



PREPARATION AND EVALUATION OF HERBAL OINTMENT FROM SELECTED MEDICINAL PLANTS AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Medicinal plants represent an important source of bioactive compounds with potential therapeutic applications. The present study aimed to formulate and evaluate a herbal ointment containing extracts of turmeric (*Curcuma longa*), eucalyptus (*Eucalyptus globulus*) leaves, and ginger (*Zingiber officinale*) and to assess its antimicrobial potential. The collected plant materials were washed, processed into a fine paste, and extracted with ethanol by maceration for 3–5 days. The resulting crude extract was filtered and subjected to qualitative phytochemical screening. The screening revealed the presence of several phytoconstituents, including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, flavonoids, terpenoids, phlobatannins, lignins, resins, lactones, anthocyanins, and betacyanins. A semi-solid herbal ointment was prepared using white wax and yellow paraffin as the base, followed by incorporation of the plant extract. The formulated ointment was evaluated for physicochemical characteristics, including color, odor, consistency, pH, spreadability, and stability. Its antimicrobial activity was evaluated against three bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, and three fungal strains, *Aspergillus niger*, *Candida albicans*, and *Penicillium* sp., using the agar well diffusion method. The formulated ointment exhibited inhibitory activity against the tested bacterial and fungal strains, indicating its potential antimicrobial properties. The observed activity may be associated with the combined effects of the bioactive phytochemicals present in the selected plant extracts. Overall, the findings suggest that the formulated herbal ointment has potential as a plant-based topical formulation for further investigation against microbial skin infections. Further studies involving quantitative phytochemical analysis, optimization of formulation, toxicity assessment, and statistical evaluation are warranted to establish its efficacy and safety.

Keywords: *Curcuma longa*; *Eucalyptus globulus*; *Zingiber officinale*; herbal ointment; phytochemical screening; antimicrobial activity; topical formulation.

1. INTRODUCTION

Medicinal plants have been used for centuries as a primary source of treatment for various diseases. Traditional systems of medicine such as Ayurveda, Siddha, and Unani rely heavily on the therapeutic properties of plants. These plants contain a wide range of bioactive compounds known as phytochemicals, which are responsible for their medicinal properties. Phytochemicals are naturally occurring secondary metabolites produced by plants that help them defend against pathogens and environmental stress. These compounds also play an important role in human health due to their diverse pharmacological activities. [1-3].

Phytochemicals include different classes of compounds such as alkaloids, flavonoids, tannins, phenols, saponins, glycosides, steroids, and terpenoids. These compounds possess a variety of biological properties including antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, and anticancer activities. Preliminary phytochemical screening is an important step in identifying the presence of these compounds in plant extracts. This screening provides valuable information about the medicinal potential of plants and serves as the foundation for further pharmacological studies. [4,5].

Turmeric (*Curcuma longa*) is one of the most important medicinal plants widely used in traditional medicine. It contains a bioactive compound known as curcumin, which possesses strong antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. Turmeric has been extensively used in herbal formulations for the treatment of skin infections and wounds. Eucalyptus (*Eucalyptus globulus*) leaves are another important medicinal plant known for their antimicrobial and antiseptic properties. The leaves contain essential oils such as eucalyptol, which exhibit strong antibacterial and antifungal activities. Eucalyptus extracts are commonly used in herbal preparations for respiratory infections, skin diseases, and wound healing. Ginger (*Zingiber officinale*) is also a well-known medicinal plant widely used for its therapeutic benefits. Ginger contains several bioactive compounds including gingerol, shogaol, and zingerone, which possess antimicrobial, antioxidant, and anti-inflammatory activities. These compounds contribute to the medicinal properties of ginger and make it an important ingredient in herbal formulations. [6-8]

Another important advantage of herbal ointments is their ability to provide localized treatment, which reduces systemic absorption and minimizes adverse effects. The presence of natural phytochemicals enhances skin regeneration, reduces inflammation, and accelerates wound healing. Due to these beneficial properties, herbal ointments are widely used in the management of dermatological conditions such as fungal infections, burns, cuts, rashes, and microbial skin diseases. Therefore, the development and evaluation of herbal ointments using medicinal plant extracts have gained considerable attention in pharmaceutical and biomedical research. Investigating the antimicrobial efficacy and therapeutic potential of such formulations is essential for developing effective natural remedies for the treatment of skin-related infections. [9-12]

