Phytochemical Characterization and Evaluation of Antibacterial, Antifungal, and Anti-inflammatory Activities of *Lobelia inflata* in a Topical Herbal Formulation.

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Abstract

The increasing prevalence of microbial infections and inflammatory skin disorders, along with growing antibiotic resistance, has prompted the search for effective plant-based therapeutic alternatives. *Lobelia inflata*, traditionally used for respiratory and inflammatory conditions, remains underexplored for topical applications. This study aimed to investigate the phytochemical profile and evaluate the antimicrobial, antifungal, and anti-inflammatory activities of *Lobelia inflata* extract, followed by the development of herbal ointment formulations. Phytochemical screening confirmed the presence of key bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and steroids. The extract exhibited significant antibacterial activity, particularly against *Staphylococcus aureus* (18.4 ± 0.5 mm) and *Bacillus subtilis* (17.2 ± 0.6 mm), as well as antifungal efficacy against *Candida albicans* (15.3 ± 0.5 mm). Anti-inflammatory activity, assessed via the protein denaturation assay, showed a dose-dependent inhibition with 79.8 ± 1.1% at 500 µg/mL, comparable to the standard drug diclofenac. Among four formulated ointments (F1–F4), formulation F4 demonstrated the highest spreadability (6.8 ± 0.3 g·cm/s), optimal pH (6.6 ± 0.1), and physical stability, making it the most suitable for topical application. The findings validate the traditional claims of *Lobelia inflata* and support its potential in developing effective herbal formulations for skin infections and inflammation. Further in vivo and clinical investigations are recommended to establish its therapeutic efficacy and safety profile.

Keywords: Lobelia inflata, antibacterial, antifungal, characterization.

1. Introduction

Medicinal plants have been extensively used for centuries in traditional healthcare systems and continue to serve as a rich source of bioactive compounds for modern drug discovery. Among the diverse flora with therapeutic potential, *Lobelia inflata*—commonly known as Indian tobacco—is a lesser-explored herbaceous plant native to North America, traditionally used by indigenous populations as a remedy for asthma, bronchitis, and muscle pain due to its potent alkaloid content, particularly lobeline (Winston, 2007). The plant is known to contain a wide range of pharmacologically active constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids, which have been reported to exhibit antimicrobial, antioxidant, antispasmodic, and anti-inflammatory activities (Sofowora, 1993; Trease & Evans, 1989). Studies have shown that alkaloids like lobeline can inhibit the proliferation of pathogenic microbes by disrupting protein synthesis and membrane integrity (Lahlou, 2004). Furthermore, flavonoids and tannins have been linked to effective wound healing, anti-inflammatory action, and microbial inhibition through free radical scavenging and enzyme inhibition mechanisms (Cowan, 1999; Havsteen, 2002). However, despite its documented pharmacological actions, *Lobelia inflata* remains significantly underutilized in modern herbal formulations, particularly for topical therapeutic applications. In the context of increasing antibiotic resistance and rising demand for plant-based alternatives, there is a pressing need to explore novel botanicals with multifunctional properties that can effectively manage microbial infections and inflammation without causing adverse effects (Khan et al., 2013; Ventola, 2015). The novelty of this study lies in the formulation and evaluation of *Lobelia inflata*-based herbal ointments, a research area that has not been extensively reported in the existing literature. While some *Lobelia* species have been studied for internal use, systematic investigation of their external application in wound healing and dermatological conditions is still limited. Recent studies suggest that phytochemical-rich herbal formulations not only enhance therapeutic efficacy but also reduce the risk of resistance and toxicity commonly associated with synthetic drugs (Newman & Cragg, 2016; Nascimento et al., 2000). Therefore, the present work focuses on the extraction and phytochemical screening of *Lobelia inflata*, followed by in vitro evaluation of its antibacterial, antifungal, and anti-inflammatory activities. Additionally, various herbal ointment formulations were prepared and characterized for their physicochemical properties, including pH, spreadability, and centrifugation stability, to determine their suitability for topical use. By scientifically validating

the traditional claims associated with this plant, the study aims to establish *Lobelia inflata* as a promising candidate for the development of stable and effective herbal formulations for treating skin-related microbial infections and inflammation.

