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Saraca indica Eyedrop as a Therapeutic Potential for the Prevention As Well As Treatment of Diabetes Induced Cataract



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ABSTRACT

Diabetes-induced Cataract could be a major concern relating to the explanation for sightlessness in developed and developing countries. Recent basic analysis studies have emphasized the role of the polyol pathway within the initiation of the malady method. A diabetes-induced cataract is additionally noted noted cataract. Demographic studies have definitely increased our data regarding associate degree association between polygenic disease and cataract formation and have outlined the chance factors for the event of cataracts. Diabetic patients even have the next risk of complications when phacoemulsification cataract surgery as compared to non-diabetic folks. Aldose-reductase inhibitors and antioxidants are tried helpful within the hindrance or treatment of this sight-threatening condition in vitro experimental studies. Simple sugar enzyme inhibitors have important importance in the treatment and hindrance of diabetic complications. Nowadays, the employment of natural, herbal, and naturopathic medications has raised, as they need additional therapeutic potential and lesser facet effects as compared to medical care and ayurvedic medications. This analysis work provides associate degree insight into associate degree seasoning standardized extract and its ocular formulation development, Eyedrop, as an easy, sterile, safe, and different to phacoemulsification. Development of eye-drop as associate degree ocular formulation associate degreeed its standardization with an in vitro analysis of the seasoning extract for the hindrance further as treatment of Diabetic cataracts.



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INTRODUCTION

Diabetes and Cataract

The term Diabetes Miletus is derived from the Greek word for diabetes as a siphon and the Latin word for Mellitus as sweet. The Greek physician Arateus described diabetes as the melting down of flesh and limbs into excrement. Diabetes is a chronic disease where the pancreas, an organ that secretes a hormone called insulin to help glucose get into the cells of our bodies. It is not a single disease rather it's a group of metabolic diseases characterized by hyperglycemia caused by inadequate secretion of insulin with a simultaneous decrease in hormone action at its receptor. When diabetes occurs, the pancreas neither makes sufficient enough insulin nor can use the body's enough insulin. This causes sugar elevation in the blood sugar. In β -cells of the islets of Langerhans in the pancreas. Insulin is the hormone of pancreatic activity. It works like a key for glucose from the food we eat, passing from the blood into the cells to generate body energy. Hyperglycemia of diabetes is related to long-term diabetic complications such as retinopathy, neuropathy, cataract, nephropathy, atherosclerosis, and cardiovascular diseases. Recently, diabetes is an important health problem all over the world due to its frequency of complications. The blood glucose is divided into all carbohydrate foods. Insulin allows glucose to reach the cells. Diabetes mellitus can influence all eye structures, with one of the most frequent ocular complications being a cataract. Cataract seems to be the world's leading blindness cause. The occurrence of cataract formation in the diabetic population becomes increased due to many mechanisms. The diabetic population remains in danger of vision loss, including diabetic macular edema (ME), postoperative ME, and diabetic retinopathy.

Pathophysiology of Diabetic Cataract

In diabetes, the enzyme aldose reductase catalyzes the reduction of glucose to sorbitol through the polyol pathway, a process that develops a diabetic cataract. The central role of the AR pathway is that it is an initiating factor in diabetic cataract formation. Sorbitol is produced in the lens and it gets converted to fructose by the enzyme sorbitol dehydrogenase. The polarity of sorbitol prevents its intracellular removal through diffusion. The increased accumulation of sorbitol creates a hyperosmotic that results in an infusion of fluid to balance the osmotic gradient. Animal studies have shown that the intracellular accumulation of polyols leads to a collapse and liquefaction of lens fiber, which ultimately results in the

formation of lens opacities. Thus, the polyol pathway is the primary mediator of diabetes-induced oxidative stress in the lens. The polyol pathway has been described as the major mediator or link that causes diabetes-induced stress in the eye lens. Inclusively, the polyol pathway provides a means through which the enzyme AR speeds the reduction of glucose into sorbitol, leading to the development of cataracts. Increased accumulation of sorbitol causes hyperosmotic effects leading to cataracts due to the formation of hydropic lens fibers. The pathogenesis of diabetic cataracts can be explained through autoimmunity in type one diabetic patients. Various organic chemistry pathways area unit activated below hyperglycemic conditions. Among these, the polyol pathway is the most widely studied and therefore the most promising to clarify the mechanisms of diabetic complications. This pathway involves two main enzymatic steps: the first and key enzyme aldose reductase (AR), which reduces glucose to sorbitol by using NADPH as a chemical compound, and also the second catalyst, sorbitol dehydrogenase (SDH), converts sorbitol to ketohexose with nicotinamide adenine dinucleotide as a chemical compound.

In cases of diabetic complications like retinopathy, neuropathy, and nephrosis, the speed of the polyol pathway considerably will increase. As a result of this, the buildup of sorbitol and its metabolites happens in many cells together with nerves, retina, and kidneys because of the poor penetration across membranes and inefficient metabolism of sorbitol. Thus, every aerophilous stress and diffusion stress increase inside the cells relying on the event of diabetic complications. High activity of AR causes an increase within the consumption of NADPH and this causes a decrease in the activities of glutathione catalyst and gas synthase. Therefore, high activity of AR causes a reduction of the living thing GSH and NO levels. As a result, monosaccharide enzyme protein activity reduces the cellular inhibitor capability. Meanwhile, the oxidization of sorbitol to levulose by SDH results in aerophilic stress. This is a result of its co-factor NAD^+ being born again to NADH within the method, and NADH is the substrate for the NADH enzyme to get reactive gas species (ROS).

Fructose, which is generated from the polyol pathway, is metabolized into fructose-3-phosphate (F3P) and 3-deoxyglucosone (3DG). F3P and 3DG are more potent non-enzymatic glycation agents. Thus, the flux of glucose through the polyol pathway would increase the advanced glycation end product (AGE) formation. It is known that AGE leads to several noxious diseases such as Alzheimer's disease,

Atherosclerosis, diabetes, heart failure, and cancer associated with oxidative stress.

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As a result, studies on the determination of the simple sugar enzyme inhibitors area unit gaining importance a day. Inhibitors of AR are employed in therapeutic applications in diabetic complications. Some well-known AR inhibitors area unit epalrestat, tolrestat, zenarestat, sorbinil, zopolrestat, ponarestat, lidorestat, fidarestat, ranirestat, quercetin, resveratrol and ADN-158.

