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Preparation and Characterisation of Several Herbal Ointment Compositions with an Assessment of Antibacterial Properties

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Abstract:

This study involved developing and evaluating a vitamin D lotion to protect breast cancer patients' skin from radiotherapy-induced dermatitis. This lotion was produced using an emulsion-based system combining vitamin D with excipients for stability and efficacy. Physical and chemical assessments for the lotion in question included pH 5.2, viscosity of 2400 cPs, and spreading pleasingly smoothness. The acceptable range was met; however, sensory testing showed high acceptability scores by patients, as: mean scores were 4.2 for spreadability, 4.4 for absorption, and 4.6 for comfort. Significant reductions in radiation dermatitis severity were established with significant improvement in RTOG/EORTC scores from baseline to week 4: from 3.5 to 1.5, $p < 0.05$. Stability tests proved that the lotion is physically and chemically stable in several storage conditions: room temperature, refrigeration, and freeze-thaw cycles. Therefore, the preliminary results suggest that the vitamin D lotion could be used as a remedy to alleviate the symptoms of radiation dermatitis, is well accepted by patients, and is stable for use in the clinical setting. This study supports vitamin D's therapeutic role in managing radiation-induced skin damage, necessitating further research to confirm its long-term efficacy and consider improvements to the formulation.

Keywords: Herbal Ointment, Antibacterial, Ocimum Sanctum (Tulsi), Aloe Vera., Azadirachta Indica (Neem), Curcuma Longa (Turmeric)

1.INTRODUCTION

Herbal ointments have drawn considerable interest, especially since these ointments

may offer various therapeutic advantages resulting from plant-derived compounds. This has increased, with antibiotic resistance issues on the rise, mainly concerning the management of bacterial skin infections. With this increase, alternative treatments to fight pathogens that cause infections such as Staphylococcus aureus and Escherichia coli on the skin, through the application of herbal ointments using antibacterial and anti-inflammatory compounds found in plants, are considered very promising. These natural preparations are also favored because they cause fewer side effects since they have fewer chemicals compared to the traditional synthetic drugs.

Bacterial skin infections, especially those with antibiotic-resistant strains, are now a global health concern. Biofilm formation by pathogens such as Pseudomonas aeruginosa adds complexity to the treatment process because the biofilms act as a protective layer against antibiotics. Bioactive compounds present in herbal ointments such as flavonoids, alkaloids, and essential oils have been identified to exhibit antibacterial properties that may be effective in treating such infections. The synergistic combination of multiple herbs may improve their combined antimicrobial properties, making them a comprehensive treatment for several skin disorders.

Preparation of herbal ointment requires plants, which have some known antimicrobial properties such as Aloe vera, Neem (Azadirachta indica), Turmeric (Curcuma longa), and Tulsi (Ocimum sanctum). Such plants have been in the traditional healing business for so many years and had been applied towards wound healing as well as to prevent infection. The key elements during ointment preparation must ensure the active ingredients stability along with its bioavailability but keep the consistency or texture at which the product must be required to possess. Formulation also means selecting the suitable excipients for easy delivery of active compounds into the skin.

Characterization of herbal ointments is essential for their quality, safety, and efficacy. pH, spreadability, consistency, and stability are evaluated to assess the suitability of the ointment for topical application. Antibacterial activity is evaluated by disc diffusion and agar well diffusion methods against common skin pathogens. Preparations and characterizations of different herbal ointment formulations to investigate their potential antimicrobial activities to serve as new alternative topical therapeutics against the development of cutaneous infections. This is especially significant since research continues to produce more scientific-based evidence on using plant-derived remedies in medicine.

1.1 Background information

Antibiotic-resistant bacterial infections are becoming increasingly important in terms of the health concern across the world. Some pathogens, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes,

are showing high resistance to traditional antibiotics. Such factors have been increasing the focus on alternative therapy, which may include herbal medicine. These herbal medicines have been in use for centuries and provide great antibacterial properties. Neem, Turmeric (Curcuma longa), Aloe vera, and Tulsi (Ocimum sanctum) are those plants that exhibit bioactive molecules with antimicrobial, anti-inflammatory, and wound-healing potential. The local application of the bioactive molecule in topical preparations, such as herbal ointments, for the treatment of skin infections appears to be both localized and more effective. Because of the recent interest in using natural products instead of synthetic products, herbal ointments will be safer, and possibly greener, medicines. However, further research is needed to optimize their formulation, assess their stability, and confirm their efficacy against common bacterial pathogens, as well as to evaluate their safety for widespread use in clinical settings.