2. Methodology

2.1. Preparation of Plant Extracts

Fresh plant leaves of *Lobelia inflata* were thoroughly washed with distilled water, shade-dried, and coarsely powdered using a mechanical grinder. The powdered material was placed inside a filter paper thimble and subjected to Soxhlet extraction using a suitable solvent. The system was set up with a condenser and heated under reflux for several hours, allowing repeated cycles of solvent vaporization, condensation, and extraction. After the process, the solvent was evaporated under reduced pressure using a rotary evaporator to yield the concentrated plant extract, which was stored in airtight containers at 4°C for further experiments *(Harborne et al., 1998)*.

2.2. Phytochemical Screening

Phytochemical constituents of the plant extract were identified using standard qualitative procedures as described by Trease and Evans *(Trease et al., 1989)*. The presence of **alkaloids** was confirmed by the formation of a cream-colored precipitate upon treatment with Mayer's reagent. **Flavonoids** were detected through a color reaction, where the addition of sodium hydroxide followed by hydrochloric acid resulted in a distinct yellow coloration. The presence of **tannins** was indicated by a yellowish precipitate after adding 1% lead acetate to the extract. For **carbohydrates**, Fehling's A and B reagents were added and the mixture was incubated at 80°C, leading to the formation of a red precipitate, which confirmed their presence. **Steroids** were identified by the development of a red upper layer and yellow rings along the walls of the test tube after the extract was mixed with chloroform and sulfuric acid. **Saponins** were confirmed by the formation of persistent foam after vigorous shaking of the extract with water. Finally, **fatty acids** were detected by the appearance of a transparent spot on filter paper after the application of ether and subsequent drying *(Sofowora et al., 1993)*.

2.3. Evaluation of Antibacterial Activity

The antibacterial activity of the plant extract was evaluated by the agar well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, and *Bacillus albus*. Nutrient agar plates were inoculated with 20 µL of bacterial suspension. Wells of 8 mm diameter were made and filled with 50 µL of extract, standard antibiotic (Ciprofloxacin), positive control (tetracycline 1000 µg/mL), and negative control (DMSO). The plates were incubated at 37°C for 48 hours, and the diameter of the inhibition zones was measured *(Bauer et al., 1966)*.

Table: Antibacterial Activity of *Lobelia inflata* Extract (Zone of Inhibition in mm)

Microorganism	***Lobelia inflata* Extract (50 µL)**	**Ciprofloxacin (Standard)**	**Tetracycline (1000 µg/mL)**
Staphylococcus aureus	18.4 ± 0.5	32.5 ± 0.4	28.6 ± 0.6
Bacillus subtilis	17.2 ± 0.6	30.1 ± 0.3	26.7 ± 0.5
Micrococcus luteus	14.8 ± 0.7	29.3 ± 0.4	25.4 ± 0.5
Bacillus albus	12.6 ± 0.4	27.6 ± 0.5	23.8 ± 0.6

2.4. Evaluation of Antifungal Activity

The antifungal potential was assessed using the agar well diffusion method against *Candida albicans* and *Aspergillus niger*. Sabouraud Dextrose Agar (SDA) was poured into petri dishes and inoculated with fungal cultures. Wells were filled with 50 µL of extract, Fluconazole (standard), and DMSO (control). Plates were incubated at 28°C for 72 hours, and zones of inhibition were measured to determine antifungal efficacy *(Perez et al., 1990)*.

2.5. Evaluation of Anti-inflammatory Activity

Anti-inflammatory activity was assessed in vitro using the protein denaturation method. A mixture of egg albumin, phosphate-buffered saline (pH 6.4), and plant extract was prepared and incubated at 37°C for 15 minutes, then heated at 70°C for 5 minutes. After cooling, absorbance

was recorded at 660 nm using a UV-Vis spectrophotometer. Diclofenac sodium served as the standard. Percent inhibition of protein denaturation was calculated to determine anti-inflammatory potential *(Mizushima et al., 1968)*.

2.6. Formulation of Herbal Ointment

Cetostearyl alcohol and hard paraffin were melted at 70°C. The plant extract was added to the molten base with continuous stirring, followed by the incorporation of wool fat and white soft paraffin. The formulation was mixed thoroughly, allowed to cool, and stored in sterile containers *(Kokate et al., 2001)*.

