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Research Article

Preparation and Evaluation of In-Situ Gel from the Root Extract of Boerhaavia Diffusa in the Treatment of Cataract

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A B S T R A C T

Cataract remains a significant global health issue, necessitating innovative treatment approaches. This study explores the formulation of an in-situ gel derived from the roots of Boerhaavia diffusa for potential cataract therapy. Boerhaavia diffusa, renowned for its pharmacological properties, offers a promising natural source for ocular drug delivery. The gel formulation is designed to provide sustained drug release and enhanced bioavailability, addressing the limitations of conventional treatments. The preparation method involves extraction of bioactive compounds from Boerhaavia diffusa roots, followed by formulation into a thermo-sensitive in-situ gel using suitable polymers.The roots of Boerhaavia diffusa were collected, washed, and dried before extraction using a suitable solvent system. The obtained extract was then evaluated for its phytochemical composition, including alkaloids, Glycosides, tannin, terpenoid and Saponins, through standard qualitative and quantitative methods.The in-situ gel formulation was prepared using the obtained extract and suitable gelling agents. Various physicochemical parameters of the gel, such as pH, viscosity, Spreadability, and washability, were optimized to ensure stability and efficacy... The findings of this research hold promise for the development of a novel, natural-based therapeutic approach for cataract management, potentially offering improved patient outcomes and quality of life.

Keywords: Cataracts, in-situ gel, drug delivery, corneal lenses, Boerhavia diffusa, fuzzy vision, bioavalibility.

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INTRODUCTION:

Astanghriday, Charka Samhita, Susrut Samhita, Bhav Prakasha, Ras Tarang, Nayan Drastam, and Many plants are utilized in the Ayurvedic medical system to treat ocular diseases, either as single treatments or in compound formulations, as documented in old Indian texts such as. Numerous eye conditions and illnesses, such as Abhishyand (conjunctivitis), Adhimanth (glaucoma), Timir (cataract), etc., are covered by the Indian medical system known as Ayurveda[1].Ayurvedic terminology refers to cataracts as Linga Nasha or Timira. Ayurvedic principles state that the aggravation of Vayu is the cause of the development of such a disorder. Here, the fluid that keeps the retina and lens pliable is dried up by Vayu aggravation[2].

Boerhaavia Diffusa is an innovative anti-oxidant anti-inflammatory medication with diverse actions that is presently approved for the treatment of ocular diseases in numerous countries. Boerhaavia Diffusa's phytochemical components make it effective in treating cataracts. As a result, efforts have been undertaken to bring our antiquated knowledge back to life by creating contemporary dosage forms and assessing their effectiveness as treatments for different eye conditions.The current study used in-situ gel of Punarnava (Boerhaavia Diffusa) aqueous distillate to assess its effectiveness in treating cataracts[3,4,5].

The study of epidemiology: Globally, cataracts are the leading cause of vision loss. Globally, an estimated 79 million people over 50 were classified as having moderate to severe visual impairment (presenting visual acuity <6/18 to 3/60) due to cataracts in 2020, while an estimated 15 million people over 50 were classified as blind (presenting visual acuity

<3/60 or less than 10° visual field around the central fixation).1. According to these projections, there will be a 30% rise in cataract blindness and a 93% rise in[6].

Causes of cataracts:

When a coating of protein accumulates in the lens of the eye, a cataract occurs. As we age, this is a normal process that occurs gradually. It may result in fuzziness of vision and eventually blindness. Trauma to the eye can also result in the formation of cataracts.

The following are risk factors that may raise your risk of cataract development:Age: The majority of those at risk are above 50.

- Genetics: inheriting the illness from family members
- Lifestyle: consuming alcoholic beverages, smoking, and going outside in the sun without wearing sunglasses Diabetes as well as other medical disorders
- Medication: A number of medications may make cataracts more likely [7].

How do cataracts form in the eye:

The colorful part of the eye, known as the iris, is where the lens is situated. It brightens the retina, which uses the optic nerve to send a picture to the brain. When a cataract affects and clouds the natural lens, light is dispersed and the lens is unable to focus it properly, which results in fuzzy vision. The majority of the material in the lens is made up of proteins and water. Changes to the proteins and fibers that are already present in the lens may cause it to become opaque. The patient eventually begins to notice cataract or motiabind symptoms as the cataract progressively worsens with age[8].

