

Protective Effect of Ethanolic Extracts of *Ananas comosus* and *Vitis vinifera* against Glucose Induced Cataract in Isolated Goat Lens

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ABSTRACT

Background: Cataracts represent the significant basis of blindness worldwide that averts the passage of light from the clear lens to the retina. The antioxidants present in natural sources are known to delay the formation of cataracts. These antioxidants are the major constituents of fruits. **Materials and Methods:** This study aimed to evaluate the fruits *Ananas comosus* and *Vitis vinifera* for their antioxidant and anticataract activities against the cataract induced by glucose in the freshly isolated goat lenses which was extracted by extracapsular method and were split into six groups with six goat lenses each. Group I was the normal control, Group II was cataract control, Group III was *A. comosus* extract treated lens, Groups IV and V were *V. vinifera* extract (green and black grapes) treated respectively and Group VI was treated with the standard drug Enalapril. The opacity of the lens was assessed after incubation and lens homogenate was evaluated for antioxidant enzymes and also for lipid peroxidation. **Results:** The ethanolic fruit extracts of *Ananas comosus* and *Vitis vinifera* showed significant antioxidant activity in *In vitro* methods and also good anticataract activity in the isolated goat lens which could be caused by the action of the phytochemicals. **Conclusion:** From the present study on the glucose induced cataractogenesis in the isolated goat lenses, it can be concluded that the ethanolic fruit extracts of *Ananas comosus* and *Vitis vinifera* exhibit significant antioxidant and anticataract activities.

Keywords: *Ananas comosus*, *Vitis vinifera*, Antioxidants, Fruits, Cataract, Goat lens.

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INTRODUCTION

A cataract is the clouding of the lens and is one of the primary causes of irreversible blindness due to complete or partial opacification of the lens. Cataracts are progressive which leads to impaired vision which occurs with elderliness and is reversible through surgery.^[1] The oxidative stress has a direct result in the opacification of the clear lens and is caused by free radicals which contribute to impaired physiological function.^[2] Phenolics, flavonoids, and certain enzymes play a critical

role in safeguarding against oxidative stress. It is claimed that there is a substantial reduction of these antioxidant enzymes in the lens during cataract formation.^[3]

Medicinal plants have been utilized for treating various diseases for centuries and majority of the people still depend on these medicinal plants.^[4] The plant kingdom produces the most abundant sources of antioxidants and also other phytochemicals. There is a budding interest in the research of numerous indigenous plants as these plants are present with potential promising sources of antioxidants.^[5] The antioxidant profile of plants is accredited to several phenolic compounds that share the same basic structure but differ in quantity and type, depending on the source.^[6] The antioxidant potential of the plants is linked to the polyphenolic content, the greater the phenolic content, the greater the antioxidant activity.^[7]

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Ananas comosus L., belonging to the *Bromeliaceae* is an herbaceous, tropical plant that produces fleshy, edible fruits. Pineapples are considered one of the most useful fruits which are rich in antioxidants, organic acids, bromelain and phenolic compounds.^[8] *Vitis vinifera* belongs to the family of Vitaceae and is a fast-growing liana that reaches up to 12- 15m in height. It has a variety of secondary metabolites, particularly flavonoids, phenolic acids and anthocyanins. It has antioxidative, cardioprotective, hepatoprotective, anticancer, antibacterial and antiviral activities.^[9] In this context, an attempt was made to assess the antioxidant and *ex vivo* anticataract potential of *Ananas comosus* and *Vitis vinifera* ethanolic fruit extracts on isolated goat lenses.

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

The fruits *Ananas comosus* L., and *Vitis vinifera* L. (black and green grapes), were collected from Coimbatore and authenticated by the BSI (Reference no. BSI/SRC/5/23/2023/Tech-162) and (Reference no. BSI/SRC/5/23/2023/Tech-163). These fruits were washed, shade-dried and powdered. Five grams of the powdered fruit materials were macerated with 50 mL of ethanol and incubated at 40°C for 48 hr in a shaker incubator. The solvent was then evaporated to obtain a dry extract and stored at -4°C for further use.

