract (low concentration) aqueous humour + high concentration of Glucose
XI	n-Hexane Extract (high concentration) aqueous humour + high concentration of Glucose

Lens Morphology

Lens opacity was assessed by observing squares visibility through the lenses placed on a wire gauze. Morphological variations were graded as per Table 2.

Table 2. Lens Morphology Grading Criteria

Grade	Description	Details
0	Unchanged	Apparent squares, lens frame preserved
1	Slight	Apparent squares, nominal lens expanding, lens frame preserved
2	Medium	Slightly apparent squares and lens expanding present
3	Moderate to intense	Almost blocked square, lens frame about to be damaged
4	Intense	Invisible square, bizarre lens frame and mature cataract about to break

Preparation of lens homogenate

Lenses from each group were separated after incubation for 72 hours, and 10% w/v homogenate was made in 50 mM phosphate buffer (pH 7.4). A cooling centrifuge was used to centrifuge the homogenate for 20 minutes. Following the collection of the supernatant, biochemical parameters including total protein, superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) were measured.

Statistical analysis

The data is expressed in mean±SD and one-way ANOVA. The Dunnet multiple comparison test was applied for analysis.

RESULT AND DISCUSSION

Phytochemical analysis and molecular docking studies

The existence of diverse phytochemical components within the different solvent fractions was shown by HR-LCMS analysis. The number of compounds found in the n-hexane fraction were 19, the dichloromethane fraction had 32, the ethyl acetate fraction had 51, and the methanol fraction had 79 as we had reported in a previous study [14]. After HR-LCMS analysis was employed to identify these compounds, molecular docking studies were conducted utilizing the target protein (PDB ID: 1US0) that is associated with the formation of cataracts. In the current study, we show only the phytochemical compounds having docking score more than -10 kcal/mol (Figures 1-4) (Tables 3-7). 3D (A) and 2D (B) binding effects are seen in Figs. 5-14. Results of *In Vitro* Anti-Cataract Activity are shown in Table 8 and Figure 15. Effects of *Spirulina platensis* on lens biochemical parameters are revealed in Table 9.

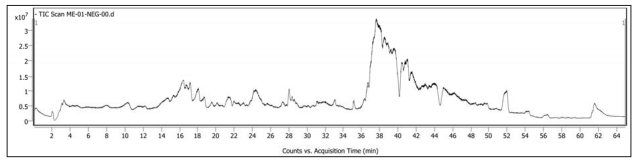


Figure 1. The phytochemical compounds found in the methanolic fraction of *Spirulina platensis* as demonstrated by the HR-LCMS Analysis

Table 3. List of phytochemical compounds showing highest docking score in methanolic fraction with retention time

Sr. No.	Name	Retention time (in minutes)	Docking Score (kcal/mol)
1	Dubamine	19.782	-11
2	Ascorbigen	19.874	-10
3	Corchoroside B	21.735	-10.4
4	dolichyl D-xylosyl phosphates	22.487	-10.3
5	Leu-leu-tyr	41.76	-10.4

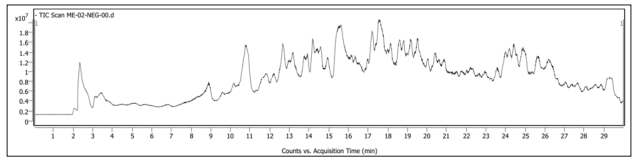


Figure 2. The phytochemical compounds found in the ethyl acetate fraction of *Spirulina platensis* as demonstrated by the HR-LCMS Analysis

Serial No	Name	Retention Time (in minutes)	Docking Score (kcal/mol)
1	Lansiumamide A	17.308	-10.6
2	(E,E)-Lansamide I	18.68	-10.5

Table 4. List of phytochemical compounds showing highest docking score in ethyl acetate fraction with retention time

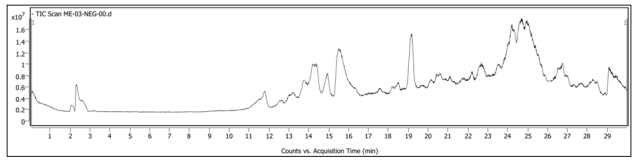


Figure 3. The phytochemical compounds found in the dichloromethane fraction of *Spirulina platensis* as demonstrated by the HR-LCMS Analysis

