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Phytochemical Screening and Development of a Polyherbal Antimicrobial Cream Containing *Adhatoda vasica*, *Bacopa monnieri* and *Cascabela thevetia*

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Abstract

Background: Herbal formulations have gained considerable attention as alternative antimicrobial agents due to their safety, efficacy, and reduced side effects. The present study aimed to perform phytochemical screening of selected medicinal plants and formulate and evaluate a polyherbal antimicrobial cream containing *Adhatoda vasica*, *Bacopa monnieri*, and *Cascabela thevetia*.

Methods: Ethanolic extracts of the selected plants were subjected to preliminary phytochemical screening. Four cream formulations (F1–F4) were prepared using the oil-in-water (O/W) emulsion method and evaluated for physicochemical parameters, including appearance, homogeneity, pH, viscosity, drug content, microbial growth, irritancy, antibacterial activity, antifungal activity, and stability.

Results: Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, phenolics, terpenoids, saponins, and glycosides. All formulations exhibited acceptable physicochemical properties with pH values ranging from 5.8 to 6.3. Among the formulations, F3 showed the highest viscosity (29500 cP) and drug content (98.1%). F3 demonstrated significant antibacterial activity against *Escherichia coli* (15.6 mm), *Pseudomonas aeruginosa* (14.9 mm), and *Staphylococcus aureus* (16.8 mm), as well as antifungal activity against *Aspergillus niger* (14.7 mm), *Macrophomina phaseolina* (15.3 mm), and *Fusarium* species (14.9 mm). Stability studies showed no significant changes over three months.

Conclusion: The optimized formulation F3 exhibited satisfactory physicochemical properties, significant antimicrobial activity, and good stability. The study suggests that the formulated polyherbal cream has potential as a safe and effective topical antimicrobial preparation.

Keywords: Polyherbal Antimicrobial Cream; *Adhatoda vasica*; *Bacopa monnieri*; *Cascabela thevetia*; Phytochemical Screening; Antimicrobial Activity

1. Introduction

Medicinal plants are abundant sources of antimicrobial molecules. Wide ranges of medicinal plants extract are used to treat several infections as they have potential antimicrobial activity.[1]Some of these bioactive molecules are screened and traded in the markets as raw material for many herbal industries.[2] Phytochemicals are chemical compounds formed during the plants normal metabolic processes, which are often referred to as “secondary metabolites”.[3]

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Herbal creams are semi-solid formulations that are used topically to treat infected skin.[4] The active ingredients in the formulations are used to create antimicrobial creams that protect the skin from microbial infections.[5]

Therefore, the present study was undertaken to perform phytochemical screening of selected medicinal plants and to formulate and evaluate a polyherbal antimicrobial cream containing *Adhatoda vasica*, *Bacopa monnieri*, and *Cascabela thevetia*. The study aimed to investigate the phytochemical constituents of the selected plants and assess the physicochemical characteristics, antimicrobial activity, and stability of the developed formulation.

2. Material and methods

2.1. Materials and equipment

The leaves of *Adhatoda vasica*, *Bacopa monnieri* and *Cascabela thevetia* were collected from local areas and authenticated by a botanist. Stearic acid, cetyl alcohol, beeswax, liquid paraffin, triethanolamine, methyl paraben, propyl paraben and other analytical grade chemicals were procured from the laboratory. The equipment used in the study included an electronic balance, pH meter, Brookfield viscometer, UV-Visible spectrophotometer, incubator and hot air oven.

2.2. Methodology

2.2.1. Collection and Processing of Plant Materials[6,7]

The collected plant materials were washed thoroughly with water and shade dried at room temperature. The dried materials were powdered separately using a mechanical grinder and stored in airtight containers for further use.

2.2.2. Preparation of Plant Extracts[8,9]

The powdered plant materials were extracted separately using ethanol by soxhlet extraction and maceration methods. The extracts were filtered and concentrated using a rotary evaporator. The dried extracts were stored in airtight containers until further use.

2.2.3. Preliminary Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening for the identification of carbohydrates, alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenolic compounds using standard procedures.

