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Research Article

**IN VITRO ANTICATARACT ACTIVITY OF TAMARINDUS
INDICA LINN. AGAINST GLUCOSE-INDUCED
CATARACTOGENESIS**S. K Godasu¹, Nimma vijayarekha², G. Anusha³, D. Varun⁴, Praveen Gujjula⁵^{1,5} Associate Professor, Sri Indu Institute of Pharmacy^{2,3} Assistant Professor, Sri Indu Institute of Pharmacy⁴ Professor & Principal, Sri Indu Institute of Pharmacy

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Abstract:

The main objective of the present study is to screen the hydromethanolic extract of *Tamarindus indica* Linn. for its in vitro anticataract activity against glucose-induced cataractogenesis using goat lenses. Phytochemical screening of the powdered leaves of *Tamarindus indica* showed the presence of phenolics, tannins, saponins, flavonoids and alkaloids. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 µg/ml) and Vitamin E (100 µg/ml) simultaneously with glucose for 72 h caused a significant ($P < 0.01$) increase in the total protein and a decrease in the level of malondialdehyde and lipid hydroperoxides. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 µg/ml) and Vitamin E simultaneously with glucose significantly ($P < 0.01$) restored the levels of both enzymatic and nonenzymatic antioxidant enzymes which is almost similar to the control group. The study suggested that the leaf extract of *Tamarindus indica* possess anticataract and antioxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further investigations on the isolation and identification of active components in the leaves may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

Key words: In Vitro, Anticataract Activity, *Tamarindus Indica* Linn, Glucose-Induced Cataractogenesis

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INTRODUCTION:

Opacification of the lens of the eye, cataract is the foremost cause of sightlessness it accounts for approximately 50% of all blindness worldwide. World Health Organization launched Vision 2020, to get rid of cataract as priority diseases [1,2]. In India, cataract is responsible for almost 80% of blindness [3,4]. Apart from senile cataract various risk factors such as nutritional deficiency, sunlight, smoking environmental factors, lack of consumption of antioxidants and diabetes are known to increase the risk of cataract [5-8]. Diabetes has been painstaking to be one of the chief threats for cataract. Numbers of in vitro and in vivo research studies hold up the scrutiny that diabetes is one of the causes of cataract [9]. Cataract is considered a major cause of visual impairment in diabetic patients. The incidence and progression of cataract is elevated in patients with diabetes mellitus. During hyperglycemia extra cellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the body parts most affected in diabetes mellitus. The proteins of the lens are extremely long-lived, and there is virtually no protein turnover, which can lead to posttranslational modification [3]. Intake of unwarranted galactose has been shown to induce the formation of cataracts in several species of experimental animals. The cataractogenic effect is primarily related to the synthesis and accumulation of excessive sorbitol (polyols) in the lens fibers and consequent osmotic stress [10]. Sorbitol is synthesized by aldose reductase utilizing NADPH and does not easily cross cell membranes; it can accumulate in cells and cause damage by disturbing osmotic homeostasis. Second Pathophysiological mechanism of cataract formation includes undersupplied glutathione levels contributing to a defective antioxidant defense system within the lens of the eye [11]. Normally the lens contains significant levels of reduced glutathione (GSH), which keeps the proteins in their reduced form. However, there are significantly decreased levels of GSH in cataractous lenses. Therefore, prevention of polyol accumulation and maintaining GSH level to prevent cataract and diabetes has received extensive interest.

A variety of therapeutic plants are reported to possess anti-diabetic and offer protection in various pathological conditions such as cardiovascular diseases, neurodegeneration [12]. A large number of plants/species are now well recognized to possess hypoglycemic potential [13,14]. Number of these hypoglycemic agents has not been investigated for their favorable effects on secondary complications of diabetes such as cataract. It would be of huge magnitude to evaluate both pharmacologically and biochemically, which might be helpful in the better management of secondary complications of diabetes.

Tamarindus indica Linn belonging to the family

Fabaceae is a large sized tree widely distributed in India. The leaves are reported to possess flavonoids, saponins and tannins. The leaves of this species are used in Indian traditional medicine for the treatment of ophthalmia and other eye infections, applied to reduce inflammatory swellings, tumours, etc (Krithikar and Basu, 1987). The main objective of the present study is to screen the hydromethanolic extract of *Tamarindus indica* Linn. for its *in vitro* anticataract activity against glucose-induced cataractogenesis using goat lenses.

