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Research

Formulation and Evaluation of Antimicrobial Cream of *Lavandula bipinnata*, *Nigella sativa*, and *Cynodon dactylon*

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Abstract

The present study aimed to formulate and evaluate an antimicrobial cream utilizing plant extracts of *Lavandula bipinnata*, *Nigella sativa*, and *Cynodon dactylon*. The formulation process involved thorough phytochemical screening, chromatographic profiling (TLC and HPLC), and FTIR spectroscopy to confirm the presence of key bioactive constituents, including flavonoids, phenolics, terpenoids, and alkaloids. Extraction yields were notably high in the aqueous extracts—12.3% (*L. bipinnata*), 10.2% (*N. sativa*), and 11.5% (*C. dactylon*)—indicating a rich content of hydrophilic phytochemicals. The cream formulations (F1-F5) were evaluated for pH, viscosity, spreadability, and thermal stability, with the polyherbal formulation (F5) showing optimal pH (6.10), higher viscosity (6175 cps), and excellent spreadability (95%). Antimicrobial activity was assessed using the agar well diffusion method against *Escherichia coli* and *Salmonella spp.*, revealing that F5 exhibited the highest activity against *E. coli* (zone of inhibition: 7 mm), while minimal activity was observed against *Salmonella spp.* (1 mm). Compared to standard antibiotics (streptomycin: 7–6 mm; tetracycline: 9–10 mm), the polyherbal cream demonstrated promising potential against *E. coli*. These findings suggest that the synergistic combination of these plant extracts in a topical formulation holds promise as a natural alternative for the treatment of skin infections caused by *E. coli*, though further optimization is needed for broader-spectrum efficacy.

Keywords: *Lavandula bipinnata*, *Nigella sativa*, and *Cynodon dactylon, Antimicrobial Cream*

1. Introduction

Infectious diseases remain one of the leading causes of global morbidity and mortality, exacerbated by the rapid rise of antimicrobial resistance (AMR). The overuse and misuse of antibiotics have significantly contributed to the emergence of multidrug-resistant (MDR) pathogens, rendering many conventional treatments ineffective. Consequently, there is a pressing need to explore novel, safer, and more sustainable antimicrobial therapies, particularly those derived from natural sources. **[1-2]**

Medicinal plants have long served as a reservoir of bioactive compounds with diverse therapeutic properties. In recent years, plant-derived antimicrobial agents have garnered renewed interest due to their efficacy, low toxicity, and reduced potential for resistance development. Essential oils and phytochemicals such as flavonoids, phenolics, terpenoids, alkaloids, and tannins demonstrate significant antibacterial, antifungal, and antioxidant activities, making them valuable candidates for formulation into topical therapies**. [3-7]**

Lavandula bipinnata, commonly known as lavender species of the Lamiaceae family, is traditionally used for its antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. It is rich in compounds such as linalool, linalyl acetate, and geraniol, which contribute to its broad-spectrum bioactivity**. [8-9]**

Nigella sativa, commonly known as black cumin, has been extensively studied in traditional systems

of medicine. Its major bioactive constituent, thymoquinone, has been shown to possess potent antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects. The seeds also contain nigellone, alpha-hederin, and various essential fatty acids that contribute to wound healing and infection control**. [10-11]**

Cynodon dactylon (Bermuda grass) is widely recognized for its therapeutic applications, including antimicrobial, antidiabetic, hepatoprotective, and anti-inflammatory effects. The plant is a rich source of flavonoids, tannins, terpenoids, alkaloids, and phenolic acids, which support its traditional use in the treatment of wounds and infections**. [12-14]**

Figure 1. lavandula bipinnata

Figure 2 Nigella sativa

Figure 3 Cyanodon dactylon

Given their pharmacological potential, the integration of these three plants into a single polyherbal topical formulation presents a novel and synergistic approach to managing skin infections. While each plant extract has demonstrated antimicrobial properties independently, limited studies exist on their combined efficacy in a cream-based formulation.

