Formulation and Evaluation of Antimicrobial Polyherbal Ointment

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***Abstract*—The present work was aimed to formulate and evaluate a polyherbal ointment with antimicrobial activity. The use of plants for healing purposes predates human history and has gained considerable attention for their applications in both traditional and modern medicine. It is getting popularized owing to its natural origin and lesser side effects. Among various dosage forms, ointments serve as an effective medium for delivering natural drugs topically. Ointment was prepare using ethanolic extracts of Banyan, Peepal, and Brahmi leaves. The extracts were obtained using the maceration method and incorporated into an ointment base via levigation. The prepared formulation was assessed for physicochemical parameters such as colour, odour, pH, spreadability, extrudability, consistency, solubility and washability. Stability studies at different temperatures indicated no significant changes in irritancy, spreadability, or antimicrobial activity. The polyherbal ointment was tested for antimicrobial efficacy against Betadine (5% w/w) as a standard, demonstrating significant activity against selected microbial species. These findings suggest that Banyan, Peepal, and Brahmi can be effectively utilized in a simple topical dosage form, offering a promising natural alternative for medicinal applications. Hence an attempt was made to formulate a polyherbal ointment and evaluate for its physicochemical parameters.**

***Index Terms*—Polyherbal ointment, Banyan, Peepal, Brahmi, antimicrobial activity, topical formulation, herbal medicine.**

I. INTRODUCTION

The practice of herbal medicine is the oldest form of healthcare which has been used for decades in developing and developed countries. Primitive people have depended on nature for food, shelter, clothing and medicine to cure ailments. These human distinguished useful herbs with beneficial effects from those that were inactive or toxic. According to literature approximately 50,000 plant species are stated to have medicinal properties. Plant based drugs awareness advanced gradually and has been passed on, therefore setting a foundation for many traditional medicine systems around the globe. The present study was focused on formulation and evaluation of polyherbal ointment for antimicrobial activity. Three herbal drugs, Ficus benghalensis, Ficus religiosa and Bacopa monnieri have been chosen for the present work. These herbs were selected, on the basis of their traditional usage in treatment for different skin infection. Ficus is a huge tropical, deciduous, evergreen tree, belonging to family Moraceae, with more than 800 species. Ficus species are rich source of polyphenolic compounds, flavonoid and tannins which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases.

It has been traditionally claimed that the whole plant (especially leaves) of Ficus religiosa and Ficus benghalensis are useful in wound healing. Moreover, the leaves of the plant are also reported to have anti-inflammatory, antioxidant, and antimicrobial properties.Brahmi (Bacopa monnieri) or water hyssop is a perennial, creeping herb whose habitat includes wetlands and muddy shores. It is a great immunostimulant, tranquilizing, mind pacifying, and neuroleptic, psychotropic herb with great action on Nervous system- CNS depressant or sedative and anaesthetic. Besides it has antiviral, anti-bacterial and antifungal effect. It has been also cited that Brahmi extracts are having anti-oxidant property. Brahmi

extract has been used as a green corrosion inhibitor. The biologically active phytochemical present in the Brahmi, are responsible for all of the above properties.

Plant Profile

1.1Ficus benghalensis [5,6]

Table 1. Plant Profile of Ficus benghalensis

Sr.No.	Parameter	Ficus benghalensis
1.	Synonym	Banyan tree, Indian fig, Ficus indica
2.	Biological Source	Leaves, bark and latex of the tree
3.	Family	Moraceae
4.	Appearance	
5.	Distribution	Widely distributed across India, Bangladesh, and parts Of Southeast Asia; often found in tropical and subtropical climates.
6.	Growth Habitat	Grows as a large tree with a broad canopy; can spread over a large area due to its aerial roots.
7.	Flowering Season	Typically flowers year-round, with peak flowering in the monsoon season
8.	Cultural Significance	Considered sacred in Hinduism and Buddhism; often associated with longevity and immortality.
9.	Conservation Status	Not currently endangered, but habitat loss can threaten local populations.
10.	Phytochemical Constituents	Flavonoids, Tannins and Terpenoids
11.	Uses	Used in traditional medicine for treating respiratory ailments, digestive issues and as an anti-inflammatory agent.

1.2 Ficus religiosa [5,7]

Table 2 Plant Profile of Ficus religiosa

Sr. No.	Parameter	Ficus religiosa
1.	Synonym	Peepal tree, Sacred fig, Bodhi tree
2.	Biological Source	Leaves, bark and latex of the tree
3.	Family	Moraceae
4.	Appearance	
5.	Distribution	Native to the Indian subcontinent and widely distributed in tropical and subtropical regions, particularly in India, Nepal and Bangladesh.
6.	Growth Habitat	A large deciduous tree that can reach heights of 30 meters or more, with a wide canopy and heart shaped leaves.
7.	Flowering Season	Typically flowers year-round, with peak flowering in the spring to early summer season

8.	Cultural Significance	Sacred in Hinduism and Buddhism; associated with enlightenment and mediation, as Siddhartha Gautama is said to have attained enlightenment under a Ficus religiosa tree.
9.	Conservation Status	Not currently endangered, but local populations can be affected by urbanization and deforestation.
10.	Phytochemical Constituents	Flavonoids, Alkaloids and Terpenoids
11.	Uses	Used in traditional medicine for treating respiratory ailments, such as respiratory issues and skin diseases.

