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PREPARATION AND EVALUATION OF BRYOPHYLLUM PINNATUM LEAF EXTRACT SPRAY FOR TOPICAL DRUG DELIVERY

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ABSTRACT

The present study focuses on the preparation and evaluation of an anti-inflammatory herbal spray using Bryophyllum pinnatum leaf extract for topical drug delivery. The plant is well known for its medicinal properties such as anti-inflammatory, analgesic, antimicrobial, and wound healing activities. Leaves were collected, authenticated, dried, and subjected to ethanolic extraction using the maceration method. The extract was further evaluated through phytochemical screening, confirming the presence of alkaloids, flavonoids, and tannins. The herbal spray was formulated using suitable excipients like Carbopol 940, propylene glycol, glycerin, and preservatives. The prepared formulation was evaluated for physicochemical parameters such as pH, viscosity, drying time, and organoleptic properties, which showed satisfactory results. The pH of the formulation was found to be skin-compatible. In-vitro anti-inflammatory activity was assessed using the protein denaturation method, and antibacterial activity was evaluated against E. coli, showing significant inhibition compared to the control. UV spectroscopic analysis confirmed the presence of active constituents in the formulation. The results indicate that the formulated Bryophyllum pinnatum herbal spray is effective, stable, and suitable for topical application. It offers a natural, safe, and cost-effective alternative to synthetic anti-inflammatory agents, with reduced side effects and good therapeutic potential.

KEYWORDS: Bryophyllum Pinnatum, Herbal spray, Anti-inflammatory activity, Topical drug delivery, Phytochemical screening, Antibacterial activity.

INTRODUCTION

An herbal spray is a natural product composed of plant-derived components such as herbs, essential oils, and botanical extracts. In contrast to chemical sprays, herbal options contain no harmful toxins and are regarded as environmentally friendly, safe for people, and less harmful to the planet. Herbal sprays are commonly utilized for various applications, including pest management, air freshening, therapeutic uses, and skincare. One of the key benefits of herbal sprays is their sustainability and low risk of side effects. They are biodegradable and do not contribute to pollution or leave chemical residues. With a growing awareness of health and environmental concerns, herbal sprays are becoming an increasingly favoured alternative to synthetic products.[1]

Herbal sprays

They use the therapeutic properties of plant-based compounds as a natural alternative to synthetic medicines. Natural medications are growing in popularity because customers are getting more interested in them and think they have fewer side effects.[2]

Need for Herbal Spray

Targeted Relief: Delivers powerful herbal compounds directly to aching muscles, tendons, and joints.

Natural alternatives: People who prefer plant-based therapies over possibly harsh pharmaceutical NSAIDs (Non-steroidal Anti-Inflammatory Drugs) are drawn to natural alternatives.

Reduces Inflammation: Menthol and curcumin (turmeric) are examples of substances that target the root cause of discomfort by suppressing inflammatory mediators.

Pain management: Provides analgesic effects by using heat (capsaicin) or cold (menthol) sensations to distract from pain.

Versatile Use: Good for back pain, bursitis, sprains, strains, arthritis, and general muscle soreness.

Convenient and gentle: Quick-absorbing, non-greasy solutions that are easy to apply and frequently appropriate for sensitive skin. [3]

Plant: Bryophyllum Pinnatum

Bryophyllum calycinum, often known as sprouting leaf, is a member of the crassulaceae family (synonyms: Kalanchoepinnatum, Bryophyllumpinnatum). B. pinnatum is often referred to as or other common name of plant is air plant, life plant, cathedral bell plant, or miracle plant.[4]

It is a succulent plant that reaches a height of one to one and a half meters. It has five bell-shaped flowers, thick, dark green leaves that are trimmed in red and have hollow branches. They are found in the various country with local name are in America ‘Air Plant, Life Plant, Miracle Leaf, Cathedral Bells, In Brazil ‘Coirama, In India Pan-futi, Parnabeeja, Ranapala, In Southern Africa Resurrection Plant, Good Luck Leaf, Sprouting Leaf.[4]

Taxonomic Classification

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta – Vascular plants
Division: Spermatophyta – seed plants
Subdivision: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Rosidae
Order: Rosales
Family: Crassulaceae – stonecrop
Genus: Bryophyllum
Species: Bryophyllumpinnatum (lam.) [4]

Vernacular Names

Sanskrit: Asthibhaksha Parnabeeja
English: Air plant
Kannada: Gandukalinga, Kadubasale
Hindi: Zakhmhaiyat, Pathharchoor
Malayalam: Elamarunga
Tamil: Malaikalli, Ranakalli
Telugu: Ranapala

Marati: Gayamari

Bengali: Koppatha, Patharkuchi. [5]

Fig.No.1: Bryophyllum Pinnatum.