In the present study, plant extracts obtained from turmeric, eucalyptus leaves, and ginger were used to prepare a herbal ointment. The extracts were first subjected to preliminary phytochemical screening to identify the presence of bioactive compounds. The prepared herbal ointment was then evaluated for its physicochemical properties such as color, odor, consistency, pH, and spreadability. Furthermore, the antimicrobial activity of the ointment was studied against selected bacterial and fungal strains using standard microbiological methods. The study aims to develop a natural herbal formulation with potential antimicrobial properties that can be used for topical applications. Thus, the present research focuses on the extraction of medicinal plants, phytochemical screening, preparation of herbal ointment, evaluation of ointment properties, and investigation of antimicrobial activity against selected microorganisms. [13,14].

2. MATERIALS AND METHODS

2.1.PREPARATION OF PLANT EXTRACT

Fresh rhizomes of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*), along with fresh leaves of eucalyptus (*Eucalyptus globulus*), were collected and thoroughly washed with distilled water to remove adhering dust and other impurities. The plant materials were cut into small pieces to facilitate extraction. Turmeric (4–8 g), eucalyptus leaves (4–8 g), and ginger (2–4 g) were weighed separately and ground using a sterile mortar and pestle to obtain a fine paste.

The prepared plant materials were transferred into a clean extraction container, and 70 mL of ethanol was added as the extraction solvent. The mixture was allowed to stand at room temperature for 3–5 days to facilitate the extraction of soluble bioactive constituents. Following the extraction period, the mixture was filtered through Whatman No. 1 filter paper to remove the plant residues. The resulting filtrate was collected as the crude ethanolic plant extract and stored appropriately until further phytochemical analysis and formulation of the herbal ointment [15,16].

2.2.QUALITATIVE ANALYSIS OF EXTRACT OF MIXTURE OF HERBAL PLANTS

Qualitative analysis is a chemical method used to determine the presence or absence of various phytochemical compounds in plant extracts by observing characteristic color changes, precipitate formation, or other visible reactions. The following testes were carried out in the plant extracts (table-1). [17-19]

Table-1: Qualitative Phytochemical Screening of Plant Extract

S.No	Name of Chemical Test	Procedure	Observation
1	Alkaloids Detection Mayer's Test	50 mg of solvent-free crude extract was dissolved in 3 ml dilute HCl and filtered. The filtrate was subjected to the following tests. 1 ml filtrate + 5 drops Mayer's reagent	Creamy white precipitate indicates alkaloids
	Wagner's Test	1 ml filtrate + 3 drops Wagner's reagent	Reddish-brown precipitate indicates alkaloids
	Hager's Test	1 ml filtrate + Hager's reagent	Yellow precipitate confirms alkaloids
	Dragendorff's Test	1 ml filtrate + Dragendorff's reagent	Yellow precipitate indicates alkaloids
2	Carbohydrates Detection	20 mg extract dissolved in 10 ml distilled water and filtered	
	Molisch's Test	2 ml filtrate + α -naphthol + conc. H_2SO_4	Violet ring indicates carbohydrates
	Fehling's Test	Filtrate + Fehling's A & B heated	Red precipitate indicates sugars
	Barfoed's Test	Filtrate + Barfoed's reagent heated	Red precipitate indicates sugars

	Benedict's Test	Filtrate + Benedict's reagent heated	Colored precipitate indicates sugars
3	Glycosides Detection	Extract hydrolyzed with HCl for 2 hrs then filtered	
	Borntrager's Test	Filtrate + chloroform + NH_4OH	Pink colour indicates glycosides
	Legal's Test	Extract + pyridine + sodium nitroprusside + NaOH	Pink colour indicates glycosides
4	Saponins Detection	Extract shaken with distilled water for 15 min	Stable foam layer indicates saponins
5	Proteins Detection	Extract dissolved in water and filtered	
	Millon's Test	Filtrate + Millon's reagent	White precipitate indicates proteins
	Biuret Test	Filtrate + CuSO_4 + KOH	Pink/violet color indicates proteins
6	Amino Acids Detection	Filtrate + Ninhydrin solution	Purple colour indicates amino acids
7	Phytosterols Detection	Extract + acetic anhydride + conc. H_2SO_4	Color change indicates phytosterols
8	Fixed Oils and Fats Spot Test		
		Extract pressed between filter papers	Oil stain indicates oils
	Saponification Test	Extract + alcoholic KOH heated	Soap formation indicates oils/fats
9	Phenolic Compounds Detection Ferric Chloride Test	Extract + FeCl_3	Dark green colour indicates phenols
	Gelatin Test	Extract + gelatin + NaCl	White precipitate indicates phenols
	Lead Acetate Test	Extract + lead acetate	White precipitate indicates phenols
10	Flavonoids Detection	Extract + magnesium + conc. HCl	Yellow fluorescence or pink colour

