



# Comparative Analysis Of A Novel Polyherbal Ointment With Standard Antibiotics Against Environmental Isolates

Dr. Khadatare Seema Vilas<sup>1</sup>, Ms. Salar Iramsaba Amjad Hussain<sup>2</sup>, Ms. Kalyani Zobia M. Toufique<sup>2</sup>, Ms. Shaikh Arshiya Hussain<sup>2</sup>, Ms. Bagwan Bushra Ejaz A.<sup>2</sup>

Department of Botany, Sangameshwar College, Solapur (Autonomous)

## Abstract

The extensive and frequently inappropriate utilization of synthetic antimicrobials has expedited the development of antimicrobial resistance, thereby diminishing the clinical efficacy of conventional antibiotics and necessitating the exploration of sustainable alternatives, such as plant-derived topical formulations with multi-target mechanisms and potential synergistic effects. This study sought to develop a polyherbal antimicrobial ointment from medicinal plants and assess its antibacterial and antifungal efficacy against microorganisms sourced from the environment. Fresh oregano, mint, Tulsi, and calendula were subjected to shade-drying (12–24 h) and extracted via coconut-oil infusion (low heat 30–40 min; standing 12 h), while aloe vera gel was aseptically prepared and mildly heated (5 min), and propolis was extracted in warmed coconut oil using a double boiler (20 min). The ointment was formulated by melting beeswax (20–25 g) into the herbal and propolis oils, followed by the incorporation of aloe gel and vitamin E at approximately 40 °C. Microorganisms were isolated from open-air exposure plates and serially diluted soil samples on nutrient agar and potato dextrose agar, purified, and standardized to 0.5 McFarland. Antimicrobial activity was evaluated using the agar well diffusion method (6 mm wells; 100 µL ointment), benchmarked against amoxicillin (10 µg), streptomycin (10 µg), and fluconazole (10 µg), with base-only negative controls; inhibition zones were measured in triplicate. The ointment demonstrated clear inhibition zones against *Staphylococcus* spp. ( $18.0 \pm 0.5$  mm) and *Bacillus* spp. ( $16.0 \pm 0.4$  mm), and inhibited *Aspergillus* spp. ( $14.0 \pm 0.3$  mm) and *Penicillium* spp. ( $15.0 \pm 0.4$  mm). Relative to the standards, it achieved approximately 81–82% effectiveness compared to streptomycin against *Staphylococcus*, approximately 72–75% compared to amoxicillin against *Bacillus*, and approximately 70–75% compared to fluconazole against fungi, albeit with slightly smaller zones than synthetic agents. The results suggest broad-spectrum activity likely driven by complementary phytochemicals (e.g., carvacrol/thymol, curcumin, allicin, eugenol, flavonoids) and an oil-beeswax matrix that may enhance stability and contact time. Overall, this eco-friendly polyherbal ointment shows promise as a supplementary or alternative topical antimicrobial, warranting further investigation into pathogen-level identification, stability and toxicity testing, and clinical validation.

**KeyWords-** Antimicrobial resistance, Plant-derived topical formulations, Polyherbal antimicrobial ointment, Medicinal plants, Phytochemicals, Broad-spectrum activity and Alternative antimicrobial

## 1. Introduction

The extensive application of synthetic antimicrobial agents in recent decades has led to a concerning rise in resistant microbial strains, posing a growing and serious threat to global public health systems. Antimicrobial resistance (AMR) diminishes the effectiveness of standard antibiotics, complicating treatment plans and resulting in higher rates of illness, death, and healthcare costs worldwide. (Agyeman et al., 2022) The rapid evolution of microorganisms in response to synthetic drugs—primarily due to their excessive and improper use in both medical and agricultural contexts—has hastened the emergence of multidrug-resistant pathogens. These resistant strains limit the range of effective treatments available and promote the transmission of infections in communities and healthcare settings, presenting a significant obstacle to contemporary medicine. (Alam et al., 2019)

As antimicrobial resistance (AMR) continues to escalate as a serious concern, there is a growing demand for novel and sustainable antimicrobial approaches. The effectiveness of synthetic antibiotics, once considered groundbreaking, is diminishing, leading to increased focus within scientific and medical communities on identifying alternative antimicrobial sources. (Ikpe et al., 2024) In Algeria and the broader Mediterranean region, *J. phoenicea* is highly valued for its ethnobotanical applications in traditional medicine. The plant's above-ground parts are frequently utilized to manage a range of health conditions, including skin issues, respiratory problems, diabetes, and digestive disorders. (Djahafi et al., 2021) Consequently, medicinal plants have garnered renewed interest due to their potential as a rich source of bioactive compounds with diverse pharmacological effects. Traditionally employed in various medical systems globally, these plants represent a promising avenue for the discovery of new antimicrobial agents capable of circumventing current resistance mechanisms. (Dantas et al., 2025)