Qualitative Phytochemical Analysis

Phytochemical analysis of the ethanolic extracts of *A. comosus* and *V. vinifera* fruits was performed to detect the types of phytochemicals present using standard methods.^[10]

Quantitative Phytochemical Analysis

The selected phytochemicals namely total alkaloids, phenolics, tannins, flavonoids and terpenoids were quantified using the standard methods.^[11-15]

In vitro Antioxidant Assays

The Total Antioxidant Capacity (TAC) of the fruits was evaluated by the phosphomolybdenum method.^[16] DPPH (2, 2'-diphenyl-2-picryl hydrazyl hydrate) was performed according to the method of Blois.^[17] The Hydrogen Peroxide (H₂O₂) scavenging activity of plant extracts was performed as per the procedure of Ruch *et al.*^[18]

Ex vivo Anticataract Activity in Goat Lens

Collection of Eye Balls and Preparation of Lens Culture

Goat eyes used in the study were freshly acquired from the slaughterhouse and were transported at 0-4°C. An extracapsular extraction method was employed to

take away the lens, which was then cultured in artificial aqueous humor (MgCl₂ 2mM, NaHCO₃ 0.5 mM, NaCl 140 mM, NaHPO₄ 0.5 mM, CaCl₂ 0.4 mM, KCl 5 mM and glucose 5.5 mM) at an appropriate temperature. NaHCO₃ was added to maintain the pH at 7.8. 32mg% of Penicillin G and 250mg% of Streptomycin were added to prevent microbial contamination.^[19]

Induction of Cataract on Goat Lens

To induce cataracts, 55 mM glucose was used. Glucose is metabolized through the sorbitol pathway at high concentrations, leading to the buildup of polyol and subsequent oxidative stress and overhydration initiating cataractogenesis.

Experimental Design

The lenses were split into six groups as follows (Table 1). The experimental period was 72 hr.

Table 1: Experimental design.

Group	Sample Size	Treatment
I	06	Normal control: Goat lens+Artificial Aq. Humor (Glucose 5.5 mM).
II	06	Cataract control: Goat lens+Artificial Aqueous Humor (Glucose 55 mM).
III	06	Treatment 1: Goat lens+Artificial Aqueous Humor (Glucose 55 mM)+150 µg/mL of <i>Ananas comosus</i> L., extract.
IV	06	Treatment 2: Goat lens+Artificial Aqueous Humor (Glucose 55 mM)+150 µg/mL of <i>Vitis vinifera</i> L., (green grapes) extract.
V	06	Treatment 3: Goat lens+Artificial Aqueous Humor (Glucose 55 mM)+150 µg/mL of <i>Vitis vinifera</i> L., (black grapes) extract
VI	06	Positive control: Goat lens+Artificial Aqueous Humor (Glucose 55 mM)+50 µg/mL of Enalapril

Assessment of Anticataract Activity

Evaluation of Lens Opacity

The lens was assessed for turbidity by placing the lens on paper and observing the visible box considering the number after 72 hr and characteristics of the grid line photographed. The degree of opacity was categorized as follows:

- No opacity: 0
- Mild opacity: +
- Diffuse opacity: ++
- Extensive thick opacity: +++

Preparation of Lens Homogenate

Following incubation, the lens was weighed and homogenized using 0.1 M potassium phosphate buffer,

pH 7.0 and centrifuged at 10000 rpm at 4°C for 15 min. The supernatant was tested for the biochemical markers.

