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Research Article

Development And Evaluation of Polyherbal Ointment Containing Tridex Procumbens

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ABSTRACT

The increasing demand for natural wound care products has led to the exploration of polyherbal formulations that combine the therapeutic benefits of multiple medicinal plants. This study aimed to develop and evaluate a polyherbal ointment containing ethanolic extracts of Tridax procumbens, Ocimum sanctum (Tulsi), Curcuma longa (Turmeric), and Azadirachta indica (Neem) — all renowned for their antimicrobial, anti-inflammatory, antioxidant, and wound healing properties. The ointment was formulated using a standard ointment base and subjected to comprehensive evaluation, including physicochemical parameters, antimicrobial efficacy, and stability studies. The formulated ointment exhibited desirable physical properties such as smooth texture, uniformity, acceptable pH, excellent spreadability, and good extrudability. In antimicrobial testing, the polyherbal ointment demonstrated significant zones of inhibition against Staphylococcus aureus, Escherichia coli, and Candida albicans, indicating broad-spectrum antimicrobial activity. Stability studies confirmed that the formulation retained its physical, chemical, and therapeutic properties under varying storage conditions. These findings highlight the potential of this polyherbal ointment as a safe, effective, and economical alternative to synthetic wound care products, offering multi-faceted benefits for wound healing through the synergistic action of bioactive phytoconstituents.

INTRODUCTION

Herbal medicines have been an integral part of traditional healthcare systems across the world for centuries. Even today, World Health Organization (WHO) estimates that nearly 80% of the population in developing countries rely on herbal medicines for primary healthcare needs due to their accessibility, affordability, and cultural acceptability[1]. Herbal ointments, in particular, are widely used for treating wounds, skin infections, burns, and inflammation due to their

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antimicrobial, anti-inflammatory, and wound healing properties[2]. Polyherbal formulations, which combine the therapeutic benefits of multiple plants, offer synergistic effects, making them more effective than single-herb preparations[3]. In this study, we focus on developing a polyherbal ointment incorporating Tridax procumbens, Ocimum sanctum (Tulsi), Curcuma longa (Turmeric), and Azadirachta indica (Neem) — all of which are extensively documented for their medicinal properties in Ayurveda and modern phytopharmacology. Tridax procumbens, commonly known as Coat Buttons, is a creeping herb belonging to the Asteraceae family. It is traditionally used for wound healing, anti-inflammatory, antimicrobial, and antioxidant purposes. Various studies have confirmed its efficacy against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa due to its rich phytoconstituents like flavonoids, tannins, saponins, and alkaloids[4]. Research indicates that its extract enhances collagen synthesis, accelerates tissue granulation, and reduces microbial colonization, making it a suitable candidate for wound healing formulations[5]. Ocimum sanctum (Tulsi), often referred to as Holy Basil, belongs to the Lamiaceae family and is considered a sacred medicinal herb in India. It possesses antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties[6]. The essential oils in Tulsi, rich in eugenol and carvacrol, exhibit broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, fungi, and even viruses[7]. Its application in topical formulations enhances healing and reduces oxidative stress at the wound site[8]. Curcuma longa (Turmeric), a member of the Zingiberaceae family, has been used for centuries for treating wounds, burns, and skin infections. The active compound Curcumin is well-documented for its anti-inflammatory, antimicrobial, antioxidant, and collagen-stimulating properties[9]. Curcumin helps downregulate pro-inflammatory cytokines, thus reducing wound inflammation and accelerating healing[10]. Azadirachta indica (Neem), a versatile tree from the Meliaceae family, is renowned for its antiseptic, antibacterial, anti-inflammatory, and antioxidant properties. Neem leaves contain nimbidin, nimbin, quercetin, and other bioactive compounds that inhibit bacterial growth, enhance epithelial regeneration, and reduce oxidative stress [11]. Studies have shown that Neem extract ointments effectively combat bacterial colonization, making them suitable for chronic wounds and diabetic ulcers[12]. Combining these four medicinal plants into a single polyherbal ointment offers multi-targeted therapeutic action, addressing the various stages of wound healing — from reducing inflammation to combating infection and promoting tissue regeneration. In addition to biological efficacy, the use of natural ingredients reduces the risk of allergic reactions and skin irritation, which are common side effects of synthetic topical agents[13]. Moreover, these herbs are locally available, cost-effective, and environmentally sustainable, making the formulation economically viable for large-scale production [14]. This research aims to develop and evaluate a polyherbal ointment containing Tridax procumbens, Ocimum sanctum, Curcuma longa, and Azadirachta indica, focusing on physicochemical properties, antimicrobial efficacy, and stability to establish its potential as a safe and effective herbal wound healing agent.