Table 5. List of phytochemical compounds showing highest docking score in dichloromethane fraction with retention time

Sr.no	Name of compound	Retention Time (in minutes)	Docking Score (kcal/mol)
1	4-Nerolidylcatechol	20.512	-10
2	Withaperuvin H	25.127	-10.1

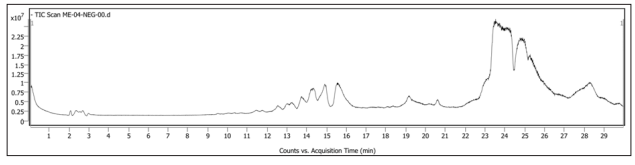


Figure 4. The phytochemical compounds found in the n-Hexane fraction of *Spirulina platensis* as demonstrated by the HR-LCMS Analysis

Table 6. List of phytochemical compounds showing highest docking score in n-hexane fraction with retention time

Sr. No.	Name of compound	Retention Time (in minutes)	Docking Score (kcal/mol)
1	Maculosin	8.963	-10.1

Molecular docking results

Table 7. An overview of the molecular docking interactions of certain compounds that have the highest binding affinity

Phytochemical	Interactive Amino acids	Bond Interaction	Docking Score
Ascorbigen	TRP111, HIS110, CYS303, LEU300, CYS80	Hydrogen Bond, Hydrophobic, Other	-10.0
Corchoroside B	GLN183, TYR209, VAL47, TRP20, PHE122	Hydrogen Bond, Hydrophobic	-10.4
Dolichyl D-xylosyl	SER210, UNL1, TYR209, PHE122, TRP111, VAL47, LEU300, CYS298, CYS303, PRO310, CYS80, TRP20, TYR48, TRP79, HIS110, PHE115, TRP219, TYR309	Hydrogen Bond, Electrostatic, Hydrophobic	-10.3
Dubamine	TRP20, CYS303, LEU300, CYS80, TRP111, VAL47	Hydrogen Bond, Hydrophobic, Other	-11.0
Leu-leu-try	TRP111, CYS298, GLN183, UNL1, CYS303, CYS80, VAL47, TRP20, TYR48, TYR209, LEU300	Hydrogen Bond, Hydrophobic, Other	-10.4
(E, E)-lansamide I	CYS303, CYS80, CYS298, TRP111, TRP20, TYR209, LEU300	Hydrogen Bond, Hydrophobic, Other	-10.5
Lansiumamide A	CYS303, TRP20, CYS80, CYS298, TRP111, TYR209, LEU300	Hydrogen Bond, Hydrophobic, Other	-10.6
4-Nerolidylcatechol	CYS80, LEU300, TRP20, CYS298, TRP111, TYR48, TYR209, TRP219, CYS303	Hydrogen Bond, Hydrophobic, Other	-10.0
Withaperuvin H	TRP20, LYS21, TRP111, PRO218, VAL47, LEU300, CYS303, TRP79, PHE122, TRP219	Hydrogen Bond, Hydrophobic	-10.1
Maculocine	TRP111, THR113, CYS80, VAL47, TRP20, TYR48, LEU300, CYS303	Hydrogen Bond, Hydrophobic, Other	-10.1

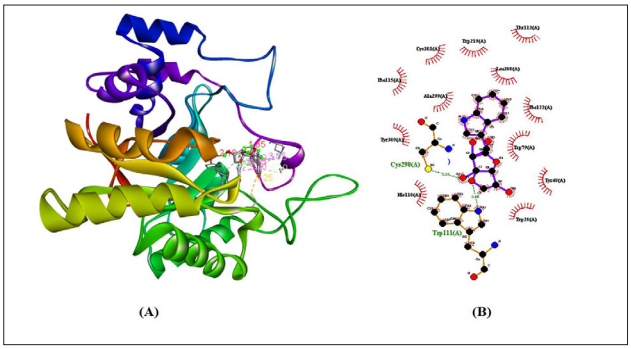


Figure 5. The 3D (A) and 2D (B) binding of Ascorbigen with Human Aldose Reductase protein

