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Investigation of In-Vitro Antioxidant and Ex-Vivo Anti Cataract Activity of Ethanolic *Extract of Solanum Surattense Burm F (Indians Nightshade)* Leaves

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***Abstract*: Objective: To investigate the in-vitro anti-oxidant and ex-vivo anti-cataract activity of ethanolic extract of *Solanum surattense* (EESS) leaves on glucose induced cataract and hydrogen peroxide induced cataract using isolated goat lenses. Methods: Anti-oxidant activity done by using specific free radical scavenging methods like DPPH free radical, H_2O_2 free radical scavenging methods and the Anti-cataract activity done by using glucose induced cataract and hydrogen peroxide induced cataract using isolated goat lenses. Results: Results of the study conducted in the ethanolic extract of leaves of solanum surattense revealed the presence of numerous phytochemicals such as alkaloids, glycosides, saponins, tannins, flavonoids, proteins etc, which revealed that the plant contains several phytochemical constituents and hence possesses good anti-oxidant activity. From all the methods gave strong evidence regarding the anti-oxidant potential of this plant. Anti-cataract activity was also investigated using isolated goat lens and promising results were obtained which speak voluminously about its anti-cataract potential and support it well prescribed use. Conclusion: Results obtained with the study clearly supported the significant anti-oxidant potential and anti-cataract activity of this plant. Further, this plant demands great attention for the development of suitable novel dosage forms for the effective treatment of cataract.**

***Keywords*: Solanum surattense, Anti-cataract, Anti-oxidant activity, H2O2, DPPH, Glucose.**

1. Introduction

Herbal medicine (also called herbalism) is the study of pharmacognosy and the use of medicinal plants, which are basis of traditional medicine. Herbal medicine also called phytomedicine or phytotherapy [1].

Traditional medicine is a very important part of health care. Most of population in the developing countries still relies mainly on indigenous traditional medicine for satisfying their primary health care needs. Traditional medicine one of the oldest medicines and widely used medicine. Materia medica of India provides lots of information on the traditional aspects and folklore practices of therapeutically important natural herbal products. Indian traditional medicine is based on various systems including ayurveda, siddha and unani. [2], [3]

Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, glycosides, phenols and flavonoids [4]. Medicinal plants continue to be a major source of drugs and natural products on the basis of their therapeutics in virtually all cultures. The plants possess potent bioactive compounds capable of preventing and treating most oxidative stress related diseases [5].

Cataract derived from Latin word cataracta meaning "water fall ". A cataract occurs when an opaque or cloudy area and develops in the normally clear lens (responsible for the focusing mechanism) of the eye. the cloudy area scatters and blocks the light as it passes through the lens, preventing a clear image from the reaching the retina. Clouded Vision caused by cataracts is often described as seeing through a frosty or foggy window. Cataracts often develop slowly and can affect one on both eyes, leads to decrease in the vision [4], [5]. when the cloudy vision interferes with daily activities such as reading books or driving a car, undergoing surgery is recommended to remove cataract. Cataract formation is mainly an age-related phenomenon, although socioeconomic and lifestyle factors such as nutritional deficiency, sunlight, smoking, environmental factors, diabetes mellitus lack of consumption of antioxidants may also influence this episode.

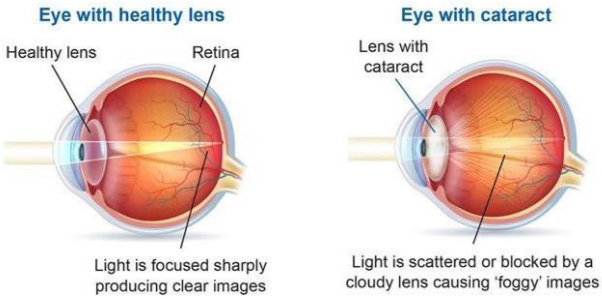

Fig. 1. Difference between healthy eye and cataract eye

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It is widely accepted that oxidative stress is a significant factor in the genesis of cataract both in experimental animal models [6] and in cultural lens [7]. The concentration of proteins damaged by the oxidative process rises with the age in the human lens and is significantly higher in a cataractous compared with the normal transparent lens. Antioxidants are one of the compounds, these Antioxidants inhibit the oxidation process that reported to be able to inhibit the progression of cataracts. Antioxidants reacts with radical and non-radical species after oxidative stress to trigger defense mechanisms that protect intercellular and extracellular components [8].