The use of those inhibitors has remained restricted because of undesirable facet effects. Therefore, the identification of recent simple sugar enzyme inhibitors and also the inhibition of simple sugar enzyme by natural chemicals area unit important. specifically, studies victimisation synthetic resin compounds as simple sugar enzyme inhibitors area unit extremely popular. In recent years, there are several studies on the isolation of synthetic resin compounds from ancient plants and their effects on aldohexose metabolism, significantly the polyol pathway.

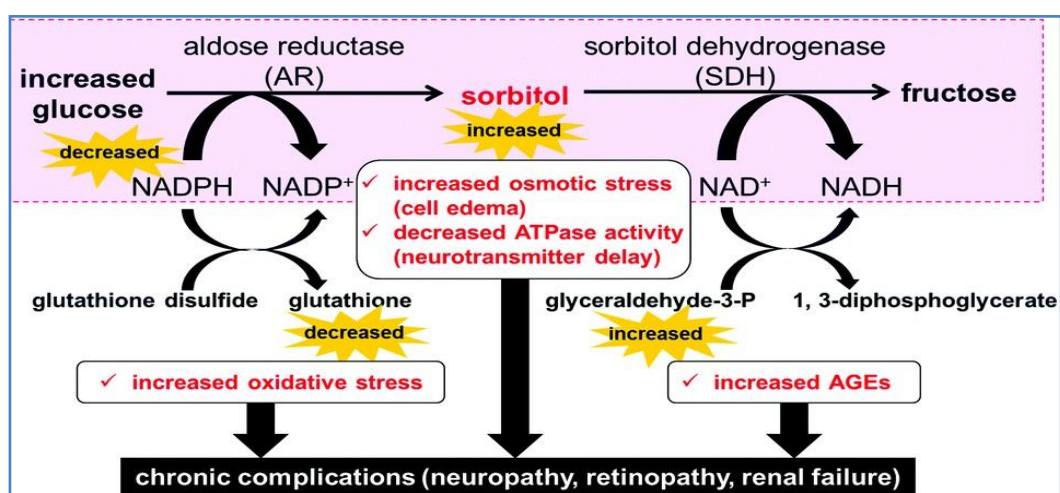


Fig-1: Mechanism of polyol pathway

Epidemiology of Diabetic Cataract

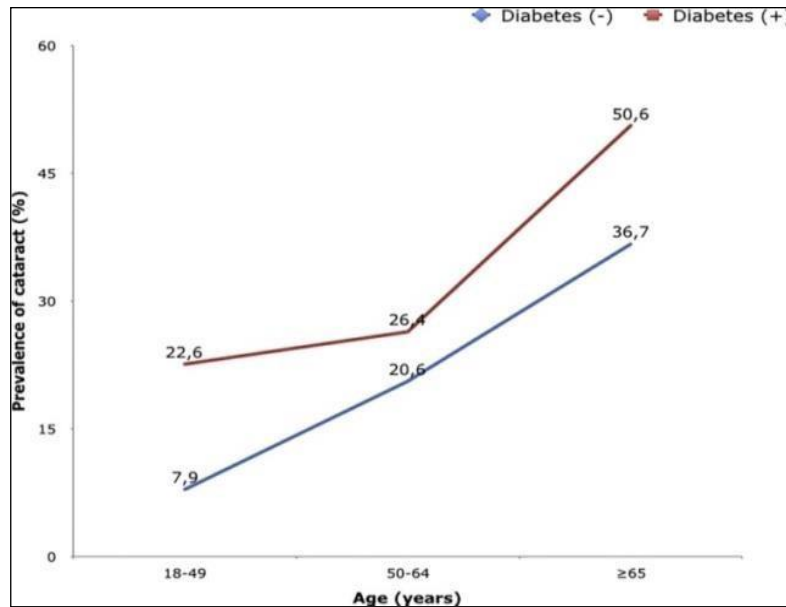


Fig-2: Prevalence of Diabetic cataract along with age factor.

Worldwide over 285 million individuals square measure laid low with DM. This variety is predicted to extend to 439 million by 2030 consistent with the International polygenic disease Federation.

Cataract in diabetic patients is a condition that causes blurring of vision and is one of the major causes of blindness. The prevalence of diabetic cataracts continues to increase on a daily basis. Several studies and bodies of data have associated cataracts with diabetes. As the number of type 1 and type 2 diabetic patients increases, there is also a steady rise in the incidents of cataract conditions. Worldwide, cataract is the major cause of vision problem in the general public. According to Kiziltoprak (2019), it is estimated that there will be over 439 million diabetes mellitus patients by 2030. Diabetic patients are reported to be five times more likely to develop diabetic cataracts, especially at an early age. The Centers for Disease Control and Prevention indicates that over 32.2% of diabetes patients above the age of 45 have cataracts. According to the data from the International Diabetes Federation, approximately 382 million people globally suffer from diabetes and this number is expected to reach 592 million an increase of 55% by the year 2030. Among all diseases, diabetes-induced death ranks eighth within the world. it's been reportable that five.1 million individuals everywhere the globe died from polygenic disease and its complications in 2013.

Additionally, to being a chronic sickness that threatens human health, {diabetes|polygenic disorder|polygenic sickness} is sort of a expensive disease for each people and country. To reduce the burden of diabetes on the individual and society, early diagnosis and the appropriate treatment of diabetes and its complications should be affected. Therefore, the prevention of diabetes and its complications has become very important.

