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Original Research Article

Ocular Formulation Development of a Standardized Herbal Extract for the Prevention and Treatment of Diabetic Cataract

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Abstract

Diabetes-induced Cataract is a major concern regarding the cause of blindness in developed and developing countries. Recent basic research studies have emphasized the role of the polyol pathway in the initiation of the disease process. Diabetes induced cataract is also referred to as "Diabetic cataract". Demographic studies have certainly enhanced our knowledge concerning an association between diabetes and cataract formation and have defined the risk factors for the development of cataracts. Diabetic patients also have a higher risk of complications after phacoemulsification cataract surgery as compared to non-diabetic people. Aldose-reductase inhibitors and antioxidants have been proven beneficial in the prevention or treatment of this sight-threatening condition in in vitro experimental studies. Aldose reductase inhibitors have a vital importance in the treatment and prevention of diabetic complications. Nowadays, use of natural, herbal, and naturopathic medications has increased, as they have more therapeutic potential and lesser side effects as compared to the allopathic and ayurvedic medications. This research work provides an insight into herbal standardized extract and its ocular formulation development, Eyedrop, as an easy, sterile, safe, and alternative to phacoemulsification. Development of eyedrop as an ocular formulation and its standardization with an in vitro evaluation of the herbal extract for the prevention as well as treatment of Diabetic cataract.

Keywords: Diabetic cataract, *Saraca Indica*, Herbal, Standardization, Eyedrop. Chemicals: Gallic acid, Apigenin, Quercetin, Luteolin, and Pelargonidin.

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 urine. 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 to rise in the blood. In β-cells of the Langerhans islets, 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 the 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 see, including diabetic macular edema (ME), postoperative ME, and diabetic retinopathy.

Pathophysiology of Diabetic Cataract

In diabetes, the enzyme aldose reductase catalyses 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. Inreased 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 biochemical pathways are activated under hyperglycemic conditions. Among these, the polyol pathway is the most widely studied and the most promising in order to explain the mechanisms of diabetic complications. When there are excessive glucose levels, they are metabolized via the polyol pathway. This pathway involves two main enzymatic steps: the first and key enzyme aldose reductase (AR), which reduces glucose to sorbitol by using NADPH as cofactor, and the second enzyme, sorbitol dehydrogenase (SDH), converts sorbitol to fructose with NAD as a cofactor.

In cases of diabetic complications such as retinopathy, neuropathy and nephropathy, the speed of the polyol pathway csignificantly increases. As a result of this, accumulation of sorbitol and its metabolites occurs in several cells including nerves, retina and kidneys due to the poor penetration across membranes and inefficient metabolism of sorbitol. Thus, both oxidative stress and osmotic stress increase in the cells depending on the development of diabetic complications. High activity of AR causes an increase in the consumption of NADPH and this causes the decrease of the activities of glutathione reductase and nitric oxide synthase. Therefore, high activity of AR causes a reduction of the intracellular GSH and NO levels. As a result, aldose reductase enzyme activity reduces the cellular antioxidant capacity. Meanwhile, the oxidation of sorbitol to fructose by SDH leads to oxidative stress. This is because its co-factor NAD+ is converted to NADH in the process, and NADH is the substrate for NADH oxidase to generate reactive oxygen species (ROS).

Fructose, which is generated from the polyol pathway, is metabolized 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 products (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.

As a result, studies on the determination of the aldose reductase inhibitors are gaining importance every day. Inhibitors of AR have been used in therapeutic applications in diabetic complications. Some well-known AR inhibitors are epalrestat, tolrestat, zenarestat, sorbinil, zopolrestat, ponalrestat, lidorestat, fidarestat, ranirestat, quercetin, resveratrol and ADN-158. Use of these inhibitors has remained limited due to undesirable side effects. Therefore, the identification of new aldose reductase inhibitors and the inhibition of aldose reductase by natural chemicals are very important. In particular, studies using phenolic compounds as aldose reductase inhibitors are very popular. In recent years, there have been many studies on isolation of phenolic compounds from traditional plants and their effects on glucose metabolism, particularly the polyol pathway.