Global estimates shows that approximately 2.2 billion people suffers from total are partial vision loss, out of which about 65 million is due to Cataract. According to the National eye institute in the United States (US), the number of people with the condition in the US is expected to double from the 24.4 million to in the US is expected in about 15 million by years of 2050[25]. and nearly 60% of the blindness in your parts of Africa and South America. Additionally, they are more common in women and less common in Hispanic and black people. Global prevalence: According to WHO this 1 billion people include those with moderate or severe distance vision impairment or blindness due to unaddressed Cataract.

Fig. 2. *Solanum surattense* plant

Solanum surattense (Solanaceae) is perennial wild growing medicinal herb used as a traditional and folklore medicine. Ancient Indian text Materia medica mentioned the use of *S. surattense* leaves to cure a wide range of illness. Solanum usually called as Indians nightshade (English), Nelamulaka (Telugu) and Kantakari (Sanskrit). It is a distributed in Australia, India, Malaysia (Parmar et al, 2010; 2017). Commonly found as wild growing plant along the dry wastelands and roadsides. Several research studies shows *S.surattense* plant leaves contains several Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols. The clinical studies on anti-asthmatic and curing dental inflammation revealed the effective therapeutic potential of S. surattense. *S surattense* have the several pharmacological activities. Proved by scientific observations of experimental works. The bark, leaves, roots and fruits[12] extensively used in traditional medicine due to presence of several phytochemical constituents. Scientific evaluation of isolated bio-compounds has ethano medical and novel pharmacological effects [13] such as antibacterial activity, antifungal activity, anti-helmenthic activity, antimalarial activity, anticancer activity, antidiabetic activity, antioxidant activity, mosquito larvicidal activity, anti-inflammatory activity, reducing the tooth pain (Analgesic activity), hepatoprotective activity, antiulcer activity etc.

2. Materials and Methods

Collection& identification of plant material:

The fresh plant leaves of *Solanum surattense burm f* was collected from the Sri Venkateshwara university, Tirupati, Balaji district, Andhra Pradesh, India. It has been recognised and authenticated by K. Madhava Chetty, plant taxonomist, Division of Botany; Sri Venkateshwara University, Andhra Pradesh.

Drying and grinding:

The fresh leaves of these species were collected and separated from undesirable material and then washed under running tap water for remove dust particles and allowed to shade dry for 10 – 15days for long term storage purpose. The shade dried leaves were ground into a coarse powder with a suitable grinder. The powder was stored in airtight container.

Fig. 3. Grained plant material

Preparation of extract:

Ethanolic extraction:

30gms of the coarsely powdered plant material was weighed accurately and extracted with 95% ethanol by using soxhlet extractor. The solvent was completely evaporated under reduced pressure at 50^0C and dried in vacuum. The material thus obtained was filtered and dried and this was used as an extract to carry out phytochemical screening and experimental studies.

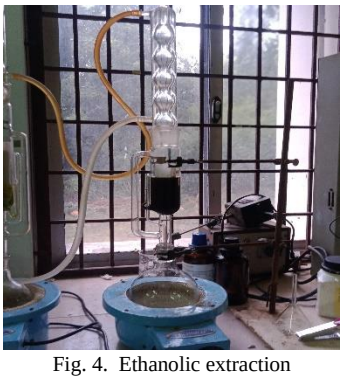

Fig. 4. Ethanolic extraction

Phytochemical screening for ethanolic extracted plant material:

Many phytochemical screening methods have identified the presence of numerous phytochemicals such as alkaloids, glycosides, saponins, tannins, carbohydrates, proteins and flavonoids [9].

1. Test for Alkaloids:

Dragondroff's test:

2ml of dilute hydrochloric acid was added to the 5 ml of extract then treated with Dragondroff's reagent, appearance of an orange brown precipitate showed the presence of alkaloids.