Test for Carbohydrate:[10]

Benedict's Test: Take 0.5 mL of filtrate and 0.5 mL of Benedict's reagent in a test tube. Heat the mixture on a boiling water bath for 2 minutes. Formation of red precipitate shows the presence of sugars.

Fehling's Test: Take a test tube and add 1ml of Fehling's A, Fehling's B solution and 2mL of extracts. Heat the mixture in boiling water bath for 10 min. Appearance of red precipitate shows the presence of sugars.

Test for Alkaloids:[11]

Wagner's test: Take a few ml of plant extracts and add drops of Wagner's reagent along the sides of test tube. Appearance of reddish-brown precipitate confirmed the presence of alkaloids.

Dragendorff's test: Take 3mL of plant extract and add 1mL of Dragendorff's reagent (potassium bismuth iodine). Appearance of brick red precipitate confirmed the presence of alkaloids.

Test for Phenolic compound and tannins:[12]

Ferric Chloride Test: Take 1mL of plant extract and add few drops of neutral 5% ferric chloride solution. A dark green or black indicate the presence of phenolic compound.

Test for Saponins: [13]

Foam Test: Take 2g extract and 10 mL of water. After shaking vigorously persistent foam is observed this indicates the presence of saponins.

Test for Terpenoids:[14]

Take 1mL of extract and 0.5 mL of chloroform followed by an addition of few drops of concentrated sulphuric acid. Formation of reddish-brown precipitate indicates the presence of terpenoids.

Test for Triterpenoids:[15]

Salkowski Test: Take 2mL of extract and add 5 drops of concentrated sulphuric acid, shake well and allow it to stand. Appearance of greenish blue colour indicates the presence of triterpenoids.

Test for Flavonoids:[16]

Take 2mL of plant extract with concentrated sulphuric acid, appearance of orange to crimson colour indicate presence of Flavonoids.

Test for Steroids:[17]

Liebermann – Burchard reaction: Mix 2mL of extract with chloroform and add 1-2 mL acetic anhydride and 2 drops of concentrated sulphuric acid from the side of test tube. Appearance of green or dark green colour indicate presence of steroids.

Test for Cardiac -Glycosides:[18]

Keller- Killani test: Take 2mL of extract with few ml of glacial acetic acid and one drop 5% ferric chloride and concentrated sulphuric acid. Appearance of reddish brown colour at the junction of two liquid layer indicates presence of cardiac glycosides.

Legal test: Take 2mL of the extract with 1mL of pyridine and 1mL of sodium nitroprusside. The appearance of pink or red colour indicates the presence of cardiac glycosides.

2.2.4. Formulation development of Anti-Microbial Herbal Cream[19]

Procedure: Polyherbal antimicrobial cream was prepared by the fusion method using an oil-in-water emulsion system. The oil and aqueous phases were heated separately to 70°C and mixed with continuous stirring. Herbal extracts were incorporated into the formulation, followed by the addition of triethanolamine and preservatives. The mixture was stirred until a smooth, homogeneous cream was obtained.

Table 1 Composition of Polyherbal Antimicrobial Cream (30g)

	Sr. No.	**Ingredients**	**Quantity IN gm (30gm)**			
			F1	**F2**	**F3**	**F4**
Aqueous Phase	1.	*Adhatoda vasica* extract	0.15g	0.30g	0.45g	0.60g
	2.	*Bacopa monnieri* extract	0.15g	0.30g	0.45g	0.60g
	3.	*Cascabela thevetia* extract	0.075g	0.075g	0.15g	0.15g
	4.	Glycerine	1.50 mL	1.50mL	1.50mL	1.50mL
	5.	Methyl paraben	0.045g	0.045g	0.045g	0.045g
	6.	Propyl paraben	0.015g	0.015g	0.015g	0.015g
	7.	Triethanolamine	0.3 mL	0.3 mL	0.3 mL	0.3 mL
	8.	Purified water	q. s to 30 gm	q. s to 30 gm	q. s to 30 gm	q. s to 30 gm
Oil Phase	9.	Stearic acid	3.60g	3.60g	3.60g	3.60g
	10.	Cetyl alcohol	0.90g	0.90g	0.90g	0.90g
	11.	Liquid paraffin	1.80mL	1.80mL	1.80mL	1.80mL

2.2.5. Evaluation of Polyherbal Antimicrobial Cream

Visual Examination[20]

The formulated creams were evaluated for colour, appearance, consistency, homogeneity and phase separation by visual inspection.