MATERIALS AND METHODS:**Plant material**

The plant material consists of dried powdered leaves of *Tamarindus indica* Linn. belonging to the family Fabaceae.

Plant collection and authentication

The leaves of *Tamarindus indica* Linn were collected from Telangana. The plant was identified and authenticated.

Preparation of extraction

The fresh leaves of *Tamarindus indica* Linn are collected, dried in shade under room temperature, powdered mechanically and sieved through No. 20 mesh sieve. The finely powdered leaves were kept in an airtight container until the time of use. The extraction was carried out by continuous hot percolation method using Soxhlet apparatus. The solvent used was a mixture of methanol: water in the ratio of 7:3. About 100 g of powder was extracted with 600 ml of solvent. The extract was concentrated to dryness under controlled temperature between 40-50 °C.

Drugs and chemicals

Glucose and vitamin E were obtained from SD fine chemicals, Mumbai. Liquid paraffin was obtained from Fisher Chemicals Ltd., Chennai. Thiobarbituric acid, Trichloro acetic acid, Butylated hydroxyl toluene, oxidized glutathione, epinephrine and 5,5'-Dithiobis-2 nitrobenzoic acid were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. 2,2'-dipyridyl and *O*-dianisidine were obtained from Himedia Laboratories Ltd., Mumbai. Goat lenses were obtained from the slaughterhouse Coimbatore. All other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

Phytochemical screening

Chemical tests were carried out for the extract of *Tamarindus indica* Linn for the presence of phytochemical constituents (Trease and Evans, 2002).

Test for tannins and phenolics

To the solution of the extract, a few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins

About 10 ml of the extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent-froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of an emulsion.

Test for flavanoids

- a) To a portion of the extract, concentrated H₂SO₄ was added. A yellow colouration observed indicates the presence of flavanoids. The yellow coloration disappeared on standing.
- b) Few drops of 1% AlCl₃ solution was added to a portion of extract. A yellow coloration indicates the presence of flavonoids.

Test for terpenoids

About 5 ml of the extract was treated with 2 ml of chloroform and 3 ml concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown coloration of the interface formed shows the presence of terpenoids.

Test for alkaloids

A small portion of the extract was stirred with few drops of dil HCl and filtered.

- a) To the filtrate, Mayer's reagent was added and a cream precipitate indicates the presence of alkaloids.
- b) To the filtrate, Dragendorff's reagent (potassium bismuth solution) was added and an orange brown precipitate indicates the presence of alkaloids.

In vitro experimental model of cataract

In vitro experimental model of cataract was induced in goat lenses using glucose at a concentration of 55 mM, incubated for 72 h at room temperature. At high concentrations, glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This leads to cataractogenesis.

Experimental protocol

A total of 30 goat lenses were used and divided into the following 5 groups (n = 6 in each group),

- Group I: Artificial aqueous humor alone (solvent control)
- Group II: Glucose 55 mM alone (Negative control)
- Group III: Plant extract (100 µg/ml) + glucose 55 mM

Group IV: Plant extract (200 µg/ml) + glucose 55 mM

Group V: Vitamin E (100 µg/ml) + glucose 55 mM (Standard drug)

In vitro lens culture

Fresh goat eyeballs were collected from slaughterhouse, immediately after slaughter and transported to the laboratory. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH(PO₄)₂ 0.5 mM, CaCl₂ 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination (Langade, 2006).

Preparation of lens homogenate

After 72 h of incubation, homogenate of lenses (10% w/v) was prepared in Tris buffer (0.23 mM, pH 7.8) containing 0.25x10⁻³ M EDTA. The homogenate was centrifuged at 10,000 g for 1 h and the supernatant was used for estimation of total protein (TP), determination of the end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), and glutathione peroxidase (GPx), and the non-enzymatic antioxidant reduced glutathione (GSH).