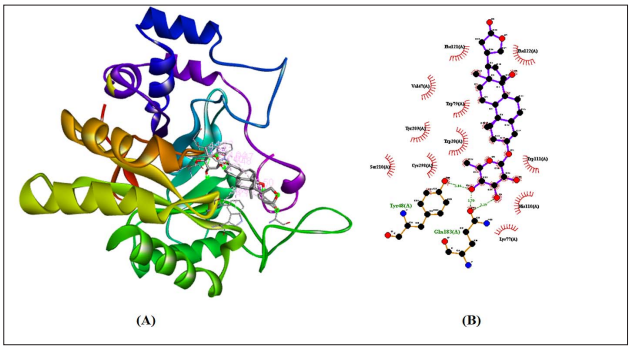


Figure 6. The 3D (A) and 2D (B) binding of Corchoroside B with Human Aldose Reductase protein

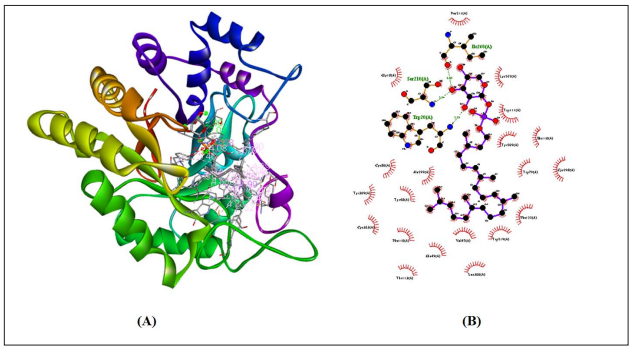


Figure 7. The 3D (A) and 2D (B) binding of Dolichyl D-xylosyl with Human Aldose Reductase protein

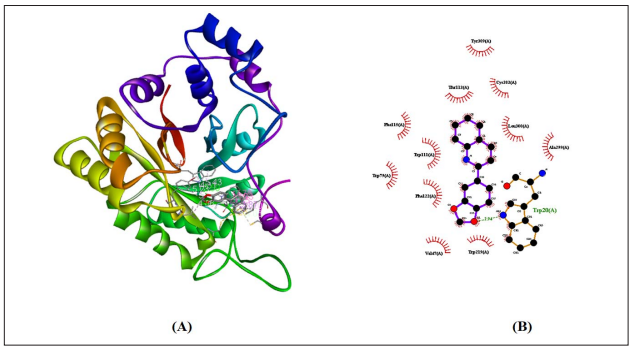


Figure 8. The 3D (A) and 2D (B) binding of Dubamine with Human Aldose Reductase protein

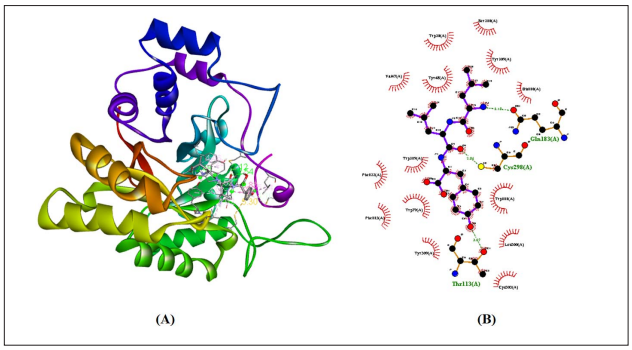


Figure 9. The 3D (A) and 2D (B) binding of Leu-leu-try with Human Aldose Reductase protein

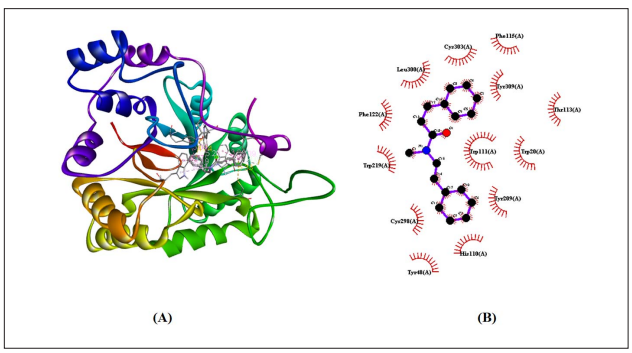


Figure 10. The 3D (A) and 2D (B) binding of (E, E)-lansamide I with Human Aldose Reductase (PDB ID: 1US0) protein

