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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF *Lagenaria siceraria* (WILD BOTTLE GOURD), AND DEVELOPMENT OF A NASAL DROP FORMULATION FOR THE TREATMENT OF JAUNDICE

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Abstract

Lagenaria siceraria (wild bottle gourd), a medicinal plant of traditional importance, is well known for its hepatoprotective and antioxidant activities. Its therapeutic potential in this research was investigated through the development of a nasal drug delivery system for liver protection, especially for the management of jaundice. Bioactive principles were obtained from the Soxhlet extractor using a series of increasing polar solvents—petroleum ether, chloroform, ethyl acetate, ethanol, and water—to provide maximum phytochemical recovery. Phytochemical analysis of the concentrated extracts all validated the occurrence of flavonoids, alkaloids, phenolic compounds, saponins, and triterpenoids, all documented to promote liver function and antioxidant protection. The extract's antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, which showed potent free radical scavenging activity with IC_{50} value 62.35 µg/mL. Due to the nasal route's capacity for evasion of hepatic first-pass metabolism and for quick systemic delivery, a nasal drop formulation was created from the most active extract. The preparation was made isotonic, pH-neutral (5.6–6.2), and stable and was analyzed for pH, clarity, viscosity, and uniformity of drug content, all of which were in accordance with pharmacopeial specifications. This work combines phytochemical analysis with pharmaceutical development to present a new, non-invasive nasal delivery system of Lagenaria siceraria as a potential adjuvant therapy for hepatic diseases, opening avenues for future preclinical and clinical research.

Keywords: Lagenaria siceraria, nasal drops, jaundice, hepatoprotective, phytochemicals

Figure 1: Wild Bottle Gourd (*Lagenaria siceraria*) fruits hanging from the vine in a cultivated field.

Introduction

Lagenaria siceraria (Molina) Standley, commonly known as wild bottle gourd, is a climber belonging to the Cucurbitaceae family. It is widely distributed across tropical and subtropical regions, and in India, it thrives in semi-arid and dry deciduous zones, particularly in Gujarat, Rajasthan, Maharashtra, Madhya Pradesh, and parts of Uttar Pradesh and Chhattisgarh.[1] The plant is characterized by its soft-stemmed vines, large lobed leaves, and bottle-shaped fruits, and has long been valued for its wide array of therapeutic applications in traditional medical systems such as Ayurveda, Siddha, and various tribal healing practices.[2]

Classical Ayurvedic texts, including the *Charaka Samhita* and *Bhavaprakasha Nighantu*, reference *Ikshu-putra*—a term believed to be synonymous with *Lagenaria siceraria*—for the treatment of *Kamala* (jaundice) and *Yakrit roga* (liver ailments). The plant has been ascribed a range of pharmacological activities including hepatoprotective, anti-inflammatory, antioxidant, diuretic, and cardiotonic effects, which have made it a prominent part of traditional hepatotherapy.[1,3]

Among its most culturally entrenched traditional applications is its use in the management of jaundice, a condition marked by elevated bilirubin levels due to hepatic dysfunction. In many tribal and rural communities, the dried fruit is powdered and consumed early in the morning on an empty stomach, followed by a strict prohibition on water intake for at least one hour. This regimen, preserved and passed down by community elders and folk healers, is believed to enhance hepatic detoxification by allowing the plant's active constituents to be efficiently absorbed and act directly on the liver.[2] Anecdotal reports consistently cite significant improvement in jaundice symptoms with this practice.

Phytochemical studies of *Lagenaria siceraria* reveal the presence of a wide spectrum of bioactive compounds, including flavonoids, saponins, tannins, alkaloids, sterols, triterpenoids, and phenolic acids. These constituents exhibit potent antioxidant and free-radical scavenging properties, which are crucial in reducing oxidative stress in hepatocytes—a key mechanism in the pathogenesis of liver damage.[1] Additionally, the inhibition of lipid peroxidation and normalization of liver enzyme markers further underscore the hepatoprotective potential of the plant.

Interestingly, traditional administration was not confined to the oral route alone. In several tribal and folk medicine systems, the nasal route was employed for systemic delivery of herbal preparations—a practice known in Ayurveda as *Nasya Karma*. Decoctions or paste preparations of *Lagenaria siceraria* were sometimes administered intranasally, particularly in cases of acute hepatic distress. This traditional insight coincides with modern pharmaceutical advances recognizing the nasal route as a highly vascularized, rapid,

and non-invasive delivery method that bypasses first-pass metabolism and enables efficient systemic absorption.[2,3]

Despite its widespread traditional use and promising phytochemical profile, standardized pharmaceutical formulations of Lagenaria siceraria, particularly for nasal administration, remain largely unexplored. This highlights a significant gap in the scientific validation of ancient knowledge. The present research aims to address this gap by formulating and evaluating a nasal drop delivery system based on Lagenaria siceraria fruit extract, inspired directly by ethnomedical practices. To support this development, a comprehensive pharmacognostic and phytochemical analysis of the fruit has been undertaken to authenticate plant material and ensure chemical consistency.

In uniting traditional wisdom with modern drug delivery technology, this study not only revives an age-old natural remedy but also paves the way for the integration of culturally relevant, plant-based novel drug delivery systems (NDDS) into contemporary healthcare.

Plant Material Collection

Fruits of wild *Lagenaria siceraria* were collected from farms and the local market in the village of Arni, located in Yavatmal district, Maharashtra, India. The plant was identified and authenticated by a botanist and local residents of the area. After authentication, only the fruits were separated for the study. The collected fruits were shade-dried and subsequently subjected to hot air oven drying at 35 °C for 30 minutes to ensure complete moisture removal. The dried fruits were coarsely powdered using an electric grinder, and the resulting powder was stored in airtight containers for further analysis.