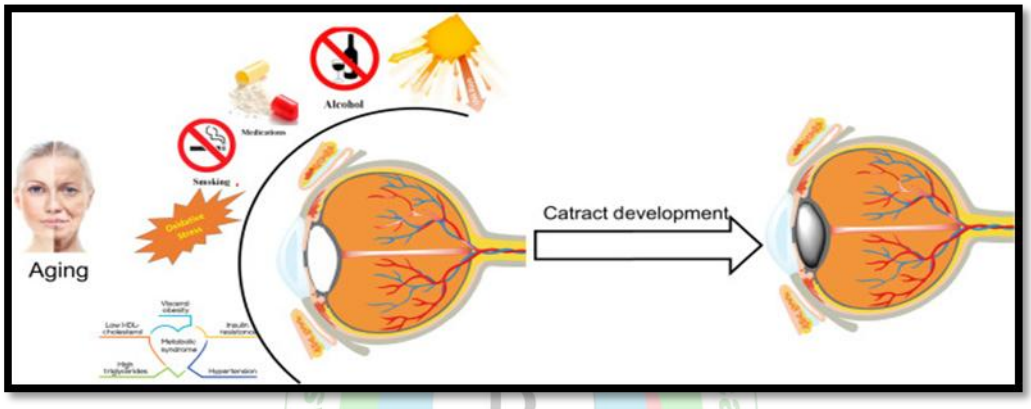

Figure 2: Cataract form in the eye

Role of medicinal plants in the treatment of ocular diseases:

Antioxidant or secondary anti-inflammatory metabolite anti-cataract agents are composed of natural substances. Comprehensive research has also been done on the function of plant polyphenols in anti-cataractogenic properties, both in vitro and in vivo. A total of forty-one plants were examined for potential anti-cataract properties. In folk medicine, sixty-six herbs have been reported to treat conjunctivitis. After screening 262 million plants for ocular disorders, including various forms ofconjunctivitis, Sandhu and colleagues (2011) discovered that 51 of the plants might effectively treat conjunctivitis. Glass containing extracts of antioxidant medicinal plants against ocular surface disease using the ocular surface disease index (OSDI) score was found to be effective in improving DED both subjectively and objectively in a multi-center, prospective, randomized, double-blind, placebo-controlled trial.

Medicinal plants and the bioactive chemicals found in them are utilized to regulate and restore abnormal cellular processes that contribute to the development of diabetic retinopathy (DR). The positive benefits of many phytoconstituents with retinal cytoprotective properties were studied. evidence from studies and clinical settings, emphasizing its application in the treatment of DR. There aren't many herbs that have been shown to treat eye cancer. Additionally, researchers at Washington University's School of Medicine claim that a naturally occurring plant ingredient has opened up a new chapter in the treatment of eye cancers such uveal melanoma [9].

In-situ gelling system:

Environmentally sensitive polymers used in ophthalmic in-situ gelling will change structurally in response to even slight variations in certain environmental parameters, including as pH, temperature, and ionic strength. Liquids are injected into the eye to create in-situ forming gels, which quickly gel in the eye's cul-de-sac to create viscoelastic gels in reaction to changes in the environment and finally release the medicine gradually under physiological settings. As a result, the medication is delivered gradually and the residence period of the gel generated in-situ is prolonged. This improves patient compliance by minimizing systemic absorption, enhancing bioavailability, and reducing the frequency of dosing regimen. Additionally, a few additional possible benefits include a straightforward production procedure, simplicity in administration, and accurate dosage delivery[10].

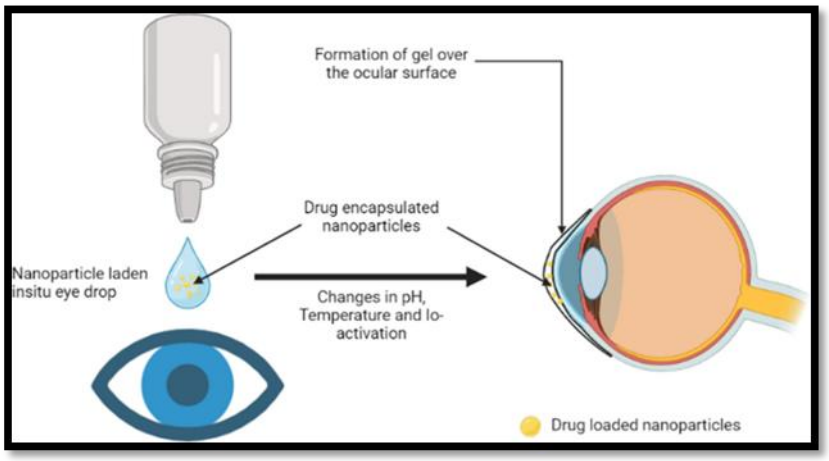

Figure 3 : Ocular drug delivery ofin-situ gel

Advantages of in-situ gel:

- less hazy vision than with an ointment.
- Diminished nasolacrimal drainage of the medication, perhaps leading to unfavorable systemic adverse effects as a result of absorption.the ability to deliver precise and repeatable dosages, as opposed to formulations that have already gelled, and to further enhance precorneal retention.
- Drug release that is prolonged and sustained while keeping the plasma profile largely consistent.
- Decreased application frequency leads to increased patient comfort and compliance.
- More pleasant than soluble or insoluble insertion, in general.
- Greater precorneal residence time and absorption lead to improved local bioavailability.
- Its less sophisticated production process reduces investment and manufacturing costs[11].

Polymers used in the formulation of in-situ gel:

polymers including gellatin, HPMC, are the most often employed polymers for in situ gels based ocular administration. Several substances, including antimicrobials, anti-inflammatory medicines, and autonomic medications used to reduce intraocular tension in glaucoma, have been administered locally via the eye. Traditional delivery methods frequently lead to inadequate bioavailability and therapeutic response due to the drug being rapidly eliminated from the eyes due to high tear fluid turnover and dynamics. In order to address issues with bioavailability, ocular in situ gels were created[12].

Figure 5 : Boerhavia diffusa plant (root)

Figure 4 : Boerhavia diffusa plant (root)

Boerhavia Diffusa is a type of flowering plant belonging to the four o'clock family. It is also referred to as red spiderling, spreading hogweed, punarnava, or tarvine in Ayurvedic medicine. Among other things, it is used in herbal medicine to relieve pain.According to Ayurveda, Boerhaavia diffusa is used to cure a wide range of ailments. In honor of Hermann Boerhaave, a well-known Dutch surgeon from the 18th century, the plant was given the name Boerhaavia diffusa. Punarnava is the name (The Sanskrit phrase punah punarnava bhawati iti means "that which becomes fresh again and again.") is most likely a result of the plant's perennial lifestyle, which sees it stay dry and dormant throughout the summer and regenerate from the same old root stock during the rainy season. (The Sanskrit phrase karotiri shariram punarnavam means "that which rejuvenates the body"). Punarnava is the name by which it appears in both the Rasayana Prakrana and the Charaka Samhita Vayahsthapana Mahakashaya. It is explained in Vidarigandhadi gana, Vatasansamana, and Tiktavarga in the Sushruta Samhita. According to Ayurvedic texts, there are two types of Punarnava: the Swetha/white (Boerhavia diffusa L.) and Raktha/red (Boerhavia verticillata Poir.) forms. Nela (blue) Punarnava is an additional type that

can also be found, as noted in Rajanighantu. With its mystic properties, the various components of B. diffusa, such as the root, leaves,aerial parts, or the entire plant, are utilized in Ayurveda and other medical systems to cure a wide range of ailments.

Table 1: Hierarchy classification of Boerhavia diffusa linn.

Scientific Name	Boerhaavia Diffusa Linn.
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Caryophyllales
Family	Nyctaginace
Group	Dicotyledons
Phylum	Angiosperms.
Other Synonyms	B. adsendens, B. caribaea, B. coccinea, B.paniculata, B. repens

Common Names:

Languages	**Names**
Sanskrit	Punarnava, Raktakanda, Shothaghni, and Varshabhu
Telugu	Punarnava, Atikamamidi, Galigeru
English	Spreading horse purslane (White) with hog weed (Red)
Hindi	Sathikari
Gujarati	Moto-satoda, Dholia saturdo
Tamil	Mukaratee-Kirei
Kannada	Kommegida
Marathi	Khapara, Ghetula,Punarnava vasu
Bengali	Rakta purnarnava

Morphology:

Boerhaavia diffusa is a perennial herb that spreads and grows up to one meter in length. It can be prostrate or ascending. branches. The stem is cylindrical, hairy, prostrate, woody or succulent, and thickened at the nodes. It is also frequently purplish. Simple, thick, fleshy, hairy leaves that are grouped in unequal pairs are green and glabrous above and typically white underneath[13]. The leaves have a wide range of shapes: smooth above and ovate-oblong, round, or subcordate at the base . The flowers are tiny and borne in clusters. These are white, pink, or pinkish-red in hue, hermaphrodite, and pedicellate. Strong and fusiform, the roots have a woody texture. In India, this trailing herb is primarily harvested following the wet season[14-15].