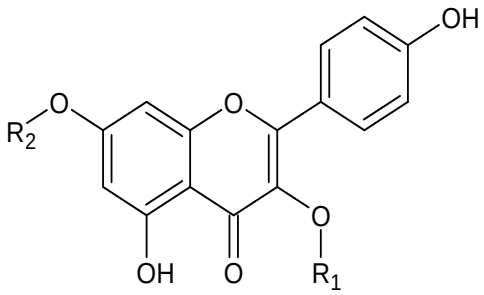
Biological Name

The biological source Bryophyllum Pinnatum (syn. A lush, everlasting plant native to Madagascar, kalanchoe pinnata was first used in anthroposophic medicine around the beginning of the 20th century. It is made out of the fresh leaves of the Bryophllum Pinnatum shrub.[5]

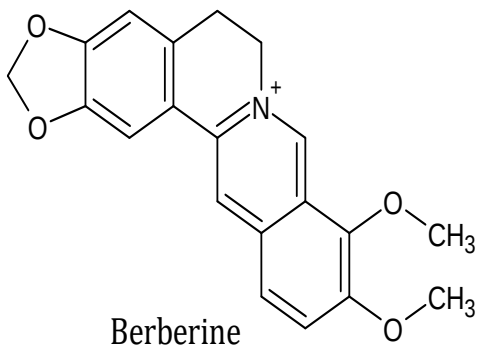
Chemical Constituent

Phytochemical tests revealed that the plant included alkaloids, phenols, flavonoids, tannins, anthocyanins, glycosides, and saponins. Pharmacological research has shown a wide range of pharmacological effects, including anticancer, antioxidant, antibacterial, antiprotozoal, neurological (sedative and anticonvulsant), anti-inflammatory, analgesic, antidiabetic, and wound healing. This review's goal was to offer specific details about Bryophyllum pinnatum's chemical makeup and pharmacological effects.[6]

Alkaloids

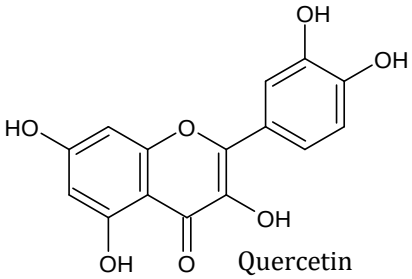


Bryophylline

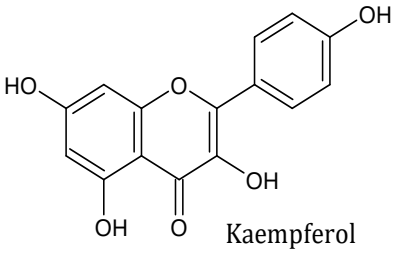


Berberine

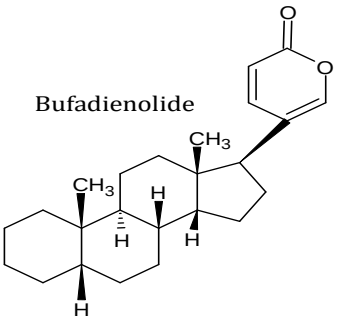
Flavonoids



Glycosides



Phenolic Compounds



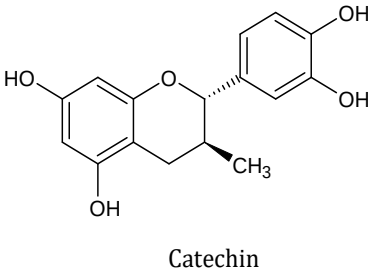


Fig. No.2: Active Chemical Constituents of Bryophyllum Pinnatum.