Smear Type and Emolliency[21]

A small quantity of cream was applied to the skin and examined for smear characteristics and emollient properties, such as smoothness and moisturizing effect.

Determination of pH[22]

The pH of the formulations was determined using a digital pH meter. Approximately 5 g of cream was dispersed in distilled water and the pH was recorded at room temperature.

Dye Solubility Test[23]

The type of emulsion was confirmed by mixing the cream with Scarlet Red dye and observing under a microscope. Uniform distribution of dye indicated an oil-in-water (O/W) emulsion.

Viscosity[24]

Viscosity was measured using a Brookfield viscometer (RV spindle, 20 rpm) at 25°C and the average value was recorded.

Drug Content Determination[25]

The drug content of the polyherbal cream was estimated using a UV–Visible spectrophotometer. Approximately 1 g of the cream was accurately weighed and transferred into a 50 mL volumetric flask. The volume was made up to the mark with phosphate buffer (pH 5.5). The solution was stirred thoroughly to extract the active constituents of *Adhatoda vasica*, *Bacopa monnieri* and *Cascabela thevetia*. The mixture was shaken and filtered through Whatman filter paper. From the filtrate, 0.1 mL was further diluted to 10 mL with the same solvent and the absorbance was measured at a suitable wavelength using a UV spectrophotometer.

Calculation of Drug Content

$$\text{Drug content} \frac{\text{Concentration} \times \text{Dilution factor}}{1000}$$

$$\%\ \text{Drug content} \frac{\text{Practical yield} \times 100}{\text{Theoretical yield}}$$

Uniform drug content indicated proper distribution of herbal extracts in the cream formulation.

In-vitro Drug Diffusion[26]

Drug diffusion was evaluated using an egg membrane diffusion model. The membrane was prepared by dissolving the egg shell in 3 M HCl, washed with phosphate buffer (pH 5.5), and mounted on a glass diffusion cell. One gram of cream was placed in the donor compartment, while 140 mL phosphate buffer (pH 5.5) was used as the receptor medium maintained at 37 ± 1°C with continuous stirring. Samples (2 mL) were collected at predetermined intervals for 3 h and analysed at 220 nm using a UV spectrophotometer.

2.3. Determination of Microbial Growth

2.3.1. In-Vitro Antibacterial Activity[27]

Preparation of Nutrient Agar Medium: Nutrient agar medium (28 g/L) was prepared in distilled water, sterilized by autoclaving at 121°C for 15 min, and aseptically poured into sterile Petri plates for solidification.

Determination of Zone of Inhibition : The nutrient agar plates were inoculated with *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus* using sterile cotton swabs. Wells were prepared with a sterile cork borer, and 1 g of the formulated polyherbal cream was added to each well. A standard antibacterial formulation was used as the

positive control. The plates were incubated at 37°C for 24–48 h, and antibacterial activity was determined by measuring the zone of inhibition (mm).

2.3.2. In-Vitro Antifungal Activity [28,29]

Preparation of Sabouraud Dextrose Agar (SDA) Medium: Sabouraud Dextrose Agar (65 g/L) was prepared in distilled water, sterilized by autoclaving at 121°C for 15 min, cooled to 45–50°C, and poured aseptically into sterile Petri plates.

Determination of Zone of Inhibition : The SDA plates were inoculated with fungal cultures of *Aspergillus niger*, *Macrophomina phaseolina*, and *Fusarium* spp. Wells were prepared using a sterile cork borer, and 1 g of the formulated cream was added to each well. A standard antifungal formulation served as the positive control. The plates were incubated at 28–30°C for 5–7 days, and antifungal activity was evaluated by measuring the zone of inhibition (mm).

3. Results and Discussion

3.1. Homogeneity

Visual appearance and touch are two commonly used methods to assess the homogeneity of a cream.