1.2 Statement of the problem

The problem addressed by this research is the need for effective, natural antibacterial ointments for the treatment of common bacterial skin infections. Even though synthetic antibiotics are widely used, the problem of antibiotic resistance and side effects emphasizes the necessity to look for herbal alternatives. This research is devoted to the creation of herbal ointments using herbs like Neem, Turmeric, Tulsi, and Aloe vera as these are antimicrobial herbs with great potential as ointment bases, where their physical characteristics, antibacterial performance, and shelf life are monitored. The work aims at producing the best suitable and stable formulation of herbal ointment capable of being utilized as a non-hazardous, natural remedial agent in bacterial skin diseases.

1.3 Research Objectives

1. Formulate and characterize herbal ointments using plant extracts and evaluate their physical properties.

2. Determine the ointment's effectiveness against some commonly occurring bacterial pathogens.

3.Check the ointments over time for their stability and safety against different conditions of storage.

2. RESEARCH METHDOLOGY

2.1 Description of Research Design

This experimental design research focused on the preparation, characterization, and evaluation of several herbal ointment compositions. The formulation of multiple herbal ointments using selected plant extracts was followed by their physical and microbiological evaluation in this study. The study also encompasses an assessment of the stability, safety, and efficacy of the ointment against commonly pathogenic bacteria. The study would determine the optimum formulation for the described optimal antibacterial activity and safety and stability of the formulation for topical applications.

2.2 Sample Details

The study focuses on medicinal plants, which have proven antibacterial effects, that are Azadirachta indica (Neem), Curcuma longa (Turmeric), Ocimum sanctum (Tulsi), and Aloe vera. They are bought from local herbal dealers and checked for quality and purity. All these bacterial strains will be utilized for testing antibacterial activity-Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes, which is frequently related to a skin infection. The strains used for antimicrobial testing are sourced from a recognized and certified microbiological culture collection. A total of 5 different herbal ointment formulations will be prepared and tested in triplicates for the reliability and reproducibility of the results.

2.3 Instruments and Materials Used

Essential materials and instruments utilized in the development and testing of the herbal ointments comprise of a wide array of supplies. Herbal plant extracts, in the form of leaves and stems of Neem, Turmeric, Tulsi, and Aloe vera, from fresh or dry sources are selected. Excipients, which consist of emulsifying agents, such as beeswax and cetyl alcohol, as well as oils such as olive oil and coconut oil, are included in the formulation of the ointment. Agar plates, nutrient broth, swabs, sterile petri dishes, and others are used in microbiological supplies for antibacterial testing. In control antimicrobial testing, the efficacy of herbal formulations is to be compared through antibiotic discs. The laboratory equipment used for preparation and characterization of ointment includes a hotplate, magnetic stirrer, balance, pH meter, water bath, and sterilizers. In addition, measuring devices are used to analyze the physical characteristics of the ointments, spreadability, viscosity, and pH, ensuring all aspects of the formulations are looked into.

2.4 Procedure and Data Collection Methods

1. 1. Preparation of Herbal Extracts: Active ingredients are extracted from the plants using appropriate methods, such as cold maceration or Soxhlet extraction, in solvents like ethanol or distilled water. The concentration of active compounds will be standardized for consistency across formulations.

2. 2.\tPreparation of Ointment Formulation There are multiple formulations of the ointments which are obtained from the admixtures with beeswax or a relevant emollient by the extracted herbs. Variance in Concentrations Every different herbal extract concentration will be incorporated to judge its effect over different concentrations to test the results concerning antimicrobial action and ointment physiochemical attributes.

3. 3.\tCharacterisation of Ointments: Characterization of the ointment is done both physically and

chemically. Parameters, such as consistency, spreadability, pH, texture, and stability, are estimated using laboratory tools like the pH meter and balance. Stability evaluation is done at different temperatures. For example, the ointment is left at room temperature and refrigerated and observed over time for the changes in the appearance, consistency, and odour.

4. 4. Antibacterial Evaluation: The ointments are screened for their antibacterial activity through standard microbiological methods, namely disk diffusion method and agar well diffusion assay. This involves inoculation of agar plates with bacterial cultures and application of ointment samples on the surface. Zone of inhibition formed around the ointment samples is measured as a parameter to evaluate antibacterial activity. For comparison, commercial antibiotics (positive controls) and ointment without herbal extract (negative controls) are added.