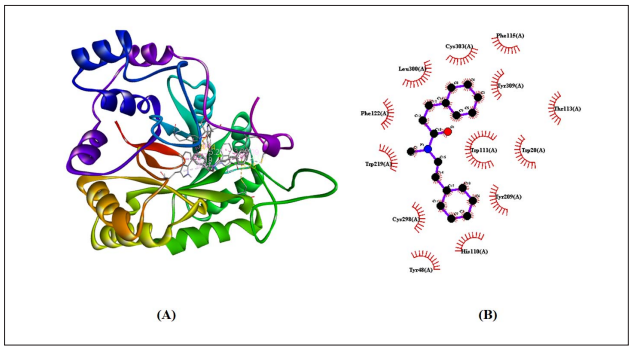


Figure 11. The 3D (A) and 2D (B) binding of compound Lansiumamide A with Human Aldose Reductase protein

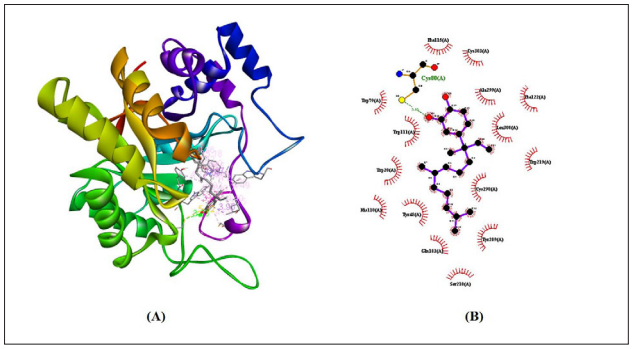


Figure 12. The 3D (A) and 2D (B) binding of compound 4-Nerolidylcatechol with Human Aldose Reductase protein

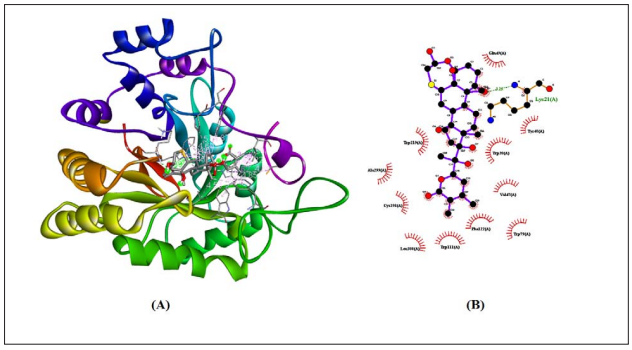


Figure 13. The 3D (A) and 2D (B) binding of Withaperuvin H with Human Aldose Reductase protein