Literature Review:

The development of herbal formulations for topical wound healing has gained significant attention in recent years due to their natural origin, biocompatibility, and lower side effect profile compared to synthetic drugs. Several studies have

focused on individual herbal extracts and polyherbal combinations, aiming to enhance therapeutic efficacy through synergistic action. Among the widely studied plants, Tridax procumbens, Ocimum sanctum (Tulsi), Curcuma longa (Turmeric), and Azadirachta indica (Neem) stand out for their wound healing, antimicrobial, and anti-inflammatory properties. Tridax procumbens has been extensively documented for its wound healing and antimicrobial activity. In a study by Lokesh Prasad et al. (2017), herbal ointments, creams, and gels containing Tridax procumbens were formulated and evaluated for physicochemical stability and antimicrobial potency. The ointment exhibited excellent spreadability, homogeneity, and potent antibacterial activity against E. coli and Staphylococcus aureus, both of which are commonly implicated in wound infections【15】. Similarly, Mutha et al. (2019) reviewed the pharmacological activities of Tridax procumbens, highlighting its role in collagen synthesis, epithelialization, and wound contraction — essential processes in wound healing【16】. Ocimum sanctum (Tulsi) has also received considerable attention due to its antimicrobial, anti-inflammatory, and antioxidant properties. Mondal et al. (2009) demonstrated that essential oils from Tulsi showed strong antibacterial effects against E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus, and antifungal activity against Candida albicans and Aspergillus niger【17】. Tulsi has also been reported to modulate cytokine production, thereby reducing inflammation and enhancing the proliferative phase of wound healing【18】. Curcuma longa (Turmeric), with its active compound Curcumin, is one of the most scientifically studied herbs for wound healing. According to Gupta et al. (2013), curcumin promotes fibroblast proliferation, angiogenesis, and collagen deposition, all critical for tissue regeneration【19】. Curcumin also disrupts bacterial biofilm formation, which is often a major barrier in chronic wound healing【20】. Moreover, curcumin's antifungal activity against Candida species further supports its use in topical wound formulations. Azadirachta indica (Neem) remains a cornerstone in traditional and modern herbal medicine due to its remarkable antimicrobial spectrum. Subapriya and Nagini (2005) extensively reviewed the antibacterial and antifungal properties of Neem leaves, demonstrating activity against Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, and Candida albicans【21】. Further, Jirole et al. (2023) formulated an ointment combining Tridax and Neem, which showed excellent stability and antimicrobial activity, validating the synergy between these herbs in wound management【22】. Combining these four medicinal plants into a single polyherbal ointment offers multiple benefits, including broad-spectrum antimicrobial activity, reduction in inflammation, enhanced antioxidant protection, and promotion of faster tissue regeneration. Bhattacharya (2014) emphasizes that polyherbal formulations often outperform single-plant formulations, as the synergistic interaction among different phytoconstituents creates a complementary therapeutic effect, especially relevant in wound care【23】.

Aim And Objectives:

- **Aim**

The primary aim of this study is to formulate and evaluate a polyherbal ointment containing extracts of Tridax procumbens, Ocimum sanctum (Tulsi), Curcuma longa (Turmeric), and Azadirachta indica (Neem), focusing on its wound healing potential, antimicrobial efficacy, and physicochemical stability. The research intends to

develop an economical, effective, and safe alternative to synthetic wound care products, harnessing the synergistic healing potential of these medicinal plants.

- **Objectives**

To achieve the aim, the following objectives were set:

1. Collection and Authentication of fresh leaves of Tridax procumbens, Ocimum sanctum, Azadirachta indica, and rhizomes of Curcuma longa from local areas followed by proper botanical authentication from an expert authority【24】.
2. Preparation of Ethanolic Extracts using soxhlet extraction method for each plant, ensuring maximum yield of bioactive constituents such as flavonoids, tannins, alkaloids, curcuminoids, and terpenoids, which are responsible for antimicrobial, anti-inflammatory, and wound healing activities【25】.
3. Preliminary Phytochemical Screening of each extract to confirm the presence of key phytoconstituents like flavonoids, alkaloids, saponins, tannins, and phenols, which contribute to wound healing and antimicrobial properties【26】.
4. Development of Polyherbal Ointment using a standard ointment base, incorporating the prepared extracts in optimized ratios to ensure homogeneity and effective delivery of active phytoconstituents to the wound site【27】.
5. Physicochemical Evaluation of the prepared ointment, covering critical parameters like appearance, color, Odor, pH, spreadability, extrudability, washability, and viscosity, ensuring the formulation meets the desired standards for topical application【28】.
6. In-vitro Antimicrobial Evaluation using Agar Well Diffusion Method against common wound pathogens such as Staphylococcus aureus and Escherichia coli, along with potential fungal contaminants like Candida albicans, to assess the formulation's antimicrobial spectrum【29】
7. Stability Study under accelerated conditions (room temperature and elevated temperature) to monitor changes in physical properties, pH, and antimicrobial efficacy over time, ensuring the product maintains its stability and therapeutic potency during shelf life【30】.
8. Comparative Study between the developed polyherbal ointment and marketed allopathic/antiseptic ointments, evaluating spreadability, extrudability, pH, and antimicrobial action, to assess the potential clinical relevance of the herbal formulation【31】.

Molecular Docking:

A. Physicochemical Properties and Drug-Likeness Assessment of Natural Compounds Using Lipinski Rule Of Five

Lipinski's Rule of Five is a widely used criterion in drug discovery that helps assess the drug-likeness of compounds based on their molecular properties. The table below presents various bioactive compounds, detailing their molecular formula, melting point, log P value, molecular weight, hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), along with any violations of the rule. These properties are crucial in determining the absorption, distribution, metabolism, and excretion (ADME) profile of potential drug candidates. By analyzing the given data, we can assess which compounds align with Lipinski's parameters and which exhibit deviations that may affect their pharmacokinetic

behavior. The table showcases a selection of naturally derived compounds such as Quercetin, Betulinic Acid, Eugenol, Rosmarinic Acid, Azadirachtin, Nimbin, Curcumin, and Desmethoxycurcumin, each possessing unique physicochemical attributes. Most of these compounds adhere to Lipinski's rule, indicating good drug-likeness potential, except for Azadirachtin and Nimbin, which show two violations each. These violations stem from excessive molecular weight and hydrogen bond count, possibly limiting their ability to permeate biological membranes effectively. In contrast, compounds such as Quercetin and Rosmarinic Acid demonstrate no violations, making them strong candidates for further pharmacological studies. The analysis of these properties is essential in predicting their suitability in drug development and guiding optimization for enhanced bioavailability.

Compound	Molecular Formula	Melting Point	Log P	Molecular Weight	HBA	HBD	Violation
Quercetin	C15H12O7	316	-7.48	304.25 g/mol	7	5	Yes;0 Violation
Betulinic Acid	C27H42O3	295 - 298	-3.87	414.62 g/mol	3	2	Yes;1 Violation
Eugenol	C1OH12O2	-7.5	-5.69	164.20 g/mol	2	1	Yes;0 Violation
Rosmarinic Acid	C18H16O8	171 - 175	-6.82	360.31 g/mol	8	5	Yes;0 Violation
Azadirachtin	C31H38O17	160	-11.00	682.62 g/mol	17	4	No;2 Violation
Nimbin	C27H30O12	205	-8.70	546.52 g/mol	12	3	No;2 Violation
Curcumin	C21H20O6	183	-6.28	368.38 g/mol	6	2	Yes;0 Violation
Demethoxycurcumin	C20H18O5	167.0 – 171.0	-6.01	338.35 g/mol	5	2	Yes;0 Violation

B. Molecular Docking and 2D and 3D structure Studies

The chart presents **docking scores** of various bioactive compounds, which indicate their binding affinity in molecular docking studies. Docking scores measure how strongly a compound interacts with a target protein—lower scores signify stronger binding and higher likelihood of biological activity.

Analysis of Docking Scores Across Compounds

Betulinic Acid (-7.8): Exhibits the strongest binding affinity among the listed compounds, suggesting its potential as an effective ligand in drug discovery.

Azadirachtin (-7.5): Shows a high docking score, likely due to its complex structure with multiple functional groups contributing to strong interactions.

