EXPLORING THE POTENTIAL OF *SPIRULINA PLATENSIS*: PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF ITS ANTI-CATARACT ACTIVITY IN A GOAT LENS MODEL

Dhamak Vikrant M[1]*, Dr Amrutkar Sunil V[2], Thorat Atmaj[3], Thopate Onkar[3], Tapikre Pradnya[3], Thorat Shashikant[3], Valvi Ganesh[3]

Abstract

A cataract is a leading cause of vision impairment and blindness worldwide, necessitating the search for effective preventive and therapeutic interventions. This study evaluated the methanolic extract of *Spirulina platensis* family *Spirulinaceae* for its potential anti-cataract activity using an in vitro goat lens model. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinones, and glycosides. The anti-cataract activity of the extract was assessed by inducing cataract formation in goat lenses through exposure to a high-glucose environment. The photographic evaluation demonstrated significant activity at 750µg/ml, significant activity at 500µg/ml, and non-significant activity at 250µg/ml of the extract.

Additionally, protein estimation data revealed a dose-dependent increase in protein levels with the extract, suggesting its potential to minimize protein utilization during cataract formation. Furthermore, the extract exhibited dose-dependent increases in the levels of superoxide dismutase (SOD) and glutathione (GSH), important biomarkers involved in counteracting oxidative stress. This indicates the extract's ability to mitigate oxidative stress and potentially slow down the progression of cataracts. Overall, these findings highlight the promising anti-cataract potential of Spirulina platensis methanolic extract.

Keywords: - *Spirulina platensis*, Phytochemical test, Anti-cataract activity

[1]Department of Pharmaceutical Chemistry, SNJB SSDJ College Of Pharmacy, Jain Gurukul, Neminagar, At/p. Tal. Chandwad Dist. Nashik, Maharashtra, India 423 101.

[2]Department of Pharmaceutical Chemistry, GES's Sir Dr M. S. Gosavi College of Pharmaceutical Education and Research Nashik, College Road, Nashik, Maharashtra, India 422005.

[3]Department of Pharmaceutical Chemistry, Dr VVPF's College of Pharmacy, Vilat Ghat, Ahmednagar, Maharashtra, India 414111.

*__Correspondences Author:-__ Dhamak Vikrant M

*Department of Pharmaceutical Chemistry, SNJB SSDJ College Of Pharmacy, Jain Gurukul, Neminagar, At/p. Tal. Chandwad Dist. Nashik, Maharashtra, India 423 101, Email: vikrantdhamak@gmail.com,

Phone Number: +917588169491

DOI: 10.48047/ecb/2023.12.si10.0028

1. Introduction

Cataract, characterized by the clouding of the lens in the eye, is a prevalent age-related ocular disorder and a leading cause of visual impairment and blindness worldwide[1]. It is estimated that by the year 2030, over 32 million individuals will be affected by cataracts globally[2]. The pathogenesis of cataracts involves accumulating damaged proteins and oxidative stress, leading to lens opacification and loss of vision[3].

The prevention and treatment of cataracts have become major challenges in ophthalmology, prompting the exploration of natural compounds with potential anti-cataract properties[4]. Medicinal plants and algae have gained considerable attention due to their diverse bioactive constituents and traditional use in various therapeutic applications[5]. Among these, *Spirulina platensis*, a filamentous blue-green algae belonging to the *Spirulinaceae* family, has emerged as a promising candidate for its potential health benefits[6].

Spirulina platensis is rich in nutrients, including proteins, vitamins, minerals, and bioactive compounds such as phycocyanin, chlorophyll, carotenoids, and polyphenols[7]. It possesses antioxidant, anti-inflammatory, and immune modulatory properties, making it a subject of interest for various health-related research areas[8].

In the context of cataracts, exploring natural compounds from *Spirulina platensis* for their potential anti-cataract activity holds great promise. Previous studies have reported the presence of bioactive compounds in *Spirulina platensis* that possess antioxidant and anti-inflammatory properties, which are crucial for counteracting oxidative stress and inflammation associated with cataract formation[9]. However, more scientific research has yet to be conducted to evaluate the anti-cataract potential of *Spirulina platensis* extracts.