			indicates flavonoids
11	Gums and Mucilage	Extract + ethanol	White precipitate indicates gums
12	Volatile Oils	Hydro-distillation of plant powder	Oil layer separation confirms volatile oils
13	Terpenoids Detection (Salkowski Test)	Extract + chloroform + conc. H_2SO_4	Reddish-brown interface indicates terpenoids
14	Phlobatannins Detection	Extract + HCl	Red precipitate indicates phlobatannins
15	Ascorbic Acid Detection	Extract + $NaHCO_3$ + $FeSO_4$ + H_2SO_4	Violet colour disappearance indicates ascorbic acid
16	Anthraquinone s Detection	Extract + benzene + NH_4OH	Pink colour indicates anthraquinones
17	Lignin Detection	Extract + phloroglucinol + HCl	Pink colour indicates lignin
18	Resins Detection	Extract + acetic anhydride + H_2SO_4	Orange to yellow colour indicates resins
19	Lactones Detection		
	Feigel's Test	Extract + ether + alcoholic KOH + $FeCl_3$	Light pink colour indicates lactones
	Baljet's Test	Extract + sodium picrate	Yellow-orange colour indicates lactones
20	Anthocyanin Detection	Extract + HCl heated	Persistent colour indicates anthocyanin
21	Betacyanin Detection	Extract + NaOH heated	Yellow colour indicates betacyanin

2.3. PREPARATION OF HERBAL OINTMENT [16-19]

The herbal ointment was prepared using a simple fusion method.

Ingredient	Quantity
Whitewax	3 g
Yellow paraffin	7 g
Plant extract	0.5 g
Distilled water	100 mL

PROCEDURE

A beaker containing 100 mL of distilled water was heated on a hot plate. Whitewax (3 g) and yellow paraffin (7 g) were added into the beaker and heated until completely melted. After complete melting, 0.5 g of the prepared plant extract was added to the mixture. The mixture was heated and stirred continuously for 1 hour until a uniform gel-like mixture was formed. The mixture was then allowed to cool and kept undisturbed overnight. On the next day, the preparation solidified to form a semi-solid herbal ointment (gel form) suitable for topical application.

2.4. EVALUATION OF OINTMENT PROPERTIES

The prepared herbal ointment was evaluated for its physicochemical properties.

2.4.1. COLOR AND ODOR

The color and odor of the ointment were observed visually and recorded.

2.4.2. CONSISTENCY

The consistency of the ointment was determined by visual inspection and touch to evaluate its smoothness and homogeneity.

2.4.3. pH DETERMINATION

The pH of the ointment was measured using a digital pH meter by dissolving a small quantity of ointment in distilled water.

2.4.3. SPREADABILITY

Spreadability was determined by placing a small quantity of ointment between two glass slides and applying light pressure. The ease with which the ointment spreads was observed.

2.4.4. STABILITY STUDY

The prepared ointment was stored at room temperature and observed for any changes in color, odor, or consistency over a period of time.

2.5. ANTIMICROBIAL ACTIVITY OF HERBAL OINTMENT:

2.5.1. PREPARATION OF MICROBIAL CULTURES

Pure cultures of the selected microorganisms were obtained from a standard microbial culture collection. The bacterial strains used in the study included *Streptococcus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, while the fungal strains included *Candida albicans*, *Aspergillus niger*,

and Trichophyton species. These organisms were selected because they are frequently involved in skin and wound infections. The bacterial cultures were maintained on nutrient agar slants, while the fungal cultures were maintained on potato dextrose agar (PDA) slants. Prior to the antimicrobial assay, the bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24 hours to obtain actively growing cultures. Similarly, fungal cultures were incubated in potato dextrose broth at 28°C for 48 hours to obtain sufficient growth. The microbial suspensions were then adjusted to obtain uniform inoculum density for the antimicrobial testing.

