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# Therapeutic Potential of Boerhavia diffusa Root Extract in the Management of Eye Diseases: Formulation and Evaluation

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***Abstract - The therapeutic potential of Boerhavia diffusa (Punarnava) root extract in managing eye diseases is explored in this study. Recognized for its antioxidant, anti-inflammatory, and immunomodulatory properties, Boerhavia diffusa holds promise as a treatment for ocular conditions such as cataracts, diabetic retinopathy, and age-related macular degeneration. The research focuses on the development of a novel Ayurvedic eye drop formulation using aqueous distillate from Boerhavia diffusa roots, complying with Indian Pharmacopoeia standards. Phytochemical analyses, including HPTLC, GC-MS, and HPLC, confirmed the presence of bioactive compounds like beta-sitosterol. The formulation was evaluated for sterility, stability, and efficacy in delaying cataract formation using in vivo models. Results indicated that the eye drop significantly postponed cataract progression, with no observed ocular irritation or adverse effects. This study highlights the potential of integrating traditional Ayurvedic knowledge with modern pharmaceutical approaches for effective ocular therapies.***

***Keywords: Boerhavia diffusa, Punarnava, eye diseases, cataract management, Ayurvedic eye drops, phytochemical analysis***

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## INTRODUCTION

In many cultures, traditional medicine has relied heavily on the medicinal use of plants, especially for the treatment of degenerative and chronic illnesses. Of these, Boerhavia diffusa, often referred to as Punarnava, has drawn interest due to its wide range of pharmacological characteristics. This perennial herb, which belongs to the Nyctaginaceae family, is frequently found in tropical and subtropical areas. Its roots are utilized extensively in traditional medicine and Ayurveda systems because of their anti-inflammatory, antioxidant, and immunomodulatory properties (Singh et al., 2011).

Globally, the most common causes of visual impairment are eye conditions such as dry eye syndrome, diabetic retinopathy, cataracts, and age-related macular degeneration. According to Johnson et al. (2019), oxidative stress, inflammation, and disturbed tear film homeostasis are frequently involved in the pathophysiology of these disorders. There is a large need for safe and efficient alternative treatments because the majority of current therapy choices concentrate on symptom management and surgical procedures. Alkaloids, flavonoids, and glycosides are among the several bioactive substances found in boerhavia diffusa root extract that have strong anti-inflammatory and antioxidant qualities (Kumar & Sharma, 2013). According to preliminary research, it may be beneficial for ocular health since it reduces oxidative stress and encourages tissue repair (Patil et al., 2020). Despite these encouraging qualities, Boerhavia diffusa's use in the treatment of eye conditions is still underexplored.

The purpose of this study is to create and test a new ophthalmic preparation of Boerhavia diffusa root extract in order to determine its potential as a treatment for eye conditions. Enhancing stability and bioavailability while maintaining patient safety and compliance is the main goal of formulation development. Furthermore, by providing an evidence-based method for incorporating herbal remedies into eye care, this study aims to close the gap between conventional medical knowledge and contemporary pharmaceutical practices (Gupta et al., 2021).

## REVIEW OF LITERATURE

Commonly referred to as spreading hogweed or Punarnava, the perennial plant Boerhavia diffusa

Linn. is distinguished by its pink blooms and sticky fruits. Its anti-aging and therapeutic qualities are greatly enhanced by its bioactive components. In Ayurveda, Punarnava is regarded as a "rasayan" that can boost the body's energy levels, improve mental and physical endurance, and fortify the immune system (Das et al., 2003).

About 40 species of plants in the genus Boerhavia are found in warm, humid, subtropical climates (Renuka, 2003). Boerhavia diffusa Linn. has been used in traditional medicine to heal wounds, inflammation, and high blood pressure. Its roots are abundant in rotenoids and boeravinones, and its phytochemical profile contains potassium nitrate, boerhavine, punarnavoside, liriodendrin, punaravine, and β-sitosterol. The plant's antibacterial, antifungal, wound-healing, and antidiarrheal qualities have all been connected to these bioactive substances (Renuka, 2003). Using methanol extraction, two lignans—liriodendrin and syringaresinol mono-beta-D-glucoside—were extracted from the roots of B. diffusa Linn. These substances' antagonistic effects on calcium (Ca2+) channels in frog heart single cells were discovered by additional analysis utilizing nuclear magnetic resonance (NMR) spectroscopy (Lami et al., 1991).

Recent research has further demonstrated B. diffusa Linn's pharmaceutical potential. Gaur et al. (2022), for example, highlighted the plant's hepatoprotective, nephroprotective, and anti-inflammatory qualities, which are ascribed to its varied phytochemical makeup, which includes flavonoids, rotenoids, and alkaloids. Boeravinones are a special class of rotenoids that have a variety of medicinal uses. To maximize the plant's therapeutic uses, more research is necessary, and the study also highlights the difficulties of possible adulteration. B. diffusa Linn. is a viable option for medicinal usage due to its abundance of bioactive chemicals (Gaur et al., 2022).