Active Chemical Constitutes Responsible of Anti-inflammatory Activity

Flavonoids

- Quercetin: A well-known antioxidant and anti-inflammatory compound.
- Kaempferol: An additional flavonoid with anti-inflammatory, antioxidant, and antibacterial properties.

Alkaloids:

- Bryophylline: An alkaloid with potential analgesic and anti-inflammatory effects.
- Berberine: Although it is more commonly found in other plants, some study suggests that Bryophyllum species ma y contain it.

Essential Oils

The plant's scent and medicinal properties, including as its antibacterial and anti-inflammatory properties, are further enhanced by its abundance of various essential oils.[7]

Application-Specific Applications of Bryophyllum Pinnatum

Skin-Related Uses

Wound Healing: The pulp or juice from the leaves is good for wound care since it reduces bleeding and speeds up healing. The hemostatic and wound-repairing qualities of this plant are well known in traditional medicine.

Inflammation: Bryophyllum pinnatum is well-known for its ability to effectively cure ulcers, infections, and inflammation. Gastritis, burns, boils, wounds, and ulcers are just a few of the conditions for which it has long been used both internally and externally.

Skin Disorders: This plant can be used to treat a variety of skin problems, such as burns and boils, because of its antibacterial, anti-inflammatory, antiallergic, and anti-fungal qualities. Additionally, it has antihistamine, antiulcer, and anticancer qualities.

Uses Associated with the Renal System

Kidney Stones: Kidney stones can be treated naturally with this plant. Changes in lifestyle, such as food and industrialization, are associated with the rising incidence of urinary calculi, especially calcium oxalate stones. Bryophyllum pinnatum and other medicinal herbs offer a safer and more potent substitute for manufactured medications. Urinary stone management can be approached holistically by combining conventional and natural therapies.

Reproductive System-Related Applications

Bleeding Disorders: Bryophyllum pinnatum has long been used to treat hemorrhoids, heavy menstrual bleeding (menorrhagia), and bleeding disorders. Its anti-inflammatory, analgesic, and uterine muscle-relaxing qualities are highlighted by ethnomedical research. Its position as a natural therapeutic alternative is supported by preliminary clinical observations that point to its potential for reducing dysmenorrhea.

Applications of the Renal System

Kidney Stones: This plant is a natural remedy for kidney stones. The increasing prevalence of urinary calculi, particularly calcium oxalate stones, is linked to changes in lifestyle, including diet and industrialization. A safer and more effective alternative to pharmaceuticals is provided by Bryophyllum pinnatum and other therapeutic herbs. Combining conventional and alternative therapy is a holistic approach to managing urinary stones.

Applications Associated with the Reproductive System

Bleeding problems: Hemorrhoids, heavy menstrual bleeding (menorrhagia), and bleeding problems have long been treated with bryophyllumpinnatum. Ethnomedical study highlights its anti-inflammatory, analgesic, and uterine muscle-relaxing properties. Preliminary clinical observations that indicate its potential to reduce dysmenorrhea strengthen its position as a natural treatment alternative.[8]

MATERIALS AND METHODS

1. Collection and authenticating Bryophyllumbpinnatum leaf plant material

In January 2026, leaves of Bryophyllum Pinnatum, a member of the Crassilaceae D.C. family, were gathered from Radhanagri Wildlife Sanctuary in Kolhapur district, Maharashtra state, India. The leaves of the plant are classified as Bryophyllum Pinnatum. "The Flora of Kolhapur District" was used to confirm the family Crassilaceae. Dr. J. M. Patil, Associated Professor Head of the Botany Department at Doodhsakhar Mahavidhyalaya in Bidri, Kolhapur, Maharashtra, verified the sample.

2. Sample Drying Procedure

After being dried in the shade, the leaves were ground into a coarse powder. This powder was used for extraction and kept in an airtight container.