Estimation of Biochemical Parameters

Estimation of Antioxidant Enzymes

The Super Oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) levels were assessed using standard methods.^[20-22]

Estimation of Proteins

The total protein content was assessed as per the procedure of Lowry *et al.*^[23]

Estimation of Aldose Reductase Activity

The aldose reductase activity was determined by the NADPH oxidation method.^[24]

Estimation of Lipid Peroxidation

Lipid peroxidation was assessed following the procedure of Ohkawa *et al.*^[25]

Statistical Analysis

The results of the antioxidant analysis are expressed as Mean±SD ($n=3$). The results of *ex vivo* anticataract activity were statistically analyzed by one-way ANOVA followed by Tukey's multiple comparison test and are represented as a Mean±SEM ($n=6$). 'p' value of less than 0.5 was considered significant.

RESULTS

Extraction Yield

The extraction yield of pineapple, green and black grapes was found to be 17.6%, 20.6% and 20.3% respectively.

Qualitative Phytochemical Analysis

Preliminary phytochemical analysis indicated alkaloids, tannins, phenolics, flavonoids, terpenoids,

carbohydrates, quinones, saponins, and glycosides in the ethanolic extract of the fruits *Ananas comosus* and *Vitis vinifera* (Table 2).

Table 2: Phytochemical Analysis of *Ananas comosus* and *Vitis vinifera* (Green and Black Grapes).

Phytochemical	Ethanolic extract of Pineapple	Ethanolic extract of Green grapes	Ethanolic extract of Black grapes
Phenolics	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Carbohydrates	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+

(+ Presence, - Absence).

Qualitative Phytochemical Analysis

The total quantity of phenols, flavonoids, terpenoids, alkaloids and tannins was analyzed (Table 3).

The pineapple extract had the highest total phenolic content of 258.6±8.17 mg gallic acid equivalents/g and alkaloid content of 99±0.81 mg caffeine equivalents/g when compared with the green and black grape extracts. The high amounts of total flavonoid, total terpenoids and total tannin content of 254.6±8.80 mg quercetin equivalents/g, 53.2±1.07 mg/g and 107.33±11.81 mg tannic acid equivalents/g respectively were found in black grape extracts.

In vitro Antioxidant Assays

The ethanolic fruit extracts of black grapes showed higher antioxidant capacity when compared with the pineapple and green grape extracts (Table 4).

Table 3: Quantitative Phytochemical analysis of *Ananas comosus* and *Vitis vinifera* (Green and black grapes).

Fruit extract	Total Alkaloids (mg CFE/g extract)	Total Phenolic compounds (mg GAE/g extract)	Total Flavonoids (mg QE/g extract)	Total Tannins (mg TAE/g extract)	Total Terpenoids (mg/g extract)
Pineapple	99±0.81	258.6±8.17	243.3±6.79	149±2.16	49±0.81
Green Grapes	93.3±1.24	250.3±11.95	248.3±8.99	105.33±10.4	51.66±1.24
Black Grapes	93.66±0.471	257.1±9.19	254.6±8.80	107.33±11.81	53.2±1.07

*Values are mean±SD ($n=3$) (CFE- Caffeine equivalents, GAE- Gallic acid equivalents, QE- Quercetin equivalents, TAE- Tannic acid equivalents).

Table 4: Total Antioxidant Capacity of Fruit Extracts.

Fruit extracts	TAC (mg/g AAE) *
<i>Ananas comosus</i>	157.53±8.96
<i>Vitis vinifera</i> (Green grapes)	177.46±8.49
<i>Vitis vinifera</i> (Black grapes)	180.23±3.43

*Values are Mean±SD of triplicates; AAE- Ascorbic acid equivalents.

DPPH scavenging activity was found to be higher in the green grape extracts at 70.58% at 1000 µg/mL followed by pineapple extract at 67.05% and black grape extracts at 52.94% at 1000 µg/mL. Ascorbic acid had an activity of 84.70% at 1000 µg/mL. The results are given in Figure 1.

