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Formulation and Evaluation of Polyherbal Ointment for Anti-Microbial Property

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ABSTRACT:

Background: In the medical field, wound healing and antibiotic resistance present serious obstacles that call for the creation of substitute medicinal formulations. The anti-microbial and wound-healing qualities of *Tridax procumbens, Acalypha indica,* and *Mimosa pudica* have been utilized historically. **Aim:** The purpose of this work was to create and test a polyherbal ointment that included of *Acalypha indica, Tridax procumbens*, and *Mimosa pudica*. Its stability, anti-microbial activity, and physicochemical parameters were evaluated **Methods:** The plant materials were gathered, verified, and ground into a fine powder. White beeswax, white soft paraffin, and liquid paraffin were used as the basis ingredients in the levigation procedure to create the polyherbal ointment. The formulation's color, odor, consistency, spreadability, pH, viscosity, extrudability, and loss upon drying were all assessed physicochemically. In accordance with ICH norms, stability studies were carried out during a six-month period. The agar well diffusion method was used to evaluate the antibacterial activity against *Aeromonas hydrophila, Propionibacterium acnes, Escherichia coli,* and *Staphylococcus aureus.* **Results and Discussion:** The dark green ointment had a pH of 6.0, a smooth consistency, good spreadability (5 seconds), and non-irritating qualities. Over the course of six months, stability studies verified that there were no appreciable changes in the physicochemical properties. Significant activity was found in antimicrobial testing, with the petroleum ether extract (PS) showing higher efficacy against Gram-negative bacteria and the methanolic extract (MS) being more efficient against Gram- positive bacteria. The greatest inhibition against *P. acnes* was shown by MS (15.1±0.14 mm), and the highest inhibition against *E. coli* was shown by PS (15.6±0.56 mm). These results imply that the prepared ointment may be used to treat skin conditions and wound infections. **Conclusion:** *Tridax procumbens, Acalypha indica,* and *Mimosa pudica* were used to create the polyherbal ointment, which showed encouraging anti-microbial efficacy and stability. Its appropriateness for topical application is supported by its physicochemical characteristics. To determine its therapeutic efficacy for usage in pharmaceutical and cosmetic applications, more in vivo research and clinical trials are advised.

Keywords : *Acalypha indica*, Anti-microbial property, *Mimosa pudica*, Polyherbal ointment, *Tridax procumbens*.

1. INTRODUCTION

Plant materials such as roots, leaves, and seeds are used to make herbal remedies, which have been used for centuries in medicine. Different plant components serve distinct medicinal purposes, requiring specific extraction methods. Herbal medicines remain widely used and hold economic and pharmaceutical significance. Their acceptance in society, compatibility with the human body, and lack of negative side effects are the main reasons for their appeal. India, home to Ayurveda, Siddha, and Unani, has a rich history of herbal medicine. Eighty percent of people in underdeveloped nations use traditional medicine, according to the WHO. Ayurveda employs single herbs and polyherbal formulations (PHFs) to enhance therapeutic efficacy[1].

1.1 Single Herbal Formulation:

Single herbal formulations use one herb for therapeutic benefits, each containing unique phytochemicals. Examples include turmeric for inflammation and ashwagandha for stress relief. However, limitations include:

- **Potency:** Insufficient active constituents for desired effects.
- **Target Specificity:** Limited scope in treatment.

- **Side Effects:** Higher doses may lead to adverse effects[2].

1.2 Polyherbal Formulations:

In contrast, polyherbal formulations combine multiple herbs to create a more holistic approach to treatment. This method exploits the concept of synergism, where the combined effect of the herbs exceeds the sum of their individual effects[3].

Advantages:

- **High effectiveness:** Because PHFs include a variety of phytoconstituents, they show notable efficacy in treating a broad range of illnesses, which are further enhanced through synergistic interactions among the herbs [4].

- **Wide therapeutic range:** PHFs exhibit remarkable effectiveness in treating a wide range of ailments since they include a variety of phytoconstituents [5].