Numerous plants and their extracts exhibit promising antifungal properties against *Aspergillus* species, potentially offering safer and more effective treatment alternatives. Essential oils derived from various plants have demonstrated significant anti-*Aspergillus* activity, with some proving more effective than traditional antifungal medications. Furthermore, the study identified the presence of *S. aureus*, *E. coli* O157:H7, and *K. pneumoniae* in both raw and processed meat samples, observing a higher incidence in processed meats. It assessed the antibacterial effects of essential oils and spice powders from cumin, black seeds, cloves, cinnamon, and marjoram against these pathogens. (Meshaal et al., 2021)

Enhancing the effectiveness of plant extracts can be achieved through meticulous formulation and their combination with other substances. Research has shown that mixing various plant extracts or incorporating them with additional compounds can lead to synergistic effects, thereby amplifying their biological activities beyond what individual extracts can accomplish. Polyherbal formulations have demonstrated improved antioxidant properties. For instance, blends of aqueous extracts from *Strobilanthes crispus*, *Phyllanthus niruri*, *Orthosiphon aristatus*, and *Stevia rebusiana* have shown synergistic radical scavenging activity, with some combinations significantly enhancing antioxidant potential compared to single extracts. (Özkan et al., 2003). In a comparable way, the integration of extracts from the *Bauhinia kockiana* flower and stem has led to superior antibacterial effects, effectively combating pathogens and minimizing biofilm formation through a range of mechanisms, such as the disruption of cell membranes. (Soulaimani, 2025)

The integration of plant extracts with antibiotics or other pharmaceutical agents has shown promise in addressing antimicrobial resistance. Research suggests that the combination of plant extracts and antibiotics can produce synergistic antibacterial effects, thereby enhancing their effectiveness against resistant bacteria. For example, when extracts from *Psidium guajava* and *Calotropis procera* are used alongside antibiotics, they significantly increase the zones of inhibition against drug-resistant pathogens, highlighting the potential for improved therapeutic outcomes through such combinations. (Ko et al., 2017). Similarly, the combination of herbal extracts with antibiotics led to heightened antibacterial activity against periodontal pathogens, highlighting the advantage of integrating plant-based compounds with traditional medications for better therapeutic results. Furthermore, the combination of specific herbal extracts with other bioactive compounds, such as curcumin, has demonstrated enhanced antimalarial effects, both *in vitro* and *in vivo*, without causing toxicity. This illustrates how strategic combinations can lead to improved therapeutic outcomes. (Ozma et al., 2022)

Plant extracts contain antimicrobial compounds that exert their effects through a variety of potential mechanisms, often differing from conventional synthetic antibiotics by targeting unique sites or processes within microbial cells. Numerous phytochemicals derived from plants work synergistically to enhance antimicrobial effectiveness rather than functioning as individual agents.

A prevalent approach involves disrupting the structural integrity of microbial cell membranes or walls, leading to increased permeability, leakage of cellular substances, and ultimately, cell death. For instance, extracts from *Trifolium baccarinii* have exhibited antibacterial effects by causing bacteriolytic damage that weakens bacterial cell walls and reduces antioxidant defenses within the cells, thereby making them more prone to oxidative harm (Sun et al., 2017)

An alternative strategy involves obstructing microbial enzymes or biochemical pathways essential for pathogen survival or replication. The structural diversity of plant compounds enables them to target multiple pathways simultaneously, such as inhibiting nucleic acid synthesis, disrupting energy metabolism, or interfering with quorum sensing, thereby limiting microbial growth or reducing virulence.