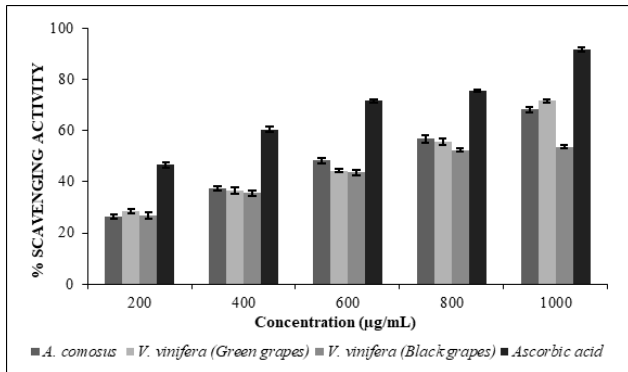


Figure 1: DPPH Radical Scavenging Activity of Fruits.

Values are Mean±SD of triplicates.

The hydrogen peroxide scavenging activity of *A. comosus*, *V. vinifera* (green and black grapes) and standard ascorbic acid at 1000 µg/mL was 70.90%, 77.49%, 78.26% and 80.32% respectively. The hydrogen peroxide scavenging activity of *Ananas comosus* and *Vitis vinifera* is given in Figure 2.

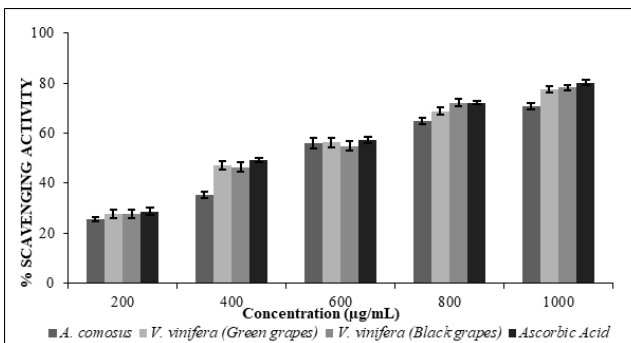


Figure 2: Hydrogen Peroxide Radical Scavenging Activity of Fruits.

Values are Mean±SD of triplicates.

Photographic Evaluation of the degree of opacity of the lens

After a 72 hr incubation period, lens turbidity was assessed by placing it on a grid and the changes in lens transparency were observed. After the incubation time, the opacity of the lenses had formed from outward to inward direction. Compared to the normal and treatment group lenses, complete opacity was seen in Group II (Cataract control) lenses. The enalapril-treated Group V showed a significantly lesser degree of opacity. Treatment Groups IV and V show the best clarity which was incubated with the fruit extract of green and black grapes in comparison with the lenses treated with the pineapple extract. The images of the lens belonging to normal control, cataract control and treatment groups are shown in Figure 3 and the photographic evaluation of the lens opacities is given in Table 5.