Limitations:

- **Misconceptions of Safety:** A common misconception is that Ayurvedic PHFs are usually safe., which is misleading. Inappropriate preparation or use can lead to adverse effects, as noted in historical texts like the *Charaka Samhita*[6].

- **Drug-Herb Interactions:** Increasing concurrent use of PHFs with allopathic drugs raises concerns about potential drug-herb interactions that may adversely affect health outcomes, especially when patients fail to disclose their complete treatment regimens[7-10].

1.3 Wound:

An injury to the body is referred to as a wound, that occurs due to various forms of trauma, which can be physical (like cuts or lacerations), chemical (such as burns from acids), microbial (infections that damage tissue), or electrical (injuries caused by electric shocks). This trauma leads to a disruption in both anatomical and functional tissue, which can impair the body's ability to perform its usual functions[11].

Stages of Wound Healing 12:

1. Haemostasis (Immediate Response)
2. Inflammation (First 3-4 Days)
3. Proliferation (Days 5-7 to 4 Weeks)
4. Remodelling/Maturation (Weeks to Years)

1.4 Ointment:

A homogeneous, semi-solid, viscous substance intended for external application to the skin or mucous membranes is called an ointment. It frequently has a high viscosity and is thick and greasy (oil 80% water 20%). The water number of an ointment indicates the maximum amount of water it can contain. When preferred, they are used as emollients or to apply active substances to the skin for medicinal, protective, or preventive purposes. Ointments are topically applied to various body areas. These include the skin and mucous membranes of the eye, the nose, vulva, anus, and chest. An ointment may or may not contain medication. Ointments are generally very moisturizing and helpful for dry[13].

Ointment should have the following qualities:

1. Stability.
2. The active component shouldn't be hampered by the base.

3. The medication ought to be dispersed evenly throughout.

Benefits of the Ointment

1. They apply the medication to the damaged area site-specifically, preventing needless out-of-target drug exposure and thereby preventing unwanted effects.

2. They steer clear of the drug's first pass metabolism.

3. Practical for patients who are unconscious and have trouble taking medication orally.

The Drawbacks of Ointment

1. These greasy semisolid formulations are less aesthetically pleasing and stain-prone.

2. Using your fingertip to apply the product could contaminate it or irritate it.

3. The semisolid preparation is more difficult to handle than the solid dose forms.

Ointment Bases:

An ingredient or component that serves as a vehicle or carrier for the medication is called the base of an ointment. When selecting an ointment foundation, factors to be taken into account include the intended action, the kind of medication to be used, and the stability of the ointment.[14].

The following factors determine which base is best:

1. The desired rate at which the specific drug substance is released from the ointment base.

2. Desirability for improvement based on the drug's percutaneous absorption base.

3. The wisdom of the base obstructing moisture from the skin.

4. The drug's stability in the ointment base, both in the short and long term.

5. Any impact the medication may have on the ointment base's consistency or other characteristics[15].

Classification of Ointment Bases:

1. **Hydrocarbon Bases:** These lipophilic bases, like petrolatum and white ointment, are water- immiscible, non-absorbable, and minimally hydrating. They form a waterproof film, enhancing medication absorption but are mainly recommended for chronic skin disorders due to their high occlusiveness[16].

2. **Absorption Bases:** Hydrocarbon mixtures with polar groups (such as sulfate or hydroxyl) that allow for the assimilation of water are known as absorption bases. They fall into two categories: those that create water-in-oil emulsions (like hydrophilic petroleum) and those that already have water-in-oil emulsions (like lanolin), which permit trace amounts of extra watery solutions.[17].

3. **Water-Removable Bases:** These are emulsions of oil and water that may be washed with water and have a cream-like consistency. They can absorb dermatological discharges, dilute with water, and improve the way some drugs are absorbed via the skin.[11].

4. **Water-Soluble Bases:** Water-soluble bases, made from polyethylene glycol mixtures, have an ointment-like consistency and melt on skin contact. They are greaseless, non-toxic, non-irritating,non-occlusive, and easily washable, making them ideal for mixing with exudates without staining[18].