2.5.2. PREPARATION OF AGAR PLATES

Sterile nutrient agar medium was prepared for bacterial studies and potato dextrose agar medium was prepared for fungal studies. The media were sterilized by autoclaving at 121°C for 15 minutes and then allowed to cool slightly. After sterilization, approximately 20–25 mL of the molten agar medium was poured into sterile Petri plates under aseptic conditions and allowed to solidify at room temperature. Once the agar plates were solidified, the microbial cultures were spread evenly on the surface of the agar plates using a sterile cotton swab. This step ensured the formation of a uniform microbial lawn on the agar surface, which is essential for accurately measuring the antimicrobial activity of the test samples.

2.5.3. PREPARATION OF HERBAL OINTMENT SAMPLE

The herbal ointment prepared from the plant extract was used for antimicrobial testing. The ointment was dissolved in dimethyl sulfoxide (DMSO) to obtain a suitable test solution. Different concentrations of the ointment extract were prepared to evaluate the dose-dependent antimicrobial activity. In this study, 25 µL, 50 µL, 75 µL, and 100 µL of the ointment extract were used for the assay. DMSO was used as a solvent because it dissolves plant extracts effectively and does not interfere significantly with microbial growth at low concentrations. A control sample containing the standard antimicrobial agent was also included in the experiment to compare the effectiveness of the herbal ointment with known antimicrobial drugs.

2.5.4. AGAR WELL DIFFUSION METHOD

The antimicrobial activity of the herbal ointment was determined using the agar well diffusion technique. After inoculation of the microbial cultures on the agar plates, wells were created on the agar surface using a sterile cork borer. Each well had a uniform diameter of approximately 6 mm to ensure consistency in the experimental procedure. Different volumes of the herbal ointment extract (25 µL, 50 µL, 75 µL, and 100 µL) were carefully introduced into the wells using a micropipette. The control sample containing the standard drug was also added to a separate well. The plates were then kept at room temperature for a short period to allow the diffusion of the extract into the agar medium.

2.5.5. MEASUREMENT OF ZONE OF INHIBITION

Following incubation, the antimicrobial activity of the herbal ointment was determined by measuring the zone of inhibition around each well. The zone of inhibition represents the clear area surrounding the well where microbial growth has been prevented due to the action of antimicrobial compounds present in the sample. The diameter of the inhibition zone was measured in millimeters (mm) using a transparent ruler or Vernier caliper. A larger inhibition zone indicates stronger antimicrobial activity. The results obtained from different concentrations of the herbal ointment extract were recorded and compared with the control to evaluate the effectiveness of the formulation.

3. RESULTS AND DISCUSSION

3.1.1. PHYTOCHEMICAL SCREENING RESULTS

Preliminary phytochemical screening was performed on the ethanolic extract prepared from turmeric (*Curcuma longa*), eucalyptus (*Eucalyptus globulus*) leaves, and ginger (*Zingiber officinale*) to determine the presence of different classes of primary and secondary metabolites. The qualitative analysis included tests for alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, flavonoids, terpenoids, phlobatannins, lignins, resins, lactones, anthocyanins, betacyanins, fixed oils, volatile oils, anthraquinones, and ascorbic acid.

The extract showed a positive reaction for alkaloids in Mayer's, Wagner's, Hager's, and Dragendorff's tests, indicating the presence of alkaloidal constituents. Carbohydrates were detected by Molisch's, Fehling's, Barfoed's, and Benedict's tests. Glycosides showed positive reactions in Borntrager's and Legal's tests, while the formation of persistent foam in the foam test confirmed the presence of saponins.

Proteins and amino acids were detected by Millon's and ninhydrin tests, respectively. Phenolic compounds and flavonoids were also detected in the extract through their respective qualitative reactions. Terpenoids exhibited a strong positive reaction in the Salkowski test. Positive reactions were additionally observed for phlobatannins, lignins, resins, lactones, anthocyanins, and betacyanins.