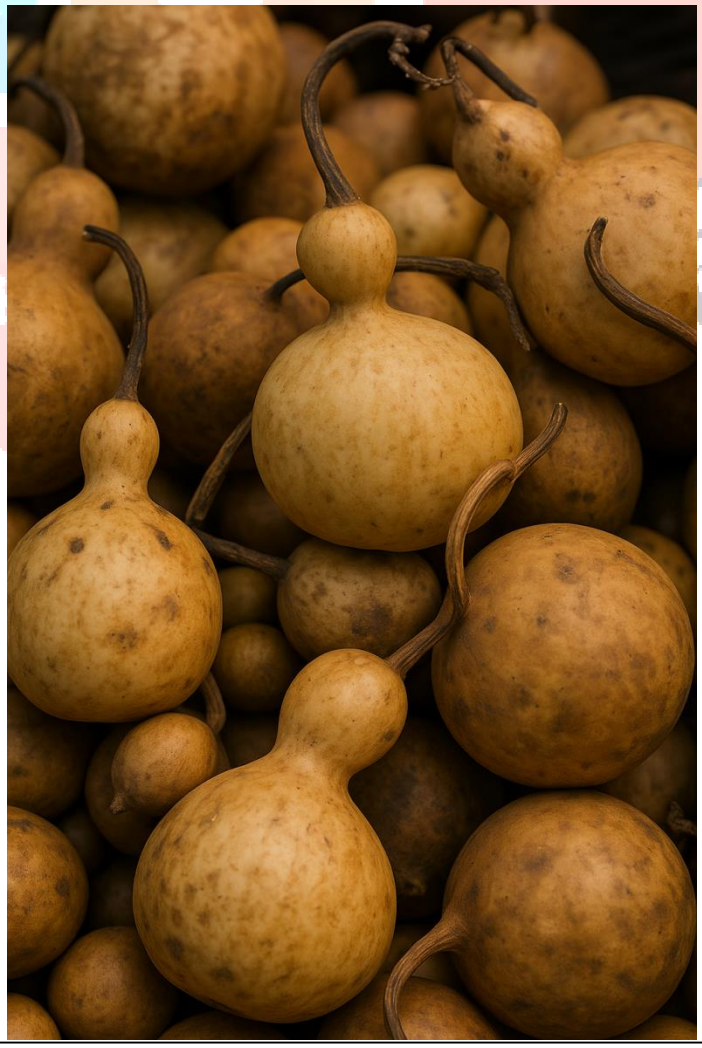


Figure 2: Dried Hard-Shelled Brown *Lagenaria siceraria* Gourds

Figure 3: Dried Fruit Pulp of Wild Bottle Gourd (*Lagenaria siceraria*) Stored in Airtight Container

Figure 4: Dried Fruit Pulp of Wild Bottle Gourd (*Lagenaria siceraria*) Displayed on Paper Sheet

Preparation of Plant Material

Approximately 500 g of powdered material was obtained from the dried fruits. The powder exhibited a pale yellow-brown color, a slightly bitter taste, and a characteristic odor.

Morphological Characteristics wild bottle gourd fruit

Characteristic	Description
Shape	Elongated, club-shaped, or bottle-shaped; irregular in wild forms
Size	Small to medium (10–30 cm in length)
Surface Texture	Smooth, hard, and waxy at maturity; may be pubescent when immature
Color	Green when immature; pale green to light brown or tan at maturity
Pericarp	Hard and woody when mature; fleshy when immature
Pulp	White, spongy, and fibrous; bitter in taste
Seeds	Numerous, flat, ovate; white to light brown; slimy when fresh
Taste & Smell	Bitter and pungent due to cucurbitacins

Table no 1: Morphological Features of Wild Bottle Gourd Fruit (*Lagenaria siceraria*)

Crude Drug Extraction

Extraction of the crude drug was performed to remove the phytochemical constituents from the dried fruit powder of Lagenaria siceraria. The extraction procedure was intended to yield a wide variety of bioactive compounds using solvents with varying polarity. This method improves the efficiency of isolating varying

classes of phytoconstituents like alkaloids, flavonoids, phenolics, and saponins. To achieve this, successive solvent extraction was conducted using a Soxhlet apparatus, as explained in the next section.

Successive Solvent Extraction (Soxhlet Method)

Figure 5: Soxhlet Apparatus for Successive Solvent Extraction

The above setup illustrates the Soxhlet extraction method, a classical and efficient technique used for isolating phytoconstituents from plant materials. In this process, solvents of increasing polarity (e.g., petroleum ether,benzene chloroform, ethyl acetate, ethanol, and water) are used successively to extract a wide range of bioactive compounds based on their solubility. The solvent is continuously heated, evaporated, and condensed back into the thimble containing the plant powder, allowing repeated washing without the need to replace the solvent manually. This method ensures maximum recovery of both polar and non-polar phytochemicals and is particularly useful for preparing plant extracts for phytochemical screening and biological evaluation.

The powdered sample (7 g) was extracted successively with the following solvents in increasing order of polarity:

Sr.no	Solvent Used	Yeild	Yeild%
1	Petroleum Ether	0.170 g	2.6% w/w
2	Benzene	0.160 g	2.3% w/w
3	Chloroform	0.140 g	2% w/w
4	Ethyl Acetate	0.110 g	1.69% w/w
5	Ethanol	0.300 g	4.46% w/w
6	Water	0.270 g	4.1% w/w

Table no 2: Yield of Successive Solvent Extracts of Lagenaria siceraria Fruit

The extracts were concentrated using an electric water bath, and the resulting residues were accurately labeled with the name of the solvent from which they were obtained.

