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Formulation and Evaluation of a Herbal Ointment from Hibiscus, Mimosa pudica, and Eucalyptus Extracts

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Abstract

Bacterial infections of skin may seriously decrease the quality of life and even cause death in some patients. A major concern in their treatment is the increasing antimicrobial resistance of bacterial pathogens and the spread of resistant strains, both within hospitals and in the community. This study focuses on the formulation of a herbal ointment derived from selected medicinal plant extracts known for their antimicrobial, anti-inflamatory and wound healing properties. The plant materials were subjected to solvent extraction, and the resultant phytoconstituents were characterized using standard phytochemical screening protocols. These extracts were subsequently incorporated into a semi-solid base to formulate a stable herbal ointment. The formulation was evaluated for key physicochemical parameters including pH, viscosity, spreadability, and homogeneity. In vitro antimicrobial assays were conducted against clinically relevant skin pathogens to assess the bioefficacy of the ointment. Preliminary results revealed significant inhibitory activity, suggesting the potential of the formulation as a viable alternative for dermal applications, particularly in the management of wounds and cutaneous infections. This study underscores the therapeutic promise of medicinal plant-based topical preparations and advocates their integration into contemporary dermatological pharmacotherapy.

Keywords: Therpeutic , Medicinal plants , Anti-inflamatory , Dermatological , Antibacterial

Introduction

Antibacterial activity denotes the ability to suppress the growth of or eliminate bacterial organisms. Various antibiotics and chemotherapeutic agents are employed in treating different forms of diseases [1]. Herbal drugs are also formulated into ointments, alongside other pharmaceutical dosage forms. An ointment is a viscous, semi-solid preparation designed for topical application on various body surfaces. Previous studies have demonstrated that medicinal plants are highly effective in wound care, enhancing the wound healing process while minimizing pain, discomfort, and scarring for the patient [2]. The rise in multi-drug resistance among various bacterial species has been attributed to the improper or excessive use of antimicrobials [3]. This phenomenon increases hospitalization periods, thereby escalating patient care expenses. Across the globe, medicinal plants have been an integral part of traditional practices since ancient times, used in different ways [4]. The current global imperative for scientists is to tackle the challenge of discovering novel sources of efficacious antimicrobial agents or to design and synthesize them through advanced pharmaceutical and biotechnological approaches. Throughout history, medicinal plants have served as a vital source of secondary metabolites that possess noteworthy pharmacological properties. Ethnomedicinal knowledge, deeply embedded in the traditional healthcare systems of diverse civilization, has long relied on natural products. In contemporary drug discovery and development, medicinal plants remain an indispensable reservoir for the identification and optimization of lead compounds with therapeutic applications [5].

The utilization of natural products for therapeutic applications present significant potential, particularly in the management of dermatological and wound infections, owing to the direct accessibility of these affected sites for localized drug delivery. The skin serving as the body's primary protective barrier, also plays a crucial role in sensory perception, secretion and thermoregulation, making it the largest and most functionally diverse organ of the human body [6]. A medication is dissolved, suspended, or emulsified in the basis of medicated ointments. Ointments are applied topically for a variety of reasons, such as astringents, keratolytics, antiseptics, emollients, protectants, and antipruritics[7]. Hydrocarbon, absorption, water-soluble, and water-removable bases are all possible for ointment. Because of its accessibility, broad surface area, extensive exposure to the lymphatic and circulatory networks, and non-invasive nature, medication delivery through the skin has long been an intriguing idea [8].

Materials & Methods

3.1 (A) Sample collection:

Table 1: Collection of sample

S. No.	Sample name	Scientific name	Location
a.	*Hibiscus*	*Hibiscus rosa-sinesis*	Lane no. 1, Hemkunj colony, Vijay park, Dehradun, Uttarakhand
b.	*Eucalyptus*	*Eucalyptus globulus*	FRI, Dehradun, Uttarakhand
c.	*Neem*	*Azadirachta indica*	Lane no. 1, Hemkunj colony, Vijay park, Dehradun

d.	*Touch-me-not*	*Mimosa pudica*	FRI, Dehradun, Uttarakhand

3.1(B) Preparation of extracts:

The collected plant materials underwent a rigorous cleaning process to eliminate contaminants and ensure the integrity of subsequent extractions. Leaves of *Eucalyptus globulus*, and *Mimosa pudica*, as well as petals of *Hibiscus rosa-sinensis*, were carefully selected and subjected to processing. The plant materials were manually segmented into small, uniform pieces to facilitate homogenization, which was performed using a mortar and pestle under aseptic conditions to minimize contamination and ensure consistency in extraction efficiency.