Table 1: Herbal Ointment Formulation Composition

S. No	Ingredients	F1	F2	F3	F4
1	Extracts	0.5 g	1 g	2 g	4 g
2	Wool fat	5 g	5 g	5 g	5 g
3	Hard paraffin	5 g	5 g	4 g	3 g
4	Cetostearyl alcohol	4.5 g	4 g	4 g	3 g
5	White soft paraffin	85 g	85 g	85 g	85 g

7. Physicochemical Characterization of the Herbal Formulation

The physicochemical characterization of the herbal ointment was conducted through pH determination, centrifugation, and Spreadability assessment. For pH measurement, 0.5 g of the ointment formulation was accurately weighed and dispersed in 50 mL of distilled water. The dispersion was allowed to stand undisturbed for 2 hours to ensure equilibration, after which the pH was measured using a calibrated digital pH meter *(Indian Pharmacopoeia, 2014)*. To evaluate formulation stability, a centrifugation test was performed by subjecting 10 mL of the sample to 10,000 rpm for 10 minutes using a Remi centrifuge, and any signs of phase separation or instability were observed. The Spreadability of the ointment was assessed by placing 1 g of the formulation between two glass slides and applying a weight of 50 g for a fixed duration of 5 minutes. The extent of spread of the sample under the applied pressure was measured and recorded, indicating the ease of application and uniformity of the formulation *(Mutimer et al., 1956)*.

3. Result

Table 2: Phytochemical Screening of *Lobelia inflata* Extract

Phytochemical Test	Observation	Inference
Alkaloids	Cream precipitate with Mayer's reagent	Present
Flavonoids	Yellow coloration with NaOH + HCl	Present
Tannins	Yellowish precipitate with lead acetate	Present
Carbohydrates	Red precipitate with Fehling's A & B	Present
Steroids	Red upper layer, yellow tube walls	Present
Saponins	Persistent froth after shaking	Present
Fatty Acids	Transparent spot on filter paper	Present

Table 3: Antifungal Activity of *Lobelia inflata* Extract (Zone of Inhibition in mm)

Fungal Strain	*Lobelia inflata* Extract (50 µL)	Fluconazole (Standard)	DMSO (Negative Control)
Candida albicans	15.3 ± 0.5	28.7 ± 0.6	0
Aspergillus niger	12.1 ± 0.6	26.2 ± 0.5	0

Table 4: Antifungal Activity of *Lobelia inflata* Extract (Zone of Inhibition in mm)

Microorganism	***Lobelia inflata* Extract (50 µL)**	**Ciprofloxacin (Standard)**	**Tetracycline (1000 µg/mL)**
Staphylococcus aureus	18.4 ± 0.5	32.5 ± 0.4	28.6 ± 0.6
Bacillus subtilis	17.2 ± 0.6	30.1 ± 0.3	26.7 ± 0.5
Micrococcus luteus	14.8 ± 0.7	29.3 ± 0.4	25.4 ± 0.5
Bacillus albus	12.6 ± 0.4	27.6 ± 0.5	23.8 ± 0.6

Table 5: Anti-inflammatory Activity of *Lobelia inflata* Extract (Protein Denaturation Assay)

Concentration (µg/mL)	**% Inhibition of Protein Denaturation (Mean ± SD)**
100	38.5 ± 1.2
200	51.7 ± 1.5
300	63.2 ± 1.3
400	71.4 ± 1.4
500	79.8 ± 1.1
Diclofenac (Standard, 100 µg/mL)	83.6 ± 0.9

Table 5: Physicochemical Characterization of *Lobelia inflata* Ointment Formulations

Formulation	pH (Mean ± SD)	Centrifugation Stability	Spreadability (g·cm/s)
F1	6.4 ± 0.1	No separation	6.2 ± 0.3
F2	6.2 ± 0.1	No separation	5.9 ± 0.4
F3	6.3 ± 0.1	No separation	6.5 ± 0.2
F4	6.6 ± 0.1	No separation	6.8 ± 0.3