1.1 Rationale and Novelty of the Study

This study is designed to formulate and evaluate a polyherbal antimicrobial cream using extracts of *Lavandula bipinnata*, *Nigella sativa*, and *Cynodon dactylon*. The novelty lies in the combination of these three plant extracts, aiming to achieve enhanced antimicrobial efficacy through synergistic interaction. The formulation is assessed through detailed physicochemical evaluation, chromatographic profiling, spectroscopic characterization, and in vitro antibacterial activity, offering a scientific basis for its future development as a natural, effective alternative to synthetic topical antibiotics.

2. Materials and Methods

2.1 Chemicals and Reagents

All solvents and chemicals used were of analytical grade. Ethanol, methanol, petroleum ether, ethyl acetate, acetone, and distilled water were procured from certified suppliers. Phytochemical reagents included Dragendorff's reagent, Wagner's reagent, Shinoda reagent, sulfuric acid, ferric chloride, Folin–Ciocalteu reagent, aluminium chloride, and others as required for qualitative tests. TLC solvents such as toluene, ethyl acetate, formic acid, chloroform, and methanol were used. For HPLC analysis, HPLC-grade acetonitrile and water, potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid, and triethylamine were utilized.

2.2 Plant Material Collection and Authentication

Fresh aerial parts of *Lavandula bipinnata*, Cynodon *dactylon,* and seeds of Nigella *sativa* were collected from Sitapur district in mid-March. The plant materials were authenticated by the Botanical Survey of India (BSI). Voucher specimens were deposited for future reference.

2.3 Pharmacognostic Studies

2.3.1 Morphological and Macroscopic Examination

The plant parts were observed for physical characteristics such as color, odor, taste, size, venation, and texture using a simple microscope. Leaf sections and seed samples were examined and photographed using a Nikon Lab Photo 2 microscope.

2.3.2 Microscopic and Histochemical Analysis

Transverse sections of plant materials were cut (10–12 µm) using a rotary microtome. The sections were stained with appropriate reagents to visualize anatomical structures and histochemical

constituents such as lignified elements, mucilage, calcium oxalate crystals, and starch grains.

2.3.3 Quantitative Microscopy

Quantitative parameters such as stomatal index, palisade ratio, vein islet number, and vein termination number were calculated to support species identification.

2.3.4 Extraction of Plant Materials

Powdered plant materials were defatted with petroleum ether and then extracted using a Soxhlet apparatus with solvents of increasing polarity (ethanol, methanol, water). The extracts were filtered, concentrated using a rotary evaporator, and dried under reduced pressure.

2.3.5 Determination of Extractive Values

Petroleum ether, 50% hydroalcoholic, and aqueous soluble extractive values were determined. Stock solutions were prepared at a concentration of 2 mg/mL for each solvent extract.

2.3.6 Phytochemical Screening

Standard qualitative tests were conducted to identify alkaloids, flavonoids, saponins, glycosides, tannins, phenols, terpenoids, steroids, and carbohydrates.

2.3.7 Physicochemical Evaluation

Moisture content (loss on drying), total ash, acid-insoluble ash, and water-soluble ash values were determined as per standard protocols to assess the quality and purity of crude drugs.

2.4 Chromatographic Studies

2.4.1 Thin Layer Chromatography (TLC)

TLC plates coated with silica gel-G were used. Extracts were applied and developed using appropriate mobile phases:

1. *Lavandula bipinnata*: Toluene: Ethyl acetate: Formic acid (5:4:1)
2. *Nigella sativa*: Chloroform: Methanol (9:1)
3. *Cynodon dactylon*: Chloroform: Methanol (10:1)

Spots were visualized under UV (254 and 366 nm) and after spraying with anisaldehyde and ninhydrin reagents. Rf values were recorded.

2.4.2 High-Performance Liquid Chromatography (HPLC)

Analysis was performed using a C18 column (4.6 × 250 mm, 5 µm). Mobile phase consisted of acetonitrile and water (50:50) with pH adjusted to 6.0 using phosphoric acid. Flow rate was maintained at 2.0 mL/min with detection at 220 nm. Standard and sample solutions were injected (20 µL) for chromatographic profiling.

2.4.3 FT-IR Spectroscopy

FT-IR analysis was conducted using a Thermo Nicolet 8700 FT-IR spectrometer. Extracts were mixed with potassium bromide (1:100) and scanned between 4000–400 cm^{1} at a resolution of 4 cm^{1}. Spectral peaks were interpreted to identify functional groups.