3. Using ethanol and the maceration extraction method to extract dried Bryophyllum pinnatum leaves

After thoroughly cleaning the plant parts with tap water, they were allowed to dry in the shade. After being dried, Bryophyllum pinnatum leaves were powdered into a coarse powder. For extraction, the cold maceration process was employed. Ethanol was used to soak powdered plant material, which was then stored at room temperature. After three days, the extracts were vacuum-filtered using Whatman filter paper No. 1. The residue was once more immersed in ethanol for three days prior to filtering. The ethanol was evaporated in a water bath heated to 50°C after the filters were mixed. Until more research could be done, the dried extracts were kept at room temperature.[9]

4. Phytochemical Screening

Examining and identifying the chemical components found in plants, with an emphasis on secondary metabolites, is known as phytochemical screening.

Sample solution: Before being filtered, 100 mg (0.1 gm) of the extract was diluted in 5 ml of distilled water. In the ensuing tests, the filtrate served as a sample solution.

- **Alkaloids**

1. Wagner's test: The extract was dissolved in two milliliters of methanol. A few drops of 1% HCl were added. After that, the mixture was heated, kept in steam, and then cooled. The mixture was then supplemented with a few drops of Wagner's reagent. The sample showed a reddish-brown precipitate.[10]

2. **Tannic acid test:** A drop of tannic acid was combined with the extract. Alkaloids that produced a yellow hue were verified by the development of crystalline solids.

Fig.No.3: Wagner's test.

Fig.No.4: Tannic acid test.

➢ **Tannins**

1. **Ferric test:** Add three drops of ferric reagent after hydrating the sample with water and using filter paper to remove extra water. Tannins are present if the solution turns grey or black.
2. **Bromine water test:** In this qualitative test, the presence of condensed tannins in plant extract solution is determined by adding bromine water.

Fig.No.5: Ferric test.

Fig.No.6: Bromine water test.

➢ **Flavonoids**

1. **Lead Acetate Test:** To check for flavonoids, add a few drops of liquid lead acetate to the plant extract; a yellow precipitate indicates the presence of flavonoids.

2. **NaOH Test:** The sodium hydroxide test is a first step in determining whether a material contains flavonoids. A sample extract is mixed with a few drops of sodium hydroxide, and the color change is monitored.[8]

Fig. No.7: Lead Acetate Test.

Fig. No.8: NaOH Test.

PROCEDURE

Formulation Table

Table No. 1: Formulation Table.

Sr. No.	Ingredients	Quantity
1.	BryophyllumPinnatum	2.5gm
2.	Carbopol 940	0.25gm
3.	Propylene Glycol	1.5ml
4.	Glycerin	1.25ml
5.	Distilled Water	qs
6.	Triethanolamine	qs
7.	Methyl Paraben	0.05gm
8.	Tween 80	0.35ml
9.	Ethanol	2.5ml
10.	Eucalyptus Oil	qs

Method of Preparation[11]

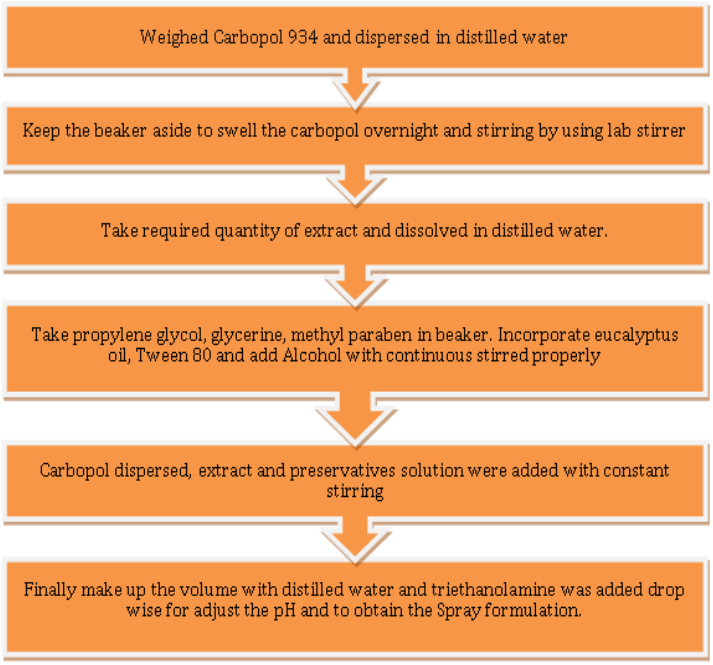


Fig.No.9: Method of Preparation

Evaluation of herbal spray

a. Assessment of herbs used organoleptic evaluation

Color: Depending on the ethanolic extract concentration, the colour was found to be light green by eye inspection.