Microscopic characters:

The powder exhibits characteristics such as surface-view cork cells, acicular calcium oxalate crystals up to 50µ in length, prismatic calcium oxalate crystals about 25µ in length, thin, long, narrow threads withSharp points and a narrow lumen up to 800µ long; simple to 5-compound oval to spherical starch grains up to 15µ long; simple pitted vessels up to 200µ long; and a few starch-grain-containing parenchymas and arteries.Both the upper and lower epidermis of plant leaves exhibit the presence of anomocytic stomata and a multitude of multicellular glandular hairs. A palisade is a single layer of spongy parenchyma that is 2-4 layers thick, with distinct intercellular gaps and polyhedral or isodiametric-shaped cells[16].

Biological and pharmacological action:

Pharmacological research has shown that the roots of B. diffusa have a variety of qualities, including laxative, antiurethritis, diuretic, and anti-inflammatory. actions that areanticonvulsant, antinematodal, antifibrinolytic, antibacterial, antihepatotoxic, anthelmintic, antileprotic, antiasthmatic, antiscabby, and antistress. Research has demonstrated the hepatoprotective, antioxidant, antinociceptive, antibacterial, and antidiabetic effects of B. diffusa leaf extracts. Extensive toxicological research on B. diffusa revealed no mutagenic or teratogenic effects[17].

Phytochemistry:

Boerhaavia diffusa is known to contain a variety of secondary metabolites, including lignins, lipids, alkaloids, hormones, triterpenoids, carbohydrates, proteins, and glycoproteins. Proteins and lipids abound in the roots and herbs. There are 15 amino acids in the herb, including 6 necessary amino acids, and 14 in the root, including 7 essential amino acids. Numerous investigations have been conducted to separate the chemical component and determine its function in the plant's pharmacological activity. These many ingredients might work in concert and are hardly likely to be divided into separate active ingredients. Similar variations exist in the chemical components based on drying procedures, plant origins, harvest seasons, and other variables. Flavonoids glycosides, 3,4-Dihydroxy-5-methoxycinnamoyl rhamnoside, quercetin 3-O-rhamnosyl (1 → 6)galactoside (quercetin 3-O-robinobioside), eupalitin 3-O-galactosyl (1 → 2) glucoside, and kaempferol 3-

O-robinobioside are illustrations of secondary metabolites. The extract of the leaves and roots of Boerhaavia diffusa revealed the presence of eupalilitin-3-O-[-D-galactopyranoside]. In a different publication, five rotenoids compounds—known as boeravinone D, boeravinone E, and C5 as well as two newly identified compounds called boeravinone G and boeravinone H—were extracted from the methanol extract of the roots of Boerhaavia diffusa L using bioassay. Compound 5, boeravinone G, and boeravinone E all demonstrated spasmolytic efficacy. Different amounts of chemical components, such as lkaloids, are distributed throughout the herb. From the roots, stems, and leaves, two quinolizidine alkaloids known as punarnavine-I and punarnavine-II have been isolated[18].

Punarnava in eye diseases:

1. Topical application of the Punarnava leaf juice is done on the eyes on a regular basis.
2. Applyingpunarnava roots to a honey area unit designated locally for inflammation, persistent redness, and cataract.
3. Applying the juice of the Punarnava root to the eyes can help with conditions including vitaminosis and redness[19].