2.5 Cream Formulation

Five formulations (F1–F5) were prepared using phase inversion emulsification. The oil phase included olive oil (7.5%), sesame oil (1.5%), cetyl alcohol (3.5%), stearic acid (4.75%), sorbitan monooleate (1.75%), and sorbitan monostearate (0.75%). The aqueous phase included distilled water, propylene glycol (4%), glycerine (3.5%), and plant extracts (0.5–1.5%). Honey (2%) was added during cooling. F1 was the base; F2–F4 contained individual plant extracts; F5 combined all three extracts.

2.5.1 Evaluation of Cream Formulations

The prepared creams were evaluated for:

1. **pH**: 1 g of cream dispersed in 100 mL of water, measured using a digital pH meter.
2. **Viscosity**: Measured using a Brookfield viscometer.
3. **Spread ability**: Evaluated using the glass slide method under standard weight.
4. **Acid and Saponification Values**: Determined by the titration method as per the Indian Pharmacopoeia.
5. **Thermal Stability**: Assessed at 40°C for 48 hours.
6. **Microbial Load**: Colony-forming units (CFU/g) determined via nutrient agar plating.

2.6 Antibacterial Activity

Targeted microbes for antimicrobial activity:

a. Escherichia coli -Gram-Negative Bacterial strain

b. Salmonella typhi Gram-Negative Bacterial strain

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Figure 4.(a) E.coli; (b) Salmonella spp.

2.6.1 Antimicrobial Screening

The agar well diffusion method is used to determine the antimicrobial properties of F1, F2, F3, F4, and F5. This method was recommended by the National Committee for Clinical Laboratory Standards (NCCLS-1999). Different formulations were individually used to screen the organisms for susceptibility. Tetracycline and Streptomycin were used as control drugs. These strains were collected from the Department of Microbiology, CSIR-NBRI, Lucknow. The zones of inhibition were measured using a transparent meter rule.

2.6.2 Media Preparation and its Sterilization

In the Agar Well-diffusion method, antimicrobial susceptibility was performed on solid Agar-Agar) media Petri plates. For bacterial assay, nutrient agar (NA) (28gm/L) and for fungus, PDA (24 gm/L) was used for developing surface colony growth. Both media were prepared and then sterilized by autoclave at 121^0C for 15 minutes.

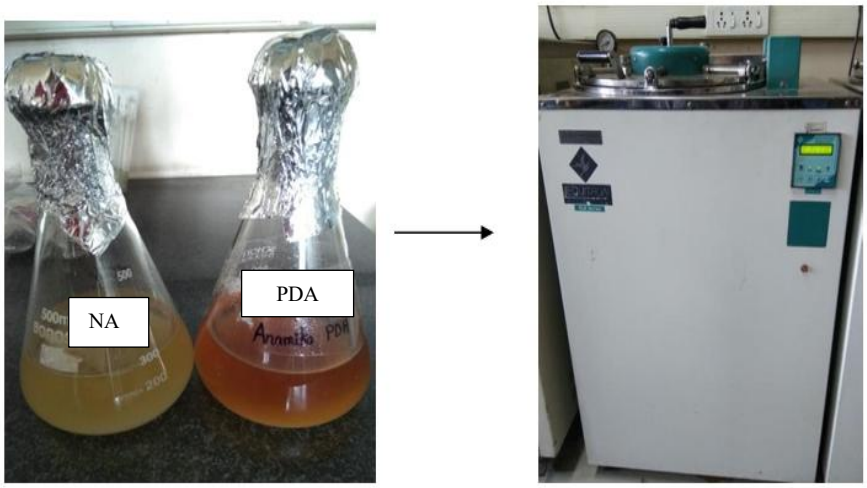


Figure 5: Media preparation and its sterilization

2.6.3 Agar Well Diffusion Method

In this method (NCCLS-1999), Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media were swabbed and poured onto Petri plates, and kept for 12-24 hours. The Petri plates (90 mm * 15 mm well diameter) had the formulation poured into the wells and allowed to diffuse. Control experiments with inocula were set up. The plates were incubated at 28°C for 24 hours. The diameter of the inhibition zone was measured in mm, and the activity index was also calculated.