Odour: Smell spray was used to identify the odour, which is a subtle, strong plant-like scent.

Touch: The spray has a light, watery sensation and is typically an aqueous (water-based) extract.[10]

b. pH

The digital pH meter was used to measure the pH of the optimized spray solution. Phosphate buffer pH was used to modify the pH meter in order to determine the pH of the optimized formulation. The pH of the spray solution was determined.After measuring each formulation three times, the results were computed. The mean values were computed.[10]

c. Viscosity

Place the sample of herbal spray in a beaker to prevent air bubbles. Use an appropriate spindle to set up the Brookfield Viscometer. Keep the temperature at 25°C. Run the spindle at a set speed (such as 50 rpm) while submerged in the sample. After stabilisation, take note of the viscosity reading. Calculate the average after two or three repetitions.[10]

d. Drying Time

The time required for the spray coating to dry is known as the evaporation time. In order to quantify it, the formulation was sprayed on paper, and the drying time was recorded.[10]

Evaluation of container

a. Spray angle

Initially, the paper-to-nozzle distance was set. The size of the circle was then measured after one actuation was sprayed onto paper.

Spray angle is calculated as

Spray angle (O) =tan-1(h/r)

Where, h =paper's distance from the nozzle =15cm and d= diameter of circle =8cm

Radius of circle (r) =d/2=8/2=4cm

1. Identify half angle ratio: R=r/h =4/15=0.2667
2. Calculate half angle: $\tan^{-1}(0.2667) = 14.93°$
3. Determine the total spray angle: 2× 14.93°= 29.86°[10]

b. Spray patterns

A pH-sensitive paper was made by dipping Whatman filter paper in a methyl red solution. This paper was sprayed with the formulation (one actuation). The concentrates were sprayed both vertically and horizontally to evaluate the spray pattern.[10]

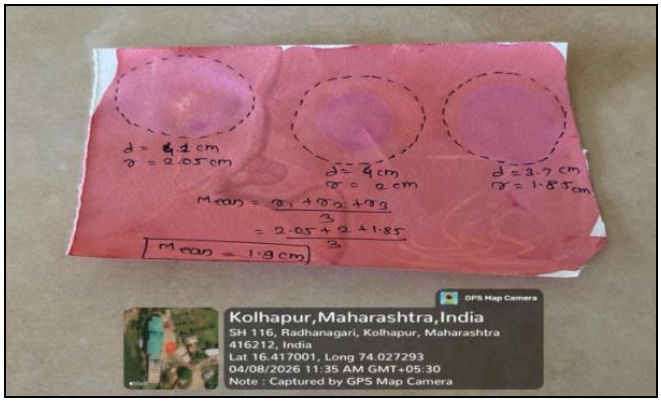


Fig.No.10: Spray Pattern.

c. Leakage test

Leakage of the canisters was verified by passing them at 55°C and monitoring changes in weight in the water bath. Selected samples were tested. Batches of people passed this exam.[10]

Fig. No.11: Leakage Test.

In vitro evaluation

a. Anti-inflammatory Activity

0.4 mL of egg albumin (from a fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4), and 100 µL of a sample with varying concentrations made up the reaction mixture (10 mL). The control was a similar volume of double-distilled water. The mixes were then heated to 700°C for five minutes after being incubated at 370°C ±2 for fifteen minutes.

Following cooling, the vehicle was used as a blank to measure their absorbance at 660 nm. For the purpose of determining absorbance, diclofenac sodium at the concentration was utilized as a reference medication and handled similarly. The following formula was used to determine the percentage inhibition of protein denaturation.[12, 13]