Preliminary Phytochemical Investigation

Phytochemical screening of the successive solvent extracts of wild Lagenaria siceraria fruit powder was carried out using standard qualitative tests as described by K.R. Khandelwal (2008) in Practical Pharmacognosy: Techniques and Experiments. The results revealed a diverse range of phytoconstituents distributed among the different solvent extracts based on polarity. Non-polar solvents like petroleum ether and chloroform primarily extracted lipophilic compounds such as terpenoids and steroids, while polar solvents like methanol and water were more effective in extracting alkaloids, flavonoids, phenols, tannins, saponins, and glycosides.[22]

Ethanolic and aqueous extracts showed strong positive reactions for phenolics and flavonoids, indicating their potential antioxidant and hepatoprotective properties. Alkaloids were detected in the ethanol, chloroform, and aqueous extracts using Mayer's and Wagner's tests, while the presence of tannins and saponins was confirmed in the aqueous and ethanolic fractions via ferric chloride and foam tests, respectively. These secondary metabolites are pharmacologically important due to their reported anti-inflammatory, antimicrobial, and hepatoprotective activities.

The systematic screening not only confirms the therapeutic potential of Lagenaria siceraria but also supports its ethnomedicinal application in liver-related disorders. These findings justify further investigation for active constituents and formulation development.

The following tables summarize the results of the preliminary phytochemical screening performed on each extract. The tests aimed to identify major classes of secondary metabolites present in the Lagenaria siceraria fruit, which may contribute to its hepatoprotective potential. Each test was conducted using standard protocols, and the presence or absence of phytochemicals was recorded based on characteristic color changes or precipitate formation.

- **Detection of Alkaloids:**

Alkaloids are nitrogenous organic compounds known for their potent biological activity and are commonly found in wild bottle gourd. They were detected using Mayer's, Dragendorff's, Wagner's,& Hager's tests. [4,5] The following reagents were used to test their presence:

Solvent Extract	Mayer's Test	Dragendorff's Test	Wagner's Test	Hager's Test
Petroleum Ether	+	-	-	-
Benzene	+	+	-	-
Chloroform	+	+	-	-
Ethyl Acetate	-	+	-	-
Ethanol	+	-	-	+
Water	+	+	+	+

Table no 3: Alkaloid Detection in Solvent Extracts of Lagenaria siceraria

- **Detection of Carbohydrates and Glycosides**

Carbohydrates and glycosides were detected using Molisch's, Fehling's, Benedict's, Legal's, and Libermann-Burchard's tests. [4,5] Carbohydrates provide energy, while glycosides often exert cardiac, hepatoprotective, and anti-inflammatory effects. The following reagents were used to test their presence:

Solvent Extract	Molisch's Test	Fehling's A & B Test	Benedict's Test	Legal's Test	Libermann-Burchard Test
Petroleum Ether	-	-	-	-	-
Benzene	-	-	-	+	-
Chloroform	-	-	-	+	-
Ethyl Acetate	-	-	-	+	-
Ethanol	-	-	-	-	-
Water	-	-	-	+	-

Table no 4: Carbohydrates and Glycosides Detection in Solvent Extracts of *Lagenaria siceraria* Lagenaria

- **Detection of Saponins**

Saponins, detected using the foam test, were present in polar extracts. [4,5] They are known for their surfactant properties and immunomodulatory potential. The following reagents were used to test for their presence:

Solvent Extract	Foam Test
Petroleum Ether	-
Benzene	-
Chloroform	+
Ethyl Acetate	+
Ethanol	++
Water	++

Table no 5: Saponins Detection in Solvent Extracts of *Lagenaria*

- **Detection of Phenols and Tannins**

Phenols and tannins, detected using Ferric chloride, Gelatin, Lead acetate, and Bromine water, were found in ethanol and water extracts. [4,5] These compounds possess significant antioxidant and astringent properties. The following reagents were used to test for their presence:

Solvent Extract	Ferric Chloride	Gelatin Test	Lead Acetate Test	Bromine Water Test
Petroleum Ether	-	+	+	-
Benzene	+	-	+	-
Chloroform	+	+	+	-
Ethyl Acetate	+	-	+	-
Ethanol	++	+	+	+
W				

Table no 6: Phenols and Tannins Detection in Solvent Extracts of *Lagenaria siceraria*

- **Detection of Amino Acids and Proteins**

Amino acids and proteins were detected using Millon's, Biuret, and Ninhydrin tests. [4,5] Their presence suggests nutritional value and potential enzyme-modulating actions. The following reagents were used to test for their presence:

Solvent Extract	Millon's Test	Biuret Test	Ninhydrin Test
Petroleum Ether	-	-	-
Benzene	-	-	-
Ethyl Acetate	+	-	-
Chloroform	-	-	-
Ethanol	-	-	-

Table no 7: Amino Acids and Proteins Detection in Solvent Extracts of *Lagenaria siceraria*

- **Detection of Flavonoids**

Flavonoids, evaluated using the Shinoda and alkaline tests, were predominantly present in ethanolic extracts. [4,5] These compounds are well-known for their antioxidant, anti-inflammatory, and hepatoprotective roles. The following reagents were used to test for their presence:

Solvent Extract	Shinoda Test	Aqueous Alkali Test
Petroleum Ether	-	-
Benzene	-	-
Ethyl Acetate	-	-
Chloroform	-	+
Ethanol	-	+
Water	-	+

Table no 8: Flavonoids Detection in Solvent Extracts of *Lagenaria*

- **Detection of Triterpenoids**

Terpenoids were assessed using the Liebermann-Burchard, Salkowski, and Copper Acetate tests. [4,5] These constituents are known to support liver health and modulate inflammatory responses. The following reagents were used to test their presence:

Solvent Extract	Liebermann–Burchard Test	Salkowski Test	Copper Acetate
Petroleum Ether	-	+	+
Benzene	-	-	+
Ethyl Acetate	-	+	+
Chloroform	-	+	+
Ethanol	-	+	-
Water	-	-	-

Table no 9: Triterpenoids Detection in Solvent Extracts of *Lagenaria*

- **DPPH Radical Scavenging Assay**

The antioxidant activity of the ethanolic extract of *Lagenaria siceraria* was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, following the protocol described by Blois (1958), with slight modifications. This assay is based on the reduction of the DPPH radical, a stable free radical with a deep violet color, upon interaction with antioxidant compounds that donate hydrogen atoms or electrons.[5,6]

Preparation of Solutions

A 0.1 mM DPPH stock solution was freshly prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol. The solution was stored in a dark container at 4 °C and used within 24 hours.

The ethanolic extract of Lagenaria siceraria was dissolved in methanol to prepare sample concentrations of 100, 200, 300, and 400 µg/mL. Ascorbic acid was used as a reference standard at the same concentrations.

Assay Procedure

In a 1:1 ratio, 2.0 mL of the DPPH solution was mixed with 2.0 mL of each concentration of the extract in separate test tubes. The mixtures were vortexed and incubated in the dark at room temperature (25 ± 2 °C) for 30 minutes to ensure complete reaction.

The absorbance was measured at 517 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan) against methanol as a blank. A control (DPPH + methanol) was used to determine maximum absorbance, and the scavenging activity was calculated using the following equation:

$$\text{Radical Scavenging Activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

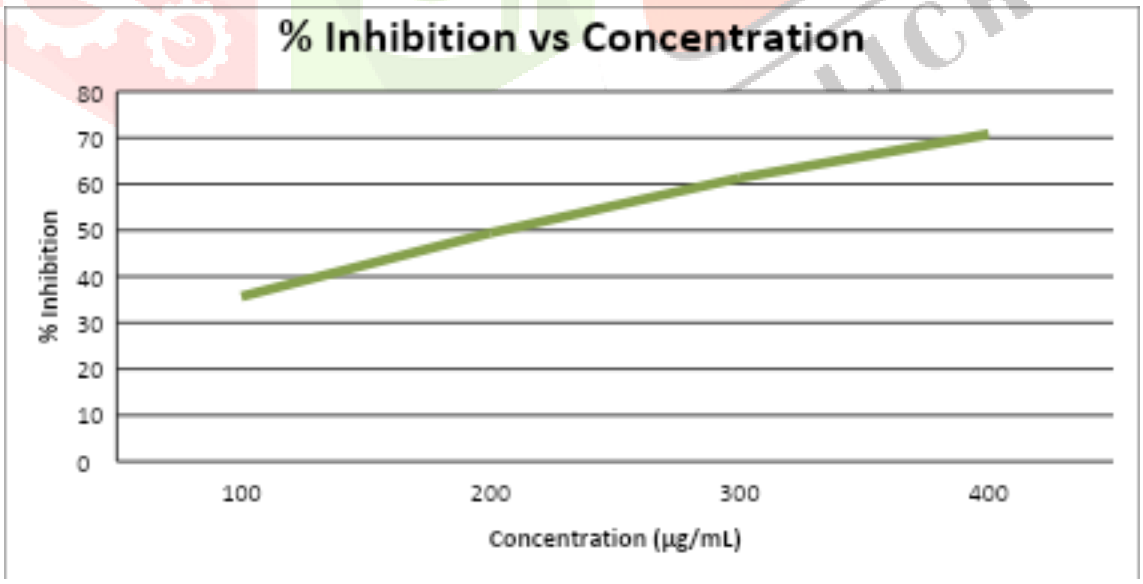
Where:

- $A_{control}$ is the absorbance of the DPPH solution without extract,
- A_{sample} is the absorbance of the DPPH solution with extract.

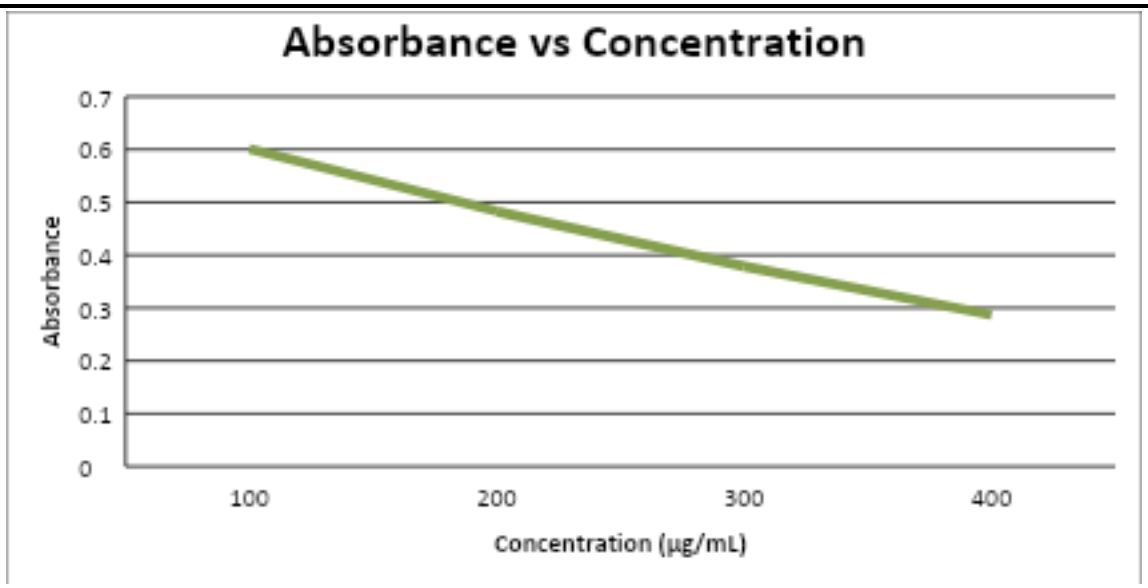
Each test was performed in triplicate, and the results are expressed as mean ± standard deviation (SD).