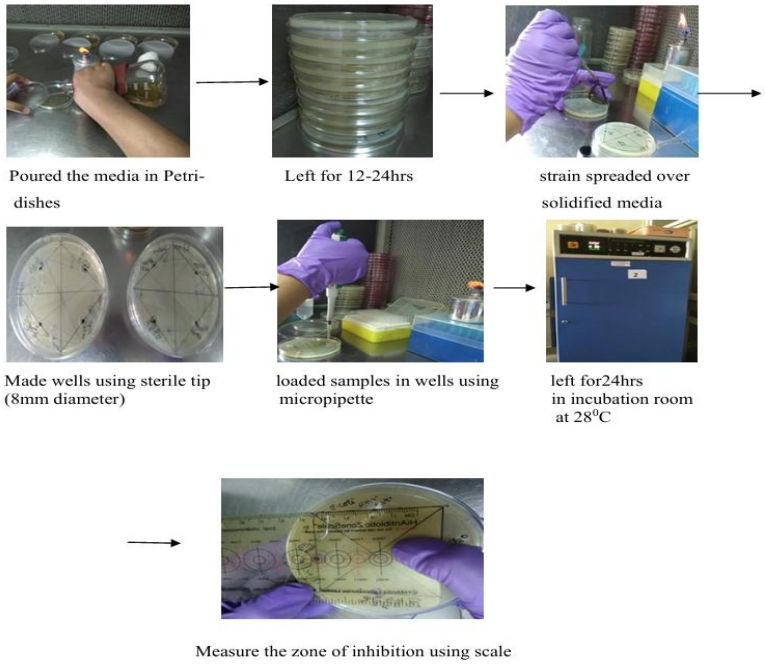


Figure 6: Method of Antimicrobial assay

3. Results and Discussion

3.1 Pharmacognostic Evaluation

3.1.1 Morphological and Microscopic Analysis

Lavandula bipinnata exhibited narrow lanceolate leaves with a greenish-grey hue and a distinct aromatic odor. *Nigella sativa* seeds were small, angular, black, and aromatic. *Cynodon dactylon* had linear, pointed leaves and green stems with a grassy scent. Microscopic analysis confirmed dorsiventral leaf structure in *L. bipinnata*, monocot characteristics in *C. dactylon*, and oil glands and aleurone grains in *N. sativa*.

Quantitative microscopy revealed the following:

1. **Stomatal Index**: *L. bipinnata* (23.3%), *C. dactylon* (17.7%)
2. **Palisade Ratio**: *L. bipinnata* (2.5), *C. dactylon* (1.8)
3. **Vein Islet Number**: Higher in *C. dactylon*, indicating adaptive efficiency

These features collectively validate species identity and support their physiological robustness, which is particularly relevant for topical formulations.

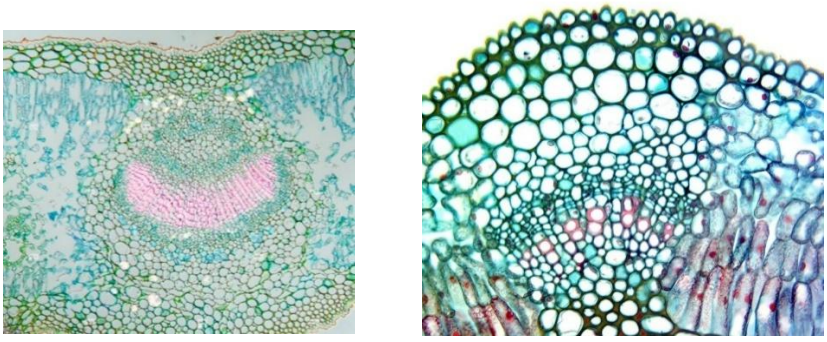


Figure 7 (a) *L. bipinnata* **(b)** *C. dactylon*

3.2 Extraction Yield

Extraction with solvents of increasing polarity produced the following yields.