Estimation of total protein (TP)

The amount of total protein present in the tissue homogenate was estimated by the method of Lowry *et al.*, 1951. To 0.1 ml of tissue homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min for colour development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue (Lowry *et al.*, 1951).

Estimation of malondialdehyde (MAD)

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) were measured by the method of Nieshus and Samuelsson, 1986. About 0.1 ml of tissue homogenate (Tris HCl

buffer, pH 7.4) was treated with 2 ml (1:1:1 ratio) of TBA – TCA-HCl reagent (Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 min, cooled and centrifuged at 1000 g at room temperature for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The values are expressed as nmoles of MDA /min/mg lens protein.

Estimation of lipid hydroperoxides (LH)

About 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (188 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90 ml of methanol and 10 ml 250 mM sulphuric acid) and incubated for 30 min. The colour developed was read at 560 nm using a colorimeter. The values are expressed as nmoles/mg lens protein (Nieshus and Samuelsson, 1986).

Determination of enzymatic antioxidants Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of the homogenate at 480 nm. The reaction mixture contained 150 µl of lens homogenate, 1.8 ml of carbonate buffer (30 mM, pH 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate (Kakkar *et al.*, 1984).

Estimation of catalase (CAT)

The catalysis of H₂O₂ to H₂O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1 ml of tissue homogenate and was incubated at 37°C for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H₂O₂. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzyme activity. Activity was expressed as µmoles/mg tissue protein (Abei, 1984).

Estimation of glutathione reductase (GSSH)

The enzyme activity was determined spectrophotometrically by the decrease in absorbance of NADPH at 340 nm. The reaction mixture contained 2.1 ml of 0.25 mM, potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione and 0.1 ml (10 mg/ml) of bovine serum

albumin (BSA). The reaction was started by the addition of 0.02 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 min against a blank. Glutathione reductase activity was expressed as nmoles NADPH oxidized /min/mg lens protein at 30°C (Racker, 1955).

Estimation of peroxidase (Px)

Peroxidase activity was measured spectrophotometrically by following the change in absorbance at 460 nm due to *O*-dianisidine oxidation in the presence of H₂O₂ and enzyme. Reaction mixture contained 0.2 ml of 15 mM *O*-dianisidine, 0.1 ml of tissue homogenate and 2.5 ml of 0.1M potassium phosphate buffer pH 5.0 and were incubated at 37°C for 15 min and the reaction was started with the addition of 0.2 ml of hydrogen peroxide and the absorbance at 460 nm was followed against a blank, spectrophotometrically for about 3-5 min at 37°C. Unit of enzyme activity defined as µmoles of *O*-dianisidine/min at 37°C (Lobarzewski and Ginalska, 1995).

Estimation of glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by the procedure of Paglia and Valentine, 1967. The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1 ml of sodium azide, 0.1 ml of hydrogen peroxide, 0.2 ml of glutathione and 0.2 ml of supernatant incubated at 37°C % TCA and the absorbance was measured at 340 nm. Activity was expressed as nmoles/min/mg lens protein.

Determination of non-enzymatic antioxidant Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. To the homogenate, 0.1 ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellmans reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid DTNB in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412 nm. Activity was expressed as nmoles/min/mg lens protein (Ellman, 1959).

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM

of six lenses in each group. P values < 0.05 were considered significant.

Phytochemical screening of the powdered leaves of *Tamarindus indica* showed the presence of phenolics, tannins, saponins, flavonoids and alkaloids.

RESULTS:

Phytochemical screening

Table 1. Phytochemical screening

Phytochemicals	<i>Tamarindus indica</i>
Alkaloids	+
Flavonoids C-glycosides	+
Saponins	+
Tannins and phenolics	+
Terpenoids	+

Effect of the extract of *Tamarindus indica* leaves on lens protein and lipid peroxidation in experimental groups

There was a significant ($P < 0.01$) decrease in the level of total protein and an increase in the level of malondialdehyde and lipid hydroperoxides in glucose-induced cataractous lenses when compared to normal control. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 $\mu\text{g/ml}$) and Vitamin E (100 $\mu\text{g/ml}$) simultaneously with glucose for 72 h caused a significant ($P < 0.01$) increase in the total protein and a decrease in the level of malondialdehyde and lipid hydroperoxides (Table 2).