Concentration (µg/mL)	Absorbance (Mean ± SD)	% Inhibition (Mean ± SD)
100	0.601 ± 0.004	35.67 ± 0.72
200	0.483 ± 0.006	49.43 ± 0.65
300	0.379 ± 0.005	61.31 ± 0.83
400	0.287 ± 0.003	70.74 ± 0.91
Control (DPPH only)	0.931 ± 0.002	—

Table no 10: In-vitro Antioxidant Activity of Ethanolic Extract of Lagenaria siceraria Assessed by DPPH Radical Scavenging Method



Graph 1: DPPH Radical Scavenging Activity (% Inhibition) of Extract at Various



Graph 2: Absorbance Values of Extract at Different Concentrations in DPPH Assay

Formulation: Development of Nasal Drops

- **Rationale for Nasal Formulation**

The traditional use of *Lagenaria siceraria* (wild bottle gourd) in the treatment of jaundice involves the inhalation of smoke from the dried fruit during early morning hours, a practice believed to aid in liver detoxification and improve jaundice symptoms.[14]This ethnomedicinal application highlights the therapeutic potential of delivering active compounds via the nasal route, which is further supported by the fruit's known antioxidant and hepatoprotective properties.[7]

Leveraging this traditional knowledge, a nasal drop formulation was developed to deliver the antioxidant-rich ethanolic extract directly via the nasal mucosa. This route ensures rapid absorption of the bioactive compounds into systemic circulation while bypassing the hepatic first-pass metabolism, allowing for more efficient pharmacological action. The nasal route offers significant advantages, including high permeability, rich vascularization, and non-invasiveness, making it an ideal alternative for the delivery of hepatoprotective phytoconstituents.

The formulation mimics the traditional practice of sniffing the dried fruit in the morning, enhancing its therapeutic potential by providing a controlled and convenient method of administration.

- **Literature Review on Nasal Drug Delivery Systems**

Nasal drug delivery systems (NDDS) have gained significant attention in recent years as an alternative route for the administration of pharmaceutical agents. The nasal route offers several advantages, including rapid absorption, bypassing the hepatic first-pass metabolism, and direct delivery of drugs into the systemic circulation. The nasal mucosa, with its rich vascularization, ensures that drugs administered via this route are rapidly absorbed and achieve high bioavailability.[7] Furthermore, the nasal route offers a non-invasive method of delivery, which increases patient compliance compared to other invasive routes like injections.[12]

The nasal cavity's anatomy is well-suited for drug delivery. It is highly permeable to small molecules and has a large surface area for absorption..[7] The olfactory region, in particular, allows for direct drug entry into the

systemic circulation, bypassing the blood-brain barrier and first-pass hepatic metabolism.[13] These features make nasal drug delivery systems an attractive option for the delivery of therapeutic agents, especially those requiring rapid onset of action or targeting specific organs such as the liver.

Despite these advantages, challenges remain, such as nasal mucosal irritation, drug deposition, and formulation stability. However, recent advances in nasal formulation technologies, including the use of biocompatible excipients and optimized formulation designs, have made this route more viable for both small molecules and herbal extracts.[12]

Selection of *Lagenaria siceraria* as the Active Ingredient

Lagenaria siceraria (wild bottle gourd) has been traditionally used in ethnomedicine, particularly for the treatment of liver-related diseases such as jaundice. The dried fruit of the plant is commonly inhaled, and the smoke is believed to possess hepatoprotective properties.[14]This traditional use forms the basis for developing a modern nasal drop formulation, where the bioactive compounds from *Lagenaria siceraria* can be directly delivered via the nasal mucosa for enhanced therapeutic efficacy.

The pharmacological properties of *Lagenaria siceraria* are attributed to its rich phytochemical composition. The plant contains bioactive compounds, including **alkaloids**, **flavonoids**, **triterpenoids**, and **phenolic compounds**, which are known for their **antioxidant**, **anti-inflammatory**, and **hepatoprotective** activities.[7] Among these compounds, **lupenol**, a triterpenoid, has been specifically identified in *Lagenaria siceraria* and has demonstrated significant **antioxidant** properties, protecting the liver from oxidative stress.[15]

The ethanolic extract of *Lagenaria siceraria* is rich in these compounds and has been shown to exhibit potent antioxidant activity, making it an ideal candidate for the development of a **hepatoprotective nasal formulation**. The nasal route will allow for **direct absorption** of these bioactive compounds, maximizing their therapeutic effects and bypassing the first-pass metabolism, which is a significant advantage in liver-targeted therapies.

Justification for the Nasal Route of Administration

The **nasal route** of drug delivery is an attractive alternative for hepatoprotective formulations due to its rapid absorption and high bioavailability. When drugs are delivered via the nasal mucosa, they enter the bloodstream directly, avoiding the first-pass metabolism in the liver. This feature is particularly beneficial for compounds that require rapid systemic circulation to exert their therapeutic effects on the liver, such as those in *Lagenaria siceraria*.[7]

In addition to bypassing the hepatic first-pass effect, the nasal route offers several other advantages. The nasal cavity is richly vascularized, providing an efficient mechanism for drug absorption. Furthermore, this route is non-invasive and convenient, enhancing patient compliance, especially for individuals with liver conditions who may benefit from frequent or regular doses of hepatoprotective agents .[13] The traditional method of sniffing dried fruit smoke in the morning for liver detoxification further supports the use of the nasal route for delivering *Lagenaria siceraria*'s bioactive compounds.