%Inhibition= C–T/ C

Where, T=absorbance of test sample C = absorbance of control

b. Antibacterial activity

The bacterial cultures were used to create the microorganism's inoculum. Clean, sterile Petri dishes were filled with 15 milliliters of nutritional agar (Hi media) medium, which was then left to cool and harden. Using a spreading rod, 100 µl of the bacterial strain's broth was pipetted out and evenly distributed over the medium until it dried completely. A sterile cork borer was used to drill wells with a diameter of 6 mm. Compound solutions (100µl/ml) were made in water, and the wells were filled with 100µl of prepared test solutions (1 mg/ml) and standard. For a whole day, the petri plates were incubated at 370C. DMSO was used as the negative control, and streptomycin (1 mg/ml) was generated as the positive control. The diameters of the zone of inhibitions (ZI) were measured to assess the antibacterial activity.[14,15]

c. UV Spectrum and Absorbance

Weigh a certain amount of dried plant extract. dissolve in an appropriate solvent (such as distilled water or methanol). To get a clear solution, filter To a known concentration, dilute. Measure out the amount of spray composition. Use the same extracting solvent to dilute To get rid of insoluble particles, filter if needed. As a blank, use the solvent (methanol/distilled water). Turn on the UV-visible spectrophotometer. Set the wavelength range to 200–400 nm. Use a blank to calibrate the instrument. Pour the extract solution into the cuvette. Scan between 200 and 400 nm. Note the absorbance and λmax, which is around 280 nm. For spray formulation, follow the same steps. Take note of the highest absorbance (λmax). Examine the spectra of the extract and spray. At around 280 nm, note the absorption values.[16]

RESULT

1. Authentication

The plant is authentication of *Bryophyllum Pinnatum* leaves. Family *Crassilaceae* and confirmed with the help of "The Flora of Kolhapur District''. The sample was authenticated

by Dr. J. M Patil, Associated Professor Head Department of Botany, Doodhsakhar Mahavidhyalaya, Bidri. Kolhapur, Maharashtra.

2. Collection and Identification of Plant Material

From Radhanagari, fresh Bryophyllum Pinnatumleaf was collected. The Botanical Department of Bhogawati Mahavidyalaya, Kurukali. recognized the plant.

3. Drying and Extraction

After being carefully cleaned with tapping water, the plant material was dried and then powdered into a course powder.

4. Phytochemical Screening of Extracts of Bryophyllum Pinnatum Leaf Extract

Ethanolic extract of Bryophyllum Pinnatum leaves was screened for various chemical ltests as per the reported methods and was found to be contains Alkaloids, Flavonoids, and Tannins.

Tab. No. 2: Phytochemical screening.

Sr. No.	Tests	Observations	Inference
1.	Wagner's Test	Reddish brown Color	+
2.	Tannic Acid Test	Yellow Color	+
3.	Ferric Test	Bluish Black Ppt	+
4.	Bromine Water Teat	Discoloration	+
5.	Lead Acetate Test	Yellow PPt	+
6.	NaOH Test	Dark Yellow	+

[**Note:** Positive (+), Negative (-)]

Fig. No.12: Phytochemical Screening.

5. Formulation of herbal spray

Formulation of anti-inflammatory spray by using the Bryophyllum Pinnatum leaves.

Fig. No.13: Herbal Spray.

6. Evaluation of herbal spray

a. Assessment of Herbs in Organoleptic Analysis

Tab.No.3: Organoleptic Analysis.

Sr. No.	Test	Observation
1.	Color	Light Green
2.	Odour	Mildly Pungent
3.	Touch	Light & Watery

b. pH

The optimum spray solution's pH was determined to be 6.10 using the digital pH meter.

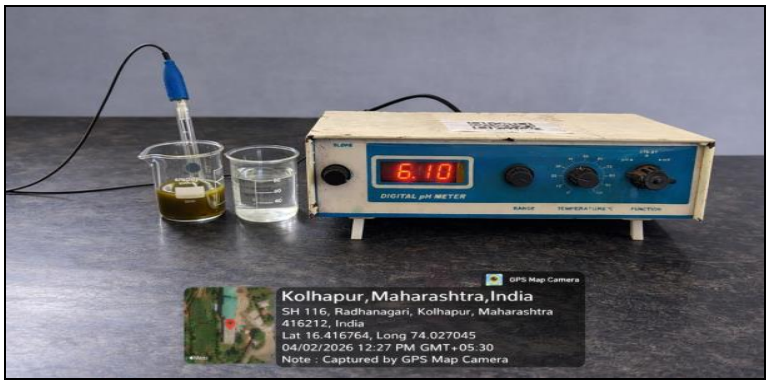


Fig.No.14: Digital pH Meter.

c. Viscosity

Tab.No.4: Measurement of Viscosity.