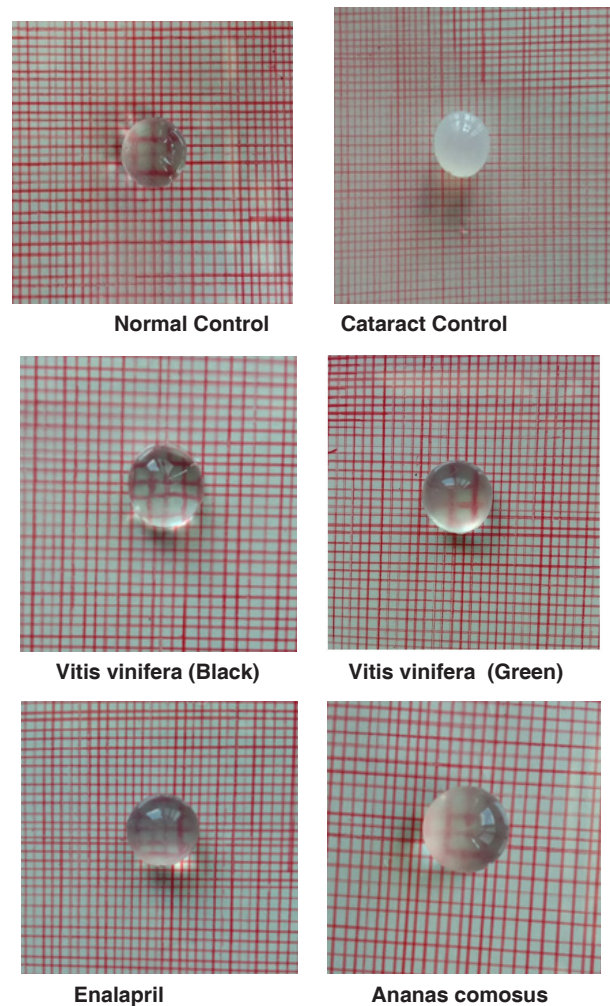


Figure 3: Assessment of Lens Opacity.

Group	Sample size	Treatment	Degree of Opacity
I	06	Normal control- Glucose 5.5 mM.	0
II	06	Cataract control- Glucose 55 mM.	++++
III	06	Treatment 1-150 µg/mL <i>A. comosus</i> fruit extract+Glucose 55 mM.	++
IV	06	Treatment 2-150 µg/mL <i>V. vinifera</i> (green) fruit extract+Glucose 55 mM.	+
V	06	Treatment 3-150 µg/mL <i>V. vinifera</i> (black) fruit extract+Glucose 55 mM.	+
VI	06	Positive control-50 µg/mL of Enalapril+Glucose 55 mM.	+

Evaluation of the Biochemical Parameters

The levels of the biochemical markers in the goat lens homogenate are given in Table 6. A significant reduction

Table 6: Estimation of Biochemical Parameters in Lens.

Experimental groups	SOD (unit/mg of protein)	Catalase (Ku/L)	Glutathione Peroxidase ($\mu\text{mol/g}$)	Total Protein (mg/dL)	Aldose Reductase ($\mu\text{g/mL}$)	Malondialdehyde (moles/mg)
Control	6.22 \pm 0.20 ^c	11.07 \pm 0.20 ^d	50.20 \pm 0.90 ^d	102.96 \pm 0.57 ^a	2.3 \pm 0.35 ^a	1.74 \pm 0.10 ^a
Cataract control	1.75 \pm 0.09 ^a	4.02 \pm 0.16 ^a	18.52 \pm 0.91 ^a	199.4 \pm 0.31 ^d	8.78 \pm 0.44 ^c	15.10 \pm 0.40 ^d
Pineapple (150 $\mu\text{g/mL}$)	3.04 \pm 0.21 ^b	7.04 \pm 0.29 ^b	27.03 \pm 1.30 ^b	143.1 \pm 0.29 ^c	3.64 \pm 0.30 ^b	10.46 \pm 0.38 ^b
Green Grapes (150 $\mu\text{g/mL}$)	3.34 \pm 0.31 ^b	8.68 \pm 0.32 ^c	34.32 \pm 1.15 ^c	140.8 \pm 1.87 ^c	2.38 \pm 0.04 ^{ab}	11.56 \pm 0.34 ^{bc}
Black Grapes (150 $\mu\text{g/mL}$)	3.14 \pm 0.19 ^b	9.03 \pm 0.30 ^c	37.96 \pm 0.74 ^{bc}	126.7 \pm 1.09 ^b	3.70 \pm 0.33 ^b	11.31 \pm 0.13 ^{bc}
Enalapril (50 $\mu\text{g/mL}$)	3.38 \pm 0.28 ^b	9.16 \pm 0.29 ^c	38.81 \pm 0.85 ^c	124.6 \pm 1.77 ^b	2.21 \pm 0.20 ^a	12.47 \pm 0.26 ^c

*Values are expressed as Mean \pm SEM (n=6). Values having different letters of alphabets in the same row differ significantly at $p < 0.05$ (One way ANOVA followed by Tukey's multiple comparison test)

Superoxide Dismutase: 1 Unit: Amount of enzyme that causes a 50% reduction in NBT oxidation, Catalase: 1 Unit: μM of H_2O_2 consumed/min/mg protein, Glutathione Peroxidase: 1 Unit: μg of glutathione consumed/min/mg protein.