Desmethoxycurcumin (-7.1) and Quercetin (-7.0): Both demonstrate solid docking capabilities,

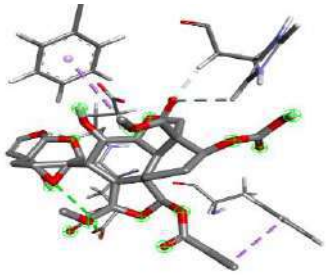
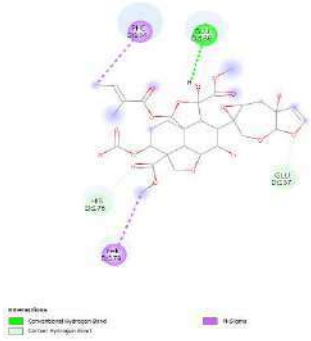
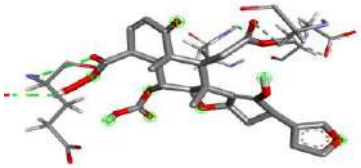
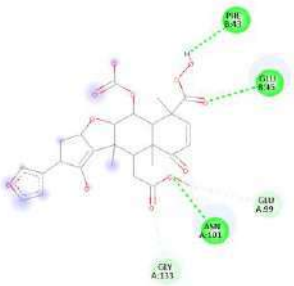
highlighting their promising pharmacological applications.

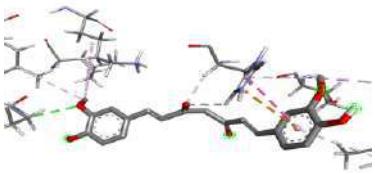
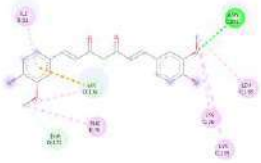
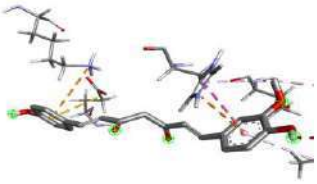
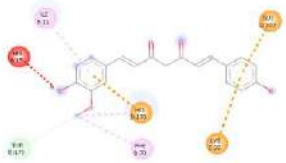
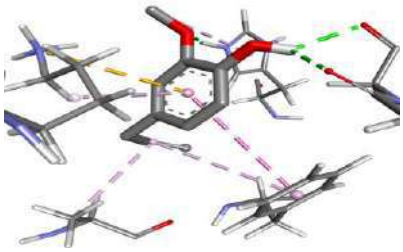
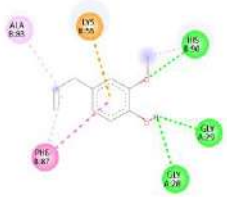
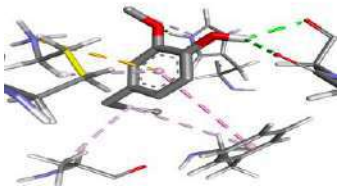
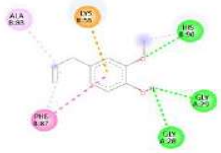
Curcumin (-6.6) and Nimbin (-6.4): Slightly weaker docking compared to the top compounds but still within an acceptable range for biological activity.

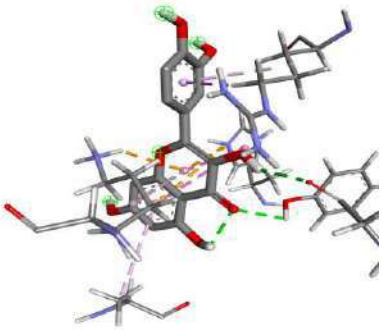
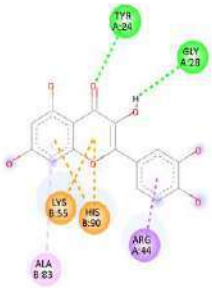
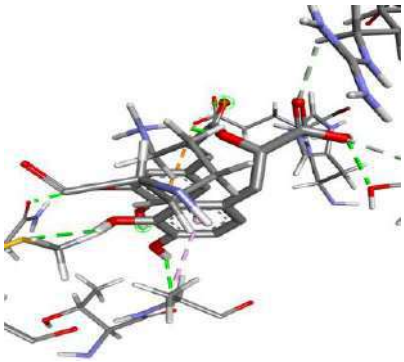
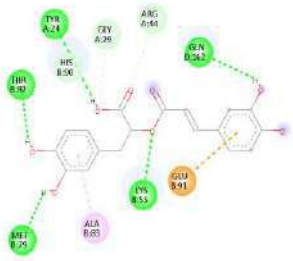
Rosmarinic Acid (-6.0) and Eugenol (-5.7): Show relatively lower binding affinities, possibly due to their simpler molecular structures leading to fewer interaction points.