2. Test for carbohydrates:

Benedicts test:

To the 5ml of Benedict's reagent, add 8 drops of solution under examination. Mix well, boiling the mixture on water bath for 2 mins, and then cool, Red precipitate is obtained.

3. Test for Saponins:

Forth test:

15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth is obtained this indicates the presence of saponins in plant extract.

4. Test for triterpenoids and steroids:

Salkowski test:

Crude extract was mixed with 2ml of chloroform and a few drops of conc.H2SO4, a shaken well and allowed to stand for some time. Red color appeared at lower layer indicated the presence of steroids and formation of a yellow-colored layer indicated the presence of triterpenoids.

5. Test for Glycosides:

Legaltest:

The extract was hydrolyzed with dilute hydrochloric acid for few hours on a water bath. 1ml of pyridine and a few drops of sodium nitroprusside solution were added and then add 2 drops of sodium hydroxide. Pink color produced which turn into red indicated presence of glycosides.

6. Test for flavonoids:

Alkaline reagent test or sodium hydroxide test:

The extracts were treated with few drops of sodium hydroxide separately. Formation of intense Yellow color, which becomes colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

7. Test for phenols:

Fecl3 test:

The crude extract mixed with 2ml of 2%fecl3 solution to give blue green or black coloration, indicates presence of phenols.

8. Test for proteins:

Ninhydrin test:

Add 2 drops of freshly prepared 0.2% ninhydrin reagent to the extract and heat. A blue color develops indicating the presence of proteins.

In-Vitro Antioxidant Activity of Ethanolic Extract of Plant:

Quantitative measurement of antioxidant potential of a drug using specific free radicals like DPPH free radical, H2O2 free radical.

1. DPPH (2,2- diphenyl-1-picrylhydrazyl) free radical scavenging assay:

DPPH Free radicals scavenging assay is popular in natural product antioxidant studies. The free radical scavenging potential of different extracts were determined according to the procedure of Kulisic with some modifications [10]. An aliquot of 50µL of sample solution of various concentrations (20-100µg/mL) were mixed with 950µl of methanolic solution of DPPH (3.4mg/100ml). The reaction mixture was incubated at 37^0C for 1hr in the dark. The free radical scavenging potential of extract expressed as disappearance of the initial purple colour. The absorbance of the reaction mixture was recorded at 517nm using UV-spectrophotometer (Agilent 8453, Germany). Ascorbic acid was used as positive control. DPPH scavenging capacity was calculated by using the following formula:

Scavenging activity (%) = [Absorbance ^{control} - Absorbance ^{sample}/ Absorbance ^{control}] ×100

Fig. 5. DPPH assay

2. H_2O_2 (Hydrogen peroxide) free radical scavenging assay:

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch et al [11]. Aliquot of 0.1ml of extract (20-100µg/mL) was transferred into the eppendrof tubes and their volume was made up to 0.4mL with 50 mM phosphate buffer (pH 7.4) followed by addition of 0.6 mL of hydrogen peroxide solution (2 mM). The reaction mixture was incubated at 37^0C for 1hr in the dark and after 10 min of reaction time, its absorbance was measured at 230nm. In this Ascorbic acid Antioxidant was used as positive control. H_2O_2 scavenging capacity was calculated by using the following formula:

Scavenging activity (%) = [Absorbance ^{control} - Absorbance ^{sample}/ Absorbance ^{control}] ×100

Fig. 6. Hydrogen peroxide assay

Ex-Vivo Evaluation of Anti-Cataract Activity of Ethanolic Extract of S.Surattense

Collection of goat eyeballs: Goat eye balls were used in the present study. They were obtained from the slaughter house immediately after slaughter and transported to laboratory at 0-4 degree Celsius.

Preparation of lens culture:

The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor.