4. Discussion

The present study explored the phytochemical composition and biological efficacy of *Lobelia inflata* extract, including its antimicrobial, antifungal, anti-inflammatory activities, and suitability in topical formulation. The phytochemical screening (Table 2) confirmed the presence of several bioactive compounds such as alkaloids, flavonoids, tannins, steroids, saponins, fatty acids, and carbohydrates. These secondary metabolites are known to contribute significantly to the therapeutic potential of medicinal plants. The findings are consistent with the work of **Trease and Evans (1989)** and **Sofowora (1993)**, who emphasized that alkaloids, flavonoids, and tannins exhibit potent antimicrobial and anti-inflammatory properties. The presence of saponins and flavonoids further suggests antioxidant and membrane-stabilizing effects that support wound healing and infection control.

The antimicrobial efficacy of *Lobelia inflata* extract was assessed against four Gram-positive bacteria using the agar well diffusion method (Table 4). Among the tested organisms, *Staphylococcus aureus* and *Bacillus subtilis* were most susceptible to the extract, showing inhibition zones of 18.4 ± 0.5 mm and 17.2 ± 0.6 mm, respectively. These results are in line with the findings of **Suthar et al. (2010)**, who reported significant antibacterial activity of Lobelia species against pathogenic bacteria. The

observed activity can be attributed to the flavonoids and alkaloids, which may disrupt bacterial cell walls and inhibit enzymatic functions.

Antifungal activity was also demonstrated by the extract against *Candida albicans* and *Aspergillus niger* (Table 3). The extract showed moderate inhibition zones of 15.3 ± 0.5 mm and 12.1 ± 0.6 mm, respectively, as compared to the standard drug Fluconazole. These results are in agreement with **Khan et al. (2013)**, who indicated that plant-derived flavonoids and saponins interfere with fungal cell membrane integrity and spore germination, contributing to antifungal activity. The better response observed against *Candida albicans* may also reflect the susceptibility of yeast-type fungi to polyphenolic compounds present in the extract.

The anti-inflammatory potential of *Lobelia inflata* was evaluated using the protein denaturation assay, a reliable in vitro model to assess membrane stabilization and inflammatory control (Table 5). The extract demonstrated a concentration-dependent inhibition of protein denaturation, reaching 79.8 ± 1.1% at 500 µg/mL, which was comparable to the inhibition shown by the standard drug Diclofenac (83.6 ± 0.9%). These results align with the findings of **Mizushima and Kobayashi (1968)** and **Chandra et al. (2012)**, who showed that plant extracts containing flavonoids and tannins significantly inhibit heat-induced protein denaturation by stabilizing protein structures, thereby reducing inflammatory responses.

Together, the biological results strongly support the therapeutic potential of *Lobelia inflata*, which can be further developed into topical formulations for treating microbial infections and inflammatory conditions. The presence of robust phytoconstituents (Table 2), strong antibacterial (Table 4), promising antifungal (Table 3), and potent anti-inflammatory activities (Table 5) validate its traditional medicinal applications. The results indicate that *Lobelia inflata* could serve as a natural alternative for managing skin-related microbial infections and inflammation.

5.Conclusion

The present study highlights the significant therapeutic potential of *Lobelia inflata* through comprehensive phytochemical screening and biological evaluations. The extract

was found to be rich in secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, and fatty acids, which contribute to its pharmacological activity. The extract demonstrated potent **antibacterial** and **antifungal** activity, particularly against *Staphylococcus aureus* and *Candida albicans*, supporting its traditional use in treating microbial infections. Furthermore, the **anti-inflammatory** potential of the extract, as evidenced by a high percentage of inhibition in the protein denaturation assay, indicates its usefulness in managing inflammation-related skin conditions.

Among the formulated ointments, **Formulation F4**, containing the highest concentration of *Lobelia inflata* extract, showed optimal **physicochemical properties** including suitable pH, excellent spreadability, and stability, making it the most promising for topical application.

Overall, the findings validate the ethnomedicinal relevance of *Lobelia inflata* and support its further development into effective herbal formulations for the treatment of skin infections and inflammation. Future studies involving **in vivo testing, stability studies**, and **clinical trials** are recommended to confirm its efficacy and safety for therapeutic use.

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