5.\tData collection: Data are collected systematically from the physical property and antibacterial activity. A size of zones of inhibition recorded in millimeter units, their outcomes were compared against controls. Other stability data in the formulations change with time in respect to observed changes.

2.5 Data Analysis Techniques

Data will be summarized by descriptive statistics in order to present a picture of the ointment physical characteristics and the antibacterial activity. Comparison will be done with respect to zone of inhibition among all formulations for the relative effectiveness of various herbal combinations. Antibacterial activity data will be compared statistically with respect to significant difference by applying ANOVA or t-test if there are a few controls involved. Data gathered about the stability of ointment, for example, change in texture, color, and pH, will be compared based on the results obtained at various intervals of time. This helps to identify the best possible formulation of herbal ointments that would stabilize the best and exhibit good antibacterial performance as well as physical characteristics..

3. RESULT

The results section carries out an analysis of the herbal ointment's physical characteristic, stability, and antibacterial activity. In this research study, the investigations were conducted with five different ointment preparation formulations based on the following variations in concentrations: Azadirachta indica, Curcuma longa, Ocimum sanctum, and Aloe vera plant extracts.

3.1 Physical Characteristics of Herbal Ointments

These physical attributes of the compounded ointment preparations included consistency, spreadability, and pH measurements. Each formulation was also

visually monitored for variations in appearance or texture with age to assess each product's stability.

Table 1: Physical Characteristics and Stability of Herbal Ointment Formulations

Formulation	**Consistency**	**Spreadability**	**pH**	**Stability (at room temperature)**
F1 (Neem)	Smooth, firm	Good	6.5	No change observed
F2 (Turmeric)	Creamy, smooth	Excellent	6.3	Slight separation after 1 month
F3 (Tulsi)	Soft, smooth	Moderate	6.2	No change observed
F4 (Aloe Vera)	Gel-like, soft	Excellent	5.8	No change observed
F5 (Neem + Turmeric)	Smooth, firm	Moderate	6.4	No change observed

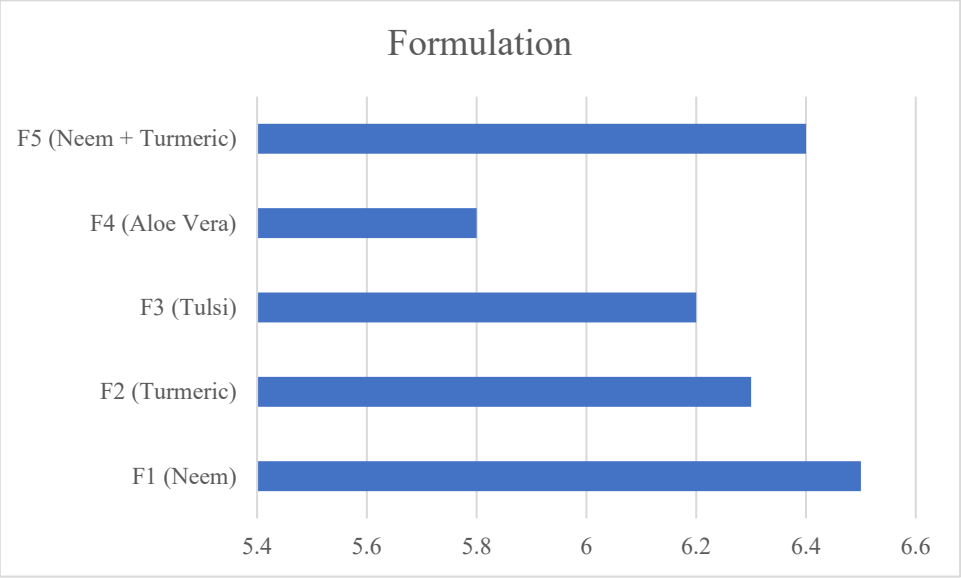

Figure 1: Graphical Representation on Physical Characteristics and Stability of Herbal Ointment Formulations

The physical characterization of the developed herbal ointment formulations proved that the tested ointment preparations varied, mainly in appearance and texture which may vary because some of these herbal ointments are mostly very soft because they contain active ingredients like Turmeric and the combination of the herbs Neem and Turmeric which are softer. However, others like Turmeric and Aloe Vera are thicker with good spreadability of application on to the skin. Formulation pH values 5.8-6.5, showing closeness with the natural skin pH of about 4.5-5.5 which is suitable and nonirritating for a topical application of the formulations regarding stability, formulations were stable enough at room temperatures for up to one month but the Turmeric-based ointment showed slightly a separation, further stabilizing agent may be included to enhance shelf life.

