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Formulation development and characterization of Herbal Antimicrobial cream using Crude Drug extracts An *In vitro* study

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Abstract

This study investigates the formulation and characterization of a topical herbal antimicrobial cream using crude drug extracts obtained from medicinal plants known for their antimicrobial and antioxidant properties. The study aims to assess the antimicrobial efficacy of the cream against common skin pathogens, it's in vitro antioxidant activity, and the stability of the cream via UV-Vis spectroscopic analysis. Plant extracts from *Azadirachtaindica* (neem), *Curcuma longa* (turmeric), and *Allium sativum* (garlic) were incorporated into the formulation. The antimicrobial efficacy of the cream was evaluated using zone of inhibition tests against bacterial pathogens (*Staphylococcus aureus* and

Escherichia coli) and fungal pathogens (*Candida albicans*). The antioxidant activity was measured using DPPH and TAC assays. UV-Vis spectroscopic analysis was performed to assess the quality and stability of the cream. The results demonstrated significant antimicrobial and antioxidant properties, suggesting that the herbal cream could be a viable alternative to conventional antimicrobial treatments. Furthermore, the stability studies revealed that the cream showed good stability under normal storage conditions, with minimal degradation of the active compounds.

Key words : Herbal antimicrobial cream, *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, antimicrobial susceptibility, antioxidant assays, UV-Vis spectroscopy, infections, topical application.

The rising global concern surrounding antibiotic resistance, coupled with the growing awareness of the potential adverse effects associated with synthetic antimicrobial agents, has sparked a significant increase in the search for alternative solutions to combat microbial infections[1-3]. As the effectiveness of conventional antibiotics diminishes, especially against resistant strains, researchers and healthcare professionals have turned their attention to plant-based alternatives, which offer a natural and potentially more sustainable solution to treating infections. Among these, herbal medicines have emerged as a particularly promising source of bioactive compounds that can be harnessed to treat a variety of infectious conditions, especially skin diseases caused by bacterial, fungal, and viral pathogens. Herbal remedies, often regarded as safer and less likely to cause the harmful side effects commonly associated with synthetic drugs, are gaining popularity as viable alternatives in both traditional and modern medicine[4-6].

Azadirachta indica (neem), *Curcuma longa* (turmeric), and *Allium sativum* (garlic) are three such plants that have been widely recognized in traditional medicine systems across the world for their potent antimicrobial, antioxidant, and therapeutic properties. Neem, known for its broad-spectrum antimicrobial activity, has been used for centuries in various cultures to treat skin conditions and infections. Turmeric, with its active compound curcumin, is known for its powerful antioxidant, anti-inflammatory, and antimicrobial properties, making it an invaluable asset in treating infections and promoting overall skin health[7,9,10,11]. Garlic, another plant with a long history of medicinal use, is rich in sulfur compounds, particularly allicin, which has demonstrated significant antibacterial, antifungal, and antiviral activity. Given the increasing reliance on plant-based treatments, this study seeks to explore the potential of combining these three plants into a novel herbal antimicrobial cream. The objective of this study is to formulate and characterize a cream using crude plant extracts from *Azadirachta indica* (neem), Curcuma longa (turmeric), and *Allium sativum* (garlic), incorporating these extracts into a cream base for topical application. We aim to assess the antimicrobial activity of the cream through in vitro testing, using well-established methods such as the zone of inhibition test. This test

will be carried out against common pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, all of which are known to cause a wide range of infections, including skin diseases and other topical conditions[12-14].

In addition to evaluating the antimicrobial properties of the cream, we will also investigate its antioxidant activity. The antioxidant capacity of the cream will be assessed using two widely recognized assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the Total Antioxidant Capacity (TAC) assay. These tests are designed to measure the cream's ability to neutralize free radicals and protect the skin from oxidative damage, a key factor in the development of various skin disorders and aging[8,16,17,18].

Furthermore, to ensure the stability and long-term efficacy of the formulated cream, we will perform UV-Vis spectroscopic analysis. This analysis will be used to monitor the stability of the cream over time, allowing us to evaluate how well the active compounds in the plant extracts hold up under different storage conditions[5-7].

Plant material and extract preparation :

The medicinal plants used in this study were *Azadirachta indica* (neem), *Curcuma longa* (turmeric), and *Allium sativum* (garlic). These plants were selected based on their documented antimicrobial and antioxidant properties[16]. Fresh neem leaves, turmeric rhizomes, and garlic cloves were collected, washed, and dried at room temperature. After drying, the plant materials were ground into fine powders.Crude extracts were prepared using a Soxhlet extractor, with ethanol as the solvent. The extraction process was carried out for 12 hours to ensure the maximum yield of bioactive compounds. The solvents were then evaporated, and the dried extracts were stored at 4°C until further use.