In contrast, fixed oils, volatile oils, anthraquinones, and ascorbic acid were not detected or showed negative reactions under the experimental conditions. Overall, the phytochemical screening demonstrated the presence of several biologically active phytoconstituents in the ethanolic plant extract. The occurrence of alkaloids, phenolic compounds, flavonoids, terpenoids, saponins, and glycosides may contribute to the biological and antimicrobial potential of the formulated herbal ointment. However, qualitative phytochemical screening indicates only the presence or absence of compound classes and does not establish their individual concentrations or directly prove their contribution to antimicrobial activity.

Table-2: Qualitative Phytochemical Screening of Plant Extract

S.No	Phytochemical Test	Observation	Result
1	Alkaloids		
	Mayer's Test	Creamy white precipitate	+
	Wagner's Test	Reddish-brown precipitate	+++
	Hager's Test	Yellow precipitate	++
	Dragendorff's Test	Yellow precipitate	++
2	Carbohydrates		
	Molisch's Test	Violet ring formed	+
	Fehling's Test	Red precipitate	+++
	Barfoed's Test	Red precipitate	+
	Benedict's Test	Colored precipitate	++
3	Glycosides	Pink colour observed	+
	Borntrager's Test	Pink colour	+
	Legal's Test	Pink colour	+
4	Saponins	Stable foam layer formed	+
5	Proteins		
	Millon's Test	White precipitate	++

	Biuret Test	No significant colour change	—
6	Amino Acids	Purple colour formed	+
7	Phytosterols	Colour change observed	+
8	Fixed Oils and Fats		
	Spot Test	Oil stain absent	—
	Saponification Test	Soap formation absent	—
9	Phenolic Compounds		
	Ferric Chloride Test	Dark green colour	++
	Gelatin Test	White precipitate	+
	Lead Acetate Test	White precipitate	+
10	Flavonoids	Yellow fluorescence/pink colour	++
11	Gums and Mucilage	No precipitate observed	—
12	Volatile Oils	Oil layer not clearly separated	—
13	Terpenoids (Salkowski Test)	Reddish-brown interface	+++
14	Phlobatannins	Red precipitate formed	++
15	Ascorbic Acid	No significant colour change	—
16	Anthraquinones	No pink colour observed	—
17	Lignin	Pink colour observed	+
18	Resins	Orange-yellow colour	+
19	Lactones		
	Feigl's Test	Light pink colour	+
	Baljet's Test	Yellow-orange colour	+
20	Anthocyanin	Persistent colour observed	++
21	Betacyanin	Yellow colour observed	+