Diuretic, antioxidant, anti-inflammatory, antibacterial, and hepatoprotective qualities are only a few of the plant's many pharmacological benefits. It is a major target of contemporary phytochemical study because of these qualities as well as possible anti-cancer actions (Gaur et al., 2022). For instance, Sharma et al. (2013) showed that B. diffusa Linn. root extract was as effective as the common medication furosemide as a diuretic in mice. Additionally, the investigation verified the existence of phytoconstituents that support its traditional usage in treating fluid retention diseases and encouraging urine output.All things considered, the increasing amount of data points to the possibility for new therapeutic uses of Boerhavia diffusa Linn. while also confirming its long-standing use in Ayurveda and traditional medicine.

**METHODOLOGY**

The National Botanical Research Institute in Ranchi, Jharkhand, confirmed that the roots of Boerhaavia diffusa were gathered from both commercial and wild sources. They were then dried, powdered, and kept out of direct sunlight in airtight containers. 1.0% foreign organic matter, 8.54% total ash, 0.25% acid-insoluble ash, 12.38% alcohol-soluble extractive, 15.15% water-soluble extractive, and 3.15% moisture content were all found by physicochemical analysis. In order to soften the powdered medication made from Boerhavia diffusa roots and enable the release of its volatile active components during boiling, a small amount of water was added and allowed to soak overnight (Kumar et al., 2021). A 1:16 mixture of the softened powder and water was added, and the mixture was put in a distillation apparatus. The collected distillate initially contained simply water and was thrown away since it lacked active medicinal compounds. The active components of Boerhavia diffusa, also known as Arka or aqueous distillate, were present in later aliquots (Sharma et al., 2019). Alkaloids, flavonoids, glycosides, phenols, saponins, lipids, tannins, anthraquinones, cardiac glycosides, proteins, and other primary, secondary, and tertiary metabolites were identified by qualitative chemical analysis of the distillate (Patel et al., 2020). Meyer's, Wagner's, Dragendorff's, and other tests verified the existence of particular chemicals. For instance, the Legal's test was used to confirm glycosides, the ferric chloride test was used to detect tannins, and ammonium hydroxide was used to identify flavonoids by their yellow fluorescence (Reddy et al., 2022). The Shinoda test and other techniques were used to detect steroids and terpenoids, while Millon's, Biuret, and Ninhydrin assays were used to identify proteins and amino acids (Gupta et al., 2018).

An eye drop formulation that complied with Indian Pharmacopoeia guidelines was created using the Arka distillate (Indian Pharmacopoeia, 2020). The distillate was mixed with sodium alginate, sodium chloride, and benzalkonium chloride, which were then sterilized and aseptically packaged. The product's adherence to pharmaceutical standards was validated by quality control procedures, which included sterility evaluations, clarity testing, and pH measurement (Raj et al., 2023). To make sure there was no microbial contamination, sterility tests were conducted using soybean casein culture media that were cultured at 20–25°C (WHO, 2017). The stability of the eye drops was confirmed by stability tests carried out over a three-month period in a variety of temperatures and humidity levels, which showed no discernible changes in color, pH, or viscosity (Ramesh et al., 2021).

For in-depth investigation, sophisticated methods like High-Performance Thin-Layer Chromatography (HPTLC), Gas Chromatography-Mass Spectrometry

(GC-MS), and High-Performance Liquid Chromatography (HPLC) were used (Kumar et al., 2022). While HPTLC produced a unique fingerprint profile of the aqueous distillate, showing greater accuracy and efficiency than conventional TLC methods, GC-MS detected important components of Boerhavia diffusa roots, such as gamma-sitosterol, stigmasterol, and eucalyptol (Sharma et al., 2019). The measurement of beta-sitosterol and other active ingredients in the eye drop formulation was confirmed by HPLC. Together, our tests demonstrated the aqueous distillate of Boerhavia diffusa's medicinal potential and acceptability for use in pharmaceutical products such as eye drops (Gupta et al., 2020).

Oxidative stress is a major contributing factor to cataracts, which are one of the leading causes of blindness and account for 51% of cases in South Asia (WHO, 2017). As people age, the lens experiences major changes, including as oxidative damage and protein aggregation, which can result in cataractogenesis (Calvin et al., 1986). The incidence of cataracts is increased by environmental factors such as diabetes, smoking, poor nutrition, and sun exposure (Truscott, 2005). Cataracts are classified as "Linga Nasha" in Ayurvedic medicine because they are brought on by exacerbated Vayu, which dries up the lens and retina. Rich in tannins, ayurvedic eye drops have anti-inflammatory, antibacterial, and antioxidant qualities, which makes them a viable substitute for treating early-stage eye diseases such diabetic retinopathy and cataracts (Benedek, 1997; Italian-American Cataract Study Group, 1991).