Table 2 Homogeneity of Different Cream Formulation

Cream Formulation	Visual Appearance	Touch
F1	Uniform color and texture, no visible particles or separation.	Smooth and consistent texture without any lumps or grittiness.
F2	Consistent texture and color throughout the cream.	Smooth and even texture with no perceptible variation.
F3	Uniform distribution of extract, no visible clumping or unevenness.	Silky texture, evenly spread extract without any irregularities.
F4	Homogeneous appearance without any visible inconsistencies	Soft and velvety texture, indicating even distribution of extract.

All formulations exhibited good homogeneity, smooth texture and uniform appearance without any signs of phase separation.

Table 3 Appearance of Polyherbal Antimicrobial Cream (F1-F4)

Formulation	Appearance
F1	No change in cream color
F2	No change in cream color
F3	No change in cream color
F4	No change in cream color

In this table, cream formulations labeled as F1 to F4 exhibit no change in the color of the cream. This indicates that the formulations were stable and the color remained consistent throughout the manufacturing process.

Table 4 After feel (F1-F4)

Cream Formulation	Emolliency	Slipperiness	Residue Amount
F1	High	Moderate	Low
F2	Moderate	High	Moderate
F3	**Moderate**	**Moderate**	**Moderate**
F4	High	High	Low

The after-feel study revealed that the formulations showed satisfactory emolliency, slipperiness and low residue, providing a pleasant feel on application.

The after feel of the formulated polyherbal antimicrobial creams was evaluated based on emolliency, slipperiness, and residue amount, as these parameters influence patient acceptability and therapeutic performance.

3.2. Type of smear

Table 5 Type of Smear of Polyherbal Antimicrobial Cream (F1-F4)

Cream Formulation	Smear Type
F1	Non-greasy
F2	Greasy
F3	Greasy
F4	Non-greasy

The formulated polyherbal antimicrobial creams (F1–F4) were evaluated by applying a small quantity on the skin surface and observing the nature of the smear formed.

The type of smear test showed that most formulations produced a non-greasy smear, which is desirable for topical creams.

Table 6 Removal

Cream Formulations	Ease of Removal
F1	Easily
F2	Moderate
F3	Moderate
F4	Easily

Ease of removal of topical formulations is an important factor influencing patient compliance. The ease of removal of polyherbal antimicrobial creams (F1–F4) was evaluated by washing the applied formulation from the skin using tap water. Ease of removal studies indicated that the formulations were easily washable with tap water.

3.3. pH of the Cream

The pH of all formulations was found to be within the acceptable range for topical application, indicating good skin compatibility.

Table 7 pH Values of Different Cream Formulations

Cream Formulation	pH Value
F1	6.2
F2	5.8
F3	6.1
F4	6.3

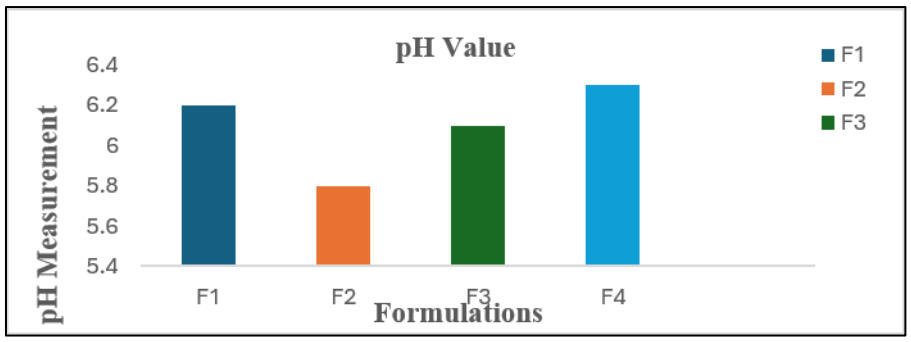

Figure 1 pH Value of Different Cream Formulations

3.4. Viscosity of the Cream

The viscosity of the formulated polyherbal antimicrobial creams was determined using a Brookfield viscometer. The viscosity values ranged from 24500 to 29500 Cp.