+ Present, ++ Moderately present, +++ Strongly present, - Absent

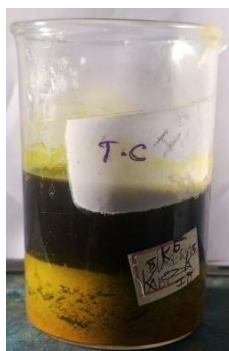


Figure-1: Extract Image

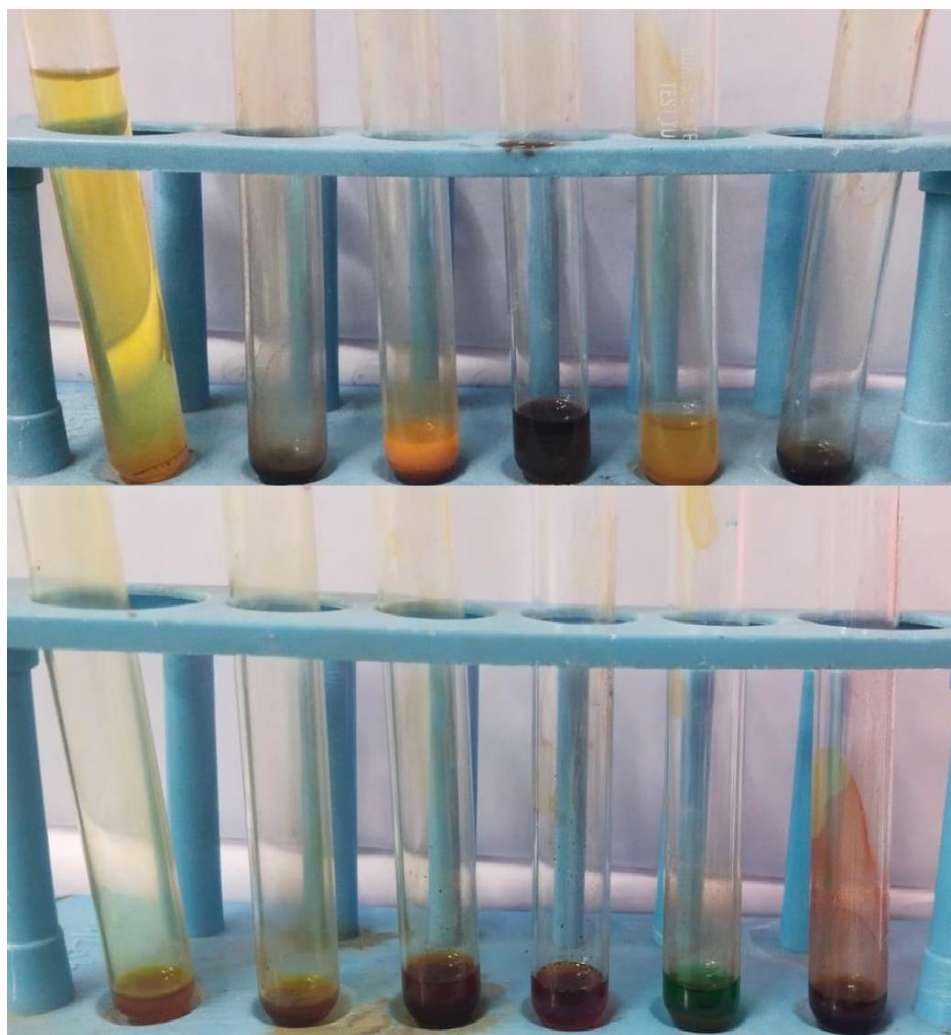


Figure-2:Qualitativeanalysisresultimage

3.1.2. EVALUATION OF OINTMENT PROPERTIES

The prepared herbal ointment was evaluated for various physicochemical properties such as color, odor, consistency, pH, and spreadability.

Table-3: Evaluation of Ointment Properties

Parameter	Observation
Color	Yellowish green
Odor	Characteristic herbal odor
Consistency	Smooth and semi-solid
pH	6.5 – 7
Spreadability	Good
Stability	Stable at room temperature

The results indicated that the prepared ointment had suitable physical properties for topical application. The pH of the ointment was found to be close to neutral, which is safe for skin application.

3.1.3. ANTIMICROBIAL ACTIVITY RESULTS OF OINTMENT:

3.1.4. ANTIBACTERIAL ACTIVITY

The ointment extract exhibited significant antibacterial activity against *Streptococcus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

- For *Streptococcus*, the zone of inhibition increased from 16 mm at 25 µL to 23 mm at 100 µL, which is higher than the control value of 20 mm, indicating strong antibacterial activity at higher concentrations.
- In the case of *E. coli*, the inhibition zone ranged from 15 mm to 25 mm, demonstrating a clear dose-dependent increase. At 100 µL, the activity (25 mm) was greater than the control (20 mm), suggesting that the ointment extract possesses potent antibacterial compounds effective against Gram-negative bacteria.
- Pseudomonas aeruginosa*, which is generally known for its resistance to many antimicrobial agents, also showed susceptibility. The zone of inhibition increased from 18 mm to 24 mm, surpassing the control value at higher concentrations.
- Overall, the results suggest that the ointment formulation has broad-spectrum antibacterial activity, particularly at higher extract concentrations.

3.1.5. ANTIFUNGAL ACTIVITY

The ointment also demonstrated notable antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton* species.

- For *Candida albicans*, the inhibition zone increased from 17 mm to 23 mm, indicating effective antifungal activity compared with the control (20 mm).
- *Aspergillus niger* showed the highest sensitivity, with inhibition zones ranging from 20 mm to 27 mm, suggesting that the ointment formulation is highly effective against filamentous fungi.
- Similarly, *Trichophyton* exhibited inhibition zones from 19 mm to 26 mm, indicating strong antifungal potential.

