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Phytochemical Characterization, Formulation, And Evaluation of Anti-bacterial activity of Kalmegh Antiseptic cream

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ABSTRACT: Andrographis paniculata Nees (commonly known as Kalmegh), a member of the Acanthaceae family, is traditionally used for managing various ailments including skin infections, liver disorders, fever, and respiratory diseases. In the present study, the aerial parts of the plant were subjected to phytochemical screening and antibacterial evaluation to support its potential use in topical formulations. Extracts were prepared using different solvents—methanol, chloroform, petroleum ether, and aqueous media—and assessed for antibacterial activity against Bacillus subtilis and Escherichia coli using the disc diffusion method. Among all, the methanolic extract showed the most significant inhibitory effect on both bacterial strains. Phytochemical analysis confirmed the presence of flavonoids, terpenoids, alkaloids, phenols, steroids, and saponins, indicating strong antimicrobial potential. Based on these findings, an antiseptic cream formulation incorporating the methanolic extract of A. paniculata was developed and evaluated for antibacterial efficacy. The results suggest that the formulated cream can serve as an effective natural alternative for topical antibacterial applications.

Keywords: Kalmegh, Andrographis paniculata, Phytochemical Screening, Antiseptic Cream, Antibacterial Activity.

I. INTRODUCTION:

The medicinal plant Andrographis paniculata (Burm.f.) Nees (family Acanthaceae), commonly known as Kalmegh, has been used for centuries in traditional systems of medicine across Asia, including Ayurveda, Unani and Traditional Chinese Medicine. It has been employed to manage a wide variety of ailments such as fever, cough, skin conditions, dysentery, liver disorders and snake-bite [1,2].

Phytochemical investigations reveal that the aerial parts of A. paniculata are rich in bioactive compoundsparticularly diterpenoid lactones such as andrographolide and neo andrographolide alongside flavonoids, phenols, terpenoids, alkaloids and saponins [1,3]. The presence of these constituents has been linked to a variety of pharmacological effects, including antibacterial, antioxidant, antipyretic, hepatoprotective, antidiabetic and anti-inflammatory activities [1,4].

In recent years, growing antimicrobial resistance has driven interest toward plants like A. paniculata as potential sources of novel antiseptic or antimicrobial agents [4]. Studies have shown that extracts of A. paniculata, especially those obtained using methanol or ethanol, exhibit inhibitory activity against both Gram-positive and Gram-negative bacteria. The efficacy of different solvents and parts of the plant appears to vary, which underlines the importance of determining suitable extraction and formulation methods[1,5].Given its ethnopharmacological relevance and promising antimicrobial profile, A. paniculata is a strong candidate for incorporation into topical dosage forms such as antiseptic creams for skin and soft-tissue applications. Developing a cream formulation that harnesses the plant's bioactive extracts would facilitate topical delivery and could provide a natural alternative to conventional antimicrobial agents. Thus, this study aims toperform phytochemical characterization of A. paniculata leaf extracts, formulate an antiseptic cream incorporating the most active extract, andevaluate its antibacterial activity against selected bacterial strains.

II. MATERIALS AND METHODS:

1. Collection of Plant Material

Fresh and healthy plants of Andrographis paniculata (Kalmegh) were collected from the

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medicinal plant garden of School of Pharmacy, Seacom Skills University, Birbhum, West Bengal, India. Identification was confirmed based on morphological features such as lanceolate leaves and small purple flowers [6]. The aerial partsmainly leaves and stemswere selected for analysis since they contain the highest concentration of bioactive diterpenoids like andrographolide. Ethical collection practices were followed to ensure environmental sustainability and prevent overharvesting [7].

Figure 1: Andrographis paniculata

2. Preparation of Plant Extract:

a. Drying and Powdering

Collected leaves were thoroughly washed with distilled water to remove debris and shade-dried for 5-7 days at room temperature to prevent the degradation of thermolabile compounds. Once dried, the leaves were pulverized using a sterile mechanical grinder to obtain a fine powder suitable for solvent extraction [8].

b. Extraction Process

Extraction was carried out using the maceration method. Approximately 22 g of powdered material was soaked in methanol at a ratio of 1:10 (w/v) for 48 hours with intermittent shaking on a rotary shaker to enhance solute–solvent interaction. The mixture was then filtered, and the filtrate was concentrated in a water bath at 40 °C to obtain a thick extract. The dried extract was stored in an airtight amber container, protected from light and moisture, until further use [9].


Figure 4: Rotary flask shaker


Figure 5: Water bath


Figure 6: Extract

3. Phytochemical Screening:

The methanolic extract was subjected to qualitative phytochemical analysis to identify secondary metabolites. Standard procedures were employed to test for the presence of alkaloids, flavonoids, phenols, saponins, tannins, carbohydrates, and terpenoids [10]

Alkaloids: Mayer's, Wagner's, and Dragendorff's reagents were used to confirm alkaloid presence.

Carbohydrates: Molisch's, Fehling's, Benedict's, and Barfoed's tests indicated the presence of sugars.