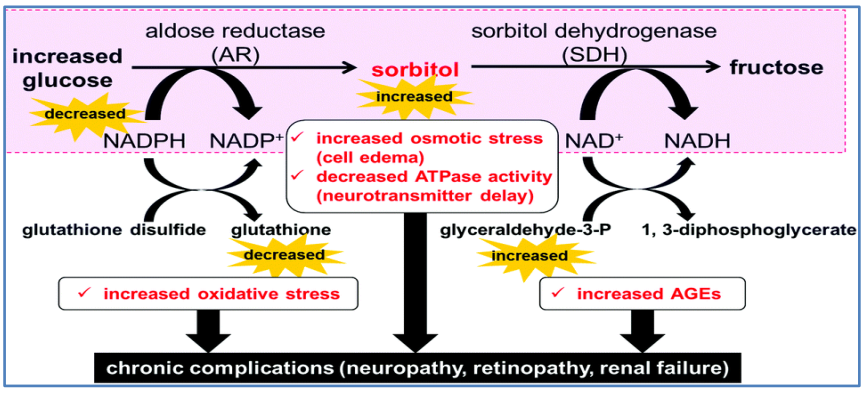

Fig-1: Mechanism of polyol pathway

Epidemiology of Diabetic Cataract

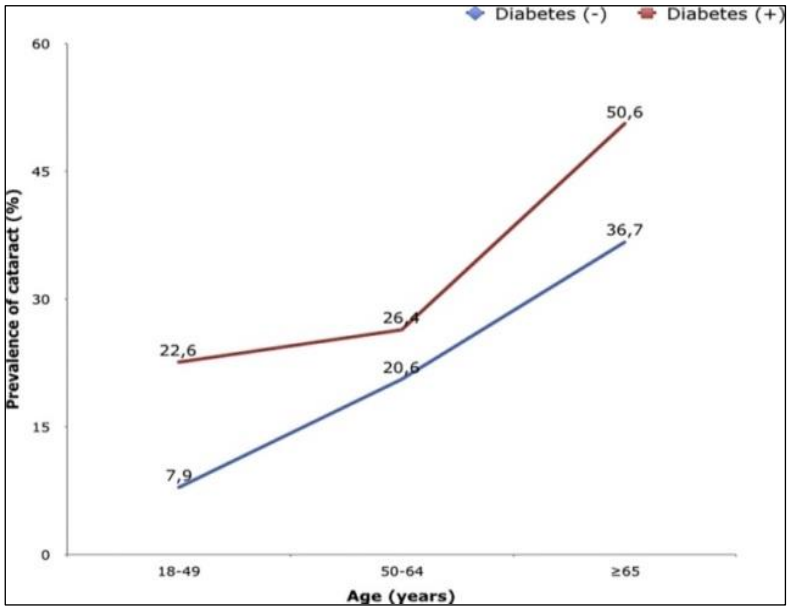

Fig-2: Prevalence of Diabetic cataract along with age factor.

Worldwide more than 285 million people are affected by diabetes mellitus. This number is expected to increase to 439 million by 2030 according to the International Diabetes 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 suffering 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 in the world. It has been reported that 5.1 million people all over the world died from diabetes and its complications in 2013. In addition to being a chronic disease that threatens human health, diabetes is quite a costly disease for both individuals and countries. 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, 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 Fabacea 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*

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. *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 dyserteriae* 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.

Anticancer activity: The anticancer activity of *Saraca Indica* flowers indicated 50 percent cytotoxicity (in vitro) in Dalton's lymphoma and Sarcoma-180 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.

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. *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.

Antioxytocic activity: Oxytocic 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 oxytocic activity. Ashoka, previously was tested twice with negative results and once with positive results.

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.

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.