The artificial humor is prepared as follows

- NaCl: 140mM
- Kcl: 5mM
- Mgcl2: 2mM
- NaHCO3: 0.5mM
- NaH(PO4)2: 0.5mM
- CaCl2: 0.4mM
- Glucose: 5.5mM.
- Hydrogen peroxide

Aqueous humor is prepared at room temperature and pH is 7.8 for 72 hours. The Pencillin- 32mg% and Streptomycin-250mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55mM was used to induce cataract.

Induction of in-vitro cataract in goat lens by using glucose:

Glucose at a concentration of 55mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes oxidative stress and over hydration causes the Cataract in the lens. These lenses were incubated in artificial aqueous humor with different concentrations of glucose (5.5mM served as normal control and 55mM served as toxic control) for 72 hours.

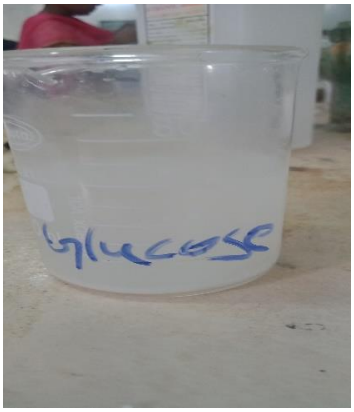

Fig. 7. Induction of cataract by using glucose in artificial aqueous humor

Experimental design:

The Anti-cataract activity was carried out with the ethanolic extracts of solanum surattense, the extract was taken in different doses. Goat lenses were divided into four groups of four lenses each and incubated as follows

Study groups:

1. Group I: Normal lens.
2. Group II: Glucose5.5mM (normal control).
3. Group III: Glucose55mM (toxic control).
4. Group IV: Glucose55mM+ Ethanolic extract 50µg/ml+ Lens culture.

Induction of in-vitro cataract in goat lens by using hydrogen peroxide free radical:

Hydrogen peroxide at a concentration of 1Mm hydrogen peroxide used to induce cataract. At high concentration of hydrogen peroxide free radical causes oxidative stress in the lens, and damage the lens proteins then causes the cataract. These lenses were incubated in artificial humor with different concentration with different concentrations of hydrogen peroxide for 72hrs.

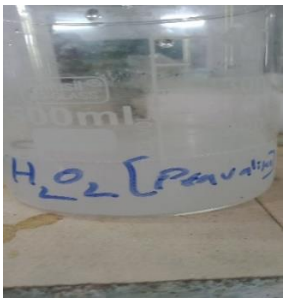

Fig. 8. Induction of cataract by using hydrogen peroxide in artificial aqueous humor

Study groups:

1. Group I: Normal lens.
2. Group II: 1Mm hydrogen peroxide (normal control).
3. Group III: 10Mm hydrogen peroxide (toxic control).
4. Group IV: 10Mm hydrogen peroxide+ Ethanolic extract 50µg/ml+ Lens culture.

Morphological examination lens:

The lenses were placed on a wired mesh or graph paper with posterior surface touching the mesh or graph paper, and the pattern of mesh (number of squares clearly visible through the lens) was observed to measure the lens opacity.

The degree of opacity was graded as follows:

1. Absence of opacity: 0
2. Slight degree of opacity: +
3. Presence of diffuse opacity: ++
4. Presence of extensive thick opacity: +++

Preparation of lens homogenate:

After incubation, lenses were homogenized in 10 volumes of 0.1M Potassium phosphate buffer and then the pH is adjusted to 7.0, then the homogenate was centrifuged at 1000rpm for 1hour and the supernatant was used for estimation of biochemical parameters. Total protein content was measured by using lowry method and the amount of sodium and potassium was estimated with the help of flame photometry.

3. Results

In this study, ethanolic extract of the plant Solanum trilobatum was used for determination of preliminary

phytochemical analysis, in-vitro antioxidant activity by using DPPH free radical scavenging method and hydrogen peroxide free radical scavenging method and ex-vivo anti-cataract activity using goat eye lens to know its potential for the effective treatment of Cataract. literature studies greatly revealed the extensive use of this plant in homeopathic systems of medicines for the prevention of free radicals from the body. The anti-cataract activity of these plant is due to the presence of flavonoids which help in combating oxidative stress which is the underlying cause of cataractogenesis in most of the cases. Therefore, the experimental study was conducted to determine the experimental antioxidant and anticataract activities.