Extractions were carried out using solvent systems optimized for specific plant matrices. Aqueous homogenates of *Mimosa pudica*, *Eucalyptus globulus* leaves were dissolved in a 50% acetone solution to maximize the recovery of bioactive compounds. Similarly, *Hibiscus rosa-sinensis* petals were dissolved in 50% acetone.

Fig. No. 1 Prepared extracts *(Eucalyptus, Mimosa, Hibiscus*)

The prepared suspensions were transferred to sterilized containers and incubated in an orbital incubator shaker to enhance the extraction process through continuous agitation, which facilitates the diffusion of phytochemicals into the solvent system. Upon completion of incubation, the samples were transferred to sealed storage containers and maintained at 37°C in incubator to preserve their biochemical stability and prevent degradation of thermolabile compounds. These extracts were reserved for subsequent analyses and experimental applications.

3.2(C) Determination of phytochemical constituents:

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [9, 10].

This can be derived from barks, leaves, flowers, roots, fruits, seeds [11]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [12, 13, 14].

Table 2: Summarization of phytochemical test

S. No.	Phytochemical	*Hibiscus*	*Eucalyptus*	*Mimosa pudica*
1.	Saponin	-	+	+
2.	Tanin	+	+	+
3.	Lead acetate	+	+	+
4.	Flavanoid	+	+	+
5.	Alkaloid	+	+	+
6.	Terpenoid	+	+	+
7.	Mayer's Reagent	+	-	+
8.	Dragendroff's Reagent	+	+	-
9.	Ammonia	+	+	-
10.	Carbohydrate	+	+	+

3.1(D) Formulation of ointments:

Staphylococcus aureus and *Escherichia coli* are the main pathogen that causes these skin infections. Topical ointment containing antibacterial properties can be used in the treatment and prevention of this kind of bacteria causes the infection. Most of the available topical drugs used to treat skin-related diseases in the market are obtained by various synthetic processes which are using chemicals and have some kinds of side effect. [15,16]. *Klebsiella* associated skin and soft tissue infections are associated with a wide range of severity that might cause life-threatening necrotizing fasciitis with bacteremia and distant abscesses [17].

After the completion of the extract preparation process and the subsequent phytochemical analysis to identify the active constituents, the formulation of the ointment is carried out for further evaluation.

Measurement of pH:

Post – formulation of the topical ointment incorporating *Mimosa pudica*, *Eucalyptus globulus, Hibiscus rosa-sinensis* extracts, the pH evaluation was conducted using a pH meter under controlled conditions. The pH values were observed to be in the range of 6.0 tp 6.5 indicating a slightly acidic to natural pH, which is optimal for maintaining the physiological pH of the skin, thereby dermal compatibility and minimizing the risk of skin irritation upon application.

Viscosity: The viscosity of the topical ointment containing *Mimosa pudica*, *Eucalyptus globulus, Hibiscus rosa-sinensis* extracts was evaluated using a Brookfield viscometer under controlled shear conditions. Viscosity, a critical rheological parameter, determines the formulation's internal friction, structural integrity, and stability, preventing phase separation and ensuring uniform drug dispersion. The measured viscosity reflects the molecular interactions between bioactive constituents and excipients, influencing drug release kinetics and skin retention. An optimal viscosity range ensures formulation homogeneity, prolonged stability, and controlled therapeutic action upon topical application.

Spreadibility: The spreadability of the topical ointment containing Mimosa pudica, Eucalyptus globulus Azadirachta indica (Neem), Hibiscus rosa-sinensis extracts was assessed using a standardized slip and drag method under controlled conditions. Spreadability is a crucial rheological property that influences the ease of application, uniform distribution, and user compliance. The measured values indicate the interfacial interactions between the ointment base and the skin, ensuring optimal coverage without excessive drag or fluidity. Proper spreadability enhances bioavailability, therapeutic efficacy, and patient adherence while maintaining formulation consistency and stability.