Therefore, this study aims to investigate the anti-cataract activity of the methanolic extract of *Spirulina platensis* using an in vitro goat lens model. The evaluation will include preliminary phytochemical screening to identify various bioactive compounds in the extract. Additionally, the study will assess the extract's impact on protein levels and key biomarkers, such as superoxide dismutase (SOD) and glutathione (GSH), known to play essential roles in counteracting oxidative stress.

Understanding the potential anti-cataract activity of Spirulina platensis extract and its underlying mechanisms could provide valuable insights into developing novel preventive and therapeutic strategies for cataract management. Moreover, it may contribute to the expanding body of knowledge on natural compounds as alternative options for ocular health promotion.

2. Methodology

2.1 Collection and Authentication

Algae, *Spirulina platensis* family *Spirulinaceae*, was collected from Pravara Institute of Research and Education in Natural and Social Sciences, Krishi Vigyan Kendra, Bhableshwar, Tal-Rahata, Dist-Ahmednagar (MH). After the collecting sample was authenticated and certified by the Department of Botany, B.P.H.E Society's Ahmednagar College, Ahmednagar 414001 (Ref No-ACA/BOT/4/5/2023, Voucher No. ACA/ BOT/Al208)[10]

.

2.2 Soxhlet Extraction

Spirulina platensis 20 gm powder was used for extraction in 250 ml of methanol by the Soxhlet extraction method. After that, the solvent was evaporated by a rotatory evaporator to remove the solvent from the extract. The dried extract was used for further phytochemical tests and anti-cataract activity[11,12].

2.3 Preliminary Phytochemical Tests[13,14,15,16,17,18]

All tests were performed using the following procedure:

a) Alkaloid Test:

To perform the alkaloid test, 2 mL of concentrated hydrochloric acid was combined with 2 mL of *Spirulina platensis* methanolic extract. Subsequently, a few drops of Mayer's reagent were added. The presence of a green colour or the formation of a white precipitate indicates the presence of alkaloids.

b) Terpenoid Test:

For the terpenoid test, 2 mL of chloroform and concentrated sulfuric acid were added to 0.5 mL of the *Spirulina platensis* methanolic extract. The formation of a reddish-brown colour at the interface indicates the presence of terpenoids.

c) Steroid Test:

To test for steroids, 2 mL of chloroform and 1 mL of sulfuric acid were added to 0.5 mL of the *Spirulina platensis* methanolic extract. The

formation of a reddish-brown ring at the interface indicates the presence of steroids.

d) Tannin Test:
In the tannin test, 1 mL of 5% ferric chloride was added to 1 mL of the *Spirulina platensis* methanolic extract. The formation of a dark blue or greenish-black colour indicates the presence of tannins.

e) Saponin Test:
For the saponin test, 2 mL of distilled water was added to 2 mL of *Spirulina platensis* methanolic extract and vigorously shaken in a graduated cylinder for 15 minutes. The formation of a 1 cm layer of foam indicates the presence of saponins.

f) Flavonoid Test:
To test for flavonoids, 1 mL of 2N sodium hydroxide was added to 2 mL *Spirulina platensis* methanolic extract. The formation of a yellow colour indicates the presence of flavonoids.

g) Phenol Test:
2 mL of distilled water, followed by a few drops of 10% ferric chloride, was added to 1 mL of the *Spirulina platensis* methanolic extract. The formation of a blue or green colour indicates the presence of phenols.

h) Coumarin Test:
To perform the coumarin test, 1 mL of 10% sodium hydroxide was added to 1 mL of *Spirulina platensis* methanolic extract. The formation of a yellow colour indicates the presence of coumarins.

i) Quinone Test:
1 mL of concentrated sulfuric acid was added to 1 mL of *Spirulina platensis* methanolic extract to perform the quinone test. The formation of a red colour indicates the presence of quinones.

j) Glycoside Test:
For the glycoside test, 3 mL of chloroform and 10% ammonium solution were added to 2 mL of the *Spirulina platensis* methanolic extract. The formation of a pink colour indicates the presence of glycosides.