The development of a nasal drop formulation offers a modern, controlled, and standardized approach to delivering these compounds in a form that is both safe and effective. This formulation not only aligns with

the traditional method of administration but also improves upon it by providing a precise dose and optimized bioavailability.

Goal of Formulation

The primary goal of this formulation is to develop a stable and highly effective nasal drop delivery system utilizing the ethanolic extract of Lagenaria siceraria, aiming to directly deliver its potent hepatoprotective and antioxidant properties via the nasal mucosa. The formulation aims to:

1. Facilitate rapid systemic absorption of the bioactive compounds, effectively bypassing the hepatic first-pass metabolism for faster therapeutic action.

2. Emulate the traditional practice of inhaling dried fruit smoke during the early morning to promote liver detoxification, while providing a modern, controlled, and reproducible method of administration.

3. Enhance the bioavailability and therapeutic efficacy of Lagenaria siceraria's bioactive compounds, particularly the antioxidants and hepatoprotective agents like lupenol, which offer protection against oxidative stress and liver damage.

This nasal drop formulation is expected to provide an innovative and scientifically advanced alternative to traditional methods of administering Lagenaria siceraria, offering improved patient compliance and ensuring consistent therapeutic benefits from this ethnomedicinal plant.

Preparation of Nasal Drop Formulation [8]

Step 1: Selection and Collection of Plant Material

1. **Plant Material :**

Lagenaria siceraria (wild bottle gourd) was selected based on its traditional use in the treatment of jaundice through inhalation of its dried fruit. This practice was leveraged to formulate the nasal drop, aiming to deliver the **hepatoprotective** and **antioxidant-rich** extract via the nasal route.

2. **Cleaning and Drying**:

Fresh, fully ripe fruits of *Lagenaria siceraria* were collected.

- They were thoroughly **washed under running tap water** to remove dirt and external contaminants.
- The fruits were **peeled**, and the pulp was discarded.
- The cleaned fruits were cut into small slices and subjected to **shade-drying** at **ambient temperature (25°C–30°C)** for **7–10 days**, to prevent any degradation of bioactive compounds that could occur with direct sunlight exposure.

3. **Powdering**:

Once dried, the fruit slices were ground into a **fine powder** using a **high-speed electric grinder**. The powdered material was sieved through a **60-mesh sieve** to ensure uniformity and to eliminate coarse particles. The final powder was stored in **airtight containers** to avoid moisture and contamination.

Step 2: Extraction of Bioactive Compounds (Maceration Method)

1. **Sample Weighing**:

A total of **50 g** of the dried and powdered *Lagenaria siceraria* fruit was accurately weighed and placed in a **500 mL glass beaker**.

2. **Maceration Process**:

The maceration method was chosen for its simplicity and efficiency in extracting bioactive compounds.

- **500 mL of ethanol (95%)** was added to the beaker containing the powdered plant material.
- The mixture was **stirred thoroughly** and allowed to **macerate** at **room temperature** for **24 hours** to facilitate the extraction of the bioactive compounds.
- During this period, the solution was stirred intermittently to ensure proper interaction between the solvent and plant material.

3. **Filtration**:

After 24 hours, the mixture was filtered through **Whatman filter paper No. 1** to separate the solid residues from the liquid extract. The filtrate was collected and stored.

4. **Concentration of Extract**:

The resulting ethanolic extract was concentrated using a **rotary evaporator** at **40°C** under reduced pressure to prevent the loss of sensitive compounds. The concentrated extract was then stored in **sterile glass containers** at **4°C** for future use in the formulation.

Step 3: Preparation of the Nasal Drop Formulation

1. **Ingredients**:

The following ingredients were selected for the formulation of the nasal drop:

- **Ethanolic extract of *Lagenaria siceraria* (2% w/v)**: The active ingredient that contains the bioactive compounds.

- **Glycerin (5%)**: A humectant that helps solubilize the extract and provides moisture.
- **Propylene glycol (10%)**: A co-solvent that enhances solubility and improves the formulation's stability.
- **Phenoxyethanol (0.5%)**: A preservative to prevent microbial growth.
- **Sterile distilled water**: Used to adjust the volume and achieve the desired formulation consistency.

Sr. No.	Ingredient	Function	Quantity (% w/v)
1	Ethanolic Extract of Lagenaria siceraria	Active herbal extract (hepatoprotective agent)	2%
2	Glycerin	Humectant and solubilizer	5%
3	Propylene Glycol	Co-solvent, enhances solubility and stability	10%
4	Phenoxyethanol	Preservative (antimicrobial agent)	0.5%
5	Sterile Distilled Water	Vehicle/base for the formulation	q.s. to 100%

Table no 11: Ingredients Used in the Formulation of *Lagenaria siceraria* Nasal Drops

2. **Weighing and Mixing**:

The ingredients were weighed accurately as per the following proportions:

- **Ethanolic extract of *Lagenaria siceraria* (2% w/v)**
- **Glycerin (5%)**
- **Propylene glycol (10%)**
- **Phenoxyethanol (0.5%)**
- The final volume was adjusted with **sterile distilled water**.

The mixture was placed in a **sterile glass beaker** and stirred using a **magnetic stirrer** for **30 minutes** to ensure uniform mixing of the components.

3. **Homogenization**:

The formulation was homogenized using a **homogenizer** to ensure the even distribution of the ethanolic extract throughout the formulation.