Number No.	Time (s) ts	Shear Stress(Pa) τPa	Shear Rate (1/s) Ẏ 1/s	Viscosity (Pa s) η Pa s	Temperature (°C) T °C
1.	168.000	3.423	95.118	5.372	28.50
2.	173.000	3.245	93.095	5.367	28.50
3.	175..000	3.108	86.198	5.319	28.50
4.	181.000	2.568	81.065	5.332	28.50
5.	185.000	2.198	78.057	5.331	28.50

d. Drying Time

Evaporation of sample by using drying time of paper was found to be 2min 40 sec.

Fig. No.15: Drying Time.

7. In vitro evaluation of Herbal Spray

a. Anti-inflammatory Activity

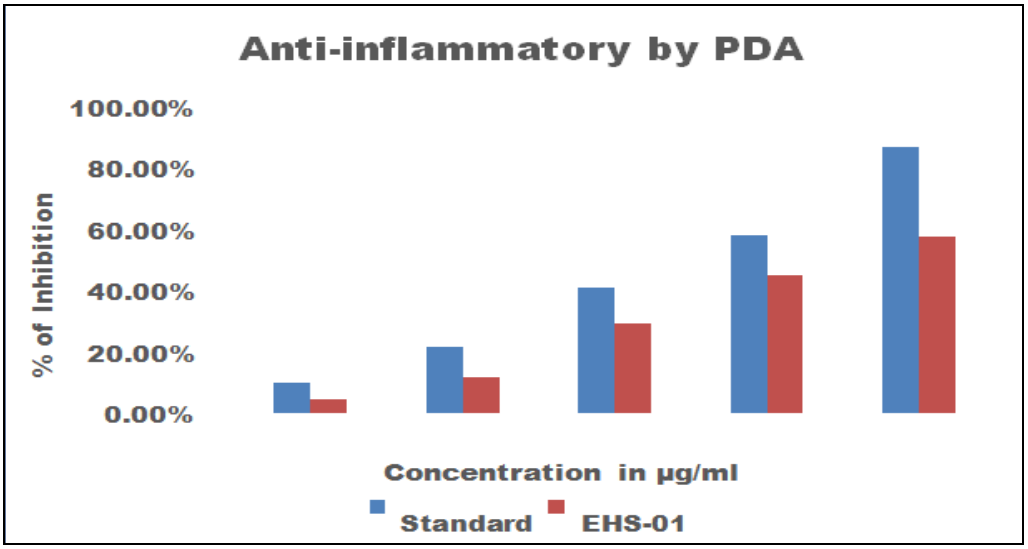


Fig. No.16: Inhibition Graph of Bryophyllum Pinnatum.

Image of the Activity

Fig.No.17: PDA Method with Standard

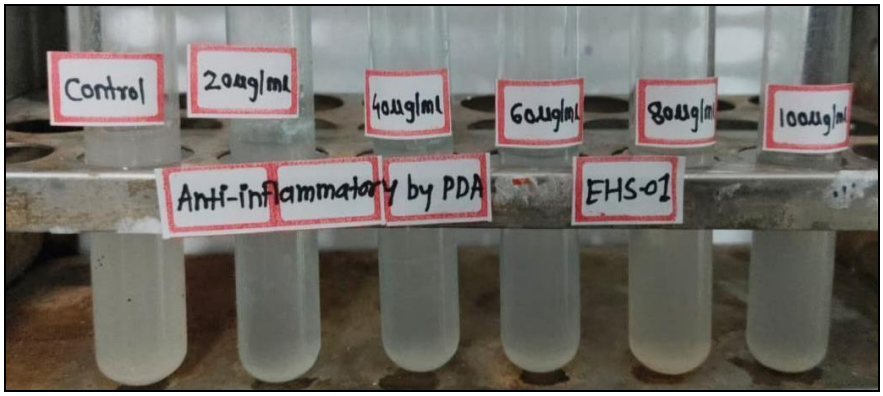


Fig. No.18: PDA Method with EHS-01.

b. Antibacterial activity

Tab. No. 5: Test compound's antibacterial effectiveness against E. coli.