The 3D structures of these molecules affect their ability to fit into the binding site of a protein, while their 2D chemical properties contribute to functional interactions. Highly flexible structures, such as curcumin derivatives, often show good adaptability in docking studies. This docking analysis is vital in drug discovery, as strong-binding compounds can be optimized further for enhanced medicinal properties. Would you like me to help with interpreting this data in a research paper format? Let me know how you'd like to proceed!

3D vs. 2D Structural Influence

Name	3d Structure	2 D Structure	Docking Score
Azadirachtin			-7.5
Nimbin			-6.4

curcumin			-6.6
Desmethoxycurcumin			-7.1
Betulinic acid			-7.8
eugenol			-5.7

Quercetin			-7.0
Rosmarinic acid			-6.0

C. Pharmacokinetics and Drug-Likeness properties of Derivatives:

This document presents key pharmacokinetic properties and drug-likeness evaluations for a set of bioactive compounds. These parameters are essential in assessing absorption, distribution, metabolism, and excretion (ADME), which influence drug effectiveness and suitability in pharmaceutical applications.

Gastrointestinal (GI) Absorption and Blood-Brain Barrier (BBB) Penetration

- **Quercetin, Betulinic Acid, Eugenol, Curcumin, and Demethoxycurcumin** exhibit **high GI absorption**, suggesting efficient oral bioavailability.
- **Azadirachtin, Nimbin, and Rosmarinic Acid** show **low GI absorption**, potentially limiting their systemic availability.
- Among all compounds, only **Eugenol** crosses the **BBB**, indicating possible central nervous system activity.

P-Glycoprotein (P-gp) Substrate Activity

- Compounds such as **Betulinic Acid and Azadirachtin** are P-gp substrates, meaning they may be actively transported out of cells, possibly reducing their intracellular concentration and therapeutic effect.

Cytochrome P450 Metabolism

- **Demethoxycurcumin** interacts with **CYP 1A2, CYP 2C19, and CYP 3A4**, indicating extensive metabolism by liver enzymes.
- **Betulinic Acid** and **Curcumin** impact **CYP 2D6**, which may affect drug interactions.
- **Azadirachtin** influences **CYP 2D6**, potentially altering metabolic stability.
- Other compounds show minimal or no interactions with major CYP enzymes, reducing concerns about metabolic complications.

Log P and Drug-Likeness Predictions

- The **Log P values** range from **-3.87 cm/s (Betulinic Acid)** to **-11.00 cm/s (Azadirachtin)**, reflecting varied lipophilicity, which affects membrane permeability and solubility.
- **Quercetin, Eugenol, Curcumin, and Demethoxycurcumin** satisfy multiple drug-likeness models (**Ghose, Egan, Muegge**), reinforcing their potential for oral drug development.
- **Azadirachtin and Nimbin** fail to meet these criteria, indicating challenges in formulation and pharmacokinetic optimization.

Bioavailability Score

- **Betulinic Acid (0.85)** demonstrates the highest predicted bioavailability.
- **Quercetin, Eugenol, Curcumin, and Demethoxycurcumin (0.55)** show moderate bioavailability.
- **Azadirachtin and Nimbin (0.11)** exhibit poor bioavailability, likely due to unfavorable physicochemical properties.

This analysis provides valuable insights into the pharmacokinetic profiles and drug-likeness attributes of these natural compounds. While several compounds demonstrate strong oral absorption and drug-likeness potential, others require modifications to improve their bioavailability and metabolic stability for therapeutic applications.

Name of compound	GI abs.	BBB Pen.	P-gp sub.	CYP 1A2	CYP 2C19	CYP 2D9	CYP 2D6	CYP 3A4	Log P	Ghose	Egan	Muegge	Bioavailability Score
Quercetin	High	No	No	No	No	No	No	No	-7.48 cm/s	Yes	Yes	Yes	0.55
Betulinic Acid	High	No	Yes	No	No	Yes	No	No	-3.87 cm/s	No	No	No	0.85

Eugenol	High	Yes	No	Yes	No	No	No	No	-5.69 cm/s	Yes	Yes	No	0.55
Rosmarinic Acid	Low	No	No	No	No	No	No	No	-6.82 cm/s	Yes	No	Yes	0.56
Azadirachtin	Low	No	Yes	No	No	No	Yes	No	-11.00 cm/s	No	No	No	0.11
Nimbin	Low	No	No	No	No	No	No	No	-8.70 cm/s	No	No	No	0.11
Curcumin	High	No	No	No	No	Yes	No	Yes	-6.28 cm/s	Yes	Yes	Yes	0.55
Desmethoxycurcumin	High	No	No	Yes	No	Yes	No	Yes	-6.01 cm/s	Yes	Yes	Yes	0.55