4. **Filtration**:

After thorough mixing, the formulation was filtered through a **0.45 µm sterile filter** to eliminate any particulate matter, ensuring the formulation was free from contaminants.

5. **Filling and Packaging**:

The final nasal drop formulation was carefully filled into **amber glass dropper bottles**.

Each bottle was filled with **10 mL** of the formulation and securely sealed with **tamper-proof caps**. The amber color of the bottles helps protect the formulation from light degradation.

Evaluation of Nasal Drop Formulation

The formulated nasal drops were subjected to a series of evaluation parameters to determine their physicochemical properties, stability, and microbiological quality.[8] The following tests were performed:

1. Organoleptic Evaluation

The formulation was observed for its color, odor, appearance, and clarity. These characteristics help ensure consumer acceptability and formulation stability.[16]

Parameter	Observation
Color	Clear solution
Odor	Characteristic herbal odor
Clarity	Clear, no turbidity
Appearance	Homogenous, free from particulate matter

Table no 12: Organoleptic Characteristics of Formulated Nasal

2. pH Determination

The pH of the formulation was determined using a calibrated digital pH meter. The pH was measured at room temperature ($25 \pm 2°C$) to ensure compatibility with nasal mucosa.[11]

Batch Code	pH Value
F1	6.12
F2	6.28
F3	6.34

Table no 13: pH Values of Formulations

3. Viscosity Measurement

The viscosity of the *Lagenaria siceraria* nasal drop formulation was determined using an **Ostwald viscometer** based on the principle of laminar flow under gravity through a capillary. The measurement was carried out at a controlled temperature of **$25 \pm 0.5°C$** using a thermostatically maintained water bath. The experiment was conducted in triplicate, and the mean value was reported.[11]

The viscometer was first cleaned with distilled water and acetone and dried thoroughly. Approximately **10 mL** of the nasal drop formulation was transferred into the viscometer and equilibrated in the water bath. Using a pipette bulb, the sample was drawn above the upper calibration mark and allowed to flow

freely. The **flow time** from the upper to the lower calibration mark was recorded using a digital stopwatch. The procedure was repeated three times for accuracy.[11]

A similar process was repeated using **distilled water as the reference liquid** under identical conditions. The **viscosity of the formulation (η_2)** was calculated using the following formula:

$$\eta 2 = \eta 1 \times (t2/t1) \times (\rho 2/\rho 1)$$

Where:

- η1\eta_1η1 = viscosity of water at 25°C (0.8907 cP)
- t1t_1t1 = flow time of water (s)
- t2t_2t2 = flow time of the formulation (s)
- ρ1\rho_1ρ1 = density of water (1.0 g/cm³)
- ρ2\rho_2ρ2 = density of the formulation (g/cm³)

Parameter	Value
Flow time for water (t_1)	38.2 sec
Flow time for formulation (t_2)	54.6 sec
Density of formulation (ρ_2)	1.02 g/cm³
Calculated viscosity (η_2)	1.30 cP

Table no 14: Viscosity of nasal drop formulation measured by

The observed viscosity of the nasal formulation was **1.30 cP**, which lies within the acceptable range for nasal drug delivery systems (generally 1–5 cP). An optimal viscosity ensures **sufficient residence time** in the nasal cavity without causing discomfort or impairing mucociliary clearance.

4. Drug Content Estimation

Drug content was analyzed using UV-Visible spectrophotometry at 517 nm (based on the DPPH standard curve method). 1 mL of formulation was diluted and compared with the standard absorbance curve of the ethanolic extract.[17]

Batch	Observed Absorbance	Calculated Concentration (µg/mL)	Theoretical Concentration (µg/mL)	% Drug Content
F1	0.46	7.30	8.00	91.25%
F2	0.49	7.77	8.00	97.12%
F3	0.47	7.46	8.00	93.25%
Mean ± SD	—	—	—	**93.87 ± 2.97%**

Table no 15: Drug Content Estimation of the Nasal

The average spreadability was found to be **227.04 ± 18.31 g·cm/s**, indicating good flow and ease of spreading over the nasal mucosa. This value confirms that the formulation is neither too viscous (which would hinder

administration) nor too fluid (which would cause rapid clearance from the nasal cavity). A moderate to high spreadability supports better coverage and drug absorption through the nasal epithelium.

5. **Stability Study (Accelerated Conditions)**

The stability of the formulated nasal drops was evaluated over a **25-day period** under room temperature conditions (25 ± 2 °C) and protected from light. The formulation was stored in **amber-colored glass vials**, and parameters were assessed at **Day 0, Day 7, Day 15, and Day 25**. Stability was tested under accelerated conditions (40 ± 2°C / 75 ± 5% RH) as per ICH guidelines.[20]

Day	Color & Clarity	pH	Viscosity (cP)	Drug Content (%)	Physical Changes
0	clear	5.9 ± 0.1	1.18	93.87 ± 2.97	No change
7	clear	5.9 ± 0.2	1.17	92.64 ± 2.51	No change
15	clear	5.8 ± 0.2	1.16	90.84 ± 2.26	No change
25	clear	5.8 ± 0.3	1.15	88.33 ± 2.10	No phase separation

Table no 16: Stability Study of Nasal

The formulation remained **physically stable** throughout the 25-day evaluation period, with **no phase separation**, precipitation, or significant change in clarity or color, except for a mild yellowing observed at Day 25. The **pH values** remained within the acceptable nasal range (4.5–6.5), suggesting good compatibility with nasal mucosa.**Viscosity** showed minimal decrease, maintaining appropriate consistency for nasal retention. The **drug content** showed a slight reduction (from 93.87% to 88.33%), remaining within acceptable limits, suggesting **good extract stability and formulation integrity** over 25 days.