MATERIALS AND METHODS

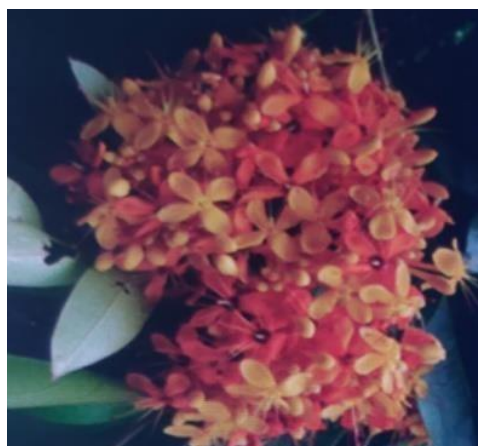


Fig-3: Flowers of *Saraca indica*



Fig-4: Bunch of *Saraca indica* flowers

Saraca indica, also known as Asoka tree or *Saraca asoca*. It is a plant belonging to the family Fabaceae and subfamily Detarioideae. The Ashoka tree belongs to the family of legumes. The Ashoka tree has been used in Traditional Indian medicine as an important ingredient in many cures and therapies. The Ashoka herb also has a beneficial effect on the circulatory system as it proves to be an effective remedy in arrhythmia and cardiac weakness. It can be used to obtain relief from burning sensations on the skin as it has anti-

inflammatory properties. It also helps to get rid of the toxins from the body. It is also naturally effective in purifying the blood and in preventing skin allergies. It has tremendous health benefits.

Flowers are orange or orange yellow turning vermillion (Brilliant red or scarlet), very fragrant and usually seen throughout the year, but it is in January and February that the profusion of orange and scarlet clusters turns the tree into a startling beauty. Pinned closely on to every branch and twig, these clusters consist of numerous, small, long-tubed flowers, which open out into four oval lobes. Young leaves are soft, red and limp and remain like a pendant even after attaining full size. The straight or scimitar shaped pods, stiff, leathery, broad and about eight inches long, is red and fleshy before ripening. It can be distinguished from *S. asoca* by its non-clasping bracteoles, a lower number of ovules, slightly smaller pods, and a more eastern geographic distribution. Ashoka is considered as one of the most legendary and sacred trees of India. Ashoka tree grows around one year life span.

Pharmacological activities of *Saraca indica*

- 1) Antimicrobial activity: *Saraca indica* was subjected to antibacterial activity (ethanol: water, 1:1) on agar plate with different organisms such as *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhosa*, *Staphylococcus aureus*, (plant pathogen). *Agrobacterium tumefaciens* showed negative activity.
- 2) Antibacterial activity: *Saraca indica* dried flower buds tested against antibacterial activity of methanol extract on agar plate against *Salmonella viballerup*, *Shigella boydii*, *Escherichia coli*, *Vibro cholera*, *Shigella flexneri* and *Shigella dysenteriae* were found active. *Saraca indica* leaves tested against antibacterial activity of ethanol (95%) and water extract on agar plate *Escherichia coli*, *Staphylococcus aureus*, *Escherichia coli* were found active; whereas tested against *Staphylococcus aureus* gave negative result. The methanolic extracts of *Saraca indica* was assayed against *Alternaria cajani*, *Helminthosporium* sp., *Bipolaris* sp., *Curvularia lunata* and *Fusarium* sp. at different concentrations. The extracts exhibited good inhibitory activity against *A. cajani*, while it was effective at lower concentrations against other fungi also.
- 3) Anticancer activity: The anticancer activity of *Saraca indica* flowers indicated 50 percent cytotoxicity (*invitro*) in Dalton's lymphoma and Sarcoma.

- 4) Tumour cells at a concentration of 38 µg and 54 µg respectively, with no activity against normal lymphocytes but preferential activity for lymphocytes derived from leukemia patients.
- 5) Antimenorrhagic activity: Ashoka dried bark has been used for the treatment of dysmenorrhea as well as menorrhagia. *Saraca indica* dried bark as well as flower is used as a tonic for ladies in case of uterine disorders. *Saraca indica* stem bark is also used to treat all the disorders associated with menstrual cycle. It is also employed in menorrhagia, as an emmenagogue, uterine sedative, uterine infections as well as used in several preparations related to female menstrual hygiene.
- 6) Astringent activity; *Saraca indica* bark is used as astringent in menorrhagia, to stop excessive uterine bleeding, also as refrigerant, demulcent, uterine disorders, regular menstrual pain in abdomen, used for uterine disorders. Aqueous extract of the bark is reported to contain active principles, stimulating as well as relaxing the muscles of the ileum of the guinea pig. It is reported to stimulate the uterus, making the contraction more frequent and prolonged. The crystalline glycoside substance is reported to stimulate uterine contraction.
- 7) Antioxytotic activity: Oxytotic activity of the plant was demonstrated in rat and human isolated uterine preparations. Estrogen primed or gravid uterus was more sensitive to the action of the alcoholic extract. Seed extract is effective against dermatophytic fungi. In vitro tests on rat uterus preparation, extracts of *Saraca indica* did not show oxytotic activity. Ashoka, previously was tested twice with negative results and once with positive results.
- 8) CNS depressant activity: The leaves of *Saraca indica* show CNS depressant activity in solvents such as petroleum ether, chloroform, methanol and water, depending upon their polarity. The activity was evaluated using phenobarbitone induced sleeping time by using actophotometer. The extract of *Saraca indica* significantly decreased the locomotor activity in mice. Thus, it was concluded that leaf of *Saraca indica* possess CNS activity.
- 9) Antiulcer activity: The aqueous suspension of *Saraca indica* flowers are used against gastric ulcer in albino rats. The major constituent of *Saraca indica* flowers contains saracasin, saracadin, waxy substance, fatty acids and flavonoids etc. The flowers of *Saraca indica* suspension exhibit an antiulcer potential activity through at least one or more possible mechanism including inhibition of basal gastric secretion, stimulation of mucus secretion and endogenous gastric mucosal prostaglandin synthesis.

10) Anti-inflammatory activity: The ethanolic extract of *Saraca indica* leaves find out the anti-inflammatory activity. The leaves of *Saraca indica* determined the anti-inflammatory activity against Carrageenan induce paw edema in animal is the most suitable test procedure to screen anti-inflammatory activity.

UV Spectrophotometry Analysis

Ultraviolet-visible (UV-Vis) spectrophotometry is mainly used for quantitative analysis, and to some extent, for qualitative analysis to characterize or identify any substance/substances in a sample. It is the most preferable technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light-weight strikes matter it will either be absorbed, reflected, or transmitted. The absorbance of radiation within the UV-Vis vary causes atomic excitation, which refers to the transition of molecules from a low-energy state to AN excited state. Before AN atom will modify excitation states, it should absorb enough levels of radiation for electrons to maneuver into higher molecular orbits. Shorter bandgaps usually correlate to absorption of shorter wavelengths of sunshine. The cuvettes used for ultraviolet analysis are created of quartz. It uses a lightweight beam that passes through the sample, and every compound within the resolution absorbs or transmits light-weight over a precise wavelength. The UV-Vis photometer uses this principle to quantify the analytes in a very sample that is supported by their absorption characteristics. According to Beer-Lambert Law, the number of sunshine absorbed is directly proportional to the concentration of the sample and also the distance the sunshine travels through the sample; the pathlength.