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 the quantitative analysis, and to some extent for the qualitative analysis to characterize or to identify any substance/substances in a sample. It is a most preferable technique used to measure light absorbance across the ultraviolet and visible ranges of the electromagnetic spectrum. When incident light strikes matter it can either be absorbed, reflected, or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state. Before an atom can change excitation states, it must absorb sufficient levels of radiation for electrons to move into higher molecular orbits. Shorter bandgaps typically correlate to absorption of shorter wavelengths of light. The cuvettes used for UV analysis are made up of quartz. It uses a light beam which passes through the sample, and each compound in the solution absorbs or transmits light over a certain wavelength. The UV-Vis spectrophotometer uses this principle to quantify the analytes in a sample based on their absorption characteristics. According to Beer-Lambert Law, the amount of light absorbed is directly proportional to the concentration of the sample and the distance the light travels through the sample; the pathlength, therefore UV-Vis spectrophotometers are used to determine the concentration of specific analytes in a microvolume by controlling the analysis wavelengths and the pathlength.

Total Phenolic Content Assay

The phenolic quantification assay is based on the folin ciocalteau method. The folin ciocalteau 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-ciocalteau 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 antioxidantactivity 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 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 the dust and grit (which is easily removable by membrane filtration). Presence of foreign particles can cause discomfort, itching and pain in eyes. It may cause blinking of 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 as 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 EA*SI* 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 EA*SI* 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 EA*SI* 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's test	Brown precipitate	Alkaloids Present
10	Hager's test	Yellow precipitate	Alkaloids Present
11	Borntrager's test	Pinkish ammonia layer	Glycosides Present
12	Modified Borntrager's 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's test	Deep Purple colour	Flavonoids Present
16	Conc. H_2SO_4 + 1 ml EA*SI* solution	Dark yellow colour	Flavonoids Present
17	Conc.H_2SO_4 + Lead acetate + EA*SI* solution	Yellow precipitate	Flavonoids Present
18	Zinc + Conc. HCl + 1 ml EA*SI*	Red colour	Flavonoids Present
19	Potassium dichromate + 1 ml EA*SI* solution	Red precipitate	Tannins Present
20	Dilute Iodine solution + 1 ml EA*SI* solution	Red colour	Tannins Present
21	Dilute HNO3 + 1 ml EA*SI* solution	Yellowish-red colour	Tannins Present
22	Dilute KmNO4 + 1 ml EA*SI* solution	Dark red ppt	Tannins Present
23	Acetic acid + 1 ml EA*SI* solution	Red colour	Tannins Present
24	Ferric chloride (5%) +1 ml EA*SI* 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 EA*SI 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.H2SO4	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

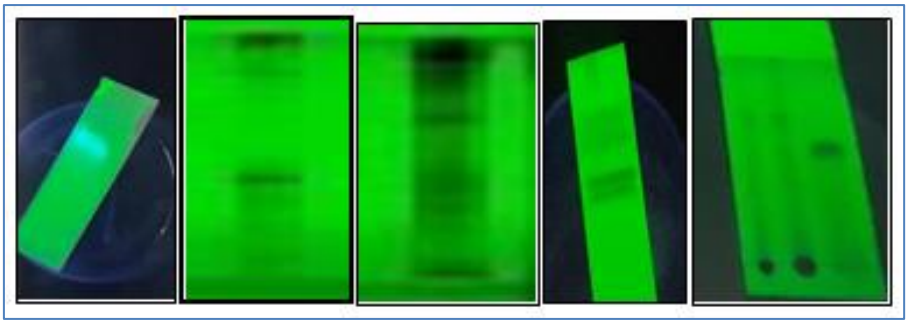

Fig-5: Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) of EA*SI* 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 (FeCl3) 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 EA*SI* extract.