2. Materials and Method:

Collection of Plants:

In June, fresh *Tridax procumbens* complete plants, fresh *Mimosa pudica* leaves, and fresh *Acalypha indica* leaves were gathered from the Salem district in India. A Research Officer verified the plants. The gathered plants were cleaned, allowed to dry in the shade for 15 days, and then powdered into a powder.

2.1 Formulation:

Methodology for Making Polyherbal Ointment:

a) The white soft paraffin, liquid paraffin, and grated white beeswax were first carefully weighed and placed in an evaporating dish on a water bath to create the ointment basis. To promote melting and even mixing, the extra components were added after the base had melted and gently stirred[1].

b) Using the levigation method, precisely weighed *Tridax procumbens, Acalypha indica*, and *Mimosa pudica* were mixed with the ointment base to create a smooth paste that was two or three times its weight. Additional bases were then added gradually until the ointment was homogenous, and it was finally transferred to an appropriate container.[19].

Formulation of Ointment Base:

Table no:1 Formulation of ointment base

S.no	INGREDIENTS	WEIGHT(gm)
1.	White bees wax	30gm
2.	White soft paraffin	50gm
3.	Liquid paraffin	20gm

Formulation of Polyherbal Ointment:

Table no:2 Formulation of polyherbal ointment

S.No	INGREDIENTS	FORMULATION(100gm)
1.	*Tridax procumbens* Powder	1%
2.	*Acalypha indica* Powder	1%
3.	*Mimosa pudica* Powder	1%
4.	Ointment base	q.s

2.2 Evaluation: Physicochemical characteristics are evaluated. The following was the preliminary assessment of formulations at various concentrations:

Colour and Odour: The prepared ointment will be visually evaluated colour, odour, and texture.

Washability: The washability of the prepare polyherbal ointment will be determine by applying the ointment on the hand and let it washed with tap water.

Loss on Drying: The ointment was placed in a petri dish on a water bath and dried at 105°C to estimate the drying loss.

pH: The pH assessment After adding 100 millilitres of distilled water to a beaker containing two grammes of the ointment, the mixture was heated to 70 degrees Celsius. Using a digital pH meter, the ointment's pH is ascertained.

Viscosity: A Brookfield viscometer was used to measure the produced ointment's viscosity.

Spread Ability : The term "spread ability" describes how readily ointments may cover a certain area when applied to skin or other vulnerable places. 9 Multimer has developed a novel tool to examine formulations' capacity for diffusion. The spread ability was expressed as the number of seconds it took for two slides to separate from the ointment and migrate between each other under a

particular stress. The time it takes to separate two slides decreases with increasing spread ability. The spread ability formula was used to calculate it.

S is equal to (M.L./T),

where M = Weight attached to the upper slide, S = Spread ability,

L = length of the glass slides, and

T = amount of time needed to separate them.

Stability Studies: The ICH Q1A (R2) guidelines define the procedure for conducting a 6-month real- time stability study for ointments in India, which falls under Climatic Zone IVa (hot and humid regions). According to these guidelines, the study must be performed using at least three different samples, stored under 30°C ± 2°C / 65% RH ± 5% RH, with the final container. Stability evaluations are conducted at 0, 3, and 6 months to monitor physical properties (such as appearance, odour, texture, and phase separation). The product must remain within the specified limits, and any deviations if present will be noted. All findings are compiled into a stability study report, which is essential for shelf-life determination and regulatory submission. If the ointment maintains its stability throughout the study, it is deemed suitable for 6-month real-time storage under Indian climatic conditions.

Extrudability: This study used a straightforward methodology. Following the setting of the ointments in the container, the formulations were put into the collapsable tubes. A 0.5 cm ribbon of ointment was extruded in 10 seconds using the weight grammes of the various ointment formulations to assess their extrudability.