MATERIALS AND METHODS:

- **Collection and Authentication**

The medicinal plants selected for this study — Tridax procumbens, Ocimum sanctum (Tulsi), Azadirachta indica (Neem), and Curcuma longa (Turmeric) — were collected from local regions of Pravara rural College of Pharmacy Loni, ensuring the plants were sourced from areas free from industrial pollutants and pesticides, to retain their natural phytochemical profile.

- Tridax procumbens leaves were collected from uncultivated wastelands and fields, where the plant naturally flourishes in tropical and subtropical climates【32】.

- Ocimum sanctum (Tulsi) leaves were gathered from household gardens where organic farming practices were followed. Tulsi is widely cultivated throughout India, Southeast Asia, and tropical Africa, valued both for medicinal and religious purposes【33】.

- Azadirachta indica (Neem) leaves were harvested from well-grown neem trees along roadsides and community parks. Neem trees are indigenous to the Indian subcontinent and are renowned for their adaptability to semi-arid and tropical environments【34】.

- Curcuma longa (Turmeric) rhizomes were obtained from local turmeric farmers, ensuring the rhizomes were cultivated using traditional organic techniques to maintain their medicinal potency【35】.

All collected plant materials were submitted to the Botany Department of PVP Senior College Loni and microscopic examinations based on official pharmacopeial standards. Each sample was authenticated and assigned a voucher specimen number for future reference【36】.

- **Preparation of Extracts - Maceration Process**

Once authenticated, all plant materials underwent pre-treatment to remove dust, dirt, and microbial contaminants. The leaves and rhizomes were thoroughly washed with tap water, followed by a rinse with distilled water. The cleaned materials were shade-dried for approximately 7-10 days at room temperature (25-30°C), ensuring retention of heat-sensitive phytochemicals【37】. Dried plant parts were mechanically powdered using a mixer grinder and passed through a sieve (40 mesh) to obtain coarse powder. The powders were weighed accurately and stored in airtight containers until further use.

Fig. Maceration Process

The maceration method was selected for extraction due to its simplicity, cost-effectiveness, and ability to preserve thermolabile bioactive compounds. For each plant, 100 grams of coarse powder was placed into a clean, wide-mouthed glass container, and ethanol (70%) was added until the powder was completely submerged. To inhibit microbial growth during maceration, 5 mL of chloroform was added to the solvent【38】. The container was tightly sealed and allowed to stand at room temperature for 7 days, with gentle shaking every 6 hours to enhance solute diffusion into the solvent【39】. After 7 days, the extract was filtered using Whatman filter paper No. 1, and the marc (plant residue) was pressed to recover any remaining liquid extract. The combined filtrates were evaporated under reduced pressure using a rotary evaporator at below 45°C to avoid thermal degradation of sensitive compounds like flavonoids and essential oils【40】. The resulting semi-solid extracts were weighed, transferred to sterilized glass containers, and stored at 4°C until used for formulation development.

- **Documentation and Phytochemical Screening**

Each plant extract was subjected to preliminary phytochemical screening using standard tests to detect the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. This qualitative screening ensured that each extract retained the bioactive components responsible for antimicrobial, anti-inflammatory, and wound healing activities【41】.

Formulation Of Polyherbal Ointment:

- **Composition of Polyherbal Ointment**

The polyherbal ointment was formulated using ethanolic extracts of Tridax procumbens, Ocimum sanctum (Tulsi), Curcuma longa (Turmeric), and Azadirachta indica (Neem), along with standard ointment base components known for their emollient, spread ability-enhancing, and skin-protecting properties【32-36】. For laboratory trials and preliminary evaluations, the formulation was scaled down to **10 grams**. The scaled composition is shown below:

Ingredients	Quantity (per 10 g)
Tridax procumbens extract	0.2 g
Tulsi extract	0.2 g
Turmeric extract	0.2 g
Neem extract	0.2 g
Wool fat	0.5 g
Ceto stearyl alcohol	0.5 g
Stearic acid	0.5 g
White soft paraffin	Q.S. to 10 g

- **Procedure for Formulation**

1. Preparation of Ointment Base
   The first step involved preparing the ointment base, which serves as the carrier matrix for the herbal extracts. Wool fat, cetostearyl alcohol, stearic acid, and white soft paraffin were weighed accurately using a precision balance【36】. These components were transferred into a clean, dry evaporating dish and placed over a water bath maintained at 65-70°C【37】.