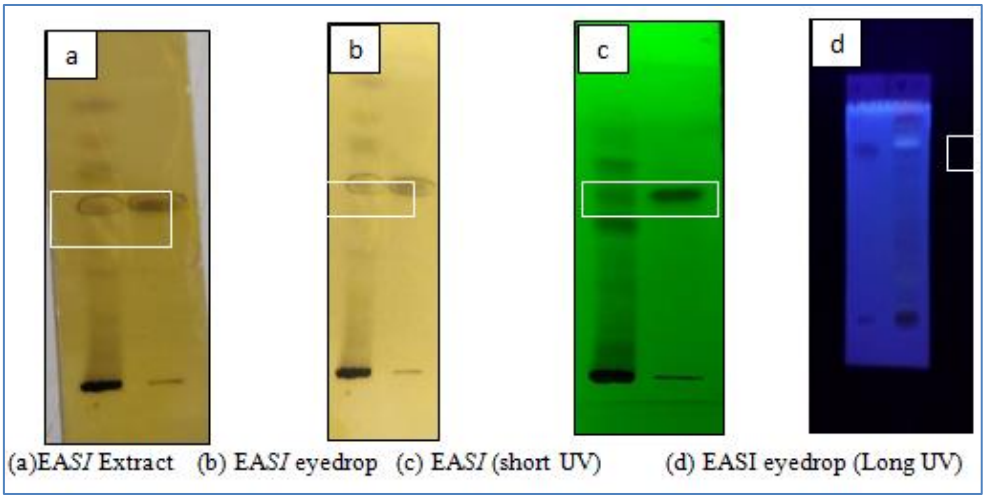

Fig-6: TLC analysis of EA*SI* (Batch I).

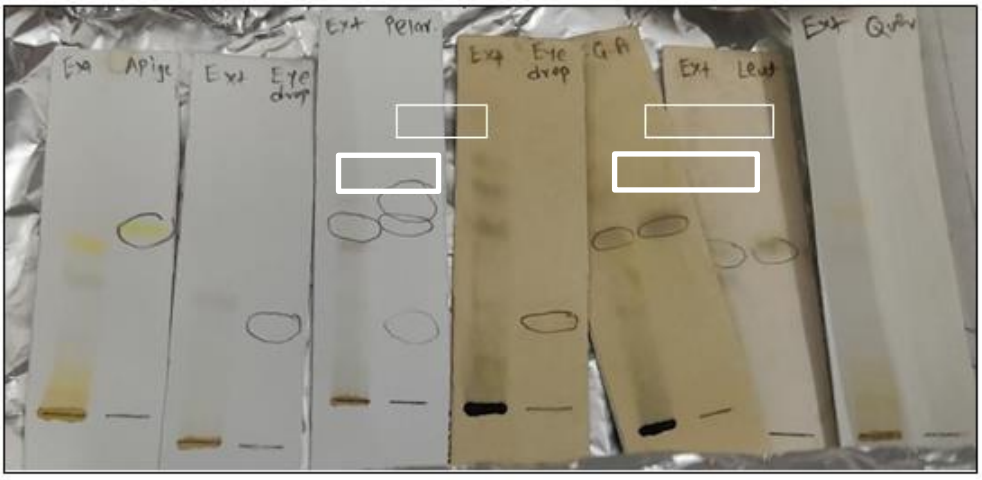

Fig-7: TLC analysis of EA*SI*, eyedrop along with standards-GA, Q, L, A and P (Batch II)

Retention Factor (R_f values) for Batch II [Obtained by calculations]:
EA*SI* + Gallic acid = 0.6
EA*SI* + Luteolin = 0.6
EA*SI* + Pelargonidin = 0.6
EA*SI* + Apigenin = 0.5
EA*SI* + 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 pelargonidin. Alcoholic solution of (5%) ferric chloride ($FeCl_3$) was used as a spraying agent for gallic acid.

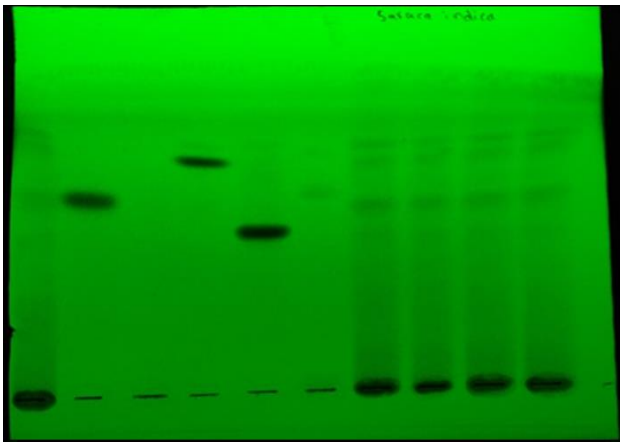

Fig-8

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 EA*SI* extract and in EASI eyedrop.