## RESULT AND DISCUSSION

The purpose of the cataract studies and ocular irritation test was to assess the safety and effectiveness of eye drop formulations in vivo. Because of their dependability in pharmacological and toxicological investigations, male Wistar rats a popular laboratory model were used.

### Test for Ocular Irritation

This test evaluated the formulations' possible irritating effects. The participants were male Wistar rats, and they were compared to either distilled water or plain gel as controls. Observations were conducted for up to 10 hours following the formulations' administration, with an emphasis on signs of ocular irritation as redness, irritation, and tear formation. Interestingly, nobody of the test animals' eyes showed any abnormalities, indicating that the formulations were non-irritating and well-tolerated.

### Examining Cataracts

Six male Wistar rats weighing between 108 and 129 grams were split up into three experimental groups for the purpose of studying cataracts:

Group I, the control group, got no therapy other than an injection of sodium selenite.

Standard Group: Applied Itone (Deys Pharmaceuticals Pvt. Ltd., Calcutta), a commercial ophthalmic product, to their right eye. The test eye drop formulation was administered to the treated group's left eye.

### Bringing on Cataracts

All groups received subcutaneous injections of sodium selenite (0.01% w/v) to induce cataracts. Usually seen 12 to 48 hours after injection, this chemical induces oxidative stress, which results in cataract development. An ophthalmoscope was used to measure the degree of lens opacity following pharmacological pupil dilatation with tropicamide (0.5%) and phenylephrine (10%).

### Lens Opacity Grading

A scale was used to classify the lens opacity:

++: Dispersed opacity; +++: Substantial diffuse opacity; ++++: Dense opacity encompassing a vast region; 0: No opacity (clear lens); +: Slight opacity.

### Therapy and Assessment

By contrasting the development of lens opacity with morphological changes, the effectiveness of the test formulations was assessed. The left eye of the treated group received the test formulation, whereas the right eye received the conventional Itone formulation. To track changes over time, visual observations and photographic recording were used. Researchers were able to evaluate the test formulations' therapeutic and preventative effects on the formation of cataracts by combining the examination of visual grading and photographic data.

The phytoconstituents of Arka, an aqueous distillate of verified Punarnava root (Boerhaavia diffusa Linn) made in accordance with the Indian Ayurvedic Formulary (2001), were assessed in this work. After being soaked for the entire night, coarsely ground roots were distilled with water in a 1:16 ratio. The resulting distillate was then kept in airtight containers in a cold, shady location. Alkaloids, glycosides, tannins, flavones, steroids, and other substances were discovered through qualitative analysis, supporting results from earlier studies.

**Table 1: Arka (aqueous distillate) of B. diffusa roots was subjected to preliminary phytochemical testing for the presence of natural components using various reagents.**

| Natural products | Test performed | Result (in triplicate) |
|---|---|---|
| Steroid | Shinoda test | +ve |
| Flavone | Alkaline test | +ve |
| Alkaloid | Dragendorff's test,Mayer's test,Wagner's test | + ve |
| Tannin | Neutral $FeCl_3$ | -ve |
| Glycoside | Noller's test | +ve |
| Glycoside | Molisch's test | +ve |
| Terpenoid | Liebermann-Burchard reagent | +ve |
| Glycoside | NaOH solution | +ve |
| Proteins | Million's test | +ve |
| Proteins | Biuret test | +ve |
| Glycoside | Brontragar,s test | +ve |
| Amino acids | Ninhydrin test | +ve |

The identification of these phytoconstituents supports earlier studies on Punarnava and its traditional Ayurvedic use. The plant's potential for treating ailments like inflammation, liver and kidney illnesses, and general detoxification is supported by the qualitative discovery of these bioactive components. Additionally, in clinical or therapeutic applications, the product's consistency and dependability are guaranteed by the standard preparation technique.

**HPTLC Analysis:**

A DESAGA HPTLC densitometer and precoated silica gel 60 F254 plates were used to conduct an HPTLC investigation of a methanolic solution of dried Arka. Toluene, acetone, and glacial acetic acid (8:0.1:1.5) were utilized as the mobile phase. With a LINOMAT V applicator, the samples (5, 10, 15 µl) were applied. There were three peaks at 10 µl with Rf values of 0.01; 0.39; and 0.58, and four peaks at 15 µl with Rf values of 0.01; 0.06; 0.38; and 0.58. At all concentrations, the peaks remained constant.