The results clearly demonstrate a concentration-dependent antimicrobial effect of the ointment extract. As the concentration increased from 25 μ L to 100 μ L, the zone of inhibition increased correspondingly for all tested microorganisms. Importantly, at 100 μ L concentration, the inhibition zones were greater than or comparable to the control, indicating that the ointment formulation possesses significant antimicrobial efficacy. Among the tested organisms, *Aspergillus niger* (27 mm) and *Trichophyton* (26 mm) showed the highest sensitivity, followed by *E. coli* (25 mm) and *Pseudomonas aeruginosa* (24 mm). This suggests that the ointment may be particularly effective in treating both bacterial and fungal skin infections. The formulated ointment containing the plant extract exhibited broad-spectrum antimicrobial activity against both bacterial and fungal pathogens. The results suggest that the formulation has promising potential for topical therapeutic applications, especially in the treatment of skin infections caused by pathogenic bacteria and dermatophytic fungi. Further studies such as minimum inhibitory concentration (MIC) determination and in vivo evaluation may be conducted to confirm its clinical applicability.

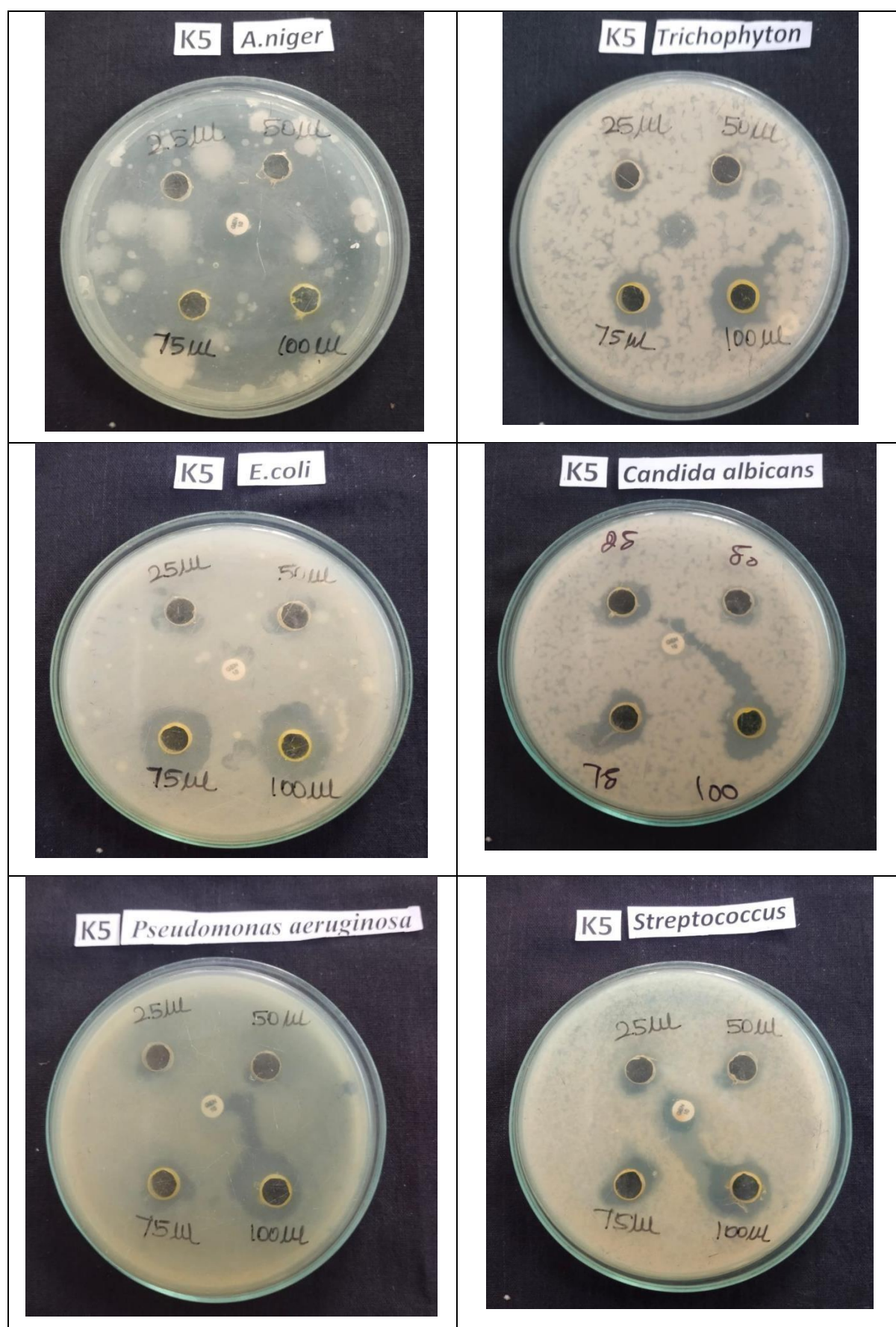


Figure-3:AntimicrobialActivityResultsofointment

Table-4:AntimicrobialActivityResultsofointment

SAMPLE	Zoneofinhibition (mm/ml)				
	25µl	50µl	75µl	100µl	Control
<i>Streptococcus</i>	16	18	20	23	20
<i>E.Coli</i>	15	18	22	25	20
<i>Pseudomonasaeruginosa</i>	18	20	22	24	20
<i>Candidaalbicans</i>	17	19	21	23	20
<i>A.niger</i>	20	22	25	27	20
<i>Trichophyton</i>	19	21	24	26	20

The combination of turmeric, eucalyptus, and ginger extracts may also produce a synergistic effect that enhances antimicrobial activity. Turmeric contains curcumin which has strong antimicrobial and anti-inflammatory properties. Eucalyptus leaves contain essential oils with antibacterial activity, while ginger contains bioactive compounds such as gingerol which contribute to antimicrobial effects. Therefore, the results of this study suggest that the prepared herbal ointment possesses significant antimicrobial potential and may be useful in treating skin infections and microbial diseases.

Following incubation, the antimicrobial activity of the herbal ointment was determined by measuring the zone of inhibition around each well. The zone of inhibition represents the clear area surrounding the well where microbial growth has been prevented due to the action of antimicrobial compounds present in the sample. The diameter of the inhibition zone was measured in millimeters (mm) using a transparent ruler or Vernier caliper. A larger inhibition zone indicates stronger antimicrobial activity. The results obtained from different concentrations of the herbal ointment extract were recorded and compared with the control to evaluate the effectiveness of the formulation.