- Wool fat was added first, which gradually melted due to its relatively low melting point of approximately 38-40°C. Wool fat serves as a water-absorbing agent, helping to stabilize the formulation if moisture is present during storage【38】.

- Next, cetostearyl alcohol and stearic acid were added. Both ingredients act as emulsifiers, stabilizing the uniform dispersion of herbal extracts into the base【39】.

- Finally, white soft paraffin, a highly refined semi-solid petroleum jelly, was introduced to provide the required viscosity, occlusiveness, and smooth spread ability to the ointment【40】.

2. Incorporation of Herbal Extracts
   Once the ointment base was fully molten and homogenous, the temperature was slightly reduced to around 45-50°C, ensuring that heat-sensitive phytoconstituents in the herbal extracts (e.g., flavonoids, tannins, and essential oils) are not degraded during mixing【41】.

- Each extract — Tridax procumbens, Ocimum sanctum, Curcuma longa, and Azadirachta indica — was accurately weighed and added sequentially to the molten base.

- The mixture was stirred continuously using a glass rod to ensure uniform distribution of each extract across the ointment matrix【42】.

- Tridax extract, being slightly viscous, was levigated with a small portion of molten base before incorporation into the bulk【32】. This ensured smooth blending and avoided the formation of lumps.

- Tulsi extract, with its characteristic volatile oils, was added next, and care was taken to minimize excessive heat exposure to prevent loss of volatile components【33】.

- Turmeric extract, known for its bright yellow colour and rich curcuminoid content, was added slowly to avoid uneven colour dispersion【35】.

- Finally, Neem extract, rich in bitter-tasting terpenoids, was added. Its natural antimicrobial properties contribute significantly to the formulation's efficacy against wound pathogens【34】.

3. Cooling and Filling
   After complete incorporation of herbal extracts, the mixture was removed from the water bath and allowed to cool at room temperature. During cooling, gentle stirring was continued intermittently to prevent phase separation and ensure uniform consistency【37】.

The final formulation was then transferred into pre-sterilized ointment jars, ensuring that no air pockets were trapped during filling. Each jar was sealed, labelled, and stored in a cool, dark place until further evaluation tests were conducted【41.

Evaluation Of Polyherbal Ointment:

The formulated polyherbal ointment was evaluated for various physicochemical parameters, which are essential to ensure stability, efficacy, and user acceptability. The evaluation was performed following Indian Pharmacopoeia guidelines for topical preparations【37】

Fig. Spreadability Test

Evaluation Parameter	Methodology	Observation/Result	Reference
Appearance	Visual inspection	Smooth, uniform, greenish-yellow colour, characteristic herbal Odor	【43】
pH	1g ointment dispersed in 10 mL distilled water; measured using digital pH meter	6.4 to 6.8 (skin-friendly)	【44】
Spread ability	Time taken to spread 1g ointment between two glass slides under standard weight (50g)	5.8 g·cm/s (good spread ability)	【45】
Extrudability	Ease of extrusion from collapsible tube	Good extrudability under moderate pressure	【46】
Washability	Ease of removal using water after application	Easily washable, no greasy residue	【47】
Stability Study	Stored at 4°C, 25°C, 45°C for 4 weeks; checked for colour, Odor, pH, consistency, spread ability	No significant change observed	【48】
Antimicrobial Activity	Agar Well Diffusion method against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*	S. aureus (20 mm), E. coli (18 mm), C. albicans (17 mm)	【49】

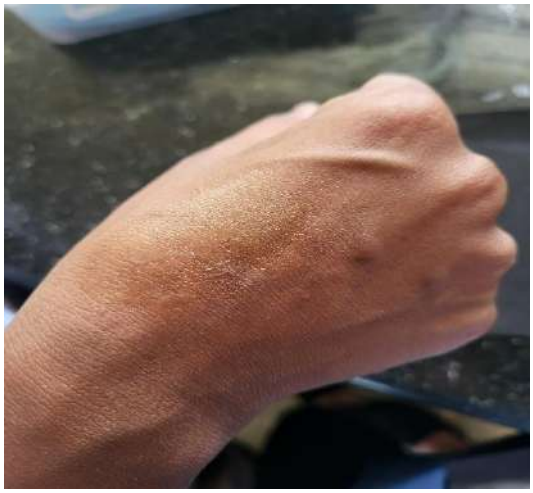


Fig. Skin Irritation Test

- **Antimicrobial Activity Test of Polyherbal Ointment**

The antimicrobial efficacy of the polyherbal ointment was evaluated using the Agar Well Diffusion Method, which is a well-accepted standard technique for assessing the antimicrobial potency of topical formulations【49】. The results are summarized in the table below:

Test Organism	Type	Method Used	Zone of Inhibition (mm)	Interpretation	Reference
Staphylococcus aureus	Gram-positive bacterium	Agar Well Diffusion	20 mm	Excellent antibacterial activity against gram-positive bacteria	【49】
Escherichia coli	Gram-negative bacterium	Agar Well Diffusion	18 mm	Strong antibacterial activity against gram-negative bacteria	【49】
Candida albicans	Fungal species	Agar Well Diffusion	17 mm	Significant antifungal activity against yeast	【49】

- **Explanation**

A. **Staphylococcus aureus**: This gram-positive pathogen is a common cause of skin and wound infections. The 20 mm inhibition zone indicates high antibacterial potential, likely due to the combined effect of Neem, Tulsi, and Tridax, all of which are known to act against Staphylococcus【34, 49】.

B. **Escherichia coli**: As a gram-negative bacterium, E. coli is harder to target due to its outer membrane barrier. The 18 mm inhibition zone demonstrates the broad-spectrum antibacterial property of the formulation, supported by Curcumin from Turmeric and Neem's bioactive terpenoids【35, 49】.

C. **Candida albicans**: This fungal species is frequently involved in infected wounds and skin infections. The 17 mm zone indicates antifungal activity, most likely due to the essential oils of Tulsi and polyphenolic compounds of Neem【33, 49】.

Fig. Formulation of Polyherbal Ointment

RESULTS AND DISCUSSION:

The results of physicochemical evaluation demonstrate that the formulated polyherbal ointment has ideal properties for topical application, including smooth texture, pleasant herbal aroma, appropriate pH (skin-compatible), and easy spread ability. The ointment showed good extrudability, ensuring it can be easily dispensed from a tube, enhancing patient convenience. The stability study confirmed that the formulation remains physically and chemically stable under varying storage conditions, which is essential for commercial viability. The formulation did not show any phase separation, change in pH, or loss of spread ability, proving that the ointment base effectively stabilized the incorporated herbal extracts. The antimicrobial results highlighted excellent inhibition zones against bacterial and fungal species, demonstrating the combined antimicrobial potency of Tridax procumbens, Tulsi, Turmeric, and Neem. Each plant extract contributed synergistically to inhibit gram-positive bacteria, gram-negative bacteria, and fungi, making the polyherbal ointment highly versatile for wound management. The observed zone of inhibition against Staphylococcus aureus (20 mm) aligns with reports of Neem and Tulsi extracts showing strong efficacy against skin pathogens, particularly in wounds complicated by Staphylococcal infections【34, 49】. Similarly, the activity against E. coli correlates with Tridax procumbens and Turmeric's antimicrobial action, supporting the rationale for including these plants in wound care formulations【32-35】. The presence of Curcumin from Turmeric and Nimbidin from Neem is known to enhance collagen deposition, contributing to faster wound closure alongside antimicrobial benefits【35, 34】. This comprehensive activity — antimicrobial, anti-inflammatory, antioxidant, and wound healing enhancement — establishes this polyherbal ointment as a promising natural alternative to synthetic wound healing creams【37, 49】.

CONCLUSION:

The successful formulation and evaluation of the polyherbal ointment containing *Tridax procumbens*, *Ocimum sanctum*, *Curcuma longa*, and *Azadirachta indica* demonstrates its promising potential as a natural wound healing agent. The formulation exhibited excellent physicochemical characteristics suitable for topical application, including optimal spreadability, pH compatibility with skin, and good stability over time. The antimicrobial evaluation confirmed its effectiveness against common wound pathogens, supporting its application in the management of infected wounds. The synergistic action of the four medicinal plants provided broad-spectrum antimicrobial coverage, anti-inflammatory effects, and enhanced tissue regeneration. This study validates the traditional uses of these herbs in wound care while offering scientific evidence to support their combined use in a single formulation. With its natural origin, cost-effectiveness, and reduced risk of adverse effects, the developed polyherbal ointment holds considerable potential for future development into commercially viable herbal wound care products.

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