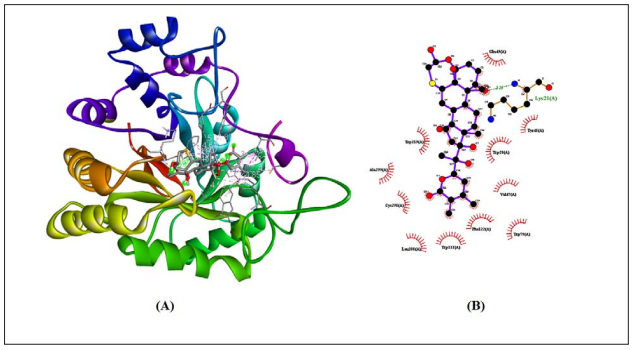


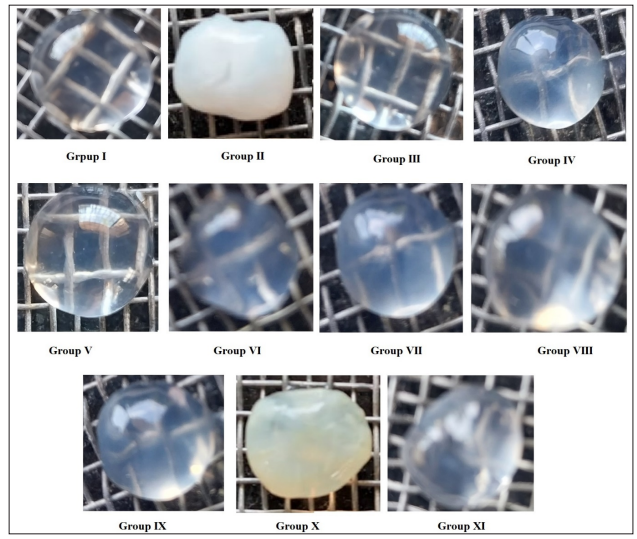
Figure 14. The 3D (A) and 2D (B) binding of Maculocine with Human Aldose Reductase protein

Results of *In Vitro* anti-cataract activity

Lens Morphology

Table 8. Lens morphology grading for *In-Vitro* anti-cataract activity

Groups	Treatment	Grades
I	Normal Control	0
II	Toxic Control	4
III	Standard	1
IV	Methanol Fraction 250 µg/ml	2
V	Methanol Fraction 500 µg/ml	1
VI	Ethyl Acetate Fraction 250 µg/ml	3
VII	Ethyl Acetate Fraction 500 µg/ml	2
VIII	Dichloromethane Fraction 250 µg/ml	3
IX	Dichloromethane Fraction 500 µg/ml	3
X	n-Hexane Fraction 250 µg/ml	4
XI	n-Hexane Fraction 500 µg/ml	4



The figure represents the lens morphology for each experimental group (Group I to Group XI) in the *in vitro* anti-cataract activity study

Figure 15. Lens morphology for *In Vitro* anti-cataract activity

Lens Homogenate analysis

Table 9. Effect of *Spirulina platensis* on lens biochemical parameter

Treatment Group	SOD activity (µ/mg of tissue)	CAT (U/mg of tissue)	GSH (nmoles/ 100 mg)	Proteins (mg/dl)
Group I - Normal Control	2.35 ±0.10	7.5 ±0.25	24.365 ±0.81	249 ±4.2
Group II - Toxic Control	0.255 ±0.03$^{\#\#\#}$	0.4605 ±0.05 $^{\#\#\#}$	3.62 ±0.53 $^{\#\#\#}$	106.5 ±3.8 $^{\#\#\#}$
Group III - Standard	1.45 ±0.15***	6.887 ±0.30***	19.01 ±0.76***	277 ±5.1***
Group IV- Methanol Fraction (250 µg/ml)	1.14 ±0.07**	4.510 ±0.20***	14.94 ±0.71***	223.5 ±3.9***
Group V - Methanol Fraction (500 µg/ml)	1.565 ±0.10***	6.59 ±0.25***	21.19 ±0.83***	255.5 ±4.7***
Group VI - Ethyl Acetate Fraction (250 µg/ml)	0.8615 ±0.05*	4.594 ±0.20***	10.765 ±0.66***	188 ±4.3***
Group VII - Ethyl Acetate Fraction (500 µg/ml)	1.0465 ±0.08**	5.653 ±0.22***	15.8 ±0.72***	212.5 ±5.0***
Group VIII - Dichloromethane Fraction (250 µg/ml)	0.693 ±0.04^{ns}	2.590 ±0.18**	7.735 ±0.47***	164 ±5.2***
Group IX - Dichloromethane Fraction (500 µg/ml)	0.905 ±0.07*	3.737 ±0.10***	11.79 ±0.63***	197 ±4.6***
Group X - n-Hexane Fraction (250 µg/ml)	0.435 ±0.04^{ns}	0.535 ±0.05^{ns}	4.72 ±0.38^{ns}	108.5 ±3.9^{ns}
Group XI - n-Hexane Fraction (500 µg/ml)	0.545 ±0.04^{ns}	1.367 ±0.07^{ns}	5.935 ±0.55*	127 ±4.8**

Data is presented as Mean±SD (n = 3). Analysis was performed using one-way ANOVA followed by Dunnett's test. Significance levels: $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ *vs*. normal control; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ *vs*. toxic control; $^{ns}p > 0.05$ non-significant *vs*. toxic control

DISCUSSION

In this study, we explored the phytochemical profiling and anticataract potential of *Spirulina platensis* using *in-vitro* assessments and molecular docking analysis. Our outcomes provide beneficial insights into the therapeutic impending of *Spirulina platensis* against cataract formation and its underlying molecular mechanisms.