1.3 Bacopa monnieri [8,9]

Table 3 Plant Profile of Bacopa monnieri

Sr. No.	Parameter	Bacopa monnieri
1.	Synonym	Brahmi, Water hyssop
2.	Biological Source	Leaves and stems of the plant
3.	Family	Plantaginaceae
4.	Appearance	
5.	Distribution	Widely distributed in tropical and subtropical regions. Commonly found in India, Nepal, China and other parts of Southeast Asia.
6.	Growth Habitat	Typically thrives in wet, marshy areas, often found along the edges of ponds, streams and wetlands.
7.	Flowering Season	Generally, occurs during the summer months
8.	Cultural Significance	In Indian culture, it is regarded as a sacred herb and is often associated with spiritual practices.
9.	Conservation Status	Not currently endangered, but habitat destruction and pollution can pose risks to its natural populations.
10.	Phytochemical Constituents	Bacosides, Flavonoids, Alkaloids and Saponins
11.	Uses	Used in traditional medicine to enhance mental health, improve memory, reduce anxiety and boost cognitive function.

II. MATERIALS AND METHOD

2.1 Ingredients

Table 4. List of Ingredients

Sr. No.	Name of Ingredients	Use of Ingredients
1.	Banyan leaf powder	Antimicrobial agent
2.	Peepal leaf powder	Antimicrobial agent
3.	Brahmi leaf powder	Antimicrobial agent
4.	Wool fat	Emollient and moisturizer
5.	Cetostearyl alcohol	Emulsifying agent
6.	Hard paraffin	Emollient and thickening agent
7.	Yellow soft paraffin	Emollient and moisturizer
8.	Methyl paraben	Preservative

2.2 Formulation of Polyherbal Ointment

2.2.1 Preparation of Extract [10,12]

ALCOHOL MACERATION METHOD

Weigh 20 gm of Banyan, Peepal and Brahmi leaf powder individually in separate containers

↓

Add 200 ml of ethanol in each container and stir the mixture gently to mix well

↓

Cover the containers to prevent evaporation

↓

Let the mixture sit undistributed for 48 hours at room temperature

↓

After 48 hours, filter the mixture using filter paper or cheesecloth

↓

Then filtrate is evaporated using a rotary evaporator

Figure.1 Banyan, Brahmi and Peepal Leaf Powder

Figure.2 Alcohol Maceration Method

Figure.3 Extracts

2.2.2 Ointment Base A. Wool Fat

Lanolin is a yellow, waxy substance obtained from sheep's wool. It is widely used as an emollient, cosmetic ingredient, and pharmaceutical aid. According to the U.S. Code of Federal Regulations (CFR), lanolin in concentrations ranging from 12% to 50% may be included in over-the-counter (OTC) skin ointments. Lanolin is used as a skin protectant to relieve dryness, irritation, and minor skin conditions such as diaper dermatitis (diaper rash).It also helps treat cracked, dry, and irritated skin, as well as minor burns. Additionally, lanolin is commonly used to prevent and treat sore nipples during breastfeeding and to protect chafed or irritated skin caused by various factors. Lanolin functions as an emollient, meaning it softens and hydrates the skin. It works by forming an occlusive oil film over the stratum corneum (the outermost layer of the epidermis), which reduces transepidermal water loss (TEWL) and helps retain moisture [17,18]

Figure.4 Wool Fat

B. Cetostearyl Alcohol

Cetostearyl alcohol is a white, waxy solid that typically appears in the form of flakes. It is oil- soluble but insoluble in water. In the pharmaceutical and cosmetics industry, cetostearyl alcohol functions as an emulsion stabilizer, opacifying agent, surfactant (foam booster), and viscosity-increasing agent. It is commonly used in creams, lotions, and other topical formulations to enhance texture and stability. Cetostearyl alcohol has a melting point of approximately 50°C (122°F) [19,20]