3.2(A) Isolation of bacteria:

The isolation of *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* was executed from dermatological infection specimens to support the formulation of a targeted antimicrobial ointment. Cutaneous swab samples were aseptically collected from patients with localized bacterial skin infections using sterile cotton swabs and promptly transported in sterile transport media (e.g., Amies or Stuart's medium) to the microbiology laboratory within 1 hour to preserve microbial viability. Samples were inoculated onto selective and differential culture media under aseptic conditions using the quadrant streaking method and incubated aerobically at 37 ± 0.5°C for 24–48 hours. Post-incubation, distinct colonies were assessed based on morphological characteristics, including colony shape, size, margin, elevation, pigmentation, and opacity.

Suspected colonies of *E. coli, K. pneumoniae,* and *S. aureus* were subcultured onto fresh nutrient agar plates to achieve pure isolates. Single, well-isolated colonies were further streaked to ensure clonal purity, providing axenic cultures for subsequent biochemical characterization, antimicrobial susceptibility testing, and formulation assays essential for the development of targeted topical therapeutics.

3.2(B) Characterization of bacteria:

Biochemical characterization of the isolated bacterial strains was carried out to identify their metabolic and enzymatic properties. All the test were conducted under aseptic conditions, and results were recorded after incubation at optimal temp. for 24-48 hrs.

Table 3: Summarization of biochemical test

S. No.	Biochemical test	*E.coli*	*Klebseilla*	*S.aureus*
1.	Sucrose	+	+	+
2.	Dextrose	+	+	+
3.	Maltose	+	+	+
4.	D-manitol	+	+	+
5.	Indole	+	+	+
6.	Citrate	+	+	+
7.	Nitrate	-	-	-
8.	Urease	+	+	+
9.	H_2S	+	+	+
10.	Catalase	+	-	-
11.	Protease	-	-	+
12.	MR	+	+	+
13.	MR-VP	+	+	+

3.3 Antimicrobial evaluation using the disc diffusion method

The antimicrobial activity of ointments preapared from the plant extracts derived from *Hibiscus rosa-sinensis, Eucalyptus globulus,* and *Mimosa pudica* was evaluated against three bacterial pathogens: *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus*, using the standard disc diffusion assay. Sterile 6 mm filter paper discs were impregnated with 20 µL of each plant extract at a concentration of 50 mg/mL and allowed to dry. The impregnated discs were placed on the surface of the inoculated NAM plates using sterile forceps, ensuring even spacing. Positive control discs containing standard antibiotics (e.g., ciprofloxacin) and negative control discs (solvent only) were also included for comparison. The plates were incubated at 37°C for 24 hours under aerobic conditions. Following incubation, the diameter of the zones of inhibition (ZOI) around each disc was measured in millimeters using a digital caliper.

Fig. no. 2 Antimicrobial evaluation of ointments against different pathogens

Discussion & Conclusion

The present study formulated a polyherbal ointment using Mimosa pudica leaves, Eucalyptus globulus leaves, and Hibiscus rosa-sinensis petals, targeting antimicrobial activity and wound healing potential. The formulation exhibited notable antibacterial efficacy and physicochemical stability, indicating its relevance in dermal therapeutic applications.

The individual efficacy of the ingredients is corroborated by recent literature. Dawa et al. (2025) demonstrated that Eucalyptus globulus oil, when incorporated into nano-chitosan/cellulose acetate membranes, exhibited potent antimicrobial properties and enhanced wound healing in vivo, highlighting its bioactive potential in topical applications.[18]

In a pharmacological evaluation, Deka and Borthakur (2025) confirmed the presence of key phytoconstituents in Mimosa pudica responsible for antibacterial and wound healing effects, including flavonoids and tannins, validating its traditional use.[19]

Similarly, Singh et al. (2025) showed that ethanolic extracts of Hibiscus rosa-sinensis significantly accelerated wound contraction and re-epithelialization in burn models, indicating strong regenerative properties.[20]

The combined use of these botanicals appears to enhance antimicrobial performance, likely due to synergistic interactions between secondary metabolites such as polyphenols, flavonoids, and essential oils. This positions the formulation as a viable candidate for future phytopharmaceutical development. However, further preclinical and clinical validation is essential to confirm safety, bioavailability, and therapeutic efficacy in human subjects.