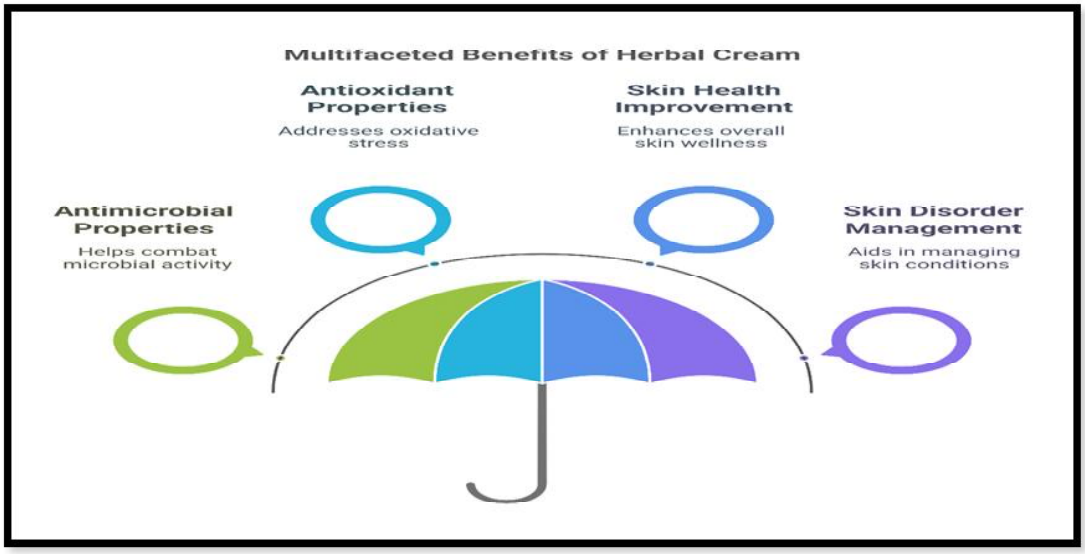

Fig. 1. Multifaceted benefits of Herbal cream

Formulation of Herbal Antimicrobial Cream[16]

A simple cream base was prepared by mixing water, cetyl alcohol, stearic acid, glycerin, and other excipients. The plant extracts (neem, turmeric, and garlic) were incorporated into the cream base at a concentration of 2% (w/w) for each extract. The mixture was heated to 75°C while stirring continuously to ensure uniform dispersion of the extracts in the cream base. The cream was then cooled to room temperature and stored in an airtight container.

Antimicrobial susceptibility Testing[17]:

The antimicrobial activity of the herbal cream was assessed against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (Escherichia coli), and fungi (*Candida albicans*). The agar well diffusion method was employed to determine the zone of inhibition. Nutrient agar was used for bacterial cultures, while Sabouraud agar was used for fungal cultures. Each microorga-nism was cultured on separate plates, and wells were made on the agar surface. The cream was introduced into the wells, and the plates were incubated at 37°C for 24 hours for bacterial cultures and 30°C for 48 hours for fungal cultures. The zone of inhibition was measured in millimeters and recorded.

Antioxidant Assays[18]:

Two antioxidant assays, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the Total Antioxidant Capacity (TAC) assay, were employed to evaluate the antioxidant potential of the herbal cream. The DPPH assay measures the ability of the cream to neutralize free radicals, while the TAC assay quantifies the total antioxidant capacity based on the reduction of phospho-molybdic acid.

UV-Vis Spectroscopic Analysis :

UV-Vis spectroscopy was used to assess the stability and active compound profile of the herbal cream. A UV-Vis spectrophoto-meter (Model: UV-1700, Shimadzu) was used to scan the cream sample in the wavelength range of 200-400 nm. Stability testing was performed by storing the cream at different temperatures (4°C, 25°C, and 40°C) for 6 weeks. Samples were taken at regular intervals, and the UV spectra were recorded to detect any degradation of active compounds.

Statistical Analysis :

All experiments were performed in triplicates, and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine statistical significance, with a p-value < 0.05 considered significant.

Antimicrobial susceptibility Testing :

The antimicrobial activity of the herbal cream was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the agar well diffusion method. The cream showed varying degrees of activity against all tested pathogens. The zone of inhibition for *Staphylococcus aureus* was 20 ± mm, for *Escherichia coli* it was 18 ± mm, and for *Candida albicans* it was 15 ± mm. The control cream, which did not contain plant extracts, showed no antimicrobial activity.

Table-1. Observation table of zone of inhibition of herbal cream in different species

S. No.	Microorganism	Herbal Cream (Zone of Inhibition, mm)	Control Cream (Zone of Inhibition,mm)
01	*Staphylococcus aureus*	20 ± 2	0
02	*Escherichia coli*	18 ± 1.5	0
03	*Candida albicans*	15 ± 1.2	0