Figure.5 Cetostearyl Alcohol

C. Hard Paraffin

Paraffin wax is a mixture of solid hydrocarbons with high molecular weight, derived from crude petroleum. It is characterized by relatively large crystals and is insoluble in water and alcohol. However, it is sparingly soluble in benzene, turpentine, chloroform, ether, carbon disulfide, and certain oils. Additionally, paraffin wax is miscible with fats. Paraffin wax is ideally suited for use in cosmetic formulations, including creams, ointments, balms, and various lip and foot care products. In ointments, paraffin wax acts as a base and consistency enhancer, providing a smooth texture, improving stability, and forming a protective barrier on the skin to help retain moisture. It is also used in pharmaceutical formulations and plays a key role in the manufacture of petroleum jelly. Paraffin wax is solid at room temperature and has a melting point starting at approximately 37°C (99°F), depending on its composition and purity [21,22]

Figure.6 Hard Paraffin

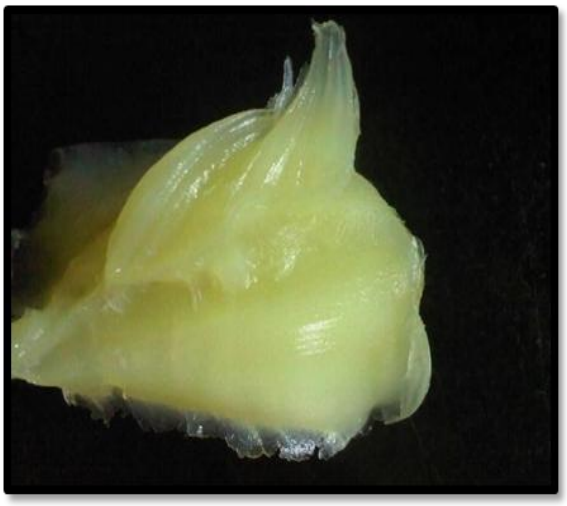

Figure.7 Yellow Soft Paraffin

D. Yellow Soft Paraffin

Yellow soft paraffin, also known as yellow petroleum jelly, is not an active ingredient but functions as an occlusive moisturizer. It forms a protective oil layer on the skin's surface, reducing transepidermal water loss (TEWL) and helping to retain moisture. Due to its high greasiness, it is particularly effective for dry, chapped, or irritated skin [23]

Table 5. Formulation of Ointment Base

Sr. No.	Name of Ingredients	Taken Quantity
1.	Wool fat	1 gm
2.	Cetostearyl alcohol	1 gm
3.	Hard paraffin	1 gm
4.	Yellow soft paraffin	17 gm

Table 6. Formulation of Polyherbal Ointment

Sr. No.	Name of Ingredients	F1 (gm)	F2 (gm)	F3 (gm)
1.	Banyan leaf extract	0.5	1	2
2.	Peepal leaf extract	0.5	1	2
3.	Brahmi leaf extract	0.5	1	2
4.	Methyl paraben	0.05	0.05	0.05
5.	Ointment base up to…	20	20	20

2.2.3 Procedure

The ointment base was prepared by accurately weighing and grating hard paraffin, which was placed in an evaporating dish over a water bath. After the hard paraffin melted, the remaining ingredients were added in sequence while stirring gently to facilitate uniform melting and mixing. Stirring continued until a homogeneous mixture was obtained. The ointment base was then cooled gradually to solidify properly. The polyherbal ointment was prepared by accurately weighing the extracts of Banyan (Ficus benghalensis), Peepal (Ficus religiosa), and Brahmi (Bacopa monnieri). These extracts were then mixed with the ointment base using the levigation method, gradually incorporating the base until a smooth, homogeneous ointment was formed. The prepared ointment was finally transferred to a suitable container for storage.

Figure.8 Polyherbal Ointment

III. EVALUATION OF POLYHERBAL OINTMENT [24,25]

3.1 Colour and Odour

The physical parameters, such as colour and odour, were examined through visual and sensory evaluation.

3.2 Consistency

The consistency of the ointment was evaluated by applying a small amount to the skin.

3.3 pH

The pH of the prepared herbal ointment was measured using a digital pH meter. A solution of the ointment was prepared by dissolving a small amount of the ointment in 100 mL of distilled water and allowing it to stand for 2 hours. The pH was determined in triplicate, and the average value was calculated.

3.4 Spreadability

The spreadability of the ointment was determined by placing an excess amount of the sample between two glass slides. A definite weight was applied to compress the ointment to a uniform thickness for a specified time. The time required to separate the two slides was measured, with a shorter time indicating better spreadability.

Spreadability was calculated using the following formula:

$S = (M \times L) / T$

Where:

- S = Spreadability
- M = Weight applied to the upper slide
- L = Length of the glass slide
- T = Time taken to separate the slides

3.5 Extrudability

The formulation was filled into a collapsible tube container. The extrudability was determined by measuring the weight of ointment required to extrude a 0.5 cm ribbon of the ointment in 10 seconds.

3.6 LOD

The loss on drying (LOD) was determined by placing the formulation in a petri dish and drying it in a water bath at 105°C.