Phenolic Compounds: Ferric chloride test showed a characteristic dark green colour.

Flavonoids: Sodium hydroxide test produced a yellow coloration confirming flavonoid content.

Tannins: Potassium dichromate test indicated positive results for tannins

4. Antibacterial Activity:

a. Preparation of Media:

Nutrient agar medium was prepared using peptone, yeast extract, sodium chloride, and agar in distilled water. The medium was sterilized at 121 °C for 15 minutes using an autoclave and poured into sterile Petri plates [11].

b. Culture Preparation:

Two bacterial strainsBacillus subtilis (Gram-positive) and Escherichia coli (Gram-negative)were selected for testing. Bacterial suspensions were prepared in peptone water and standardized to match 0.5 McFarland turbidity standards.

c. Agar Well Diffusion Assay:

Antibacterial activity was determined using the agar well diffusion method. Sterile Petri plates containing solidified Mueller Hinton Agar (MHA) were inoculated with the test organisms

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using sterile cotton swabs. Wells of 5 mm diameter were made using a sterile borer, and 0.1 mL of the plant extract (5%, 10%, and 15% v/v solutions) was introduced into each well. Plates were incubated at 37 ℃ for 24 hours, and zones of inhibition were measured in millimeters[12].

5. Formulation of Antibacterial Cream:

An herbal antiseptic cream was formulated using the methanolic extract of A. paniculata. The formulation included cetyl alcohol, xanthan gum, liquid paraffin, Tween 80, methyl paraben, ethyl paraben, glycerine, triethanolamine, perfume, and distilled water.

The oil phase (cetyl alcohol, xanthan gum, paraffin, and Tween 80) and aqueous phase (methyl paraben, glycerine, and water) were separately heated to 60 °C and then mixed with continuous stirring. After cooling to 40 °C, triethanolamine was added to adjust the pH, followed by the incorporation of plant extract and perfume. The cream (Figure 15) was stored in airtight containers for further testing [13].

6. Evaluation of Formulated Cream:

The formulated cream was evaluated for various physicochemical and functional parameters, including:

Appearance and Texture: Colour, odour, and consistency were recorded.

pH Determination: 0.5 g of cream dispersed in 50 mL of distilled water was analysed using a digital pH meter.

Irritancy Test: A small amount of cream was applied to a 1 cm^2 area on the skin to observe for irritation after 24 hours.

Washability: The cream's ease of removal with tap water was evaluated.

Stability Study: Samples were stored at 2 ℃, 25 °C, and 37 °C for four weeks to check physical stability [7].

III. RESULTS AND DISCUSSION:

The phytochemical analysis of the methanolic extract of Andrographis paniculata confirmed the presence of several bioactive constituents such as alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, and carbohydrates (Figure 7-13), which are known to contribute to its therapeutic properties. These naturally occurring compounds play a crucial role in enhancing the plant's antimicrobial and antioxidant activities. The results of the antibacterial study revealed that the methanolic extract exhibited significant inhibitory activity against Escherichia coli and Bacillus subtilis using the agar well diffusion method. Among the tested concentrations, the 10% extract showed (Figure 14) the highest zone of inhibition, indicating that the antibacterial potential of the extract increases with concentration. This suggests that methanol is an effective solvent for extracting active phytochemicals such as andrographolide, which are responsible for bacterial inhibition. The formulated herbal antiseptic cream (Figure 15) prepared using the methanolic extract was dark green in color, smooth in texture, and had a pleasant odor. It showed a pH value of approximately 7.0, which is within the suitable range for skin application and ensures non-irritancy. The irritancy test confirmed that the cream was safe for use, as no redness or itching was observed after application. The cream also demonstrated good washability and stability, maintaining its appearance, odor, and consistency under different temperature conditions for four weeks. Overall, the findings of this study indicate that Andrographis paniculata possesses strong antibacterial properties and that the formulated antiseptic cream provides a stable, safe, and effective natural alternative to synthetic antimicrobial products for the prevention and treatment of skin infections.



Figure 7: Mayer's test Figure 8: Wagner's test. Figure 9: Dragendroff's test

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Figure 10: Fehling's Test

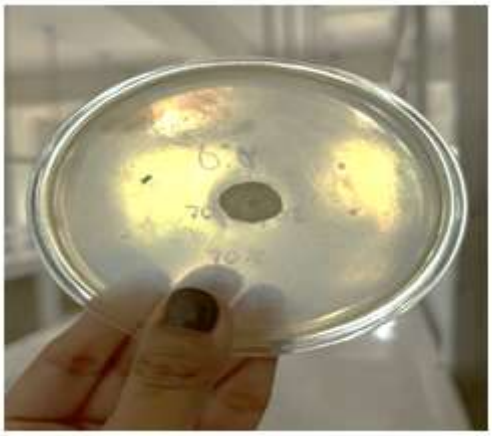

Figure 11: Ferric chloride test

Figure 12: Flavonoid Test

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Figure 13Pottasiumdichloromate Test Figure 14: Bacterial culture for 10% Solution.

Figure 15: Prepared cream with plant extract e for 10% Solution.

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