Plant	Petroleum Ether (%)	Ethanol (%)	Water (%)
L. bipinnata	4.2	8.5	12.3
N. sativa	5.1	7.8	10.2
C. dactylon	3.8	6.9	11.5

The high aqueous extractive values indicate the abundance of hydrophilic bioactives such as tannins and flavonoids, while *N. Sativa* showed higher petroleum ether extract yield, reflecting lipophilic constituents such as thymoquinone.

3.3 Phytochemical Profile

Qualitative screening confirmed the presence of multiple phytoconstituents.

Table 01. Phytochemical Profile of the extract of three plants

Phytochemical Test	Test Used	Lavandula bipinnata	Nigella sativa	Cynodon dactylon
Alkaloids	Dragendorff's & Mayer's Test	+	+	-
Flavonoids	Shinoda Test	+	+	+
Tannins	Ferric Chloride Test	+	+	+
Saponins	Foam Test	-	+	+
Glycosides	Keller-Killiani Test	+	+	-
Steroids	Salkowski Test	+	-	+
Phenols	Ferric Chloride Test	+	+	+
Terpenoids	Liebermann-Burchard Test	-	+	+
Carbohydrates	Molisch's Test	+	+	-

These findings support the antimicrobial and wound-healing potential of all three plants.

3.4 Chromatographic Analysis

3.4.1 Thin Layer Chromatography (TLC)

Distinct Rf values under UV and chemical reagents confirmed key compounds.

1. *L. bipinnata*: Rf 0.24 (flavonoids), Rf 0.72 (terpenoids)
2. *N. sativa*: Rf 0.30 (alkaloids), Rf 0.79 (phenolics)
3. *C. dactylon*: Rf 0.50 (flavonoids), Rf 0.88 (terpenoids)

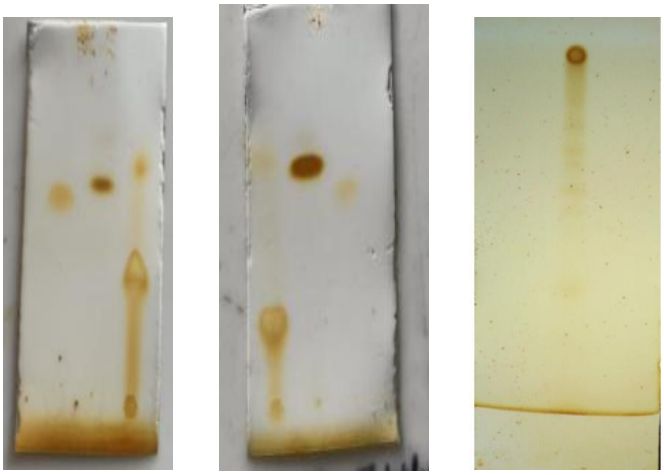
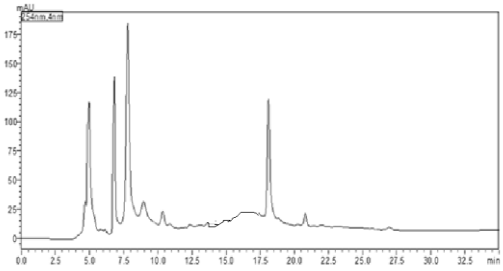


Figure 8. TLC profiling of three extracts

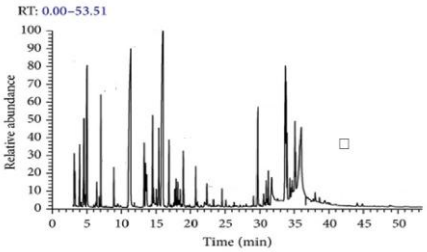
3.4.2 High-Performance Liquid Chromatography (HPLC)

1. *L. bipinnata* showed peaks at ~3–15 min, indicating the presence of gallic acid, caffeic acid, and thymoquinone.
2. *N. sativa* exhibited major peaks for thymoquinone, thymol, and carvacrol.
3. *C. dactylon* revealed flavonoids (quercetin, kaempferol) and phenolic acids.

These data affirm the presence of bioactive constituents responsible for antimicrobial and antioxidant properties.