Oxidative stress can be triggered by certain phytochemicals, which either produce reactive oxygen species (ROS) or impair the pathogen's antioxidant defenses, resulting in damage to DNA, proteins, and lipids. This process is demonstrated by the reduced activity of antioxidant enzymes in bacterial cells treated with specific plant extracts. (Sebghatollahi et al., 2025) Plant-derived antimicrobials can collaborate with standard antibiotics or interact among various phytochemical elements within the extracts, enhancing the overall antimicrobial efficacy and addressing antibiotic-resistant bacteria. This synergistic effect may arise through mechanisms such as boosting antibiotic uptake, inhibiting enzymes that break down antibiotics, or targeting several microbial pathways at once, positioning plant extracts as a promising candidate for combination therapies (Dassanayake et al., 2021). Moreover, plant extracts frequently demonstrate a wide range of antimicrobial effects, impacting bacteria, fungi, and viruses. This indicates that their compounds may target essential and conserved microbial structures or processes. Variations in extraction methods and the types of plants used can impact the scope and strength of these mechanisms, underscoring the importance of establishing standardized procedures to maximize their effectiveness. (Vaou et al., 2021) Antimicrobial agents found in plant extracts primarily operate by compromising the integrity of microbial membranes and cell walls, obstructing crucial biochemical pathways, triggering oxidative stress, and working in conjunction with other compounds or antibiotics. These varied actions target a range of microbial functions, offering a robust defense against pathogens, including those that are drug-resistant. (Mulat et al., 2025). Phytochemicals, when used alongside standard antibiotics, can amplify antibacterial potency and effectively tackle resistance mechanisms through their synergistic interactions. Plant extracts from *Aegle marmelos* and *Terminalia chebula*, when used together, demonstrate superior antimicrobial properties against MDR isolates compared to their individual use. (Wolska et al., 2012)

Moreover, plant extracts often show varying levels of effectiveness against Gram-positive and Gram-negative bacteria, typically being more effective against Gram-positive strains. For example, methanol extracts from *Caesalpinia sappan* have been found to possess stronger antimicrobial properties and lower MIC values when tested against Gram-positive bacteria like *Listeria monocytogenes* compared to Gram-negative bacteria (Anupama et al., 2017). The antibacterial properties of plant extracts can differ significantly depending on factors such as plant type, extraction method, concentration, and the specific pathogens targeted. For example, extracts from certain herbal plants, when tested in milk models, showed less potent antimicrobial effects than some antibiotics. However, they still effectively inhibited pathogens like *Staphylococcus aureus* and *Listeria monocytogenes*, suggesting their potential use as natural antimicrobial agents in food preservation or safety. (Angane et al., 2023)

Medicinal plants are rich in diverse chemical compounds, including phenolics, flavonoids, terpenoids, alkaloids, and essential oils, many of which exhibit notable antimicrobial properties against a wide range of pathogens. Unlike synthetic antibiotics, which often target specific bacterial functions, phytochemicals typically operate through multiple mechanisms, such as disrupting microbial cell walls and membranes, inhibiting enzyme activity, and interfering with nucleic acid synthesis. (Stanojevic et al., 2017) Targeting multiple mechanisms simultaneously reduces the likelihood of resistance development and enhances the therapeutic value of plant-based compounds. Evidence from studies indicates, for example, that phenolic compounds can damage bacterial membrane structure, leading to leakage of cellular contents and

subsequent cell death. Conversely, flavonoids can interfere with nucleic acid synthesis and disrupt energy metabolism in microorganisms.

Topical ointments derived from medicinal plants, a type of herbal formulation, offer several advantages over synthetic drugs. These include a lower risk of adverse side effects, superior biodegradability, and improved patient adherence, largely due to their natural origins and cultural acceptance. Direct application to the skin enables targeted therapy, minimizing overall exposure and the risk of systemic toxicity. Furthermore, integrating various plant extracts into polyherbal formulations can leverage synergistic effects of different phytochemicals, potentially enhancing antimicrobial effectiveness and broadening the range of action against diverse microbial strains. This synergy can simultaneously address multiple microbial pathways, decreasing the likelihood of resistance development and improving therapeutic outcomes.

Recent studies have highlighted the antimicrobial properties of plants like *Origanum vulgare* (oregano), *Mentha spp.* (mint), *Ocimum sanctum* (Tulsi), *Calendula officinalis* (calendula), *Curcuma longa* (turmeric), *Allium sativum* (garlic), *Aloe barbadensis* (aloe vera), and propolis.. These plants contain bioactive substances such as carvacrol, thymol, curcumin, allicin, eugenol, and flavonoids, which have demonstrated strong antibacterial and antifungal properties. For example, oregano essential oil disrupts bacterial membranes and inhibits microbial proliferation, while curcumin affects bacterial cell division and metabolism. Similarly, allicin from garlic and eugenol from Tulsi exhibit wide-ranging antimicrobial effects. Consequently, incorporating these plants into topical formulations may provide effective, natural alternatives or supplements to traditional antimicrobial treatments.

In response to the critical global health threat of antimicrobial resistance and the limitations of current synthetic drugs, the pursuit and assessment of plant-based topical antimicrobial agents represents a promising and sustainable strategy. This research aims to develop a polyherbal antimicrobial ointment utilizing extracts from renowned medicinal plants and to evaluate its antimicrobial properties against bacteria and fungi sourced from the environment. Employing established microbiological methods, this study seeks to thoroughly assess the ointment's viability as a natural, environmentally friendly, and effective topical antimicrobial solution, thereby contributing to the growing body of research on alternatives to traditional antibiotics.