**GC-MS Study:**

A mass spectrometer and a Perkin-Elmer GC Clarus 500 system were utilized to examine the aqueous distillate of Boerhaavia diffusa (Arka). According to the NIST library, the following important components were found: gamma-sitosterol, stigmasterol, stigmast-4-en-3-one, dihydrobrassicasterol, dibutyl phthalate, cetylglycidylether, n-hexadecanoic acid, eucalyptol, and glycerin.

**HPLC Study:**

Beta-sitosterol was shown to be a marker in the phytochemical analysis of Arka, which also detected beneficial terpenoids and sterols. HPLC and UV spectroscopy were used to quantitatively analyze the eye drop formulation. After eight hours, the eye drop's beta-sitosterol content was 0.45% and 0.68%, measured with a UV/VIS spectrophotometer set at 273.6 nm. With a concentration of 0.86%, the HPLC analysis revealed a retention period of 23.72 minutes for beta-sitosterol.

**Formulation Development:**

**pH**: All eye drop samples (E1–E4) had pH values between 6.9 and 7.4.

**Clarity**: There were no suspended particles in any of the formulations.

**Stability**: The pH levels stayed constant between 7.1 and 7.4, and no color fading was noticed.

**Sterility Testing**: The formulations E1, E2, E3, and E4 were found to be appropriate for ophthalmic use based on sterility tests that revealed no microbial growth.

The Arka Punarnava sample had 31 different chemical components, according to the GCMS analysis.

**Table 2: Sterility testing outcomes**

| Formulation types Aqueous Eye Drop | Development of microbes |
|---|---|
| Eye drops samples (E1, E2, E3 and E4) | Absent |

**Pharmacological Study:**

Evaluation of Visual Irritation: None of the enhanced eye drop formulations caused redness, irritation, or an increase in tear production.

**Evaluation of Photometrics and Lens Morphology:**

Over the course of 30 days after cataract induction, rats were observed for the development of cataracts. Rats in Group I (control) were given sterile distilled water, and after 30 days, they still had cataracts throughout their retina. Rats in group II (eye drop) on the other hand showed a small opacity at first, which progressively got worse and reached full opacity after 48 hours. The first day following cataract induction marked the beginning of eye drop treatment, which postponed the development of opacity. There were three categories for the degree of opacity: +, ++, and +++. In comparison to the conventional treatment, the eye drop group had a similar delay in cataract formation, according to the study. A SONY digital camera with a 25 mm wide-angle lens and a 10X optical magnification in macro mode was used to record cataract development.

The 2,500-year-old medical system known as Ayurveda treats a number of eye conditions, including as xerosis, conjunctivitis, cataracts, and glaucoma. It suggests remedies such as aqueous distillates (arka), extracts, and collyrium. In accordance with the Indian Pharmacopoeia, the goal of this study was to create a contemporary dosage form of arka (Punarnava) for the treatment of cataracts. The National Botanical Research Institute verified the authenticity of the punarnava root samples, which were subsequently utilized to make arka using conventional techniques.

Animal models were used to assess two dose forms: nasal spray and ocular drops. The eye drops were autoclaved and exposed to UV radiation after being prepared with sodium chloride, benzalkonium chloride, and phosphate buffer to maintain pH 7.2. The formulations' stability throughout a range of temperatures and humidity conditions was verified using stability testing.

Up to 31 chemicals were found in the arka by chemical analysis utilizing HPTLC, GCMS, HPLC, and UV spectroscopy; beta-sitosterol was used as the marker. The marker was present in 0.84% of the eye drop formulation. Testing on male albino rats that had cataracts caused by sodium selenite revealed that Arka Punarnava eye drops prevented the development of cataracts in a manner comparable to that of the common medication Itone. A digital camera was used to record the results, which showed that the treatment group developed cataracts later than the control group.

**CONCLUSION**

The study concludes that ark Punarnava, an Ayurvedic eye drop formulation that complies with Indian pharmacopoeia requirements, is useful in postponing the development of cataracts. The use of aqueous distillate from the roots of Punarnava (Boerhavia diffusa Linn.) as ark Punarnava is one of the traditional remedies for cataract surgery (Timir) offered by Ayurvedic medicine; nevertheless, its high irritating content frequently restricts its recommendation for cataract therapy today.

In order to comply with Indian pharmacopoeia regulations, this study created eye drops utilizing modern dosages of the Punarnava that were made following conventional Ayurvedic techniques. The standardized formulations demonstrated an equivalent delay in cataract formation to standard therapies when tested on animal models. According to these results, ark Punarnava shows great promise as a cataract treatment. However, before this Ayurvedic medication can be formally approved or marketed for the prevention and treatment of cataracts, more thorough preclinical and clinical study is needed.

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