4. CONCLUSION

The present study was carried out to prepare and evaluate a herbal ointment using extracts obtained from turmeric (*Curcuma longa*), eucalyptus leaves (*Eucalyptus globulus*), and ginger (*Zingiber officinale*). The plant materials were extracted using ethanol and subjected to preliminary phytochemical screening to identify the presence of bioactive compounds.

The phytochemical analysis revealed the presence of several important secondary metabolites such as alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenolic compounds, flavonoids, terpenoids, phlobatannins, lignins, resins, lactones, anthocyanins, and betacyanins. These compounds are known for their various biological activities including antimicrobial, antioxidant, and anti-inflammatory effects.

However, some compounds such as fixed oils, volatile oils, anthraquinones, and ascorbic acid were found to be absent in the plant

extract.

The herbal ointment was successfully prepared using white wax and yellow paraffin as the ointment base. The plant extract was incorporated into the base and heated to obtain a uniform gel-like formulation. The prepared ointment showed desirable physicochemical properties such as smooth texture, good consistency, pleasant herbal odor, and suitable pH for skin application. The ointment also exhibited good spreadability and stability.

The antimicrobial activity of the prepared herbal ointment was tested against three bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*) and three fungal strains (*Aspergillus niger*, *Candida albicans*, and *Penicillium* species). The results showed that the herbal ointment exhibited significant antimicrobial activity against both bacterial and fungal pathogens. Among the tested microorganisms, *Staphylococcus aureus* and *Candida albicans* showed comparatively higher sensitivity to the herbal formulation.

The antimicrobial activity observed in this study may be attributed to the presence of bioactive phytochemicals such as flavonoids, phenolic compounds, terpenoids, and alkaloids in the plant extract. The combined effect of turmeric, eucalyptus, and ginger may also contribute to enhanced antimicrobial activity due to their synergistic action. Overall, the results of this study demonstrate that the prepared herbal ointment possesses promising antimicrobial properties and can be considered as a potential natural topical formulation for the treatment of microbial infections and skin diseases.

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