## 2. Materials and Methods

### 2.1 Materials

All plant materials and base ingredients used in the study are listed in Table 1.

Table 1. Ingredients Used for Ointment Preparation

Category	Ingredient	Quantity
Plant materials	Fresh oregano leaves	50 g
	Fresh mint leaves	50 g
	Fresh Tulsi leaves	50 g
	Fresh calendula flowers	30 g
	Turmeric powder / fresh rhizome	20 g
	Garlic cloves	10 g
	Aloe vera gel	50 g
	Raw propolis	10–15 g

Base ingredients	Coconut oil	200 ml
	Beeswax	20–25 g
	Vitamin E-Oil	

## 2.2 Preparation of Plant Extracts

### 2.2.1 Oil Infusion Extraction

Fresh plant materials were meticulously washed with distilled water to eliminate surface contaminants and subsequently shade-dried under ambient conditions for 12 to 24 hours to reduce moisture content while preserving sensitive phytochemicals. The dried materials were finely chopped to enhance surface area and then infused into coconut oil using a sterile stainless-steel container. The mixture was gently heated on a low flame for 30 to 40 minutes, carefully avoiding oil reaching its smoking point to prevent degradation of heat-sensitive compounds. Following heating, the infusion was allowed to stand for 12 hours to maximize phytochemical extraction, after which it was filtered through sterile muslin cloth to obtain a clear herbal oil extract rich in bioactive constituents.

### 2.2.2 Preparation of Aloe Vera Gel

The aloe vera leaves were thoroughly cleansed, and the outer rind was aseptically excised to prevent contamination. The inner gel was then meticulously extracted and blended to achieve a homogeneous consistency. To reduce microbial presence and enhance safety for topical application, the gel was subjected to mild heating for 5 minutes, followed by cooling to ambient temperature before incorporation into the ointment.

### 2.2.3 Propolis Oil Extraction

The raw propolis was pulverized to enhance extraction efficiency and subsequently combined with warmed coconut oil. This mixture was heated using a double boiler for 20 minutes, facilitating the extraction of bioactive compounds while minimizing direct exposure to elevated temperatures. Following heating, the mixture underwent filtration to eliminate impurities, resulting in a propolis oil extract characterized by significant antimicrobial properties.

## 2.3 Formulation of Polyherbal Ointment

The ointment was formulated using the double boiler technique. Herbal oil extract and propolis oil were gently heated, after which beeswax (20–25 g) was added. The mixture was continuously stirred until the beeswax was completely melted. Upon cooling to approximately 40 °C, aloe vera gel and vitamin E oil were incorporated with ongoing stirring to ensure homogeneity. The final ointment was subsequently poured into sterile glass containers and allowed to solidify at room temperature, resulting in a stable, uniform polyherbal formulation suitable for topical application.

## 2.4 Isolation and Purification of Microorganisms

### 2.4.1 Preparation of Culture Media

Nutrient agar (NA) and potato dextrose agar (PDA) were prepared and sterilized by autoclaving at 121 °C for 15 minutes to maintain aseptic conditions. The sterile molten media were then carefully poured into Petri dishes under aseptic conditions and allowed to solidify prior to use.

### 2.4.2 Isolation by Open Plate Method

To capture airborne microorganisms, sterile NA and PDA plates were exposed to the laboratory atmosphere for 10 to 15 minutes. Following exposure, the plates were incubated at 37 °C for 24 hours to promote bacterial proliferation and at 28 °C for 48 to 72 hours to support fungal development, thereby facilitating the

isolation of a variety of environmental microbes. This procedure facilitated the isolation of a variety of environmental microbes.

#### 2.4.3 Soil Sample Isolation

A one-gram soil sample was suspended in 9 mL of sterile distilled water and then subjected to serial dilutions to achieve a  $10^{-4}$  dilution. From these dilutions, a 0.1 mL aliquot was systematically spread onto NA and PDA plates. The incubation conditions were consistent with those used in the open plate method.

#### 2.4.4 Purification and Standardization of Inoculum

Colonies exhibiting distinct morphological traits were isolated and purified via quadrant streaking on fresh agar plates to obtain pure cultures. These cultures were subsequently suspended in sterile normal saline (0.85% NaCl) and adjusted to a turbidity matching the 0.5 McFarland standard, thereby ensuring a consistent inoculum density for antimicrobial testing.