Table 2. Effect of *Tamarindus indica* hydromethanolic leaf extract on lens protein, MDA and LH in control and experimental groups

GROUP	DOSE	PROTEIN	MDA	LH
Normal control	–	103.6 \pm 8.89	0.68 \pm 0.024	4.13 \pm 0.37
Glucose control	55 mM	36.64 \pm 1.26 ^a	1.98 \pm 0.017 ^a	10.98 \pm 0.60 ^a
Plant extract	100 $\mu\text{g/ml}$	74.38 \pm 2.12 ^b	0.84 \pm 0.014 ^b	5.16 \pm 0.37 ^b
Plant extract	200 $\mu\text{g/ml}$	76.22 \pm 3.12 ^b	0.78 \pm 0.032 ^b	4.88 \pm 0.73 ^b
Vitamin-E	100 $\mu\text{g/ml}$	84.24 \pm 3.62 ^b	0.72 \pm 0.08 ^b	4.54 \pm 0.23 ^b

Values are mean \pm SEM; n=6 in each

^a $P < 0.01$ when compared to normal control;

^b $P < 0.01$ when compared to glucose control (one way ANOVA followed by Dunnett's test). Protein = nmoles/min/mg, MDA = nmoles/min/mg protein, LH = nmoles/min/mg protein.

Effect of *Tamarindus indica* hydromethanolic leaf extract on lens enzymatic and non-enzymatic antioxidants in experimental groups Incubation with glucose 55 mM for 72 h produced a significant ($P < 0.01$) decrease in enzymatic antioxidants like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase and the non-enzymatic antioxidant reduced glutathione in the lens homogenate when compared to normal control. Incubation with the hydromethanolic leaf extract of *Tamarindus indica* at doses of (100 & 200 $\mu\text{g/ml}$) and Vitamin E simultaneously with glucose significantly ($P < 0.01$) restored the levels of both enzymatic and nonenzymatic antioxidant enzymes which is almost similar to the control group (Table 3).

Table 3. Effect of *Tamarindus indica hydromethanoli* leaf extract on lens enzymatic and non-enzymatic antioxidants in control and experimental groups

Group	Dose	Catalase	GPx	SOD	GSSH	Peroxidase	GSH
Normal control	–	1.73±0.17	2.88±0.29	4.53±0.23	1.99±0.09	2.47±0.32	2.99±0.32
Glucosecontrol	55 mM	0.49±0.04 ^a	1.12±0.11 ^a	1.23±0.37 ^a	0.08±0.016 ^a	0.86±0.19 ^a	1.11±0.20 ^a
Plant extract	100 µg/ml	1.12±0.09 ^b	2.26±0.21 ^b	3.59±0.73 ^b	0.94±0.26 ^b	1.89±0.04 ^b	2.17±0.23 ^b
Plant extract	200 µg/ml	1.38±0.09 ^b	2.43±0.24 ^b	3.84±6.37 ^b	1.13±0.08 ^b	1.96±0.13 ^b	2.29±0.12 ^b
Vitamine-E	100 µg/ml	1.42±0.07 ^b	2.54±0.25 ^b	3.92±0.60 ^b	1.24±0.08 ^b	2.10±0.21 ^b	2.37±0.21 ^b

Values are mean ± SEM; n = 6 in each group.

^aP<0.01 when compared to normal control;

^bP <0.01 when compared to glucose control (One way ANOVA followed by Dunnett's test). CAT = µmoles/min/mg protein GPx = nmoles/min/mg protein, GSH = nmoles/min/mg protei

DISCUSSION AND CONCLUSION:

The present thesis entitled “*In vitro* anticataract activity of *Tamarindus indica* L. against glucose-induced cataractogenesis” deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Tamarindus indica* L. belonging to the family Fabaceae, which is traditionally used by the local people and tribals in India for the treatment of inflammatory swelling, tumors, ringworm; useful in disease of blood, small pox, ophthalmia and other eye diseases, earache, snake-bite (Krithikar and Basu, 1981).