($p < 0.05$) was observed in the levels of antioxidant enzymes in the cataract control group. The goat lens treated with enalapril and fruit extracts displayed a significant improvement ($p < 0.05$) in their levels. The total proteins were significantly raised ($p < 0.05$) in the cataract control whereas the treatment of the lens with the fruit extracts of pineapple, green and black grapes as well as the enalapril produced a decrease ($p < 0.05$) in the total protein level in the respective groups. The goat lens treated with the ethanolic extract of black grapes had significantly reduced ($p < 0.05$) total protein levels and it was followed by green grapes and pineapple extracts.

The activity of aldose reductase was significantly increased ($p < 0.05$) in the cataract control group. The lens treated with the *A. comosus* and *V. vinifera* extracts showed a significant ($p < 0.05$) reduction in the aldose reductase levels. Lipid peroxidation was also significantly increased ($p < 0.05$) in the lens of the cataract control group. The lens treated with the fruit extracts *A. comosus* and *V. vinifera* demonstrated a significant reduction ($p < 0.05$) in the lipid peroxidation rate.

DISCUSSION

Cataract is the main reason for loss of vision worldwide.^[26] In recent times, herbal medicine has garnered significant interest recently due to its reported safety and numerous pharmacological benefits. Natural compounds containing anti-inflammatory or antioxidant compounds could be considered possibly ideal anticataract agents, as the antioxidant effect is one of the primary mechanisms for cataract prevention in most cases.^[27] In the present study, the ethanolic extracts of *Vitis vinifera* (green and black grapes) and *Ananas comosus* (pineapple) were investigated for their

phytochemical content, antioxidant activity and *ex vivo* anticataract activity on freshly isolated goat lens.

Phytochemical analysis revealed the presence of phenolics, tannins, flavonoids, alkaloids, terpenoids, carbohydrates, saponins, quinones and glycosides in the ethanolic extract of the fruits *Ananas comosus* and *Vitis vinifera*. Higher quantities of total phenolics, tannins, terpenoids, flavonoids and alkaloids were also observed in the ethanolic fruit extracts and they could jointly play a crucial role in their antioxidant potential. Thus, it could be inferred that the antioxidant capacity of the fruits is due to the considerable amount of phenolic substances which also play a key role as anti-cataract compounds. The antioxidant capacity of the fruit extracts was estimated by *In vitro* assays. The fruit extracts exhibited significantly good antioxidant activities comparable to the standard ascorbic acid. This can be attributed to the phenolic and flavonoid compounds in the fruit extracts which support its use in the treatment of cataracts by managing oxidative stress.

The *ex vivo* anticataract activity of the ethanolic extracts of pineapple, black and green grapes was evaluated using a glucose-induced cataract model on a freshly isolated goat lens. The glucose-induced cataract model in isolated goat lenses is a valuable tool for cataract research, as it effectively mimics the pathophysiology of diabetic cataracts, including osmotic and oxidative stress. 36 lenses were taken and were split into 6 groups ($n=6$) and incubated in an artificial vitreous humor medium and cataract was induced using glucose. The evaluation of the onset of cataracts was studied after 72 hr. Based on visual assessment, the lenses of the normal control group I were determined to be of grade 0 indicating complete transparency and clarity. Lenses of group II were graded as ++++ as severe opacity was observed signifying oxidative stress and high production of free radicals which indicates cataract. Lenses belonging to

group III treated with *A. comosus* extract at 150 µg/mL were graded ++ because lenses were slightly opaque and those belonging to group IV and V treated with 150 µg/mL of green and black grape extracts were graded as + as the lenses were nearly clear with very minor opacity. The lenses of group VI treated with 50 µg/mL enalapril were also graded + as they exhibited very mild opacity and were otherwise clear. The differences in the results among different treatment groups can be credited to the antioxidant potential of the fruits as oxidative stress is the primary cause of cataracts. The fruit extract caused a decrease in oxidative stress in groups III, IV and V by preventing free radicals generated by glucose. This clearly shows that the ethanolic extracts of *A. comosus* and *V. vinifera* possess promising anti-cataract activity which could be due to a decrease in oxidative stress. Various studies have highlighted that natural antioxidants such as flavonoids and phenolics can protect the lens from oxidative damage and are crucial for the prevention of cataracts.^[28] Similar to our results, Mohandass *et al.* (2021) reported that the leaf extract of *Mentha spicata* prevented glucose-induced oxidative damage in the lenses, helping in delaying the onset of cataracts.^[29]

