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Research Paper

Formulation of Antimicrobial gel of *Adhatoda vasica* Leaf Extract

Kirti Burman*, Dr. Satish Nayak

Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal, MP-462021, India

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ABSTRACT

The present study investigates the antimicrobial activity of *Adhatoda vasica* leaves extract and its potential application in developing a topical antimicrobial gel. The study began with the preparation of ethanolic and aqueous extracts of *Adhatoda vasica* leaves, followed by phytochemical screening to identify bioactive compounds. Antimicrobial activity was evaluated against pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*, using the agar well diffusion method. The results revealed significant antimicrobial activity, particularly in the ethanolic extract, attributed to alkaloids, flavonoids, and phenolic compounds. Based on these findings, a topical gel formulation was developed using the ethanolic extract, and its physicochemical properties, stability, and antimicrobial efficacy were evaluated. The gel exhibited promising antimicrobial properties, good spreadability, and stability under various conditions. This study highlights the potential of *Adhatoda vasica* as a natural source for antimicrobial agents and provides a basis for the development of herbal topical formulations for combating microbial infections.

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health concern where microorganisms, including bacteria, fungi, viruses, and parasites, evolve to withstand antimicrobial treatments, rendering conventional drugs ineffective. This phenomenon is driven by the overuse and misuse of antibiotics, inadequate sanitation, and lack of new drug development. AMR poses a significant threat to public health, leading to prolonged illnesses, increased mortality rates, and economic burdens on healthcare systems worldwide. Natural products offer advantages such as structural diversity, lower toxicity, and synergistic effects with existing antibiotics. They can disrupt microbial biofilms, inhibit virulence factors, and modulate immune responses, making them effective alternatives in tackling resistant pathogens. However, challenges such as low bioavailability, difficulties in large-scale extraction, and the need for clinical validation

***Corresponding Author:** Kirti Burman
Address: *Bansal College of Pharmacy, Kokta, Anand Nagar, Bhopal, MP-462021, India*
Email ✉: ganugavade123@gmail.com
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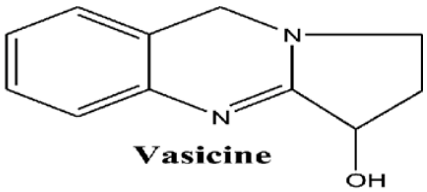
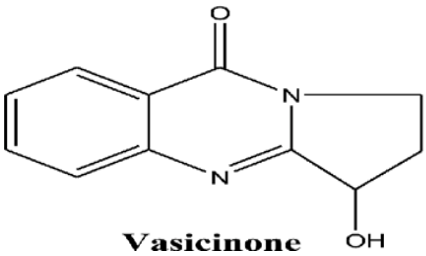
hinder their widespread application. Advanced techniques like nanotechnology, bioengineering, and synthetic modifications are being explored to enhance their efficacy and stability.

Natural products derived from plants are considered safer and more sustainable compared to synthetic drugs. Among these, *Adhatoda vasica* (commonly known as Malabar Nut), a medicinal plant extensively used in traditional systems of medicine like Ayurveda, holds considerable promise due to its rich phytochemical profile. The leaves of *Adhatoda vasica* are known to contain active constituents such as vasicine, vasicinone, and other alkaloids, which have demonstrated a wide range of biological activities, including anti-inflammatory, antioxidant, and antimicrobial effects.

Adhatoda vasica, also known as Vasaka or Arusha, is a prominent medicinal plant with a deep-rooted history in traditional medicine systems such as Ayurveda, Unani, and Siddha. Native to the Indian subcontinent and Southeast Asia, it has been revered for its wide range of therapeutic properties, particularly in treating respiratory disorders. Its historical uses include managing conditions like asthma, chronic bronchitis, tuberculosis, and even bleeding disorders, thanks to its anti-inflammatory, expectorant, and hemostatic properties.



Vasicine



Vasicinone

The bioactive compounds in *Adhatoda vasica*, primarily vasicine, vasicinone, and other alkaloids, have been extensively studied for their antimicrobial properties, which are effective against bacterial strains like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as several fungal pathogens. These compounds exhibit antibacterial, antifungal, and antiviral activity, making the plant a valuable resource for treating infections caused by multidrug-resistant microorganisms. Additionally, the leaves, flowers, and roots of the plant have been used in traditional formulations such as syrups, decoctions, and poultices to heal wounds, manage fevers, and alleviate digestive and skin disorders. The ability of *Adhatoda vasica*, to combat microbial infections while offering a range of therapeutic benefits has made it a focus of modern pharmacological research. Its integration into both traditional and modern healthcare underscores its enduring significance as a natural antimicrobial agent with immense potential for drug development. Vasicine and vasicinone are two biologically active alkaloids derived from the plant *Adhatoda vasica*, which has a long history of use in traditional medicine, particularly for treating respiratory and other ailments. These compounds, specifically vasicine (also known as vasicine) and vasicinone, have garnered significant interest in antimicrobial research due to their potential therapeutic properties.