Cataract is one of the leading causes of visual disability often leading to blindness. It is an age-related phenomenon over and above; oxidative stress also plays an important role. The situation can be remedied surgically by extirpation of the cataractous lens. The limitations of cataract surgery have stimulated experimental cataract research in laboratory animals and epidemiological studies to determine the incidence, prevalence and risk factors for the development of cataract so as to focus on the preventive aspects of cataract (Gupta *et al.*, 1997a)

Cataract was induced *in vitro* with glucose at a concentration of 55 mM in aqueous humor media and incubated for 72 h at room temperature (Langlade 2006). After incubation the lens homogenate was used for the estimation of total

protein (TP) content, determination of end products of lipid peroxidation namely malondialdehyde (MDA) and lipid hydroperoxides (LH), enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSH), peroxidase (Px), glutathione peroxidase (GPx), and non-enzymatic antioxidant reduced glutathione (GSH).

Lipid peroxidation is an autocatalytic process, which is a common cause of cell death (Bandhyopadhyay *et al.*, 1999). In order to elucidate the protective mechanism of the leaves of *Tamarindus indica*, glucose-induced goat lens was examined for lipid peroxide levels. Decomposition of lipid peroxides initiate the chain reactions that produce reactive carbonyl compounds. The by-products of lipid peroxidation are the toxic compounds malondialdehyde (MDA) and lipid hydroperoxides (LH) whose involvement in cataractogenesis has been suggested, mainly due to its cross-linking ability. Lens MDA may be the result of lipid peroxidation of the lens cell membrane or may represent the consequence of its migration from the readily peroxidizable retina or from the central compartment. In our studies, glucose-induced goat lenses showed an increase in malondialdehyde and lipid hydroperoxide levels in lens. Incubation at different concentrations (100 & 200 µg/ml) of extract of *Tamarindus indica*, simultaneously

with glucose (55 mM) for 72h caused a significant ($P<0.01$) decrease in the lens malondialdehyde and lipid hydroperoxides and an increase in total protein level. This effect was almost similar to the vitamin E treated group. phase), leading to the formation of superoxide and H_2O_2 as well as hydroxyl radicals. It has been proposed as a central mechanism of oxidative injury in some situations (Nijveldt *et al.*, 2001). Thus the determination of the lens *in vitro* antioxidant enzymes like SOD, CAT, GPx, GSSH, peroxidase (Px) and non-enzymatic antioxidant enzyme, GSH were carried out.

Catalase is present in almost all the mammalian cells localized in the peroxisomes. It catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and hydroxyl radical. The dichromate/acetic acid reagent can be thought of as a 'stop bath for catalase activity. As soon as the enzyme hits the acetic acid, its activity is decomposed on heating to give a green solution. *Tamarin dus indica* extract significantly increased ($P<0.01$) the catalase level in glucose-induced cataractous lenses.

The first enzyme involved in the antioxidant defence is superoxide dismutase. It is metalloprotein found in both prokaryotic and eukaryotic cells. The oxygen radicals, generated by intraction of Fe^{2+} and H_2O_2 are the species responsible for the oxidation of epinephrine at pH 10.2 and was strongly inhibited by superoxide dismutase (Misra *et al.*, 1972).

GPx has a major role in degrading the levels of H_2O_2 in cells. Since GPx acts on hydroperoxides of unsaturated fatty acids, the enzyme plays an important role in protecting membrane lipids, and thus the cell membranes from oxidative disintegration (Rotruk *et al.*, 1973). The enzymatic antioxidant levels in glucose-induced cataract groups were decreased when compared to normal control group. The leaf extract of *Tamarin dus indica* significantly ($P<0.01$) increased the level of antioxidant enzymes. which is almost similar to the vitamin E treated group.

GSH is an intracellular reductant which plays major role in catalysis, metabolism and transport. It protects cell against free radicals, peroxides and other toxic compounds. Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects

(Gupta *et al.*, 1997a). Extract of *Tamarin dus indica* significantly ($P<0.01$) increased the level of GSH when compared to glucose-induced cataractous lenses.

To conclude, the study suggested that the leaf extract of *Tamarin dus indica* possess anticataract and antioxidant activities, which might be helpful in preventing or slowing the progress of cataract. Further investigations on the isolation and identification of active components in the leaves may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

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