Table 8 Viscosity of Different Cream Formulations

Formulation	Viscosity (cP)
F1	24500±120
F2	26800±140
F3	29500±110
F4	28200±130

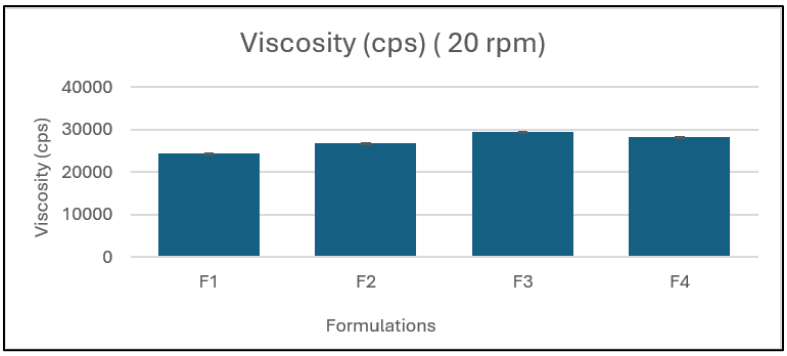

Figure 2 Viscosity of Different Cream Formulations

3.5. Drug Content

The drug content determination was carried out using UV-Visible spectrophotometry. The percentage drug content of the formulations ranged from 91.4 % to 98.1%.

Table 9 Drug Content of Different Cream Formulations

Formulation	Drug Content (%)
F1	91.4±0.5
F2	94.2±0.4
F3	98.1±0.3
F4	96.3±0.4

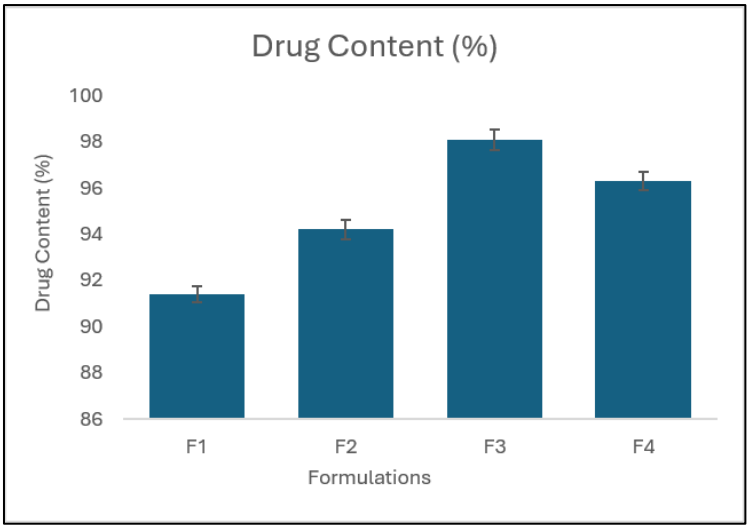

Figure 3 Drug Content of Different Cream Formulations

3.6. In vitro-studies

3.6.1. Antibacterial Activity (Standard vs F1-F4)

Among the tested formulations, F3 showed the highest zone of inhibition against both bacterial and fungal strains, indicating superior antimicrobial activity when compared to other formulations.

Table 10 Zone of Inhibition against Bacterial Strains

Formulation	*E. coli (mm)*	*P. aeruginosa (mm)*	*S. aureus (mm)*
STD (Ciprofloxacin)	18.5 ± 0.6	17.8 ± 0.5	20.2 ± 0.7
F1	11.2 ± 0.4	10.6 ± 0.3	12.8 ± 0.5
F2	12.4 ± 0.5	11.8 ± 0.4	13.6 ± 0.4
F3	15.6 ± 0.6	14.9 ± 0.5	16.8 ± 0.6
F4	13.1 ± 0.4	12.5 ± 0.3	14.2 ± 0.5