Total Phenolic Content Assay

The phenolic quantification assay is based on the Folin Ciocalteu method. The Folin Ciocalteu reagent consists of phosphomolybdic acid complex and phosphotungstic acid complex. It depends on the transfer of electrons in an alkaline medium from the phenolic compounds to form a blue chromophore from the complexes, where the maximum absorption depends on concentration of the phenolic compounds. The Folin- Ciocalteu reagent can be detected by UV Spectrophotometer, in the range of 200 to 400 nm. Gallic acid was used as a standard and results were expressed in GAE mg/ml.

Total Flavonoid Content Assay

The basic principle of flavonoid content determination is the formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride forms acid labile complexes with the orthodihydroxyl groups in the A-ring or B-ring of flavonoids. The total flavonoid content was determined according to the aluminium chloride colorimetric method. For the calculations of total flavonoid content in the plant extract, a standard calibration curve is needed which is obtained from a series of different concentrations of a standard reference flavonoid i.e. Gallic acid. The results were expressed as gallic acid equivalents (mg/ml).

Antioxidant Assays (in vitro): The anti-oxidant capacity of EASI is evaluated by its radical scavenging activity using in-vitro methods by performing the following two radical scavenging assays:-

DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging assay

Antioxidants through their scavenging power are useful for the management of certain diseases. DPPH stable free radical scavenging method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. DPPH is a stable free radical by virtue of the delocalisation of the spare electron over the molecule, so that the molecules do not dimerize, like most other free radicals. Delocalisation also gives rise to a deep violet colour, with an absorption in alcoholic solution at 517 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour. Thus, this assay is based on the measurement of the scavenging capacity of antioxidants towards DPPH. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine.

ABTS (2, 2'-azinobis- [3-ethylbenzthiazoline- 6-sulphonic acid]) radical scavenging. Assay. The ABTS assay is used to measure the total antioxidant activity of the test samples. It is based on the principle of suppression of the intensely colored (blue-green) ABTS radical cation by the antioxidant and hence the antioxidant capacity is inversely proportional to the absorbance of the radical cation. The assay was standardized using ascorbic acid as the standard antioxidant.

Evaluation of eyedrop

The following parameters were taken into consideration for evaluation of ophthalmic solution (for eyedrop):

Clarity (Refractive index)

Ophthalmic solutions should be clarified very carefully to remove fibers and other solid contaminants. Eye is very sensitive to particulate matter which can cause discomfort. Particulate matter can also cause abrasion of the corneal epithelium facilitating invasion of pathogenic micro-organisms.

pH

One of the most significant parameters is the pH. It must be maintained at normal levels (4 to 8) because a change from acidic to alkaline pH can cause serious eye injury.

Viscosity

Viscosity of an eye drop approaching that of normal tears at normal value (optimal range 0.55 to 34.5 centipoise) helps prevent blurring and discomfort during blinking. If eye drops have a viscosity similar to that of tears, they are eliminated within a few minutes. It is essential that ocular lubricants are of sufficient viscosity to maximise the bioavailability.

Free from particles or any foreign substances

Foreign substances are the particles present in the atmosphere that get into the eye because we don't see them. Foreign substances or particles present during any stage of manufacturing are considered to be contaminants and are a risk to the control of the manufacturing processes. Minor foreign particles include dust and grit (which is easily removable by membrane filtration). The presence of foreign particles can cause discomfort, itching, and pain in the eyes. It may cause blinking of the eyes (excessive). It can also lead to an ocular infection such as endophthalmitis or can even damage the lens or cornea of an eye. Therefore, it is considered a critical defect in ophthalmic products.

Soxhlet Extraction

Soxhlet Extraction is a standard and a well-established technique. The Soxhlet apparatus is

made up of borosilicate glass and consists of three parts- a round bottom flask, a condenser and thimble. The thimble has a syphon glass tube and a side arm. Sample (Solid/Semisolid), which is to be extracted is put into this thimble by wrapping the material into a filter paper. The round bottom flask is placed at the bottom and into the water bath fitted in the heating mantle. Extracting solvent is placed in round bottom flask and heated on water bath. Vapours of solvent pass through the side arm of the thimble into the condenser. On condensation, the drops of solvent falls on sample placed in the thimble and the level of solvent rises which enters the syphon tube and again to the side arm. When the liquid reaches overflow.

Phytochemical Investigation of EASI

The Phytochemical Investigation of EASI will be carried out by performing the following tests for detection of alkaloids.

Test for Reducing sugars; Test for Monosaccharides; Tests for Alkaloids; Test for saponins; Test for glycosides; Test for flavonoids; Test for volatile oils;; Test for Proteins;; Thin Layer Chromatography Analysis; High Performance Thin Layer Chromatography Analysis; UV Analysis (Spectrophotometer ; Determination of Total Phenolic Content; Determination of Total Flavonoid Content; Antioxidant Assays; DPPH Assay; ABTS Assay; Fluorescence Analysis of EASI:- Fluorescence is a phenomenon exhibited by various chemical constituents present in the plant material. It is seen in the visible range in daylight.

The ultraviolet light also produces fluorescence in many natural plants. It can often assess qualitatively some crude drugs using fluorescence and it is the most important parameter of pharmacognostical evaluation. The results of fluorescent analysis of the medicinal plants show a characteristic colouration when tested with various chemical reagents. The dried powdered flowers and EASI extract solution (dissolved in methanol), and were treated with Concentrated Sulphuric acid, concentrated hydrochloric acid, Concentrated Nitric acid. Iodine solution (2%), Ferric chloride solution, Sodium hydroxide (10%), Acetic acid+ Sulphuric acid, Sodium hydroxide(10%) + Copper sulphate solution, Sodium hydroxide (10%) + Lead acetate solution and Acetic acid.

The fluorescence analysis of EASI was observed under an ordinary visible light and under UV light (245 nm). These were observed under different wavelengths i.e. visible rays and ultraviolet rays (254 nm and 365 nm). Observation in change in the colour of the solution and R_f values were noted.