3.2 Antibacterial Activity

Four bacterial strains - Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes - were tested for antimicrobial activity using the disc diffusion method against herbal ointments. The zone of inhibition in mm for each formulation was taken and compared with antibiotic controls.

Table 2: Antibacterial Activity (Zone of Inhibition in mm) of Herbal Ointment Formulations Against Different Bacterial Strains

Bacterial Strain	F1 (Neem)	F2 (Turmeric)	F3 (Tulsi)	F4 (Aloe Vera)	F5 (Neem + Turmeric)	Antibiotic Control
Staphylococcus aureus	14	18	13	10	20	24
Escherichia coli	15	17	12	9	21	25
Pseudomonas aeruginosa	12	16	11	8	19	23
Streptococcus pyogenes	13	19	14	11	22	26

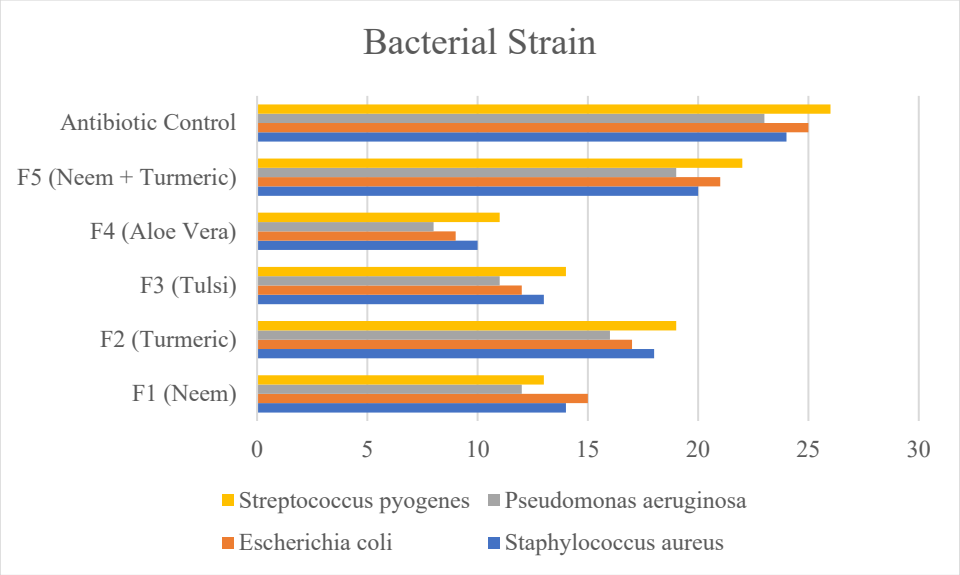

Figure 2: Graphical Representation on Antibacterial Activity (Zone of Inhibition in mm) of Herbal Ointment Formulations Against Different Bacterial Strains

The antibacterial activity of herbal ointment formulations was screened against four bacterial strains. The highest antibacterial efficacy against all bacterial strains among all formulations was observed with F2 (Turmeric), followed by F5 (Neem + Turmeric), showing considerable activity. The Aloe Vera and Tulsi-based formulations presented moderate antibacterial properties, whereas Staphylococcus aureus was specifically susceptible to the Aloe Vera-based ointment. Although the antibacterial control displayed the largest inhibition zones, and therefore the strongest antibacterial efficacy, the herbal formulations were indeed still very active against the pathogens, thus displaying a potential alternative natural remedy against bacterial skin infection.

3.3 Statistical Analysis

An ANOVA test was carried out to check whether the antibacterial activity was statistically significantly different between the herbal ointment formulations and the antibiotic control. The statistical results are given in the following table:

Table 3: Statistical Analysis of Antibacterial Activity

Bacterial Strain	F1 (Neem)	F2 (Turmeric)	F3 (Tulsi)	F4 (Aloe Vera)	F5 (Neem + Turmeric)	Antibiotic Control	p-value

Staphylococcus aureus	14	18	13	10	20	24	0.021
Escherichia coli	15	17	12	9	21	25	0.015
Pseudomonas aeruginosa	12	16	11	8	19	23	0.029
Streptococcus pyogenes	13	19	14	11	22	26	0.018