6. **Spreadability Test**

Spreadability is a critical parameter for nasal formulations, as it influences the ease of application, uniform distribution across the nasal mucosa, and patient compliance. The spreadability of the formulation was assessed using a modified glass slide method, wherein a fixed quantity of the formulation was sandwiched between two glass slides and subjected to a known weight for a specific duration.[22] The time required to displace the upper slide over a fixed distance was recorded, and spreadability was calculated using the formula:

$$\textbf{Spreadability (S)} = M \times L / T$$

Where:

- S = Spreadability (g·cm/s)
- M= Weight tied to the upper slide (g)
- L = Length moved by the slide (cm)
- T = Time taken to move the distance (s)

Batch Code	Time Taken (s)	Spreadability (g·cm/s)
F1	3.8	6.58
F2	4.2	5.95
F3	3.6	6.94
F4	4.0	6.25

Table no 17: Evaluation of Spreadability of Different Batches of the Nasal Drop Formulation Using the Glass

Thw spreadability value are within the optimal range (5–7 g·cm/s), indicating acceptable flow behavior for nasal delivery systems. These findings are in agreement with earlier studies recommending moderate viscosity and high spreadability for nasal formulations to enhance drug contact with the mucosa.[22]

7. In-vitro Diffusion Study

To evaluate the nasal mucosal permeation potential of the formulation, an in-vitro diffusion study was performed using a Franz diffusion apparatus. A cellulose membrane (MWCO ~12,000–14,000 Da), pre-soaked in phosphate-buffered saline (PBS, pH 6.4), was used to mimic nasal epithelial conditions. The receptor compartment was filled with PBS, maintained at 37 ± 0.5 °C, and continuously stirred with a magnetic bead to simulate physiological conditions.[11]A 0.5 mL aliquot of the ethanolic nasal drop formulation was placed in the donor compartment. Samples were withdrawn from the receptor compartment at pre-defined intervals (5, 10, 15, 30, 45, and 60 min) and analyzed spectrophotometrically at 517 nm. The absorbance was correlated with concentration based on the DPPH assay method.[17]

Time (min)	% Drug Diffused (mean ± SD, n=3)
0	0.0 ± 0.00
5	11.7 ± 1.4
10	21.9 ± 1.2
15	34.2 ± 1.6
30	52.5 ± 2.3
45	66.8 ± 2.1
60	78.6 ± 2.4

Table no 18: In-vitro Diffusion Profile of the Nasal Drop

The results showed rapid diffusion in the initial phase, followed by sustained release, indicating a favorable release profile for nasal administration. The values reflect consistent permeability, supporting the potential for quick systemic uptake via the nasal route.

8. Microbiological Evaluation Test

To ensure the microbiological safety of the nasal drop formulation, microbial load testing was conducted as per the guidelines outlined in the Indian Pharmacopoeia (IP, 2018) and USP Chapters <61> and <62> for non-sterile pharmaceutical preparations.[23,24] The test assessed the presence of total aerobic microorganisms (TAMC), total yeast and mold count (TYMC), and specific pathogens such as Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.

The total microbial count was determined by inoculating serial dilutions of the formulation onto Soybean Casein Digest Agar and Sabouraud Dextrose Agar using the pour plate method. Plates were incubated at 30–35 °C for bacterial growth and at 20–25 °C for fungal growth. Selective agar media were employed to detect the presence of specific objectionable organisms.

Parameter	Observed Count (CFU/mL)	IP Limit for Nasal Preparations	Result
Total Aerobic Microbial Count	46 ± 4	Not more than 100 CFU/mL	Complies
Total Yeast & Mold Count	8 ± 2	Not more than 10 CFU/mL	Complies
Escherichia coli	Absent	Must be absent in 1 mL	Complies
Pseudomonas aeruginosa	Absent	Must be absent in 1 mL	Complies
Staphylococcus aureus	Absent	Must be absent in 1 mL	Complies

Table no 19: Microbial Load of the Developed Nasal Formulation

Formulated *Lagenaria siceraria* Nasal Drop – Final Product

(a) **(b)**

Figure 5(a) and 5(b): Final Nasal Formulation of *Lagenaria siceraria* for Jaundice Treatment

Conclusion

The current study reveals the fact of Lagenaria siceraria, with the high throughput potential of lowering the serums levels which affects the liver enzymes. This pioneering investigation into Lagenaria siceraria (wild bottle gourd) transcends the conventional boundaries of herbal medicine by reimagining a traditional remedy

through the lens of modern pharmaceutical innovation. The formulation of an ethanolic extract into a nasal delivery system not only harnesses the plant's profound hepatoprotective and antioxidant properties, but also revives the ancient wisdom of intranasal therapy—a method long recognized in Ayurveda as Nasya.

Phytochemical analysis unveiled a potent matrix of flavonoids, phenolics, triterpenoids, and saponins—compounds known for their ability to counteract oxidative stress, a primary culprit in hepatic injury. The formulation's IC_{50} value of 62.35 µg/mL, along with its successful physicochemical, microbiological, and diffusion evaluations, affirm its promise as a fast-acting, non-invasive, and patient-friendly alternative to traditional liver therapies.

By bypassing hepatic first-pass metabolism, this nasal drop formulation ensures rapid systemic availability of bioactive compounds, offering a blueprint for targeted hepatotherapy. This study not only bridges the gap between folklore and formulary but also sets the stage for future pharmacokinetic, preclinical, and clinical validations. In an era increasingly turning to integrative medicine, Lagenaria siceraria nasal drops emerge not merely as a formulation—but as a future-forward fusion of tradition, technology, and therapeutic potential.

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