RESULTS AND DISCUSSION

Soxhlet Extraction and Fractionation

For Soxhlet Extraction, methanol was used as a solvent. The methanolic extract was subjected to fractionation. For fractionation, the final product-Ethyl acetate fraction of *Saraca indica* (EASI) was used for further investigations and procedures. The final outcome of fractionation was EASI extract and it was obtained in a semi-solid form. The yield was obtained in grams (mg/ml) and was calculated in percentage (%) as shown below. The yield of both the batches of both extraction methods (shown below).

In this research work, the dried powdered flowers of *Saraca indica* were appropriately weighed (on a weighing balance). Quantity 100 grams for Batch I and 200 grams for Batch II (as shown below).

Table-1: Reported and Practical Yield obtained after Soxhlet extraction and fractionation

Solvent		Practical yield		Reported yield
		Batch I	Batch II	
Methanol	9 %	28.6 %	24 %	11 %
Ethyl Acetate	1.5 %	1.1 %	2.1 %	1.5 %

Table-2: Observations and results of Phytochemical Screening of Ethyl acetate fraction of *Saraca indica*.

Sr. No	Tests	Observation	Inference
1	Molisch's test	Violet ring at the bottom of the test tube	Carbohydrates Present
2	Biuret test	Violet colour	Proteins Present
3	Millon's test	Red colour	Proteins Present
4	Barfoed test	Red precipitate	Monosaccharides Present
5	Fehling's test	Brick red precipitate	Reducing sugars Present
6	Benedict's test	Greenish yellow ppt	Reducing sugars Present
7	Mayer's test	Cream coloured ppt	Alkaloids Present
8	Dragendorff's test	Dark orange precipitate	Alkaloids Present
9	Wagner test	Brown precipitate	Alkaloids Present
10	Hager's test	Yellow precipitate	Alkaloids Present
11	Borntrager test	Pinkish ammonia layer	Glycosides Present
12	Modified Borntrager test	Pink colour at the bottom	Glycosides Present
13	Foam test	No foam	Saponins Absent
14	Paper test	Solvent evaporates Staining the paper yellow.	Volatile oil Present
15	Shinoda test	Deep Purple colour	Flavonoids Present
16	Conc. H ₂ SO ₄ + 1 ml EASI solution	Dark yellow colour	Flavonoids Present
17	Conc. H ₂ SO ₄ + Lead acetate + EASI solution	Yellow precipitate	Flavonoids Present
18	Zinc + Conc. HCl + 1 ml EASI	Red colour	Flavonoids Present
19	Potassium dichromate + 1 ml EASI solution	Red precipitate	Tannins Present
20	Dilute Iodine solution + 1 ml EASI solution	Red colour	Tannins Present
21	Dilute HNO ₃ + 1 ml EASI solution	Yellowish-red colour	Tannins Present
22	Dilute KmNO ₄ + 1 ml EASI solution	Dark red ppt	Tannins Present
23	Acetic acid + 1 ml EASI solution	Red colour	Tannins Present
24	Ferric chloride (5%) +1 ml EASI solution	Black colour	Tannins Present

Fluorescence analysis

The fluorescence analysis of dried powder of *Saraca indica* and the solution of EASI extract (dissolved in methanol) helps in assessment of crude drugs qualitatively using fluorescence

and it is an important parameter of pharmacognostical evaluation. The results of fluorescent analysis of the medicinal plants show a characteristic colouration when tested with various chemical reagents. The fluorescence analysis of dried flowers and EASI was observed under an ordinary visible light and under short UV light and long UV light (254 nm and 366 nm). After observation in visible light and in both UV lights, the powdered drug and its extract showed different colours with different chemical reagents (as shown in the table below).

Table-3: Colour observation of fluorescence exhibited with different chemicals by the powdered drug *SI* and *EASI* extract

Sr. No	Chemicals/ Reagent	Visible light	Short UV(254 nm)	Long UV(366 nm)
		Powder EASI	Powder EASI	Powder EASI
1	Concentrated Sulphuric acid	Brown Brown	Black Black	Black Black
2	Concentrated Hydrochloric acid	Grey Orange	Dark Grey Amber	Green Brown
3	Concentrated Nitric acid	Yellowish Orange	Mustard Squash	Green Phthalo
4	Iodine solution	Darkish Brown Brown	Black Black	Black Black
5	Ferric Chloride solution	Dark Green Black	Black Black	Sepia Black
6	Sodium hydroxide (10%)	Brown Beige	Dark Brown Black	Brown Violet
7	Acetic acid + conc.H ₂ SO ₄	Peach Bronze	Brown Brown brown	Copper Sienna
8	NaOH (10%) + Copper sulphate	Light blue Blue	Blue Blue	Blue Jade
9	NaOH (10%) + Lead acetate	White White	Cream Silver	Grey Plum
10	Acetic acid	Lemon Pale	Brown Yellow	Brown Maroon

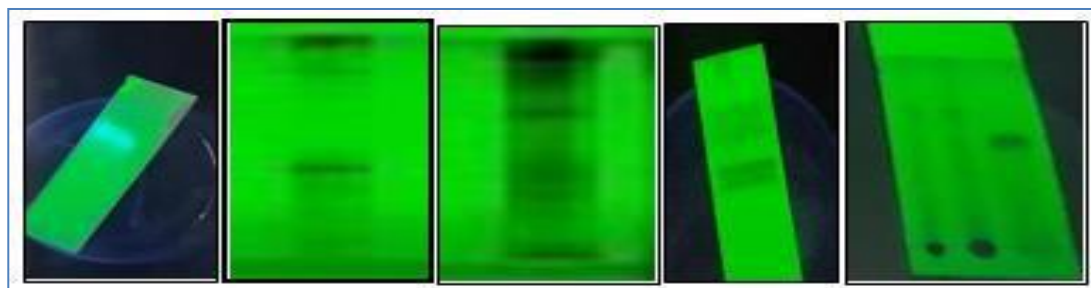


Fig-5: Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) of EASI extract and eyedrop was performed using aluminium backed plates coated with silica gel (TLC plates). A TLC chamber using toluene, ethyl acetate and formic acid in the ratio 5:4:1 was prepared for the saturation of the spotted TLC plates. After 90% saturation of the TLC plate, it was removed and dried by a spray dryer. The retention factor (R_f) values of all the spots were noted, calculated and observed under short and long UV in an UV inspection cabinet. Standard Gallic acid solution (1 mg/ml) was prepared in methanol for comparison. Alcoholic solution of (5%) ferric chloride (FeCl_3) was used as a spraying agent for gallic acid. Standard quercetin solution and standard luteolin solution (each 1 mg/ml) were also prepared in methanol for comparison with the specific compounds- quercetin and luteolin. NPPEG was used as a standard for comparison of pelargonidin and also as a spraying agent for TLC analysis of EASI extract.