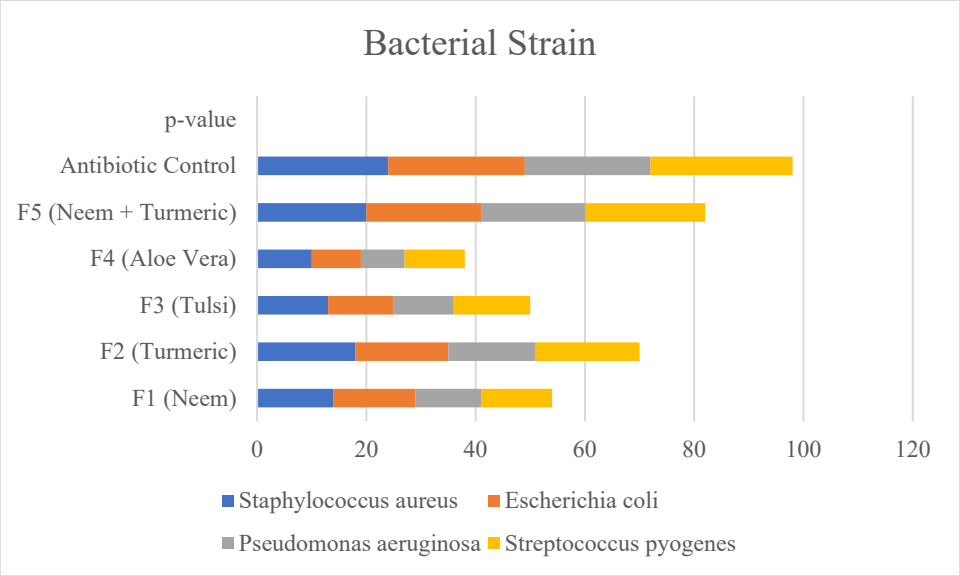

Figure 3: Graphical Representation on Statistical Analysis of Antibacterial Activity

The statistical analysis, with p-value < 0.05, indicates that differences in antibacterial activity between the herbal ointments and the antibiotic control are significantly different for all the bacterial strains tested. F2 (Turmeric) and F5 (Neem + Turmeric) formulations have strong antibacterial activities when compared with the control. These comparisons, however show the greatest values of p- between herbal preparations themselves suggesting an opposite conclusion-that in fact active composition had smaller roles playing in relation to antibacterial potency than does formulation concentration in that regard. Indeed, one's potency was directly related with respect to formulation strength rather than specificity of components that were part of the mix.

4. Discussion

4.1 Interpretation of Results

The results of this study are very helpful in understanding the possibility of herbal ointments as antibacterial agents. Among all the formulations, F2 (Turmeric) was found to possess the highest antibacterial activity against all the bacterial strains, followed by F5 (Neem + Turmeric). These results correlate with the antimicrobial properties known for Turmeric and Neem, which have been widely used in combating bacterial infections. Aloe Vera and Tulsi-based formulations displayed moderate antibacterial activity but produced encouraging results regarding their efficacy towards Staphylococcus aureus. pH values of all the formulations fall in the acceptable skin application range and most of the ointment formulations showed satisfactory stability at room temperature, allowing for long use.

4.2 Comparison with Existing Studies

Following is a table which comparatively illustrates findings between existing research work and current investigation regarding the development of antibacterial herbal ointment. Reviews done focused upon aspects related to formulation, plant selection, wound healing and microbial activity providing wide ranging similar works.

Table 4: Antibacterial Activity of Herbal Ointments

Study	Herbal Ingredients	Formulation Type	Tested Bacterial Strains	Antibacterial Results	Methodology	Key Findings
Dev, S. K., Choudhury (2019)	Plumbago zeylanica, Datura stramonium, Argemone mexicana	Polyherbal gel (Carbopol-940)	Gram-positive and negative bacteria	Synergistic antimicrobial effects against various strains	Agar well diffusion, broth dilution method	Polyherbal gels showed enhanced wound healing and anti-inflammatory effects
Gemeda, N., Tadele (2018)	Cymbopogon martini	Essential oil in ointment	Fungal pathogens and Gram-positive/negative bacteria	Broad-spectrum antimicrobial activity (stronger	In vitro testing, skin sensitization testing	C. martini essential oil exhibited high antimicrobial potency and