3.7 Solubility

The solubility of the ointment was determined by placing a small amount of the formulation in a suitable solvent and observing its dissolution over time.

3.8 Washability

The washability of the ointment was determined by applying a small amount of the formulation to the skin or a suitable surface and then washing it off with water. The ease of removal and any residual ointment left behind were observed and recorded.

3.9 Non-Irritancy Test

The herbal ointment was applied to the skin of a human volunteer and monitored for any effects, including signs of irritation or allergic reactions, over a specified period.

3.10 Stability Study

The physical stability of the herbal ointment was evaluated over a period of four weeks under various temperature conditions, including 2°C, 25°C, and 37°C. The ointment was periodically observed for changes in color, odor, consistency, pH, and any signs of separation or degradation. The results were recorded to assess the formulation's stability over time.

3.11 Antimicrobial Activity

A required quantity of nutrient agar was prepared, and microorganisms were inoculated into it. The inoculated nutrient agar solution was poured into petri dishes and allowed to stand for a few minutes to solidify. After solidification, bores of the required size were made using a sterile borer. The prepared ointments of different concentrations were then carefully filled into the bores. The entire procedure was performed in an aseptic laminar airflow chamber. The petri plates were then placed in an incubator and allowed to incubate for 24 hours to enable microbial growth. After 24 hours, the zones of inhibition were measured to evaluate the antimicrobial activity of the prepared ointment.

IV. RESULTS & DISCUSSION

The present study was conducted to formulate and evaluate the herbal ointment. Herbal extracts were prepared using the simple maceration process to obtain

a good yield while preserving the chemical constituents and their biological activity. The ointment was prepared using the levigation method to ensure uniform mixing of the herbal extract with the ointment base, resulting in a stable formulation during storage. The physicochemical properties of the ointment were evaluated, showing satisfactory results for spreadability, extrudability, washability, solubility, loss on drying, and other parameters. The formulation was subjected to a stability study under different temperature conditions (2°C, 25°C, and 37°C) for four weeks. No significant changes were observed in spreadability or irritant effects. The formulations were then evaluated for their physical parameters and compared with the marketed 5% w/w Betadine ointment for antimicrobial activity. The physical parameters of the formulations were found to be within the acceptable range.

The antimicrobial activity of the prepared ointments was compared with the 5% w/w Betadine ointment using selected microorganisms such as Staphylococcus aureus and Escherichia coli. The results showed that formulation F2 exhibited greater activity against both Staphylococcus aureus and Escherichia coli compared to the 5% Betadine. Therefore, the antimicrobial study indicates that the prepared ointments have superior activity against Staphylococcus aureus and Escherichia coli compared to the standard 5% Betadine ointment.

Table 7. Evaluation of Polyherbal Ointment

Sr. No.	Evaluation Parameters	Observation F1	Observation F2	Observation F3
1.	Color	Brownish green	Brownish green	Brownish green
2.	Odor	Mild Aromatic	Pleasantly Aromatic	Strong Aromatic
3.	Consistency	Smooth	Smooth	Greasy
4.	pH	5.8	6.5	6.0
5.	Spreadability	8.5 g.cm/sec	12.3 g.cm/sec	9.0 g.cm/sec
6.	Extrudability	0.3 gm	0.5 gm	0.4 gm
7.	LOD	22%	12%	18%
8.	Solubility	Slightly soluble in boiling water, miscible with alcohol, ether, chloroform	Slightly soluble in boiling water, miscible with alcohol, ether, chloroform	Slightly soluble in boiling water, miscible with alcohol, ether, chloroform
9.	Washability	Good	Good	Moderate
10.	Non irritancy	Non irritant	Non irritant	Non irritant
11.	Stability	Stable	Stable	Not stable

Figure 9. Labelled Polyherbal Ointment

Figure 10. Before using ointment

Figure 11. While using ointment

Figure 12. After washing

Figure 13. Colour of Ointment

Figure 14. LOD for Polyherbal Ointment

Table 8. Antimicrobial Study

Bacteria Name	Test Sample (zone of inhibition) (F2)	Standard (zone of inhibition) (Betadine 5% w/w)
Staphylococcus aureus	4.5 cm	2.5 cm
Escherichia coli	4.5 cm	2.5 cm

Figure 15. Escherichia coli

Figure 16. Staphylococcus aureus

V. CONCLUSION

The study aimed to formulate an antimicrobial polyherbal ointment using locally available plants. Ethanolic extracts of three selected plants were incorporated in an optimal ratio into a suitable base. The final formulation exhibited good spreadability, caused no irritation, demonstrated antimicrobial activity against Staphylococcus aureus and Escherichia coli, and remained stable under different temperature conditions. Thus, the formulated antimicrobial ointment showed the evaluation parameters within the acceptable range
and recommended for further pharmacological evaluations in future for further research.

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