L. bipinnata



N. sativa

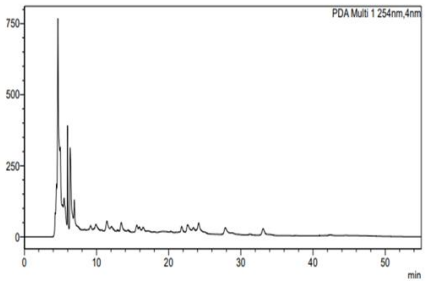
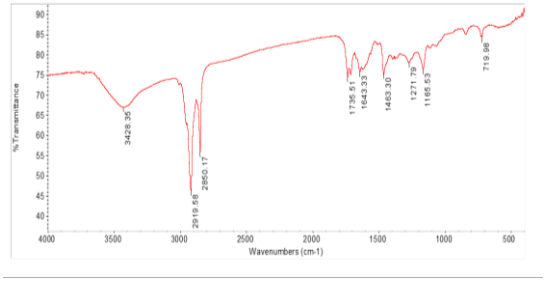


Figure 9 HPLC spectra of (a) *L. bipinnata* (b)*N. sativa* (c) *C. dactylon*

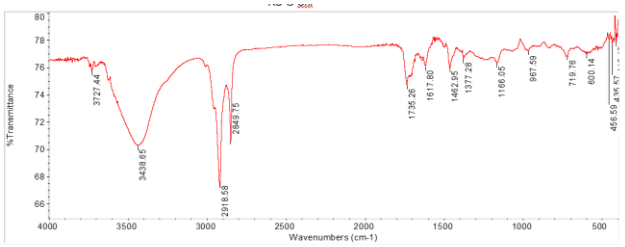
3.5 FTIR Spectroscopy

1. *L. bipinnata*: Presence of O-H, C=O, and C-H stretching bands confirmed phenols, esters, and hydrocarbons.
2. *N. sativa*: Showed strong absorption for thymoquinone, esters, and alkanes.
3. *C. dactylon*: Bands corresponding to C-O, C=C, and O-H indicated flavonoids and polyphenols.

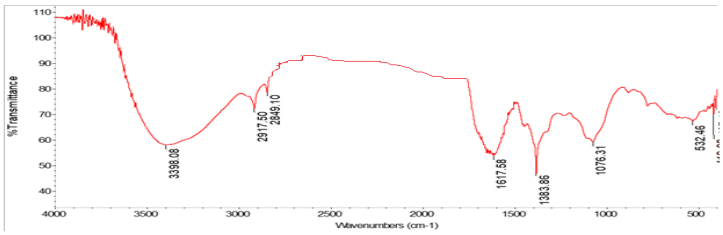
FTIR results align with HPLC data and further validate chemical complexity.



(a) *L. bipinnata*



(b)*N. sativa*



C. dactylon

Figure 10. FTIR spectra of (a) *L. bipinnata* (b)*N. sativa* (c) *C. dactylon*

3.6 Evaluation of Anti-Microbial Cream

Parameter	F1	F2	F3	F4	F5
pH	5.85	6.02	6.12	6.18	6.10
Acid Value	6.0	6.2	6.1	6.5	6.3
Saponification	15.2	17.0	17.5	18.2	17.4
Spreadability %	96	95	95	94	95
Thermal Stability (%)	98.0	96.5	96.8	95.9	97.0
Viscosity (Cps)	6100	6150	6200	6290	6175

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All formulations fell within acceptable topical application limits. The polyherbal formulation (F5) displayed ideal pH, excellent spreadability, higher viscosity, and superior thermal stability

3.6.1 Antimicrobial activity

The results of the antimicrobial activity of all formulations against the test microorganisms are summarized in Tables 2 and 3. The zones of inhibition of all formulations were measured in mm, and two antibiotics (streptomycin and tetracycline)

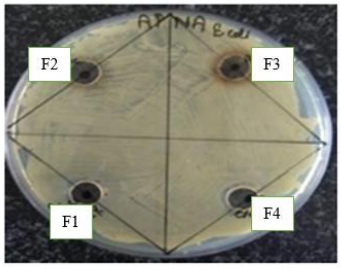
Table 02: Antibacterial activity of all formulations

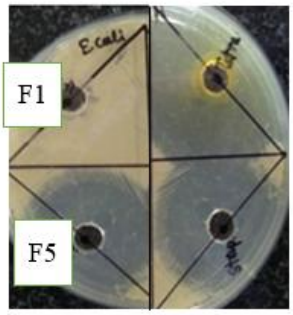
Bacterial spp.	F1	F2	F3	F4	F5
E. coli	0	1	1	2	7
Salmonella spp	0	0	0	1	1

E. coli

1) **F1** has no activity (0) against E. coli.
2) **F2 and F3** show very little activity (1).
3) **F4** shows moderate activity (2).
4) **F5** shows the highest activity (7).