### 2.5 Evaluation of Antimicrobial Activity

#### 2.5.1 Agar Well Diffusion Assay

Bacterial and fungal isolates were systematically cultured on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) plates, respectively, using a standardized inoculum. Wells, 6 mm in diameter, were formed in the agar using a sterile cork borer. Approximately 100  $\mu$ L of liquefied polyherbal ointment was then introduced into each well to evaluate its antimicrobial efficacy.

#### 2.5.2 Comparative Analysis with Standard Drugs

Aseptic placement of antibiotic discs containing amoxicillin (10  $\mu$ g), streptomycin (10  $\mu$ g), and fluconazole (10  $\mu$ g) on inoculated plates served as positive controls. Amoxicillin and streptomycin were assessed against bacterial isolates, and fluconazole was utilized for fungal isolates. A negative control, consisting of an ointment base without herbal extracts, was maintained to account for any inherent antimicrobial properties of the base components.

#### 2.5.3 Incubation and Measurement

Bacterial cultures were incubated at 37 °C for 24 hours, and fungal cultures were maintained at 28 °C for 48 to 72 hours. The zones of inhibition surrounding the wells and discs were quantified in millimeters using a digital Vernier caliper. All experimental procedures were performed in triplicate to ensure reproducibility, and the results are reported as mean  $\pm$  standard deviation.

## 3. Results

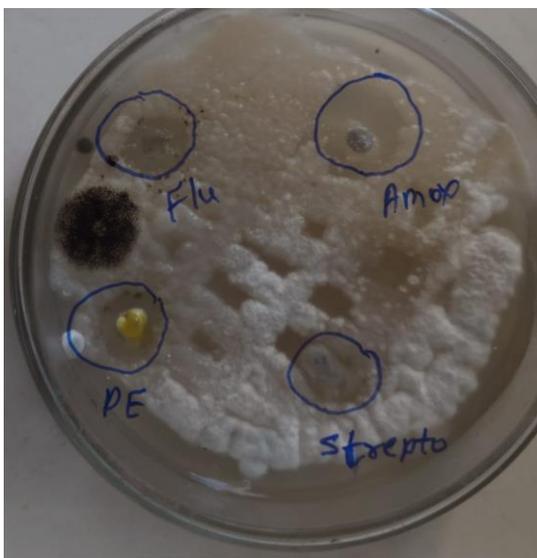
The newly formulated polyherbal ointment demonstrated strong antibacterial and antifungal properties when tested against environmental microbial isolates. The ointment consistently produced clear inhibition zones around the wells, mirroring those observed with conventional antimicrobial agents. Among the bacterial isolates, *Staphylococcus spp.* from airborne sources demonstrated the highest level of sensitivity, followed closely by *Bacillus spp.* from soil samples. Regarding antifungal efficacy, the ointment was notably effective against *Aspergillus spp.* and *Penicillium spp.*

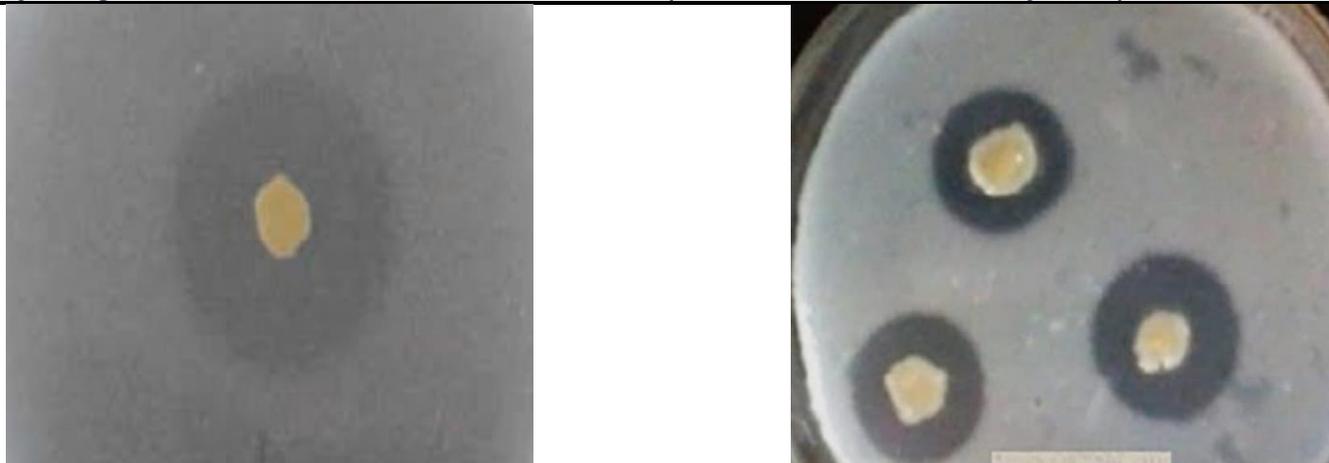
In comparison to conventional antibiotics, the polyherbal ointment demonstrated similar inhibitory effects on Gram-positive bacteria and exhibited moderate antifungal activity against Fluconazole. Although synthetic antibiotics produced slightly larger inhibition zones, indicative of their greater potency in controlled laboratory settings, the significant antimicrobial effectiveness of the polyherbal formulation suggests synergistic interactions among its phytochemical components.