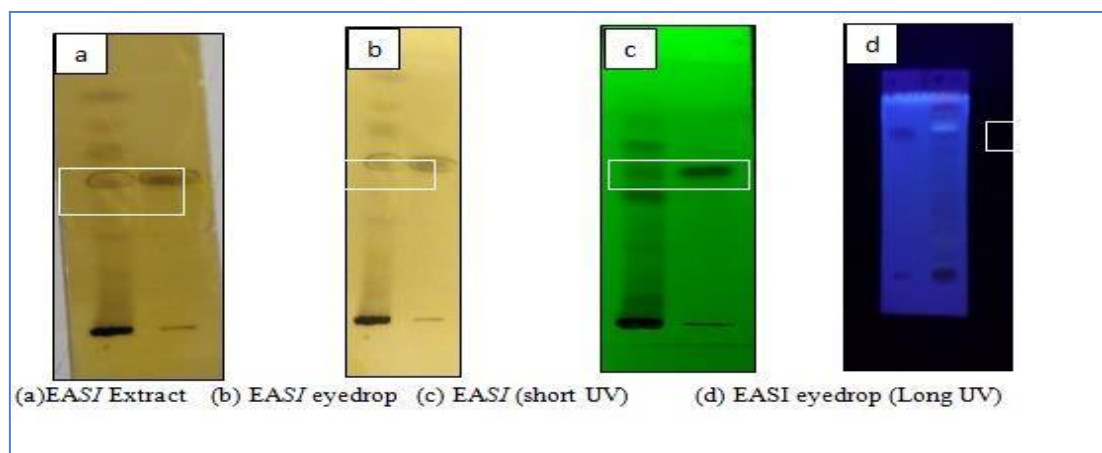


Fig-6: TLC analysis of EASI (Batch I).

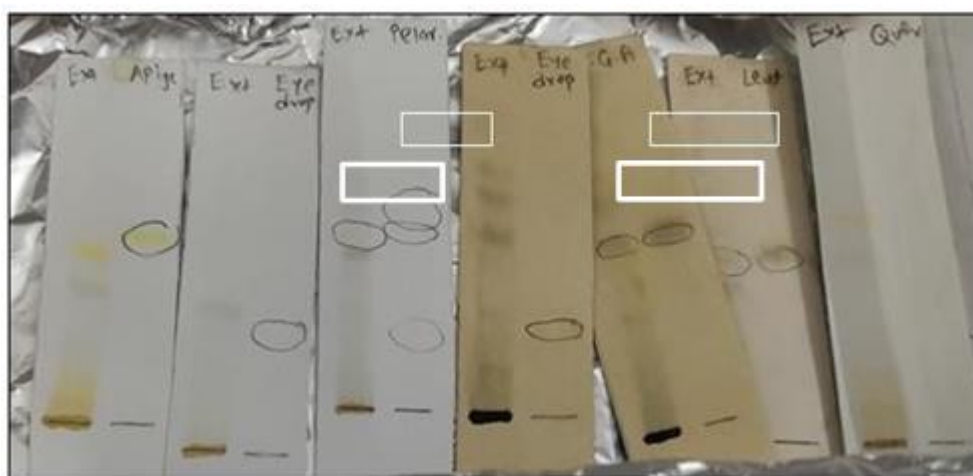


Fig-7: TLC analysis of EASI, eyedrop along with standards-GA, Q, L, A and P (Batch II)

Table no 4: Retention Factor (R_f values) for Batch II [Obtained by calculations]

]:EASI + Phytochemical component	R _f Value obtained
Gallic acid	0.6
Luteolin	0.6
Pellargonidin	0.6
Apigenin	0.5
Quercetin	0.5

The R_f values obtained by TLC analysis confirms the presence of gallic acid, apigenin, luteolin, quercetin and pellargonidin in EASI extract, in the EASI eyedrop as compared to its standards (Std. Gallic acid, Std. Quercetin, Std. Luteolin and NPPEG). It identifies the presence of these phytochemical constituents in the EASI extract and in the eyedrop.

High Performance Thin Layer Chromatography(HPTLC) Analysis

HPTLC Analysis of EASI extract and eyedrop was performed using aluminium backed plates coated with silica gel (TLC plates). It was compared with standards. HPTLC analysis was carried out without an instrument and same method as performed for TLC analysis.

The Standard Gallic acid solution (1 mg/ml) was prepared in methanol for comparison. Standard quercetin solution and standard luteolin solution (each 1 mg/ml) were also prepared in methanol for comparison with the specific compounds- quercetin and luteolin. Natural products polyethylene glycol (NPPEG) was used as a standard for comparison of pellargonidin. Alcoholic solution of (5%) ferric chloride (FeCl₃) was used as a spraying agent for gallic acid.

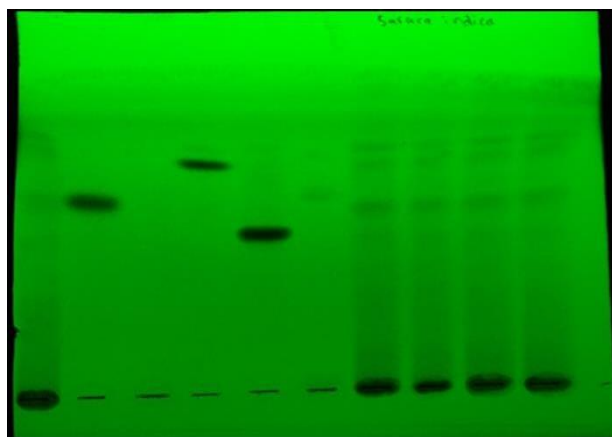


Fig-8 Observation under UV inspection cabinet [Short UV and Long UV light (254 nm and 366 nm)]

Fig. HPTLC observed under short UV light (254 nm) in UV inspection Cabinet. (Left to Right Bands- EASI Extract, Std. gallic acid, Std. Quercetin, Std. Luteolin, NPPEG and Eyedrop) The HPTLC Analysis confirms the presence and identification of phytochemical constituents such as gallic acid, luteolin, apigenin, quercetin and pellargonidin in the EASI extract and in EASI eyedrop.