Preliminary phytochemical analysis:

The phytochemical analysis of ethanolic extract solanum surattense has revealed the presence of various phytochemical constituents such as Alkaloids, Flavonoids, Saponins and other which are tabulated below in Table.

Table 1
Phytochemical screening

S.No.	Qualitative Phytochemical Test	Ethanolic Extract
1.	Test for Alkaloids	+++
2.	Test for Carbohydrates	----
3.	Test for Saponins	+++
4.	Test for Triterpenoidsq	+++
5.	Test for Sterols	----
6.	Test for Glycosides	+++
7.	Test for Flavonoids	+++
8.	Test for Tannins	+++
9.	Test for Phenols	----
10.	Test for Proteins	+++

Fig. 9. Phytochemical tests

In Vitro Antioxidant Activity:

Antioxidant activity was performed by two different methods such as DPPH, Hydrogen peroxide scavenging assays. In DPPH assay technique, Ascorbic acid was used as standard for the determination of antioxidant potential of S. surattense. Results obtained with the DPPH assay are given in Table 2. From the DPPH method, the IC50 value of ethanolic extract of plant was 79.11% shown in Fig. 11. The hydrogen peroxide method was second method which was used for the determination of antioxidant potential. In this method, ascorbic acid was used as standard. Results obtained with the hydrogen peroxide assay given in Table:4 from the hydrogen peroxide assay, The IC50 value of ethanolic extract of the plant was 100.3% shown in Fig. 13. Thus, results obtained with all the techniques show strong evidence of the antioxidant potential of the plant *solanum surattense*.

1) DPPH free radical scavenging assay

Table 2
Absorbance of both standard and plant extract by using DPPH assay

Concentration (µg/ml)	Absorbance of standard Ascorbic acid	Absorbance of Plant extract(*S.surattense*)
20	1.572	1.02
40	1.564	0.824
60	1.482	0.662
80	1.434	0.514
100	1.403	0.432

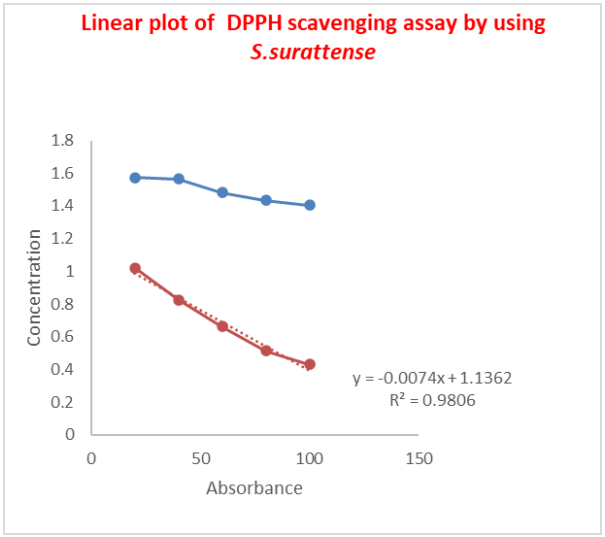

Fig. 10. Linear plot Absorbance of both standard and plant extract by using DPPH assay

Table 3
% Inhibition of Solanum surattense by using DPPH Assay

Concentration(µg/ml)	% Inhibition of *S. surattense*
20	43.66
40	53.7
60	59.05
80	64.15
100	69.06