SR.NO	SAMPLES	ZONE IN DIAMETER (mm)
1	Control	00
2	Standard (Streptomycin)	23
3	EHS-01	16

Image Activity

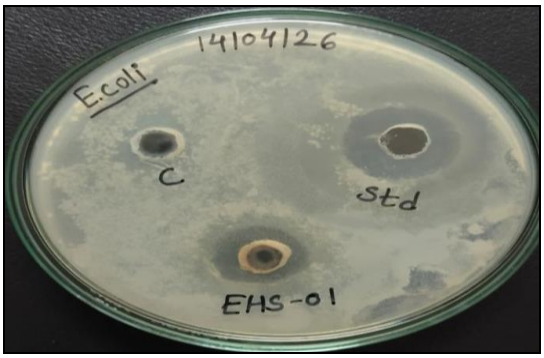


Fig.No.19: Test compound's antibacterial effectiveness against E. coli.

c. UV Spectrum

Extract absorbance is 280nm and spray formulation absorbance is 278-282nm by checking the UV absorbance range was found to be 255nm.

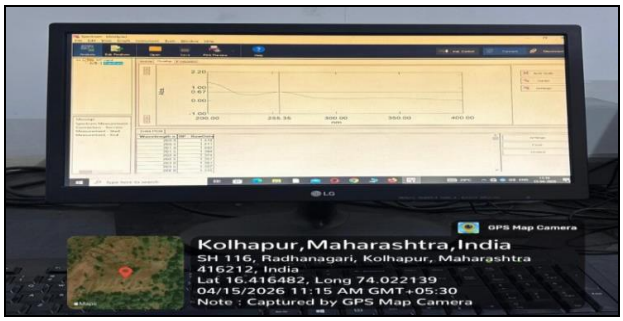


Fig.No.20: UV Spectroscopy.

DISCUSSION

The present study successfully demonstrated the formulation and evaluation of an herbal anti-inflammatory spray using Bryophyllum pinnatum leaf extract. The plant is well known for its rich phytochemical composition, which includes flavonoids, alkaloids, and tannins, all of which contribute to its pharmacological activities. The phytochemical screening results confirmed the presence of these bioactive compounds, supporting earlier reports of its medicinal importance.

The formulation of the spray was carried out using appropriate excipients to achieve desired physicochemical properties such as suitable viscosity, stability, and ease of spraying. The organoleptic evaluation indicated that the spray had a light green color, mild pungent odor, and a smooth watery texture, making it acceptable for topical application. The pH of the formulation (6.10) was found to be within the acceptable range for skin application, ensuring minimal irritation.

Viscosity and drying time are critical parameters for topical spray formulations. The prepared formulation exhibited appropriate viscosity, allowing easy spraying and uniform distribution over the skin surface. The drying time of approximately 2 minutes and 40 seconds indicates good evaporation characteristics, enhancing patient convenience and compliance.

The in-vitro anti-inflammatory activity using the protein denaturation assay revealed that the formulation exhibited significant inhibition in a dose-dependent manner. Although the activity was slightly lower compared to the standard drug diclofenac sodium, the results are promising, indicating the potential of the herbal formulation as a safer alternative. The IC50 value of the extract suggests moderate anti-inflammatory potency.

CONCLUSION

The present study concludes that Bryophyllum pinnatum is a valuable medicinal plant with significant anti-inflammatory and antimicrobial properties. The successful formulation of an herbal spray using its leaf extract demonstrates its potential as an effective topical therapEutic agent. The formulation showed satisfactory physicochemical properties, stability, and ease of application, making it suitable for practical use.

The in-vitro studies confirmed that the herbal spray possesses considerable anti-inflammatory activity, although slightly lower than standard synthetic drugs. However, its natural origin,

reduced side effects, and cost-effectiveness make it a promising alternative for the management of inflammatory conditions. Additionally, the observed antibacterial activity further enhances its therapeutic value.

This study supports the growing demand for herbal formulations and emphasizes the importance of scientific validation of traditional medicinal plants. Future research should focus on in-vivo studies, clinical trials, and long-term stability analysis to further establish the efficacy and safety of the formulation.

ACKNOWLEDGMENT

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