Adhatoda vasica has been explored for its therapeutic benefits in various clinical settings over the years. A 2023 randomized open-label study investigated its efficacy in mild COVID-19 patients. The study found that Adhatoda vasica (AV) extract, administered at 500 mg for 14 days, significantly reduced hypoxia-inducible factor 1-alpha (HIF-1α), ferritin, vascular endothelial growth factor (VEGF), and prothrombin time/international normalized ratio (PT/INR), suggesting its potential in managing hypoxia, inflammation, and thrombosis. In 2021, preclinical studies demonstrated that aqueous extracts of Adhatoda vasica attenuated inflammation and hypoxia in mouse models of bleomycin-induced pulmonary fibrosis and sepsis, highlighting its potential applications in these conditions (Respiratory Research, 2021). Earlier, a clinical trial conducted in 1983 evaluated the efficacy of Adhatoda vasica syrup for treating non-ulcer dyspepsia (Amlapitta). The trial reported symptomatic relief, supporting its potential as a treatment option for digestive disorders (LWW Journals, 1983). These studies collectively underscore the medicinal promise of Adhatoda vasica in addressing respiratory, inflammatory, and digestive health concerns.

Extraction of active principle:

The extraction of *Adhatoda vasica* was carried out using a standardized procedure to obtain a concentrated extract. Fresh leaves of *Adhatoda vasica* were collected, and if dried leaves were used, they were finely powdered using a mortar and pestle or a mechanical grinder. A specific quantity of the dried leaves (100 g) was accurately weighed for the extraction process. The powdered leaves were placed in a beaker or flask, and dimethyl sulfoxide (DMSO) was added in a solvent-to-leaf ratio of 1:10 (100 g of leaf powder per 1000 mL of DMSO), with adjustments made as needed to optimize solubility and extraction efficiency. The mixture was stirred continuously for 24–48 hours at room temperature or agitated using a mechanical shaker. In an alternative method, the flask containing the mixture was placed in a water bath maintained at 40°C–50°C to enhance the extraction process, taking care to avoid overheating the DMSO. After the extraction period, the mixture was filtered using filter paper or muslin cloth to separate the liquid extract from the residual plant material. The resulting filtrate constituted the *Adhatoda vasica* extract dissolved in DMSO, which was then used for further formulation studies.

Preparation of antimicrobial gel:

Ingredient	Function	Quantity (g)	% w/w
Adhatoda vasica extract	Active ingredient	2.0	2.0%
Carbopol 934	Gelling agent	0.8	0.8%
Triethanolamine	pH adjustment	0.3	0.3%
Glycerin	Humectant	5.0	5.0%
Neem oil	Natural preservative	0.5	0.5%
Aloe vera gel (pure)	Skin soother + stabilizer	2.0	2.0%
Distilled water	Solvent (base)	89.4	89.4%
Total		100.0	100.0%

The formulation of the topical gel was carried out through a systematic procedure to ensure consistency, stability, and effectiveness. Initially, Carbopol 934 (0.8 g) was gradually dispersed into distilled water (89.4 g) with continuous stirring to prevent lump formation and to ensure complete dissolution. The mixture was then allowed to hydrate for 2 hours to facilitate proper swelling of the polymer. After hydration, glycerin (5.0 g) was added as a humectant and mixed thoroughly to achieve a uniform gel base. Subsequently, Adhatoda vasica extract (2.0 g) was slowly incorporated into the gel base with constant stirring to ensure even distribution of the active ingredient. Aloe vera gel (2.0 g) and neem oil (0.5 g) were then added one after the other, and the mixture was blended thoroughly after each addition to obtain a homogeneous composition. To adjust the pH to a suitable level for topical application and to enhance gel stability and viscosity, triethanolamine (TEA, 0.3 g) was added dropwise while stirring continuously. The formulation was stirred until a clear, smooth, and consistent gel was formed. Finally, the prepared gel was transferred into a clean container and stored in a cool, dry place away from direct sunlight to preserve its quality. The formulated gel was subjected to various evaluation parameters to assess its physicochemical properties, stability, and suitability for topical application. These parameters included organoleptic characteristics such as color, odor, and texture, which were visually and manually examined to ensure aesthetic acceptability. The pH of the gel was measured using a digital pH meter to confirm compatibility with the skin and to avoid potential irritation. Viscosity was evaluated using a Brookfield viscometer to determine the gel's consistency and ease of application. Spreadability was assessed to ensure uniform application over the skin surface, which is crucial for therapeutic effectiveness. The gel was also tested for homogeneity by visual inspection to ensure uniform distribution of the active ingredients. Additionally, the formulation underwent stability studies under different temperature and storage conditions to evaluate any changes in color, phase separation, or consistency over time. These evaluation parameters provided critical insights into the quality, effectiveness, and safety of the formulated topical gel.

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