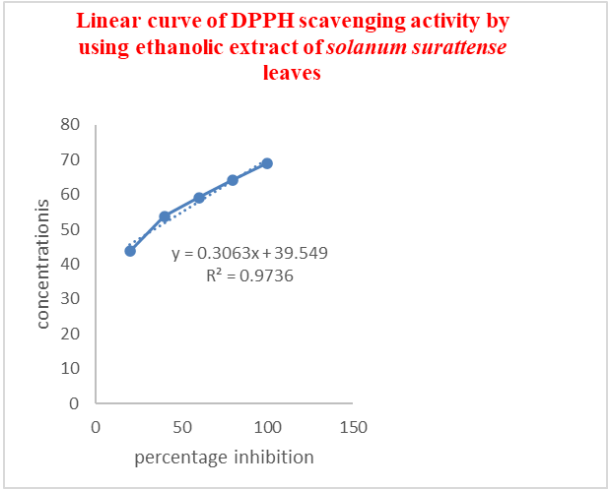

Fig. 11. Linear plot of % Inhibition of *Solanum surattense* by using DPPH Assay

2) Hydrogen peroxide free radical scavenging assay

Table 4
Absorbance of both standard and plant extract by using H_2O_2 Assay

Concentration (µg/ml)	Absorbance of standard Ascorbic acid	Absorbance of Plant extract (*S.surattense*)
20	1.579	1.08
40	1.562	0.749
60	1.491	0.701
80	1.424	0.531
100	1.401	0.4189

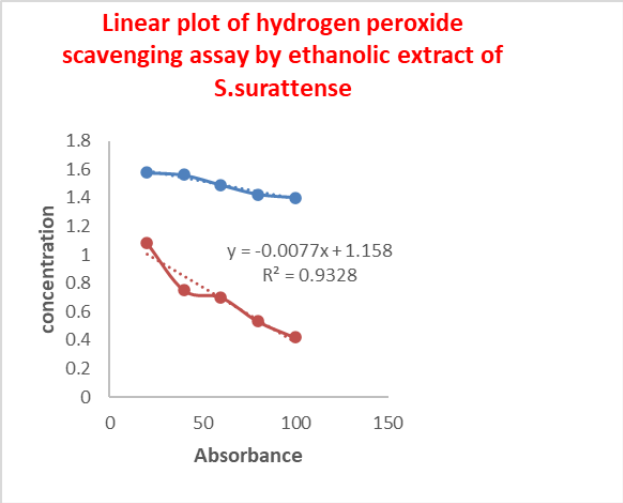

Fig. 12. Linear plot Absorbance of both standard and plant extract by using H_2O_2 Assay

Table 5
% Inhibition of *Solanum surattense* by using H_2O_2 Assay

Concentration (µg/ml)	% Inhibition of *S. surattense*
20	39.6
40	52.04
60	55.8
80	62.71
100	68.09

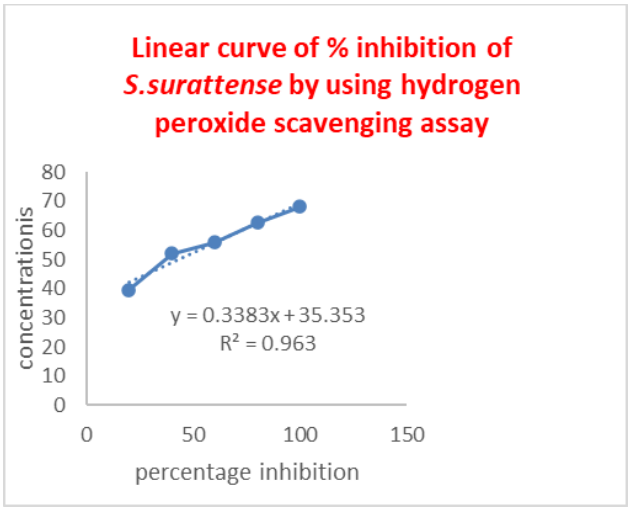

Fig. 13. Linear plot of % Inhibition of *Solanum surratense* by using H_2O_2Assay

Ex-Vivo Evaluation of Anti-Cataract Activity of Ethanolic Extract of S.Surattense:

Photographic evaluation of glucose induced lens opacity:

Photographs of the lens in normal and experimental groups are shown below in the first plate revealed that normal lens and in the second plate revealed the normal lens incubated with the artificial aqueous humor solution and glucose (5.5Mm) showed complete transparency. In the third plate the negative control in which the lens are incubated with glucose (55Mm) a complete opacification of lens was noticed in plate Four the lens simultaneously incubated with glucose (55Mm) and the ethanolic extract of *solanum surattense* (50µg/ml) showed a considerable reduction in the lens opacity of the lens. The result indicates a positive effect of the Solanum surattanse plant extract on anticataract potential by exhibiting reduction in the opacity of cataractous lenses shown in the Figure 10.