2.4 Anti Cataract Activity: In Vitro using Goat Lens Model[19,20,21,22.]

a) Collection of eyeballs
Goat eyeballs used for the study were collected from the local slaughterhouse and stored at 0-4 ^{0}C.

b) Lens Culture
Artificial aqueous humour is used for the anticataract activity. Aqueous humour was prepared using the formula NaCl 140 mM, KCl 5mM, MgCl2 2mM, NaHCO3 0.5mM, NaHPO4 0.5 mM, CaCl2 0.4 mM and glucose 5.5 mM at room temperature and maintain pH 7.4 by addition of NaHCO3. Penicillin G and streptomycin 250 mg were added to prevent bacterial growth.

c) Cataract formation
The glucose solution having a concentration of 55mM was used for the cataract formation. A higher concentration of glucose metabolizes by the sorbitol pathway. Cataract was formed due to the accumulation of polyol (Sugar + Alcohol), which causes oxidative stress and over-hydration, which forms a cataract.

d) Group Design
Goat lenses were divided into six groups, and each group had four lenses:

Table 1: Group Design

Group Name	Treatment
Group I (Normal Control)	Aqueous humour + Glucose 5.5mM
Group II (Negative Control)	Aqueous humour + Glucose 55mM
Group III (Standard)	Aqueous humour + Glucose 55mM + 100µg/ml Ascorbic acid
Group IV (Test 1)	Aqueous humour + Glucose 55mM + 250µg/ml methanolic extract of *Spirulina platensis*
Group V (Test 2)	Aqueous humour + Glucose 55mM + 500µg/ml methanolic extract of *Spirulina platensis*
Group VI (Test 3)	Aqueous humour + Glucose 55mM + 750µg/ml methanolic extract of *Spirulina platensis*

e) Incubation of Lenses
Lenses were incubated at 37 ^{o}C in Incubator for 72 hours in Incubator.

f) Photographic Evaluation
Lenses were placed on a graph paper with the posterior surface touching the paper. The pattern of graph paper squares clearly visible through the lens was observed to measure lens opacity. The degree of opacity was graded as follows:

Table 2: Showing Grades with description

Grade	Description	Details
0	No changes	Kept lens form, outline, and visible grid lines
1	Mild	Minimal lens swelling, grid lines are visible shape and lens outline preserved
2	Moderate	Gridlines are faintly visible , and there is lens swelling
3	Moderate to severe	Shape damaged and lens outline almost obstructed grid lines
4	Severe	Distorted lens ,outlines and lens-shaped mature cataracts about to ruptured and invisible grid lines

g) Preparation of Lens Homogenate

After 72 hours of incubation, the lens homogenate was prepared in tris buffer (0.23 M, pH 7.8) containing 0.25 × 10-3 M EDTA. The homogenate was adjusted to 10% w/v, which was centrifuged at 10,000 G at 4ºC for 1 hour, and the supernatant was used to estimate biochemical parameters.

f) Estimation Protein, Superoxide Dismutase (SOD), Glutathione (GSH)

The protein content was calculated from a standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue. The bovine erythrocyte standard curve calculated Superoxide Dismutase (SOD). Glutathione was estimated by Ellman's method.

3. Result

3.1 A phytochemical test performed on *Spirulina platensis* methanolic extract has shown the following result:

Table 3: Phytochemical Analysis of *Spirulina platensis* Methanol Extract. (+ = Present and - = Absent)

Sr.no	Test	Results
1	Alkaloids	+
2	Terpenoids	+
3	Steroids	+
4	Tannins	+
5	Saponins	+
6	Flavonoids	+
7	Phenols	+
8	Coumarins	+
9	Quinones	+
10	Glycosides	+