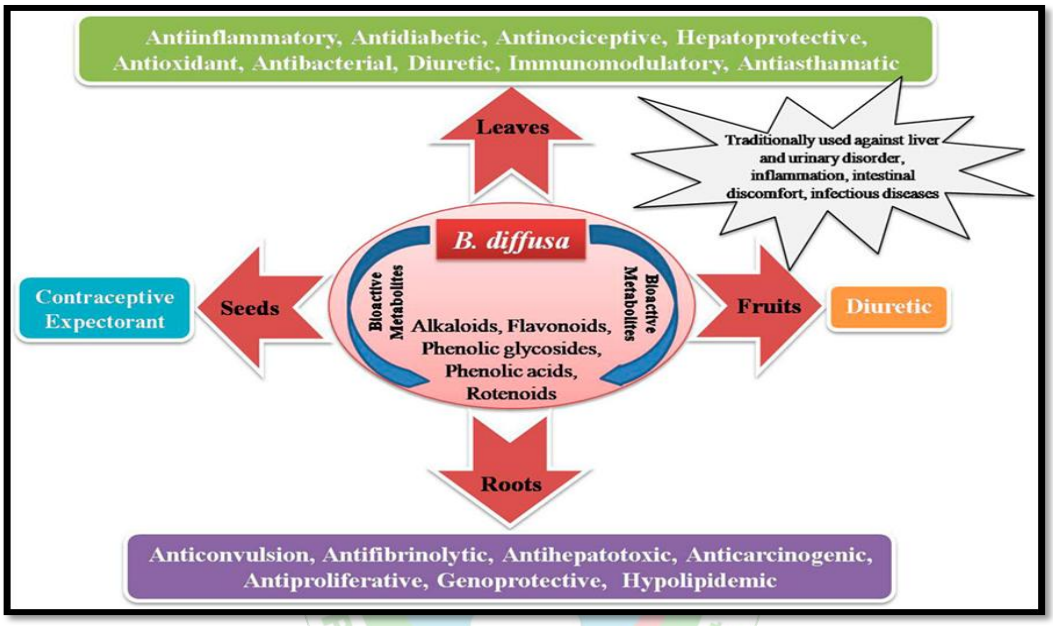

Figure 6: Ethnomedicinal values of Boerhavia Diffusa Linn

Methods and material:

Phytochemical screening of Boerhavia diffusa linn.

1. **Alkaloids test :** Procedure: 2 mL of concentrated HCl was mixed with 2 mL of the biochemical extract, a few drops of Mayer's reagent were added.

   Interpretation: The development of a greenish coloration indicated the presence of alkaloids.

2. **Glycoside test :** Procedure: The 2 mL of biochemical extract was mixed with 3 mL of chloroform and 10% NH3 solution.

   Interpretation: The presence of glycosides was confirmed by the development of a pink coloration

3. **Tannin test** : Procedure: The 1 mL of biochemical extract was mixed with 2 mL of 5% ferric chloride.

   Interpretation: The presence of tannins was confirmed by the appearance of a greenish-black coloration..

4. **Terpenoid test :** Procedure: The 0.5 mL of biochemical extract was mixed with 2 mL of chloroform and concentrated H2 SO4 .

   Interpretation: The presence of terpenoids was demonstrated by the emergence of a red-brown coloration at the interface.

5. **Saponins test :** Procedure: The 2 mL of biochemical extract was mixed with 2 mL of distilled water. It was shaken for 15 minutes or the formation of foam was observed.

   Interpretation: The presence of saponins was inferred from the observed foam formation. [20-21]

Table 3: Phytochemical test

Sr.no	Test	Observation	Inference
1.	Alkaloids	Green	Pass
2.	Glycoside	Pink coloration	Pass
3.	Tannin	Greenish-black	Pass
4.	terpenoid	Red-brown	Pass
5.	Saponins	Foam formation	Pass

Collection of plant material: Boerhavia diffusa Linn., a well-identified plant sample for the current study.In addition to the samples from the nearby market, [roots] were gathered from their natural habitats. these were dehydrated and processed in a lab to become powder. Powdered samples were gathered and kept out of direct sunlight in waterproof, airtight containers[22].

Formulation: A beaker containing two polymers was filled with 2 ml of aqueous distillate. This was let to soak for approximately one hour. After some time, 1:16 ml of Boerhaavia diffusa root (aqueous distillate) was added together with other ingredients, and the remainder (aqueous distillate) made up the remaining volume of 100 ml. To create a uniform drug dispersion in the gel, the stirring was kept up. The gels were autoclaved and exposed to UV light for 30 minutes after being buffered with phosphate buffer at a pH of 7.2 to 0.05. These were lebelled after being aseptically packed into sterile plastic containers[23].

Composition of in-situ gel:

Table 4: Composition of in-situ gel

INGREDIENT	F1	F2	F3	F4
Punarnava root	6.23gm	6.23gm	6.23gm	6.23gm
HPMC	0.1gm	0.1gm	0.2gm	0.2gm
Gelatin	1gm	1gm	2gm	2gm
Citric acid	0.407gm	0.407gm	0.407gm	0.407gm
Povidon	0.2ml	0.2ml	0.2ml	0.2ml
Sodium chloride	0.9gm	0.9gm	0.9gm	0.9gm
Disodium hydrogen orthophosphate	1.125gm	1.125gm	1.125gm	1.125gm
Aqueous distillate	q.s	q.s	q.s	q.s

Evaluation Parameters:

Physical Evalution: Color: the formulations' color was examined on a white backdrop.
Consistency: by applied to skin, the consistency was examined.
Odor: the gel's smell was measured by combining it with water and inhaling its order [23].