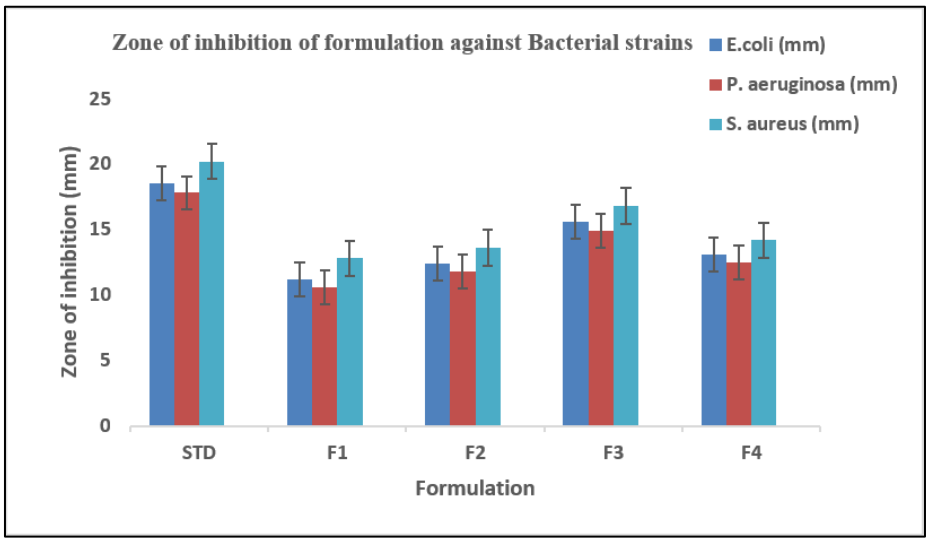

Figure 4 Zone of Inhibition of Formulation against Bacterial Strains

3.7. Antifungal Activity (Standard Vs F1-F4)

In antifungal studies, the formulations exhibited noticeable inhibition against *Aspergillus niger*, *Macrophomina phaseolina*, and *Fusarium species*. Among all formulations, F3 exhibited better antifungal activity, suggesting its suitability for treating fungal skin infections.

Table 11 Zone of Inhibition Against Fungal Strains

Formulation	*A.niger* (mm)	*M. phaseolina* (mm)	*Fusarium* (mm)
STD	17.2 ± 0.5	18.0 ± 0.6	17.6 ± 0.5
F1	10.8 ± 0.3	11.4 ± 0.4	10.6 ± 0.3
F2	11.9 ± 0.4	12.2 ± 0.5	11.8 ± 0.4
F3	14.7 ± 0.5	15.3 ± 0.6	14.9 ± 0.5
F4	12.6 ± 0.4	13.1 ± 0.4	12.4 ± 0.3

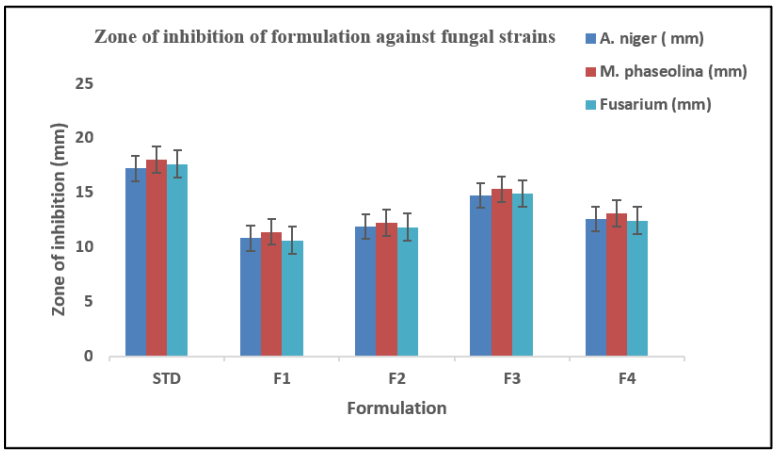

Figure 5 Zone of Inhibition of Formulation against Fungal Strains

4. Conclusion

From the present investigation, it can be concluded that the polyherbal antimicrobial cream formulations (F1–F4) prepared using *adhatoda vasica*, *Cascabela thevetia* and *Bacopa monnieri* are safe, stable and effective. Among them, F3 was identified as the best formulation due to its superior antimicrobial activity, good stability and excellent skin compatibility. The study supports the potential use of polyherbal formulations as effective topical antimicrobial agents and provides a scientific basis for their further development and commercial application.

Compliance with ethical standards

Acknowledgments

All the authors have contributed equally.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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