3.2 Photographic Evaluation: Anti-Cataract Activity

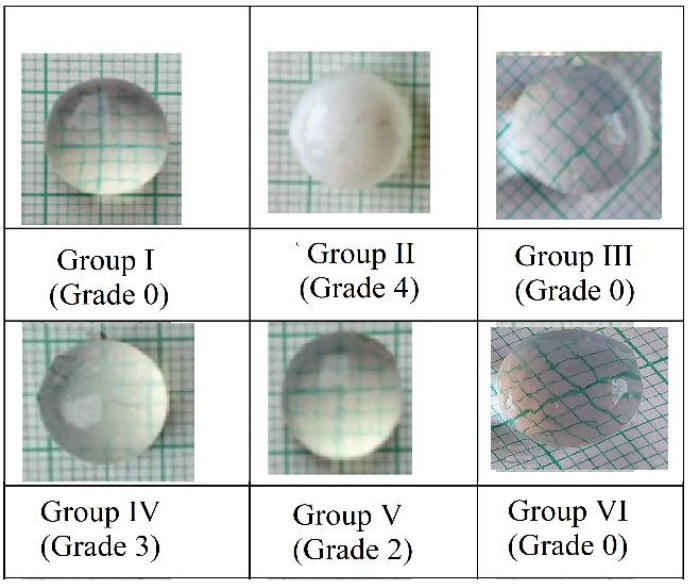

Figure 1: Anti-Cataract Activity: Photographic Evaluation

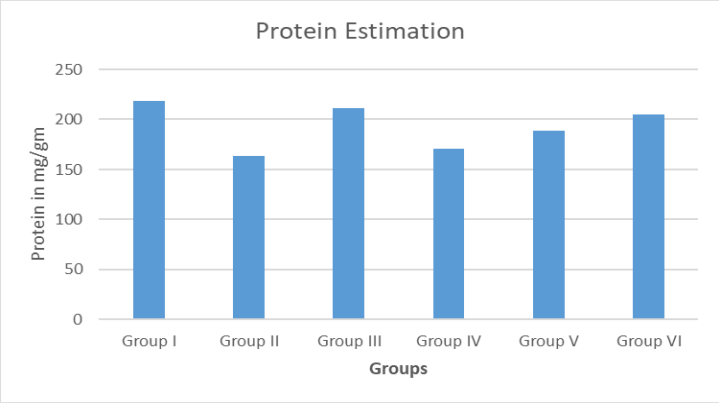

Figure 2: Protein Estimation From Lens Homogenate

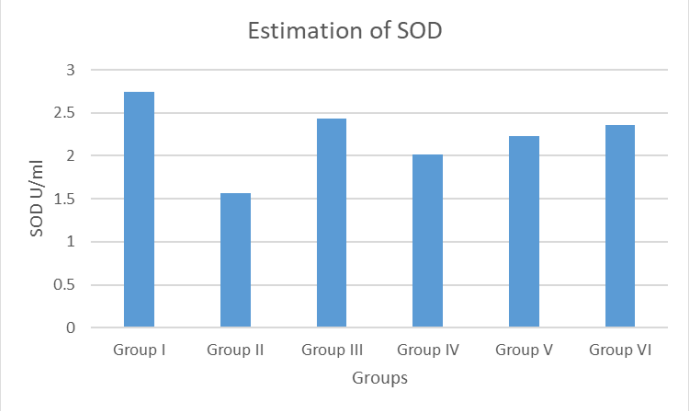

Figure 3: Estimation of Superoxide Dismutase from lens homogenate

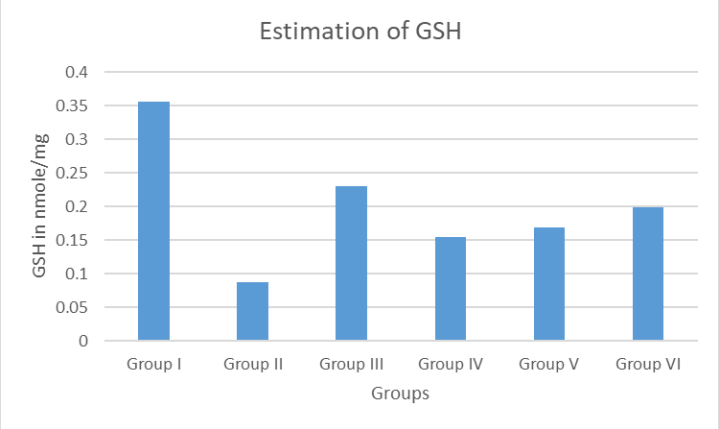

Figure 4: Estimation of Glutathione from lens homogenate

4. Discussion

The preliminary phytochemical screening of Spirulina platensis methanolic extracts revealed positive results for all tested phytochemicals, indicating the presence of a diverse range of metabolites with potential activity. In the assessment of anti-cataract activity using an in vitro goat lens model, the methanolic extract of Spirulina platensis demonstrated significant activity at 750µg/ml (Group 6), significant activity at 500µg/ml (Group 5), and non-significant activity at 250µg/ml (Group 4) based on the photographic evaluation.