Fig-9 HPTLC observed under long UV light (365 nm) in UV inspection Cabinet

Total Phenolic Content (TPC)

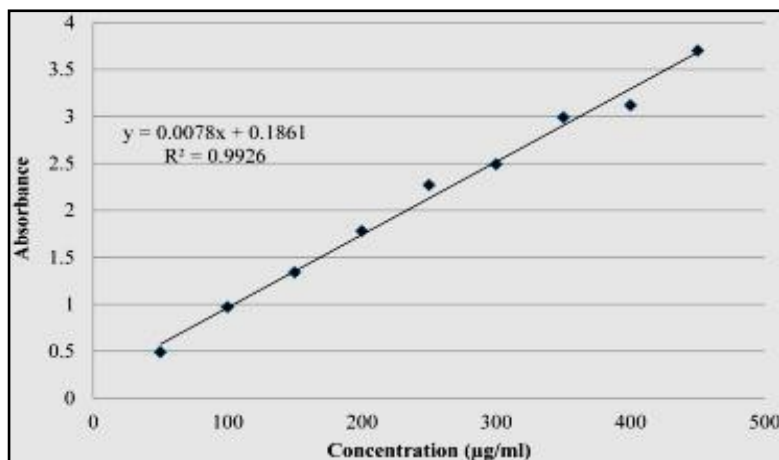


Fig-10

Total Phenolic Content (TPC) of *EASI* extract was determined using folin-ciocalteau method on the UV spectrophotometer. A standard curve was prepared using various concentrations of GA against the absorbance. It was observed that extract phenolic content. The TPC was 29.43 GAE/gm of extract and 29.43 ± 0.31 , respectively.

Total Flavonoid Content (TFC)

TFC was determined by Aluminium chloride method. A standard curve was prepared using various concentrations of GA against the absorbance. The results were estimated as 90.24 mg of QE/gm of extract and 90.24 ± 0.04 , respectively.

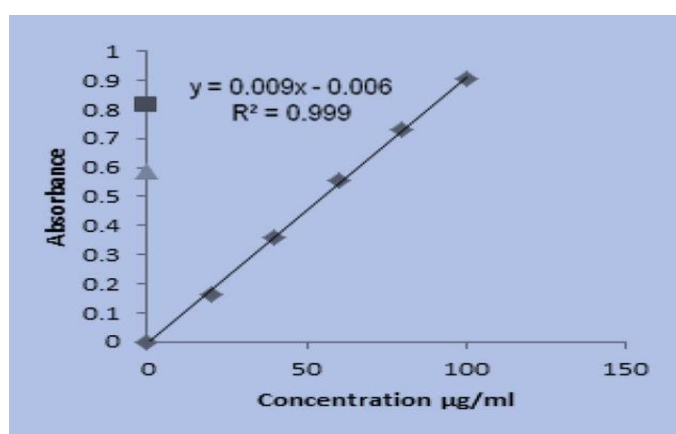


Fig-11

Antioxidant Assays DPPH assay

The standardization of this assay was done using standard ascorbic acid. Inhibitory concentration (IC₅₀) was used as a measure of comparison of antioxidant activity of test samples of concentration. IC₅₀ values were calculated with comparison to standard reference flavonoids (Gallic acid) from the graph of concentration versus % Inhibition. It was calculated from the plot of % DPPH v/s concentration.

Table-5

No.	Wavelength (nm)	Absorbance
1	729	0.055
2	302	2.035
3	236	0.538

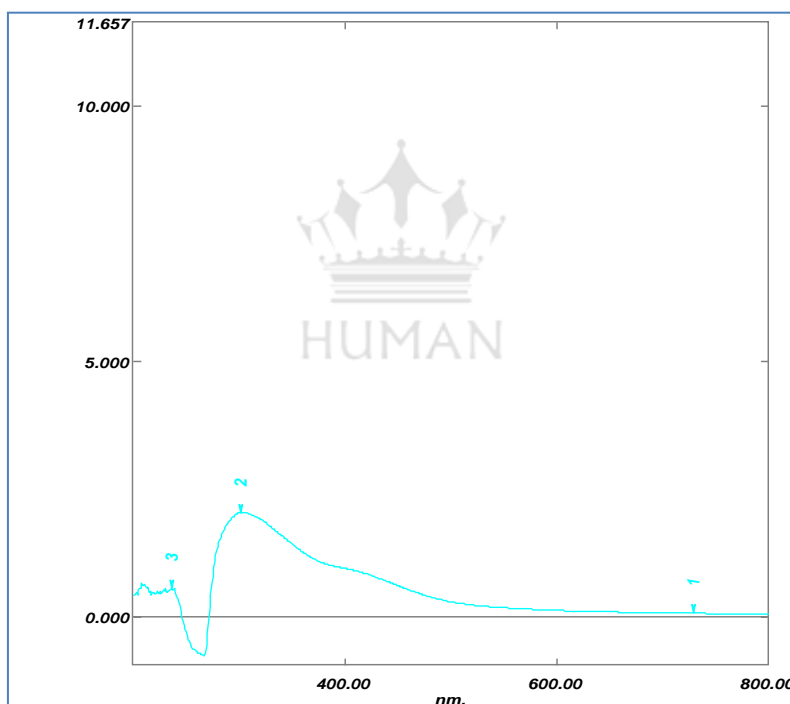


Fig-12

ABTS Assay

The method was standardized using std. ascorbic acid. The IC₅₀ value of standard ascorbic acid in ABTS radical scavenging assay was found to be $\mu\text{g/mL}$.

The IC₅₀ value of the EASI was found out to be 8.32 μg .