Table 2. Comparative Antimicrobial Activity of Polyherbal Ointment and Standard Drugs

Microorganism (Probable)	Source	Polyherbal Ointment (mm)	Amoxicillin (10 µg) (mm)	Streptomycin (10 µg) (mm)	Fluconazole (10 µg) (mm)
<i>Staphylococcus spp.</i>	Open air	18.0 ± 0.5	22.0 ± 0.4	24.0 ± 0.6	—
<i>Bacillus spp.</i>	Soil	16.0 ± 0.4	20.0 ± 0.5	22.0 ± 0.5	—
<i>Aspergillus spp.</i>	Open air	14.0 ± 0.3	—	—	20.0 ± 0.4
<i>Penicillium spp.</i>	Soil	15.0 ± 0.4	—	—	18.0 ± 0.5

Values are expressed as mean ± standard deviation (n = 3).





### Interpretation of Comparative Results

The polyherbal ointment demonstrated 81–82% effectiveness against *Staphylococcus* spp., compared to Streptomycin, and 72–75% effectiveness against *Bacillus* spp., compared to Amoxicillin. Its antifungal properties were assessed at 70–75% effectiveness compared to Fluconazole.

Although these results demonstrate greater effectiveness of synthetic antibiotics in laboratory settings, they also confirm the significant antimicrobial capabilities of the polyherbal formulation.

### 4. Discussion

The study revealed that the developed polyherbal ointment exhibits a wide range of antimicrobial properties against bacteria, including *Staphylococcus* spp. and *Bacillus* spp. and fungi, including *Aspergillus* spp. and *Penicillium* spp., isolated from environmental samples. The observed antibacterial and antifungal effectiveness is consistent with previous research highlighting the antimicrobial capabilities of the individual plant components used in the formulation.

The strong antibacterial effect against *Staphylococcus* species is primarily attributed to phenolic monoterpenes, such as carvacrol and thymol, which are abundant in *Origanum vulgare*, commonly known as oregano. Previous research has demonstrated that oregano essential oil disrupts the structure of Gram-positive bacterial membranes, leading to leakage of cytoplasmic contents and subsequent rapid cell death. This mechanism explains the increased susceptibility of *Staphylococcus aureus* to oregano oil, supporting the findings of the current study.

The suppression of *Bacillus* species is likely attributable to the combined effects of curcumin, derived from *Curcuma longa* (turmeric), and allicin, obtained from *Allium sativum* (garlic). Curcumin is known to disrupt bacterial cell division by inhibiting the formation of the FtsZ protein, essential for bacterial cytokinesis. Concurrently, allicin targets sulfhydryl groups in bacterial enzymes, thereby interfering with critical metabolic processes. These actions have previously been documented as major contributors to the antibacterial properties of garlic extracts against soil-dwelling *Bacillus* species, aligning with the inhibition zones observed in this research.

The antifungal properties against *Aspergillus* spp. and *Penicillium* spp. align with previous studies demonstrating the effectiveness of flavonoids and triterpenoids in *Calendula officinalis*, alongside phenolic acids and caffeic acid derivatives found in propolis. These substances interfere with ergosterol biosynthesis in fungal cell membranes and weaken cell wall integrity, thereby preventing spore germination and hyphal development. The observed antifungal patterns are consistent with research on plant-based topical treatments targeting filamentous fungi.

*Ocimum sanctum*, commonly known as Tulsi, is recognized for its antimicrobial properties, attributable to the presence of eugenol and ursolic acid. These compounds kill bacteria by disrupting cell membranes and inhibiting enzymes. Extracts from *Mentha* species, which are rich in menthol, also offer antimicrobial and anti-inflammatory benefits, as demonstrated in various laboratory tests. The addition of aloe vera gel, abundant in polysaccharides and glycoproteins, likely enhanced the formulation's ability to promote wound healing and provide soothing effects, while simultaneously improving its antimicrobial efficacy—a combination well-documented in dermatological studies.