In our previous study, the phytochemical composition of different fractions of *Spirulina platensis* was analyzed by HR-LCMS. The methanol fraction displayed 79 phytochemical compounds (Figure 1), ethyl acetate fraction displayed 51 phytochemical compounds (Figure 2), Dichloromethane fraction displayed 32 phytochemical compounds (Figure 3) and n-Hexane fraction displayed 19 phytochemical compounds (Figure 4) [14]. All identified phytochemical compounds were subjected to molecular docking studies to predict the potential interactions of these phytochemical compounds with human aldose reductase target protein. Only high docking score (more than -10 kcal/mol) phytochemical compounds are shown in Tables 3 to 6.

The methanol fraction dubamine exhibited the highest docking score (-11 kcal/mol), suggesting its potential for strong binding affinity towards the selected human aldose reductase target protein. Other notable compounds with high docking scores include furegrelate, maculosin, ascorbigen and nigericin, which may have promising pharmacological activities (Table 3). The ethyl acetate fraction Lansiumamide A demonstrated the highest docking score (-10.6 kcal/mol), indicating its potential for significant interaction with the human aldose reductase target protein (Table 4). Other compounds, such as sinalexin, 4-tert-butylphenyl salicylate, linalyl phenylacetate and 4-nerolidylcatechol, displayed high docking scores, suggesting their possible pharmacological relevance. In the dichloromethane fraction (Table 5), Withaperuvin H revealed the highest docking score (-10.1 kcal/mol), and, finally, in the n-Hexane fraction (Table 6), Maculocine demonstrated the highest binding affinity (-10.1 kcal/mol).

The molecular docking study is summarized in Table 8. As indicated, the compounds exhibited diverse interactions with the active amino acids of the target protein, as well as varying docking scores. Among the tested compounds, Dubamine had the maximum binding affinity, with a docking score of (-11.0 kcal/mol), followed by Lansiumamide A (-10.6 kcal/mol) and (E, E)-lansamide I (-10.5 kcal/mol). Figures 8, 11 and 10 show that these compounds formed stable complexes with human Aldose Reductase through hydrogen bonding, hydrophobic and other interactions.

Dubamine (Figure 8) created hydrogen bonds with TRP20, CYS303 and LEU300 and hydrophobic interactions with CYS80, TRP111 and VAL47. Similarly, Lansiumamide A (Figure 11) formed hydrogen bonds with CYS303, TRP20, CYS80 and CYS298 and hydrophobic interactions with TRP111, TYR209 and LEU300. (E, E)-lansamide I (Figure 10) also established hydrogen bonds with CYS303, CYS80, CYS298, TRP111, TRP20 and TYR209, and hydrophobic interactions with LEU300. Moreover, other compounds, such as Ascorbigen (Figure 5), Corchoroside B (Figure 6), Dolichyl D-xylosyl (Figure 7), Leu-leu-try (Figure 9), 4-Nerolidylcatechol (Figure 12), Withaperuvin H (Figure 13) and Maculocine (Figure 14), showed significant binding affinity to human Aldose Reductase. These compounds formed hydrogen bonds, hydrophobic and other interactions with the target protein's active amino acids, demonstrating their potential repressive effects on human Aldose Reductase. However, further experimental validation isolation of phytochemical from fraction and performance of animal studies is necessary to confirm these compounds' inhibitory potential and therapeutic efficacy.

In conclusion, the molecular docking study identified several promising candidates as potential inhibitors of human aldose reductase. Of these, Dubamine, Lansiumamide A and (E, E)-lansamide I exhibited the highest binding affinity. The potential of these compounds highlights their use as lead molecules for developing novel therapeutic agents against human Aldose Reductase-associated diseases.