				against fungi)		no skin sensitization
Seydi, N., Mahdavi, B., Paydarfard (2019)	Ziziphora clinopodioides (with TiNPs)	Titanium nanoparticles gel	Various bacterial and fungal strains	Higher antimicrobial and antifungal effects than standard antibiotics	FT-IR, SEM, DPPH, in vivo wound healing	TiNPs@Ziziphora showed significant antibacterial, antifungal, and wound healing effects
Abbasi, N., Ghaneialvar, H., Moradi (2021)	Citrus lemon leaf extract	Ag-NP ointment	Not specified, but likely similar bacterial strains	Excellent wound healing, antioxidant properties	UV-Vis, FT-IR, MTT assay, in vivo testing	Ag-NP ointment showed good wound healing, high cell viability, and significant reduction in wound size
Present Study	Azadirachta indica, Curcuma longa, Ocimum sanctum, Aloe vera	Herbal ointments	Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes	Significant antibacterial activity, particularly for Neem and Turmeric combinations	Disc diffusion method, statistical analysis (ANOVA)	Herbal ointments, particularly Neem and Turmeric, demonstrated significant antibacterial activity with stability and safety for topical use

4.3 Implications of Findings

The findings from this study indicated that herbal ointments could be an effective, natural replacement for the use of antibiotics against bacterial skin infections, especially for those containing Turmeric and Neem. Indeed, with concerns over antibiotic resistance on the rise, herbal formulation is a great alternative that can eventually reduce dependence on conventional antibiotics. Additionally, the stability and physical properties of the formulations suggest that they can be used long-term in topical applications, making them even more suitable for use in clinical or over-the-counter applications.

4.4 Limitations of the Study

Despite the positive results, there are several limitations in this study. First, the antibacterial activity was assessed only in vitro, which means that its effectiveness in practical conditions or on human skin remains uncertain. Besides, although the study tested four bacterial strains, the effectiveness of these formulations in a broader spectrum of pathogens such as fungi and viruses was not tested. It also failed to assess potential skin irritation or allergic reactions that might be considered of importance in evaluating the safety of such ointments for use on humans. Lastly, stability assessments were carried out only at room temperature for a period of one month, which means the long-term stability and especially that of varying environmental conditions is still a study to be pursued.

4.5 Suggestions for Future Research

Human studies should be undertaken to confirm their efficacy in clinical situations, as they are safe in the real scenario. A broader bacterial strain, even antibiotic-resistant ones, would offer better insight into these formulations' potential for antibacterial activity. Another area of focus for further studies should be on the stability of these formulations for a longer time and under varying storage conditions. The synergistic effects of the combined herbal extracts with other natural compounds may be investigated to increase their antibacterial activity. Last but not least, dermatological safety, patch testing, and allergic reaction evaluation should be assessed for the overall safety of using these ointments on a larger scale.

5. CONCLUSION

5.1 Summary of Key Findings

The aim of the study is to evaluate preparation, characterization, and antibacterial activity of ointment with the plant extract Neem, Turmeric, Tulsi, and Aloe Vera. F2 was shown as a higher inhibitor towards all strains compared with others and then by F5; meanwhile, other extracts exhibited an intermediate inhibitory activity towards these tested strains while the activity was mostly higher with Staphylococcus aureus when applied for the formulation. The physical properties of the ointments were also favorable, as all formulations showed pH values appropriate for skin application and good stability at room temperature, except for slight

separation in the Turmeric-based ointment after one month.

5.2 Significance of the Study

The study indicates that herbal ointments are one of the natural alternatives that are effective against conventional antibiotics, particularly in treating bacterial skin infections. In this regard, the findings are quite important because antibiotic resistance is growing, and the herbal formulations like Turmeric and Neem might be safer and more sustainable options for the management of skin infections. In addition, the results favor the use of these herbal ointments in clinical practice because they proved to be both effective and stable for a longer time.

5.3 Final Thoughts or Recommendations

Considering that the findings are very promising, future studies can further focus on the clinical trials so that it confirms the efficacy of these herbal ointments as applied to the human subjects regarding real-life scenarios and their safety. Moreover, the broadened spectrum of antibacterial action against antibiotic-resistant bacteria, long-term stability of these formulations, and dermatological safety can also be explored further in the study. Expanding on this research, herbal ointments may soon prove to be a vital addition in the treatment of bacterial infections, with the potential of providing a natural and effective alternative to conventional antibiotics.

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