Cellular antioxidants such as superoxide dismutase, catalase and glutathione peroxidase perform key roles in combating free radicals. Under cataract conditions, the activities of these antioxidants decrease, leading to an imbalance in the antioxidant-oxidant systems. Furthermore, accelerated lipid peroxidation under oxidative stress can damage and destroy the lipid bilayers of the lens cell membranes.^[30] In this study, levels of SOD, CAT and GPx, were lower in the lens of the cataract control group. Lipid peroxidation, measured by Malondialdehyde (MDA) levels was higher in the lens of the cataract control group, indicating increased lipid peroxidation. The ethanolic extracts of *A. comosus* and *V. vinifera* significantly improved the antioxidant levels in the goat lens and reduced lipid peroxidation, resulting in lower MDA levels. This suggests the extract's ability to alleviate oxidative stress, thus protecting the lens from damage and improving or preventing cataracts. Aldose reductase is involved in the polyol pathway and typically has a low affinity for glucose. However, under high concentrations of glucose, its activity increases and the subsequent production of sorbitol leads to a decrease in NADPH. Increased aldose reductase activity thus indirectly contributes to the overall redox imbalance, exacerbating oxidative stress-induced damage.^[31] The fruit extracts of pineapple, green and black grapes produced a decrease in the aldose reductase activity in the lenses, helping to balance the retinal antioxidant systems.

The current study focussing on identifying fruits as anticataract agents, highlights the importance of the natural antioxidant compounds that help combat oxidative stress in the lens, a major contributor to cataract formation. Given their non-toxic nature and wide accessibility, fruits offer a practical and cost-effective means of cataract prevention, especially in regions with limited healthcare resources. High consumption of antioxidant-rich fruits is associated with a lower risk of cataracts, emphasizing the potential of dietary interventions.^[32] Furthermore, identifying specific fruits can pave the way for developing functional foods and supplements, while recognizing synergistic effects between certain fruits may enhance their protective action, leading to more effective dietary recommendations for at-risk individuals. The limitations of the study include the absence of an *In vivo* animal model where gene expression and molecular mechanisms can be studied. However, this *In vitro* study serves as a basis for further investigation into the protective effects of fruits *Ananas comosus* and *Vitis vinifera* providing preliminary data that can enlighten further *In vivo* studies.

CONCLUSION

The outcomes of the study show that the *Vitis vinifera* L., and *Ananas comosus* L., extracts exhibited significant antioxidant and anticataract activities in the isolated goat lens which could be due to the antioxidant phytochemicals present in these fruits. These fruit extracts thus have curative and preventive effects against cataract formation. Future studies should be focused on exploring the molecular mechanisms of *Ananas comosus* and *Vitis vinifera* fruit extracts on cataracts in an *In vivo* cataract model.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

All authors equally contributed to conceptualization, methodology, experiments, investigation, writing and visualization and data analysis under the supervision of the corresponding author.

ABBREVIATIONS

SOD: Superoxide dismutase; **CAT:** Catalase; **GPx:** Glutathione peroxidase; **NADPH:** Reduced nicotinamide adenine dinucleotide phosphate; **SD:** Standard deviation; **SEM:** Standard error of the mean; **CFE:** Caffeine equivalents; **GAE:** Gallic acid

equivalents; **QE**: Quercetin equivalents; **TAE**: Tannic acid equivalents; **MDA**: Malondialdehyde.

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