When compared to standard antibiotics, Amoxicillin, Streptomycin, and Fluconazole produced larger zones of inhibition. However, the polyherbal ointment demonstrated 70–82% of their relative effectiveness. This finding supports the notion that polyherbal formulations, while sometimes slightly less potent than purified synthetic drugs, offer multi-target mechanisms of action and may reduce the risk of resistance development owing to their complex mixture of bioactive compounds.

The oil-based extraction method employed in this research likely facilitated the effective extraction of lipophilic phytoconstituents, including essential oils and curcuminoids. Literature suggests that oil infusion techniques enhance the stability and bioavailability of plant-derived antimicrobial agents in topical applications. Furthermore, the beeswax-based matrix may have contributed to sustained release and extended antimicrobial contact time, thereby boosting efficacy, as previously observed in studies of herbal ointment formulations.

In summary, these results align with existing research highlighting the antimicrobial properties of medicinal plants and demonstrate that their combination within a polyherbal system can produce synergistic effects. The increased inhibitory activity observed against bacterial and fungal environmental isolates supports the development of eco-friendly, plant-based topical antimicrobial formulations as supplementary or alternative therapeutic options.

## 5. Conclusion

This research successfully developed a polyherbal antimicrobial ointment incorporating extracts from *Origanum vulgare*, *Ocimum sanctum*, *Curcuma longa*, *Allium sativum*, and *Calendula officinalis*, alongside other supportive herbal ingredients. The ointment demonstrated notable antibacterial and antifungal properties against environmental isolates, exhibiting effectiveness comparable to that of standard antimicrobial agents in laboratory settings.

The findings suggest that the combined effect of bioactive phytochemicals enhances the ointment's broad-spectrum antimicrobial capabilities. Due to its natural origin and environmentally friendly composition, this newly developed formulation demonstrates potential as an alternative topical antimicrobial solution. Nevertheless, further research is necessary to confirm its therapeutic effectiveness and safety, including pathogen-specific identification, stability testing, toxicity evaluations, and clinical trials.

## Author Contributions

Main Author: Dr. Seema Vilas Khadatare

Conceptualization, methodology design, supervision, validation, data analysis, manuscript preparation (including original draft and final review/editing).

Co-authors: Student Researchers

Plant material collection, extraction procedures, ointment formulation, microbial isolation and culturing, experimental execution, data recording, and assistance with preliminary manuscript drafting.

## Conflict of Interest

The authors declare no known competing financial interests or personal relationships that may have influenced the reported work.

## Funding

This research was conducted without external funding, utilizing institutional laboratory facilities and self-supported resources.

## Data Availability

All data generated and analyzed during this study are included in this published article. Additional information may be obtained from the corresponding author upon reasonable request.