Formulation F5 is the most effective against E. coli.

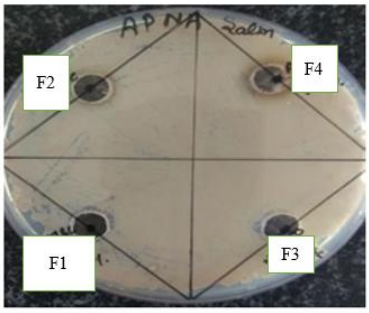




Salmonella spp.

1) **F1, F2, and F3** have no activity (0).
2) **F4 and F5** show only slight activity (1).

None of the formulations are very effective against Salmonella spp., but F4 and F5 show minimal activity.



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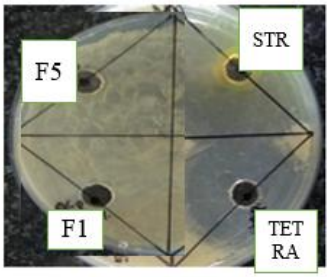
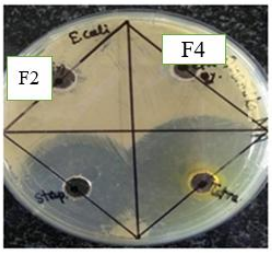
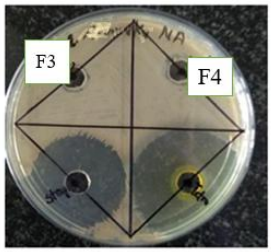


Table 03 Antimicrobial activity of some antibiotics

	E. coli	*Salmonella spp*
Streptomycin	**7**	**6**
Tetracyclin	**9**	**10**





4 Discussion

The formulation and evaluation of a polyherbal antimicrobial cream comprising *L. bipinnata*, *N. sativa*, and *C. dactylon* aimed to harness the individual and synergistic antimicrobial properties of these plants. The extraction process demonstrated that aqueous extracts yielded the highest percentages across all plants, aligning with the known hydrophilic nature of several key bioactive (e.g., flavonoids, tannins). Phytochemical screening confirmed the presence of relevant antimicrobial compounds, including alkaloids, flavonoids, phenolics, and terpenoids.

Chromatographic analysis further validated the presence of key phytoconstituents such as gallic acid, caffeic acid, thymoquinone, thymol, carvacrol, quercetin, and kaempferol, known for their antibacterial and antioxidant effects. FTIR analysis confirmed functional groups consistent with these bioactive.

Antimicrobial testing highlighted that while all single-extract formulations (F2–F4) showed limited activity against *E. coli* (1–2 mm) and negligible activity against *Salmonella spp.*, the polyherbal formulation (F5) exhibited a significant increase in antibacterial activity (7 mm) against *E. coli*. This suggests a synergistic enhancement of antibacterial efficacy when combining extracts, in agreement with the hypothesis that multi-component formulations can exhibit superior bioactivity compared to single-extract creams.

5 Conclusion

The study successfully demonstrated the feasibility of formulating a polyherbal antimicrobial cream using extracts of *Lavandula bipinnata*, *Nigella sativa*, and *Cynodon dactylon*. The polyherbal formulation (F5) showed enhanced physicochemical properties and the highest antibacterial activity against *E. coli*, confirming the synergistic potential of combining these plant extracts. Although the formulation exhibited limited activity against *Salmonella spp.*, the overall findings support the potential of the cream as a natural topical alternative for managing *E. coli*-related skin infections. Further studies focusing on formulation optimization, extended microbial testing, and *in vivo* evaluation are recommended to establish its therapeutic efficacy and clinical relevance fully.

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