Furthermore, the protein estimation data revealed an interesting correlation: as the dose of the methanolic extract of Spirulina platensis increased, the protein levels also increased. This suggests that a lower amount of protein was utilized in cataract formation. In contrast, the negative control group, which exhibited the lowest protein content, indicates that protein levels decrease as they coagulate during cataract development.

Moreover, the biomarkers SOD and GSH exhibited an increase in levels in a dose-dependent manner with the methanolic extract of Spirulina platensis. This indicates that the extract has the potential to

counteract oxidative stress, thereby potentially preventing or slowing down the progression of cataracts.

These findings suggest that Spirulina platensis may contain bioactive compounds that could be beneficial in the context of cataract prevention or treatment. However, further studies are necessary to elucidate the specific mechanisms of action and to evaluate the extract's efficacy and safety in clinical settings.

5. Conclusion:

In conclusion, the preliminary phytochemical screening of Spirulina platensis methanolic extracts demonstrated the presence of various metabolites with potential activity. The in vitro goat lens model assessment revealed significant anti-cataract activity of the methanolic extract, with higher concentrations exhibiting stronger effects. The protein estimation data also indicated that higher doses of the extract correlated with increased protein levels, suggesting a potential role in minimizing protein utilization during cataract formation. Furthermore, the extract's impact on biomarkers SOD and GSH suggested its ability to counteract oxidative stress and potentially slow cataract progression. These findings highlight the potential of Spirulina platensis as a source of bioactive compounds for cataract prevention or treatment. However, further research is required to fully understand the mechanisms involved and evaluate the extract's efficacy and safety in clinical applications.

References

1. Balyen L, Peto T. Promising artificial intelligence-machine learning-deep learning algorithms in ophthalmology. The Asia-Pacific Journal of Ophthalmology. 2019 May 1;8 (3):264-72. https://doi.org/10.1016/j.jaim.2019.08.001
2. McCormick I, Butcher R, Evans JR, Mactaggart IZ, Limburg H, Jolley E, Sapkota YD, Oye JE, Mishra SK, Bastawrous A, Furtado JM. Effective cataract surgical coverage in adults aged 50 years and older: estimates from population-based surveys in 55 countries. The Lancet Global Health. 2022 Dec 1;10 (12):e1744-53. https://doi.org/10.1016/S2214-109X(22)00419-3
3. Ahmad A, Ahsan H. Biomarkers of inflame-mation and oxidative stress in ophthalmic disorders. Journal of Immunoassay and Immunochemistry. 2020 May 3;41 (3):257-71. https://doi.org/10.1080/15321819.2020.1726774
4. Liu XY, Wang XL, Qiu MC, Ye YJ, Wang F, Xu YQ, Gong JJ, Xu ZJ. Exploring the protective effect and mechanism of Buddlejae Flos on sodium selenite-induced cataract in rats by network pharmacology, molecular docking, and experimental validation. Evidence-based Complementary and Alternative Medicine. 2022 May 14;2022. https://doi.org/10.1155/2022/7776403
5. Sachdeva V, Roy A, Bharadvaja N. Current prospects of nutraceuticals: A review. Current pharmaceutical biotechnology. 2020 Aug 1;21(10):884-96.
6. Calabrò C, Bertuccio C, Gervasi T, Lauriano ER, Leonardi M, Cicero N, Lo Cascio P. Effects of spirulina diet on the oogenesis of zebrafish: morphological analysis and immunohisto-chemical determination of the vitellogenin. Natural Product Research. 2021 Nov 18; 35(22):4454-9. https://doi.org/10.1080/14786419.2020.1727469
7. Bortolini DG, Maciel GM, Fernandes ID, Pedro AC, Rubio FT, Brancod IG, Haminiuk CW. Functional properties of bioactive compounds from Spirulina spp.: Current status and future trends. Food Chemistry: Molecular Sciences. 2022 Sep 19:100134. https://doi.org/10.1016/j.fochms.2022.100134
8. Calella P, Di Dio M, Cerullo G, Di Onofrio V, Gallé F, Liguori G. Antioxidant, immune-modulatory, and anti-inflammatory effects of Spirulina in disease conditions: a systematic review. International Journal of Food Sciences and Nutrition. 2022 Nov 17;73(8):1047-56. https://doi.org/10.1080/09637486.2022.2137785
9. Ávila-Román J, García-Gil S, Rodríguez-Luna A, Motilva V, Talero E. Anti-inflammatory and anticancer effects of microalgal carotenoids. Marine Drugs. 2021 Sep 23;19(10):531. https://doi.org/10.3390/md19100531
10. Kumar A, Ramamoorthy D, Verma DK, Kumar A, Kumar N, Kanak KR, Marwein BM, Mohan K. Antioxidant and phytonutrient activities of Spirulina platensis. Energy Nexus. 2022 Jun 16;6:100070.
11. Pinto LF, Ferreira GF, Beatriz FP, Cabral FA, Maciel Filho R. Lipid and phycocyanin extractions from Spirulina and economic assessment. The Journal of Supercritical Fluids. 2022 May 1;184:105567. https://doi.org/10.1016/j.supflu.2022.105567
12. Saefurahman G, Rahman AA, Hidayatuloh S, Farobie O, Abidin Z. Continuous extraction of Spirulina platensis biopigments using different