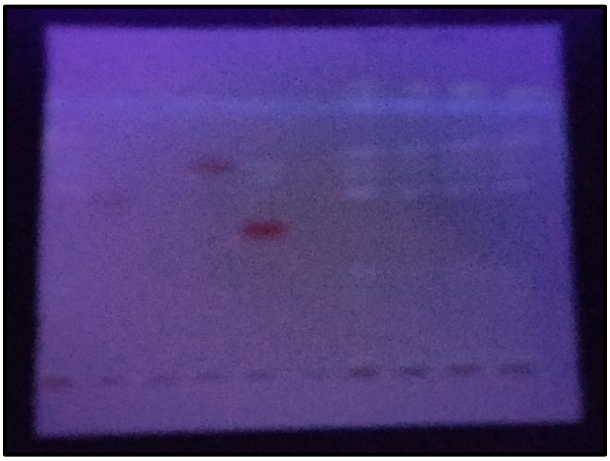

Fig-9

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

Total Phenolic Content (TPC)

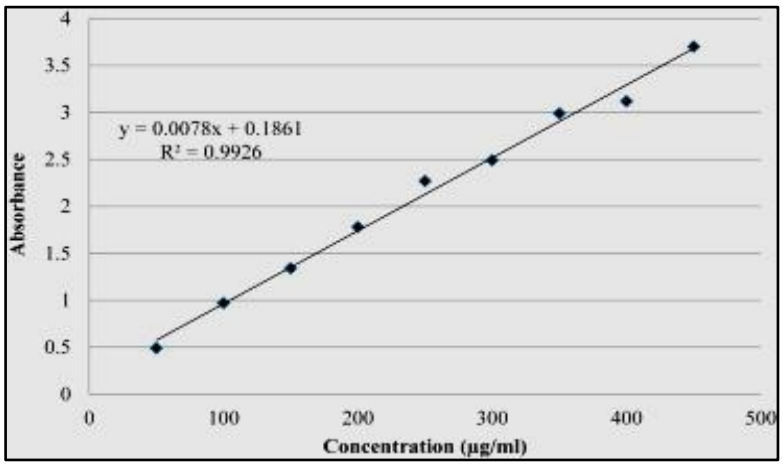

Fig-10

Total Phenolic Content (TPC) of EA*SI* 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.