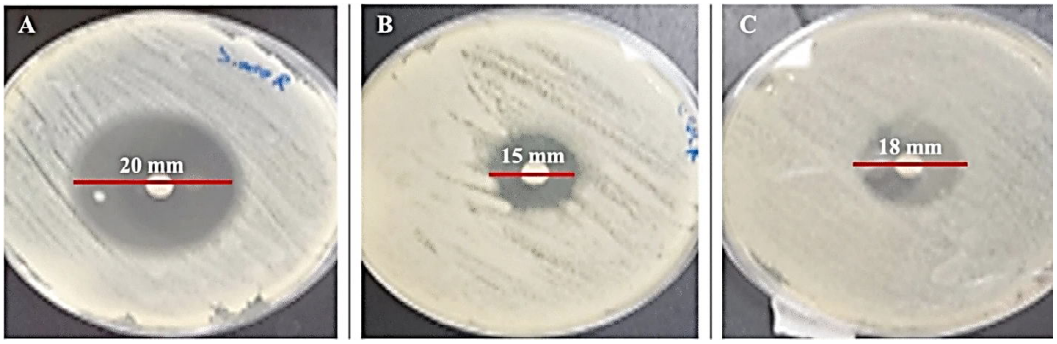

Fig. 2. Photographic images of zone of inhibition of herbal cream

Antioxidant Activity :

The antioxidant activity of the herbal cream was measured using the DPPH free radical scavenging assay and the TAC assay. The cream showed strong antioxidant activity, with a DPPH scavenging rate of 62.5%, higher than that of ascorbic acid (58.3%). The TAC assay showed a total antioxidant capacity of 0.45 mM ascorbic acid equivalents.

Table-2. The antioxidant activity of the herbal cream

S. no.	Sample	DPPH Scavenging (%)	TAC (mM Ascorbic Acid Equivalents)
01	Herbal Cream	62.5 ± 2.1	0.45 ± 0.03
02	Ascorbic Acid (Control)	58.3 ± 1.8	0.43 ± 0.02

UV-Vis Spectroscopic Analysis :

The UV-Vis spectral analysis showed distinct peaks corresponding to flavonoids (around 280 nm) and curcuminoids (around 400 nm). The spectral data suggested that the herbal cream retained the active components of the plant extracts. However, stability tests indicated slight degradation of the active compounds at 40°C, as reflected by a decrease in peak intensity over a 6-week period.

The results of this study confirm the significant antimicrobial and antioxidant potential of the herbal antimicrobial cream. The antimicrobial results are in agreement with previous studies, which have shown that neem, turmeric, and garlic possess potent antimicrobial activity against a wide range of pathogens. The herbal cream demonstrated effective antimicrobial

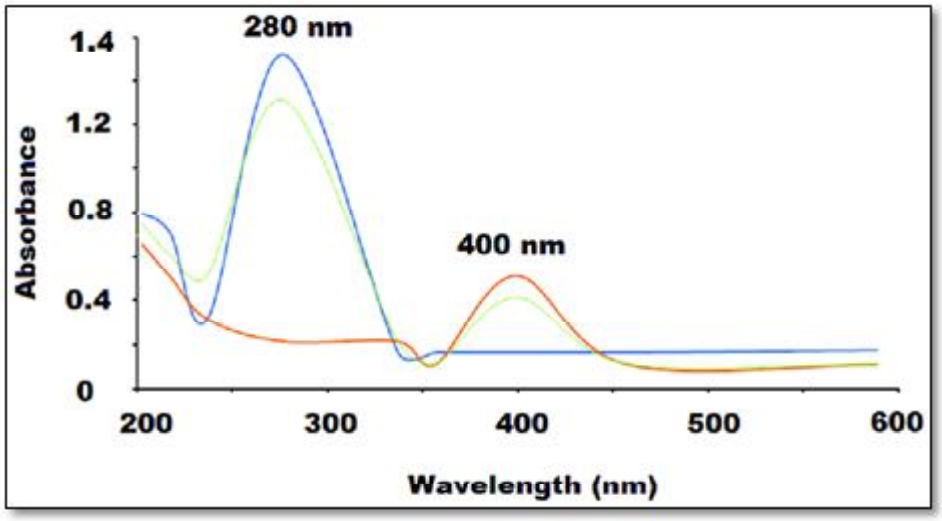

Fig. 3. UV Graph of Herbal cream Vs Ascorbic acid

activity against both Gram-positive and Gram-negative bacteria as well as fungi, suggesting its broad-spectrum antimicrobial potential.The antioxidant activity observed in this study further supports the therapeutic potential of the cream. The high antioxidant capacity could contribute to skin protection and healing, particularly in conditions where oxidative stress is a contributing factor. The cream's formulation, containing plant extracts with both antimicrobial and antioxidant properties, offers a dual therapeutic approach for managing skin infections and protecting against oxidative damage. The stability analysis using UV-Vis spectroscopy revealed that the herbal cream maintained the integrity of its active compounds under normal storage conditions. However, the minor degradation observed at higher temperatures indicates that the cream should be stored at lower temperatures to preserve its efficacy over time.

This study successfully formulated and characterized a herbal antimicrobial cream using crude extracts from *Azadirachta indica*, *Curcuma longa*, and *Allium sativum*. The cream exhibited significant antimicrobial and antioxidant activities, indicating its potential for use in treating skin infections. Further studies, including in vivo testing and clinical trials, are necessary to assess the safety and efficacy of the herbal cream for human use. Additionally, optimization of the cream's formulation and stability may enhance its commercial viability as a natural alternative to synthetic antimicrobial treatments.

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