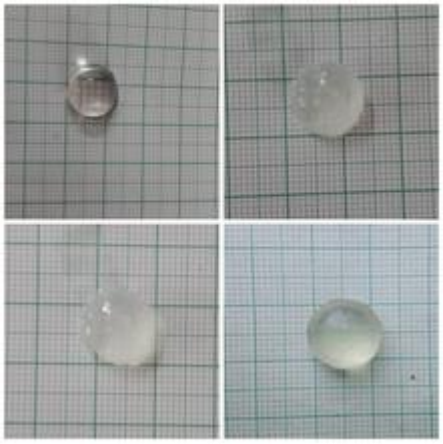

Fig. 14. Photo graphic evaluation of glucose induced lens opacity

Table 6
Morphological examination of glucose induced lens based on degree of opacity

Treated groups of lens	Degree of opacity
1. Normal lens	0
2. lens+aqueous humor + glucose 5.5 Mm	++
3. lens+ aqueous humor + Glucose 55 Mm (Negative control)	+++
4. lens+aqueous humor + Glucose 55 Mm + ethanolic extract of *solanum surattense* leaves (50µg/ml)	+

Photographic evaluation of hydrogen peroxide induced lens opacity:

Photographs of the lens in normal and experimental groups are shown below in the frist plate revealed that normal lens and in the second plate revealed the normal lens incubated with the artificial aqueous humor solution and hydrogen peroxide (1 Mm) showed complete transparency. In the third plate the negative control in which the lens are incubated with hydrogen peroxide (10Mm) a complete opacification of lens was noticed in plate Four the lens simultaneously incubated with hydrogen peroxide (10Mm) and the ethanolic extract of *solanum surattense* (50µg/ml) showed a considerable reduction in the lens opacity of the lens. The result indicates a positive effect of the *solanum surattanse* plant extract on anticataract potential by exhibiting reduction in the opacity of cataractous lenses shown in the Figure 11.

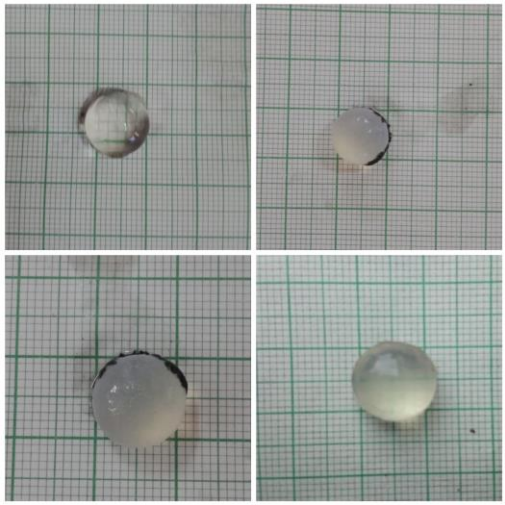

Fig. 15. Photographic evaluation of hydrogen peroxide induced lens opacity

Table 7
Morphological examination of hydrogen peroxide induced lens based on degree of opacity

Treated groups of lens	Degree of opacity
1. Normal lens	0
2. lens+ aqueous humor + 1Mm Hydrogen peroxide	++
3. lens+ aqueous humor +10 Mm hydrogen peroxide (Negative control)	+++
4. lens + aqueous humor + 10Mm hydrogen peroxide + ethanolic extract of ***solanum surattense*** leaves (50µg/ml)	+

4. Conclusion

Results obtained with the study clearly supported the significant anti-oxidant potential and anti-cataract activity of this plant. Further, this plant demands great attention for the development of suitable novel dosage forms for the effective treatment of cataract. However further studies to identify and isolate the main constituents responsible for its anti-cataract effect from phenolic and flavonoid class to comprehend the proper dosage forms for maximum benefits of plant needs to be carried out in future.

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