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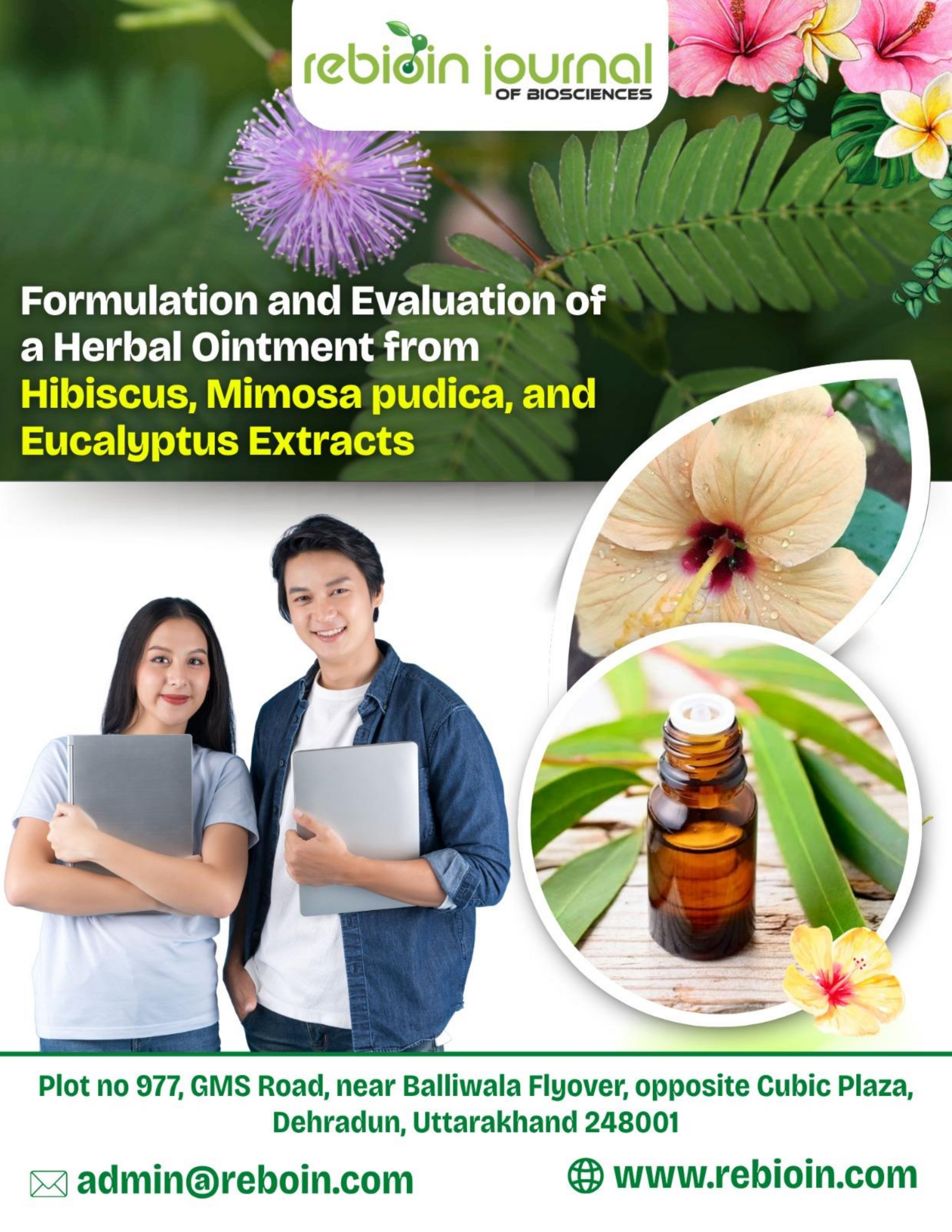

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