extraction sequences. InIOP Conference Series: Earth and Environmental Science 2021 May 1 (Vol. 749, No. 1, p. 012005). IOP Publishing.
13. Agustini TW, Suzery M, Sutrisnanto D, Ma'Ruf WF. Comparative study of bioactive substances extracted from fresh and dried Spirulina sp. Procedia Environmental Sciences. 2015 Jan 1;23:282-9. https://doi.org/10.1016/j.supflu.2022.105567
14. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. The Scientific World Journal. 2017 Jan 1;2017. https://doi.org/10.1155/2017/5873648
15. e Silva AD, Moreira LM, de Magalhães WT, Farias WL, Rocha MV, Bastos AK. Extraction of biomolecules from Spirulina platensis using non-conventional processes and harmless solvents. Journal of environmental chemical engineering. 2017 Jun 1;5(3):2101-6. https://doi.org/10.1016/j.jece.2017.04.008
16. Sivakumar J, Santhanam P. Antipathogenic activity of Spirulina powder. Recent Research in science and Technology. 2011 Mar 28;3(4).
17. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan.2008.42nd Edition.
18. Khandelwal KR,Vrunda S.Practical Pharmacognosy. Nirali Prakshan.2013.25.1-9.
19. Haroon HB, Perumalsamy V, Nair G, Anand DK, Kolli R, Monichen J, Prabha K. Repression of polyol pathway activity by hemidesmus indicus var. pubescens R. Br. linn root extract, an aldose reductase inhibitor: an in silico and ex vivo study. Natural Products and Bioprospecting. 2021 Jun;11:315-. https://doi.org/10.1007/s13659-020-00290-w
20. Kumar M, Singh T, Ali J, Tyagi LK. In vitro anticataract activity of Zingiber officinale on goat lenses. International Journal Pharmaceutical Biological Archives. 2011;2: 1430-3.
21. Hajarnavis AM, Bulakh PM. Anticataract effects of S. cumini and A. marmelos on goat lenses in an experimental diabetic cataract model. Journal of Ayurveda and integrative medicine. 2020 Oct 1;11(4):421-5. https://doi.org/10.1016/j.jaim.2019.08.001
22. Nimbalkar, V. V., Pansare, P. M., Nawale, D. S., & Nishane, B. B. (2015). Investigation of Carvedilol as an Antidenaturing and Anticataract Agent. Research Journal Pharmacy and Technology. 8(5), 511-516. https://doi.org/10.5958/0974-360X.2015.00085.2