The *in vitro* anti-cataract activity of *Spirulina platensis* extract fractions was evaluated based on lens morphology and graded as per the observations in Table 9. The normal control group (Group I) exhibited clear lenses with no cataract formation. In contrast, the toxic control group (Group II) showed significant cataract formation, indicating the successful induction of cataracts in the *in vitro* model. Among the treated groups (Group III to Group XI), varying degrees of cataract formation and prevention were observed, with the standard group (Group III) and the methanol fraction-treated groups (Group IV and V) showing the best results in terms of cataract prevention (Figure 15).

The results from Table 10 reveal the effects of *Spirulina platensis* extract fractions on lens biochemical parameters, including SOD, CAT, GSH levels and protein concentrations. The data indicate that various *Spirulina platensis* extract fractions impact these parameters differently than did the normal control and toxic control groups. Compared with the toxic control group, the standard treatment group (Group III) exhibited a significant increase in SOD, CAT, GSH levels and protein concentrations. This suggests that the standard treatment effectively prevents oxidative stress-induced damage to the lens and preserves its function.

The methanol fractions at 250 and 500 µg/ml (Group IV and V) demonstrated a dose-dependent improvement in all four parameters, with the 500 µg/ml concentration showing the most significant improvement. This indicates that the methanol fraction may contain bioactive components that effectively counteract oxidative stress and protect lens tissue. Similarly, the ethyl acetate fractions at 250 and 500 µg/ml (Group VI and VII) exhibited dose-dependent increases in the parameters related to the toxic control group, signifying that this fraction also contains active compounds with protective effects on the lens. The dichloromethane fractions (Group VIII and IX) revealed some improvement in lens parameters, but were not as effective as the methanol or ethyl acetate fractions. This suggests that the dichloromethane fraction may have less potent bioactive compounds, or their concentration may not be as high. The n-hexane fractions (Group X and XI) demonstrated minimal to no significant improvement in lens biochemical parameters. Hence, this fraction does not contain active compounds that protect the lens against oxidative stress-induced damage. In conclusion,

the results of this study suggest that the methanol and ethyl acetate fractions of *Spirulina platensis* have the most potent anti-cataract activity, effectively protecting the lens against oxidative stress by modulating SOD activity, CAT levels, GSH concentrations and protein content. Further studies are necessary to isolate the phytochemicals from the fractions to explore their potential therapeutic applications in preventing and treating cataracts.

CONCLUSION

In conclusion, this comprehensive investigation into the phytochemical profiling and anti-cataract potential of *Spirulina platensis* has provided valuable insights into its therapeutic properties against cataract formation. HR-LCMS further profiled the phytochemical composition of different fractions of Spirulina platensis. Molecular docking analysis identified promising candidates as potential inhibitors of human aldose reductase, a key factor in cataract development.

The *in vitro* anti-cataract activity evaluation demonstrated that the methanol and ethyl acetate fractions of *Spirulina platensis* exhibited potent effects in protecting the lens against oxidative stress, as evidenced by their modulation of SOD activity, CAT levels, GSH concentrations and protein content. These discoveries feature the promise of *Spirulina platensis* as a valuable source of phytochemical compounds with anti-cataract properties.

Future studies should focus on isolating and identifying the particular phytochemical compounds accountable for the observed conclusions and further discover their probable therapeutic applications in the anticipation and management of cataracts. This research sets the stage for drug discovery and development efforts aimed at harnessing the therapeutic potential of *Spirulina platensis* in combating cataract formation, ultimately benefiting individuals at risk of vision impairment.

ABBREVIATION

SOD – Superoxide Dismutase; CAT – Catalase; GSH – Glutathione; PDB – Protein Data Bank; hAR – Human Aldose Reductase; 2D – Two-dimensional; 3D – Three-dimensional; µg/ml – micrograms per milliliter; U/mg – units per milligram; nmoles/100 mg – nanomoles per 100 milligrams; mg/dl – milligrams per deciliter; µ/mg – microunits per milligram; hrs – Hours

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