Homogeneity: Every generated gel formulation was evaluated for homogeneity and the gel appearance was reported after being allowed to set in an appropriate container[24].

PH determination: A digital pH meter was used to determine the formulations' pH. Prior to every trial, the device was calibrated using standard buffers[25].

Spreadability: Spreadability is a term used to describe the area across which gel distributes easily when applied to the skin or other affected portion. The spreading value of a formulation also affects how effective it is as a medicine. Spreadability is measured in terms of the number of seconds it takes for two slides separated by gel to separate under a specific load.

It is calculated by using the formula

S = M. L / T

Where, **M** = weight tied to upper slide,

L = length of glass slides,

T = time taken to separate the slides.

Washability: After applying formulations to the skin, the degree and ease of water washing were manually assessed[26].

Stability studies: Stability testing determines how a drug substance or product's quality changes over time due to environmental factors like temperature, humidity, and light. It also establishes a re-test period, shelf life, and recommended storage conditions. Accelerated stability tests are a general method for estimating the stability of any product by subjecting it to elevated temperatures in accordance with ICH recommendations. For the prepared formulation, a one-month accelerated stability assessment was performed. The sample were held at temperatures ranging from 3 to 50C,with RH levels of 60% at 250C and 75% at 400C ±2%. Finally, the accelerated study samples were extracted and evaluated on a monthly basis. [27-29].

Phytochemical screening: The freshly prepared crude extracts of *B. diffusa* were qualitatively tested for the presence of Alkaloids, Terpenoids, Saponins, Tannins, glycosides

Viscosity: viscosity is determined by using Ostwald viscometer, Mount viscometer in vertical position on a suitable stand. Fill water in dry viscometer up to mark G. Count time required, in second for water to flow from mark A to mark B.Repeat step 3 at least 3 times to obtained accurate reading Rinse viscometer with test liquid and then fill it up to mark A, find out the time required for liquid to flow to mark B. Determination of densities of liquid as mentioned in density determination experiment [28].

Results and discussion:

Physical evaluation of Boerhaavia Diffusa Root Extract:

Table 5: Physical evalution of Boerhavia diffusa

Sr.No.	Parameters	Observations
1.	Colour	Yellowish Brown to Brown
2.	Odour	characteristics

Physical Evaluation:

The physical evaluation of in-situ gel was evaluated

Table 6: Physical evaluation of in-situ gel

Sr. No.	Parameters	Observations
1.	Colour	Yellowish Brown
2.	Odour	characteristics
3.	consistency	semisolid

Physicochemical parameters of In-Situ gel:

Table 7: Physicochemical parameters of In-Situ gel:

Sr.No.	Parameters	Result
1.	pH	7.20
2.	Viscosity	3.0108cp
3.	Washability	Easily washable
4.	Spreadability	2.42gm-cm/s
5.	Homogeneity	Uniformly distributed

Stability testing of In-situ gel:

The result of physical stability was studied. The change in color, odour and physical separation of formulated mouthwash was observed at room temperature, refrigerator, and at sunlight. The pH of formulations varied little at 40°C, as indicated by stability study results.

Table 8: Stability study

Temperature	Evaluation Parameter	Observation (Days)			
		15	30	45	2 months
3-5℃	Visual Appearance	Yellowish to brown	Yellowish to brown	Yellowish to brown	Yellowish to brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room Temperature (25℃ RH=60%)	Visual appearance	Yellowish to brown	Yellowish to brown	Yellowish to brown	Yellowish to brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40℃+2℃ RH=75%	Visual appearance	Yellowish to brown	Yellowish to brown	Yellowish to brown	Yellowish to brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

CONCLUSION:

Boerhaavia diffusa root's ophthalmic drop and in-situ gel exerted following results:

- Affirmative detectable preventive effect against cataract.
- In comparison to standard drug , ophthalmic drop, in-situ gel delay the equivalent progression of cataract best result.
- In-situ gel showed good result over ophthalmic drop
- It may help in preventing or slowing the progression of cataract.

Future scope:

Research on the preparation and evaluation of insitu gel from the roots of Boerhaavia diffusa for cataract treatment holds significant future potential.

- It could help as a convinient dosagr form for encapsulation directly into the eye.
- It could lead to the development of novel pharmaceutical formulations for efficient drug delivery to the eye, potentially improving treatment efficacy and patient outcomes.
- Additionally, exploring the mechanism of action and conducting clinical trials could further validate its therapeutic potential.

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