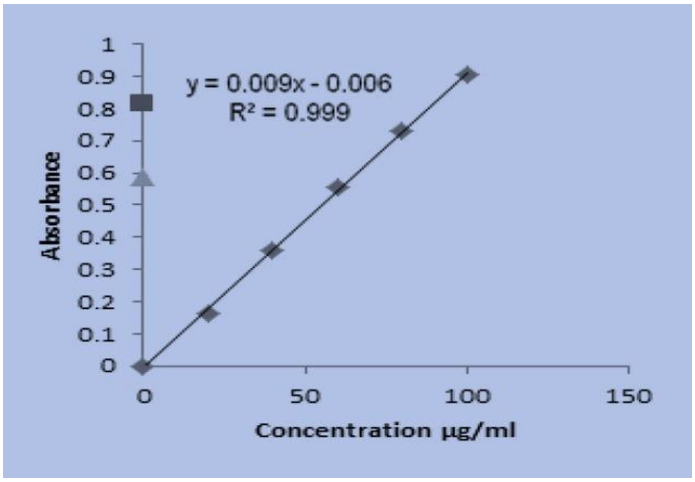

Fig-11

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-4

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

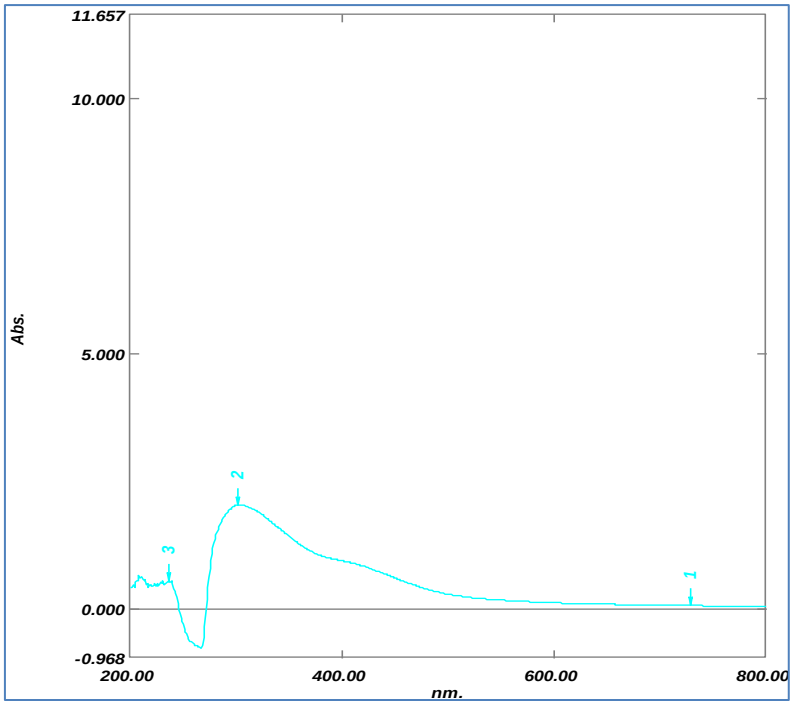

Fig-12

ABTS Assay

The method was standardized using std. ascorbic acid. The IC50 value of standard ascorbicacid in ABTS radical scavenging assay was found to be µg/mL. The IC50 value of the EASI was found out to be µg/ml.

Table-5

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

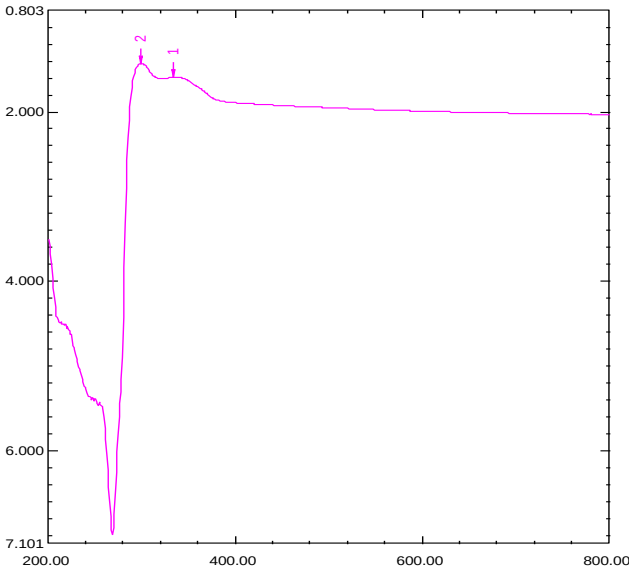

Fig-13

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

4.13 Sterilization of Eyedrops

Fig-4.16: Luer lock syringe filter

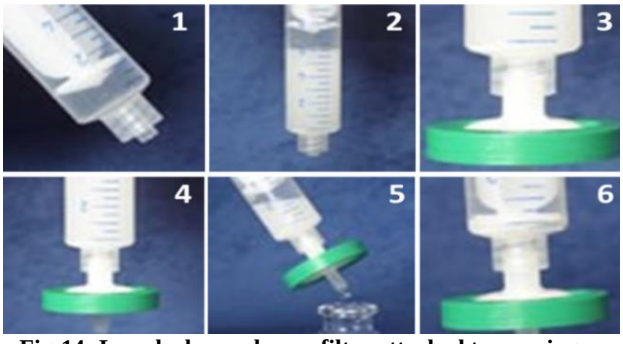

Fig-14: Luer lock membrane filter attached to a syringe

The formulated ophthalmic solution (eyedrop) was sterilized by membrane filtration (pore size ≤ 0.45µm) and luer lock syringe filtration (filter size 0.22µm).

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 remains 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 EA*SI* 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 EA*SI* 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 $\leq$ 0.45µm) and luer lock syringe filtration (syringe filter size 0.22 µm). 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, minidiscs (OTS), occuserts/ocular inserts, ophthalmic sprays or eyewash. It can be used in Novel Occular 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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