## References

- Agyeman, W. Y., Bisht, A., Gopinath, A., Cheema, A. H., Chaludiya, K., Khalid, M., Nwosu, M., Konka, S., & Khan, S. (2022). A Systematic Review of Antibiotic Resistance Trends and Treatment Options for Hospital-Acquired Multidrug-Resistant Infections. *Cureus*, *14*(10). <https://doi.org/10.7759/cureus.29956>
- Alam, M. M., Islam, M., Wahab, A., & Billah, M. (2019). Antimicrobial Resistance Crisis and Combating Approaches. *Journal of Medicine*, *20*(1), 38–45. <https://doi.org/10.3329/jom.v20i1.38842>
- Angane, M., Swift, S., Huang, K., Perera, J., Chen, X., Butts, C. A., & Quek, S. Y. (2023). Synergistic antimicrobial interaction of plant essential oils and extracts against foodborne pathogens. *Food Science & Nutrition*, *12*(2), 1189–1206. <https://doi.org/10.1002/fsn3.3834>
- Anupama, C., Kaphle, A., Udayabhanu, U., & Nagaraju, G. (2017). Aegle marmelos assisted facile combustion synthesis of multifunctional ZnO nanoparticles: study of their photoluminescence, photocatalytic and antimicrobial activities. *Journal of Materials Science: Materials in Electronics*, *29*(5), 4238–4249. <https://doi.org/10.1007/s10854-017-8369-1>
- Dantas, T. D. S., Machado, J. C. B., Ferreira, M. R. A., & Soares, L. A. L. (2025). Bioactive Plant Compounds as Alternatives Against Antifungal Resistance in the Candida Strains. *Pharmaceutics*, *17*(6), 687. <https://doi.org/10.3390/pharmaceutics17060687>
- Dassanayake, M. K., Khoo, T.-J., & An, J. (2021). Antibiotic resistance modifying ability of phytoextracts in anthrax biological agent Bacillus anthracis and emerging superbugs: a review of synergistic mechanisms. *Annals of Clinical Microbiology and Antimicrobials*, *20*(1). <https://doi.org/10.1186/s12941-021-00485-0>
- Djahafi, A., Taibi, K., & Ait Abderrahim, L. (2021). Aromatic and medicinal plants used in traditional medicine in the region of Tiaret, North West of Algeria. *Mediterranean Botany*, *42*, e71465. <https://doi.org/10.5209/mbot.71465>
- Ikpe, F., Williams, T., Orok, E., & Ikpe, A. (2024). Antimicrobial resistance: use of phage therapy in the management of resistant infections. *Molecular Biology Reports*, *51*(1). <https://doi.org/10.1007/s11033-024-09870-2>
- Ko, S. J., Kim, M. K., Bang, J. K., Seo, C. H., Luchian, T., & Park, Y. (2017). Macropis fulvipes Venom component Macropin Exerts its Antibacterial and Anti-Biofilm Properties by Damaging the Plasma Membranes of Drug Resistant Bacteria. *Scientific Reports*, *7*(1). <https://doi.org/10.1038/s41598-017-16784-6>
- Meshaal, A. K., Hetta, H. F., Yahia, R., Abualnaja, K. M., Mansour, A. T., Al-Kadmy, I. M. S., Alghamdi, S., Dablood, A. S., Emran, T. B., Sedky, H., Batiha, G. E.-S., & El-Kazzaz, W. (2021). In Vitro Antimicrobial Activity of Medicinal Plant Extracts against Some Bacterial Pathogens Isolated from Raw and Processed Meat. *Life*, *11*(11), 1178. <https://doi.org/10.3390/life11111178>
- Mulat, M., Banicod, R. J. S., Tabassum, N., Javaid, A., Karthikeyan, A., Jeong, G.-J., Kim, Y.-M., Jung, W.-K., & Khan, F. (2025). Multiple Strategies for the Application of Medicinal Plant-Derived Bioactive Compounds in Controlling Microbial Biofilm and Virulence Properties. *Antibiotics (Basel, Switzerland)*, *14*(6), 555. <https://doi.org/10.3390/antibiotics14060555>
- Özkan, G., Sağdıç, O., & Özcan, M. (2003). Note: Inhibition of Pathogenic Bacteria by Essential Oils at Different Concentrations. *Food Science and Technology International*, *9*(2), 85–88. <https://doi.org/10.1177/1082013203009002003>
- Ozma, M. A., Köse, Ş., Pagliano, P., Asgharzadeh, M., Abbasi, A., Guarino, A., & Kafil, H. (2022). Antibiotic therapy for pan-drug-resistant infections. *Infezioni in Medicina*, *30*(4). <https://doi.org/10.53854/liim-3004-6>
- Sebghatollahi, Z., Yogesh, R., Mahato, N., Kumar, V., Mohanta, Y. K., Baek, K.-H., & Mishra, A. K. (2025). Signaling Pathways in Oxidative Stress-Induced Neurodegenerative Diseases: A Review of Phytochemical Therapeutic Interventions. *Antioxidants (Basel, Switzerland)*, *14*(4), 457.

<https://doi.org/10.3390/antiox14040457>

Soulaimani, B. (2025). Comprehensive Review of the Combined Antimicrobial Activity of Essential Oil Mixtures and Synergism with Conventional Antimicrobials. *Natural Product Communications*, 20(3).

<https://doi.org/10.1177/1934578x251328241>

Stanojevic, L. P., Marjanovic-Balaban, Z. R., Kalaba, V. D., Stanojevic, J. S., Cvetkovic, D. J., & Cakic, M. D. (2017). Chemical Composition, Antioxidant and Antimicrobial Activity of Basil (*Ocimum basilicum* L.) Essential Oil. *Journal of Essential Oil Bearing Plants*, 20(6), 1557–1569.

<https://doi.org/10.1080/0972060x.2017.1401963>

Sun, Z., Li, P., Liu, F., Bian, H., Wang, D., Wang, X., Zou, Y., Sun, C., & Xu, W. (2017). Synergistic antibacterial mechanism of the *Lactobacillus crispatus* surface layer protein and nisin on *Staphylococcus saprophyticus*. *Scientific Reports*, 7(1). <https://doi.org/10.1038/s41598-017-00303-8>

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives.

*Microorganisms*, 9(10), 2041. <https://doi.org/10.3390/microorganisms9102041>

Wolska, K. I., Grześ, K., & Kurek, A. (2012). Synergy between Novel Antimicrobials and Conventional Antibiotics or Bacteriocins. *Polish Journal of Microbiology*, 61(2), 95–104. <https://doi.org/10.33073/pjm-2012-012>