Table-6

No.	Wavelength nm.	Abs.
1	333	1.591
2	299	1.428

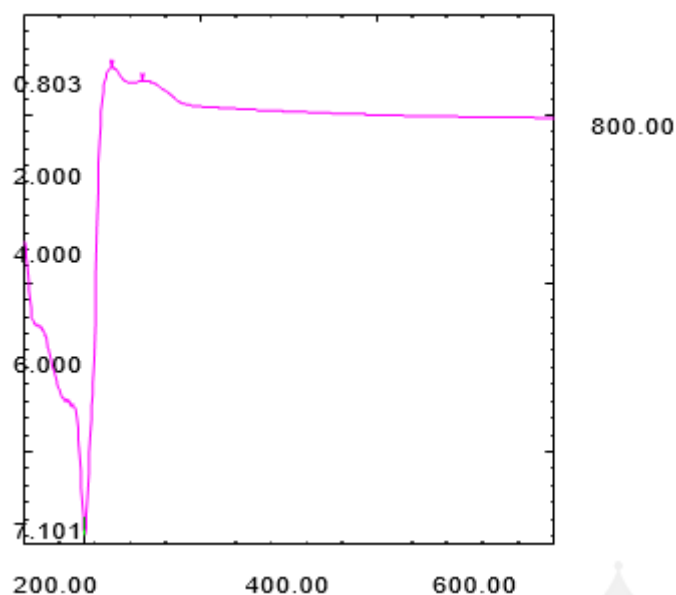


Fig-13

These assays-DPPH and ABTS proved antioxidant potential of the EASI extract.

4.13 Sterilization of Eyedrops



Fig-14: Luer lock syringe filter

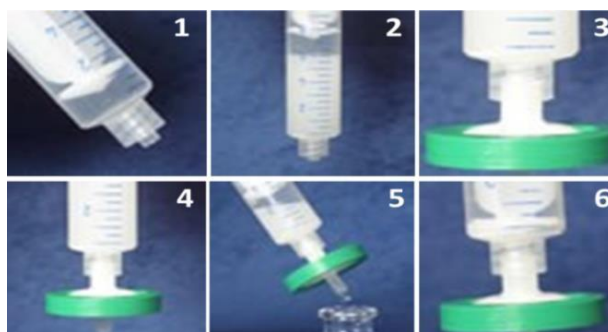


Fig-15: Luer lock membrane filter attached to a syringe

The formulated ophthalmic solution (eyedrop) was sterilized by membrane filtration (pore size $\leq 0.45\mu\text{m}$) and luer lock syringe filtration (filter size $0.22\mu\text{m}$).

SUMMARY AND CONCLUSION

Diabetic cataract is a condition that causes blurring of vision and is one of the major causes of blindness. In diabetic patients, cataract is indeed the main cause of vision disability. Removal of cataract is one of the most common procedures and increases the number of cataract surgeries every year. Cataract surgery has been advanced into enhanced surgery such as Phacoemulsification. There are certain critical surgical outcomes, as well as risks of cataract surgery of diabetics, which were found in reports. Since, herbal drugs are traditionally considered as harmless and safe and are increasingly being consumed for various diseases. The phytomedicines available in the market are standardized herbal preparation consisting of a mixture of one or more plants which are used in most of the countries. Standardization of herbal drugs consists of numerous factors which influence the safety, efficacy and reproducible therapeutic effects. Considering these all factors, this research work was an attempt to obtain a good quality herbal product, as safety was considered and care was taken right from the suitable, ideal, best and a proper extraction method to the standardization and evaluation methods till sterilization of ocular formulation. Ethyl acetate fraction of *Saraca indica* was prepared by extraction methods like Soxhlet extraction and liquid-liquid extraction (fractionation). The presence of alkaloids, tannins and flavonoids was confirmed by phytochemical analysis in the *EASI* extract. The phytochemical investigation, fluorescence analysis, TLC and HPTLC analysis has identified and confirmed the presence of phytoconstituents/phytochemicals mainly gallic acid, quercetin, luteolin, apigenin and pelargonidin in *EASI* extract.

The radical scavenging assays-DPPH and ABTS assays confirmed the antioxidant activity of EASI. Evaluation tests results for ophthalmic solution indicated good results which were ideal and are suitable in a range of ideal values for all parameters like pH, Viscosity, Clarity, Sterility and Stability. The formulated and sterilized ophthalmic solution of the standardized herbal extract EASI shows an aldose reductase inhibitory activity and gives definite and promising results, hence it can reverse the symptoms of diabetic cataracts. The formulated ophthalmic solution (eyedrop) was sterilized by membrane filtration (pore size $\leq 0.45\mu\text{m}$) and luer lock syringe filtration (syringe filter size $0.22\mu\text{m}$). Eyedrops were sterilized and were evaluated by certain parameters. The refractive index of eyedrop was 1.38, pH was 6.33 and the viscosity was found to be 1.19 cp and it was a clear transparent solution (no white or dark particles- checked with a black and white background). Thus, an eyedrop with safety, efficacy and sterility with antioxidant activity and a therapeutic potential was developed in aseptic conditions, with safety and in a sterile area. Finally, it was aseptically filled into vials and stored in refrigerator. The standardized herbal extract EASI shows an aldose reductase inhibitory activity; hence it can reverse the symptoms of diabetic cataract.

The preliminary research work on ocular formulation development as an eyedrop from for treating was carried out and results concluded that showed strong to moderate anti-oxidant potential. In future, after its *in vivo* studies on animals and *in vitro* studies using micro-organisms like *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Haemophilus influenza*, and human clinical trials, it can be prescribed by ophthalmologists for the prevention and treatment of diabetes-induced cataract. The future research scope can be *In-vitro* studies, *In-vivo* studies, *Ex-vivo* studies, *In Silico* studies, Residual Solvent Analysis by Gas Chromatography, Nuclear Magnetic Resonance (NMR) Spectroscopy, Human clinical trials, Clinical trials with other herbal drug extracts. The ophthalmic formulation can be formulated into suspensions, eyegel, microemulsion, minidisks (OTS), occlusers/ocular inserts, ophthalmic sprays or eyewash. It can be used in Novel Ocular Drug Delivery System (NODDS). It can be formulated into an eyedrop and used for the treatment as well as prevention of diabetic cataract.

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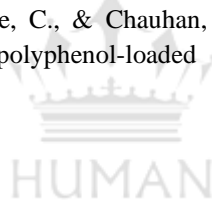
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