Antimicrobial Activity: To make Nutrient Agar Medium, dissolve 2.8 g of Nutrient agar (HiMedia) in 100 ml of distilled water, autoclave at 15 lbs pressure (121°C) for 15 minutes, mix thoroughly, and then pour 25–30 mL per 100 mm Petri plate while still molten. Nutrient Broth can be made similarly by dissolving 2.8 g of medium in 100 ml of distilled water, heating it to dissolve it, then dispensing it as needed and autoclaving it to sterilise it. Bacterial cultures are seeded with a 24-hour culture (0.5 OD according to the McFarland standard) in petri plates that contain 20 ml of solidified Nutrient agar. To each well, 500 µg/ml of the test sample (MS PS) is added once the agar has been prepared. The zone of inhibition is measured in millimetres to assess the antibacterial activity of plates after they are incubated for 24 hours at 37°C. Software from the USA called GraphPad Prism 6.0 is used to analyse the results.

Diffusion Method in an Agar well:

Principle: The antimicrobials in the supplied sample were allowed to diffuse out into the medium and interact in a plate that had just been seeded with the test organisms. Zones of inhibition will be consistently circular due to a confluent lawn of growth. Millimetres can be used to measure the zone of inhibition's diameter.

Materials Required: Required materials were acquired from MTCC in Chandigarh, India, and included S. aureus 902, P. acnes 1951, E. coli 443, and A. hydrophila 7966. Himedia, India, supplied the nutrient broth and nutrient agar medium. Beakers, conical flasks, test tubes, petri plates, and test samples came from Borosil, India. Water with double distillation and a spirit lamp.

Agar-well Diffusion Technique20,21:

a) The medium was made by dissolving 2.8 grams of commercial Nutrient Agar Medium (HiMedia) in 100 milliliters of distilled water. The dissolved medium was autoclaved for 15 minutes at 121°C and 15 pounds of pressure. The autoclaved medium was placed onto 100mm Petri plates (25–30ml/plate) while still molten after being well mixed.

b) To make the nutritional broth, 2.8 g of commercially available nutrient medium (HiMedia) was dissolved in 100 ml of distilled water. The combination was then brought to a boil until the medium was fully dissolved. After the desired dispensing, the medium was sterilised by autoclaving it for 15 minutes at 121ºC (15 lbs of pressure).

Procedure: The process involved seeding 20 ml of nutritional agar medium into petri plates with a 24-hour culture of bacterial strains that were adjusted to a 0.5 OD value in accordance with the McFarland standard. The concentration of test sample MS PS (500 µg/ml) was added after wells were cut. The plates were also incubated for twenty- four hours at 37 °C. By measuring the diameter of the inhibitory zone that developed around the wells, the antibacterial activity was evaluated. Utilising Graph Pad Prism 6.0 software (USA), the data were computed.

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3. Result And Discussion:

3.1 Physiochemical Evaluation of Ointment:

Table No 3: Physiochemical Parameters of ointment

S.NO	Physiochemical Parameters	Observation
1.	Appearance	Dark green
2.	Odour	Characteristic
3.	Consistency	Smooth
4.	Spread ability (sec)	5 sec
5.	pH	6.0
6.	Washability	Non washable
7.	Non irritancy	Non irritant
8.	Viscosity	4900 cps
9.	Extrudability	Easily extrudable
10.	Loss on drying	0.3%

The formulation exhibited a dark green appearance with a characteristic odor. It had a smooth consistency, ensuring ease of application. The spread ability recorded as five, while the pH measured at 6.0. The formulation was non washable and proved to be non-irritant upon application. It demonstrated a viscosity of 4900 cps, ensuring appropriate thickness and stability. The loss on drying was 0.3% and easily extrudable.

Anti- microbial activity:

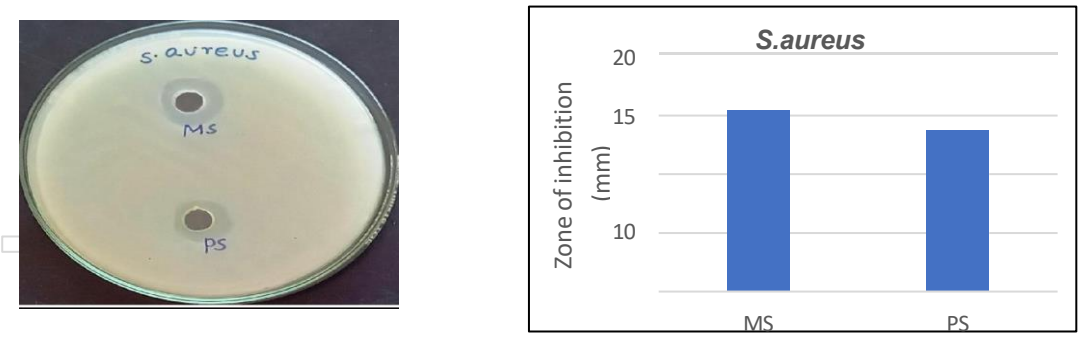

Fig no 1 : Effect of Sample MS PS Against *S.aureus.*

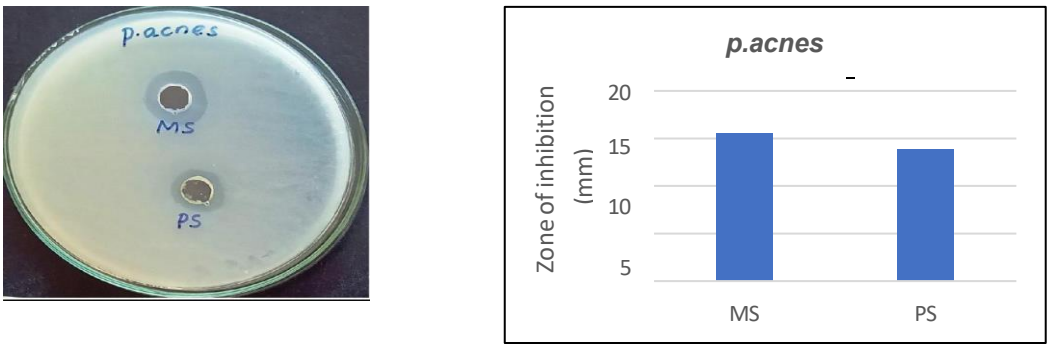

Fig no 2 : Effect of Sample MS PS Against *P.acnes.*

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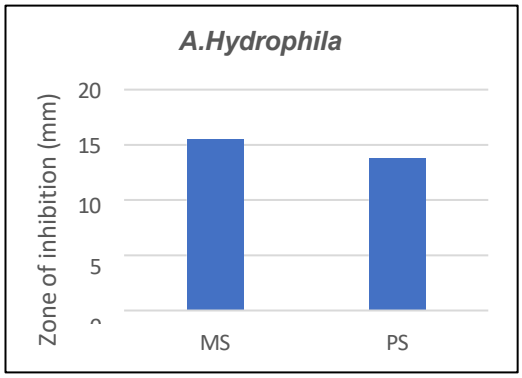

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Fig no 3: Effect of Sample MS PS Against *E.coli.*

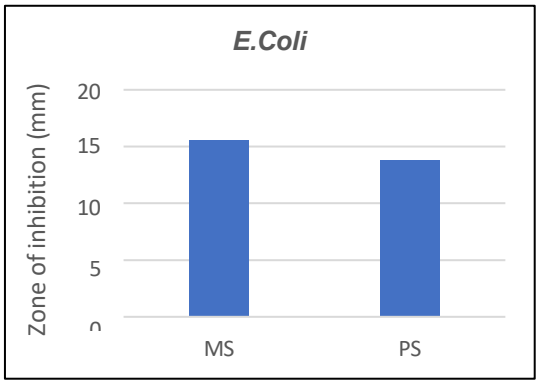

Fig no 4: Effect of Sample MS PS Against *A.hydrophila*

Means ± SD of Zone of Inhibition Obtained by Sample MS PS Against Test Organisms

Table no 4: Zone of Inhibition

S. No	Name of the Test Organism	Name of the Test Sample	Zone of Inhibition (mm) Mean±SD	
			MS	PS
1.	*S.aureus*	MS	13.6±0.56	12.75±0.35
2.	*P.acnes*		15.1±0.14	12.5±0.70
3.	*E.coli*	PS	14.5±0.70	15.6±0.56
4.	*A.hydrophila*		15.5±0.70	13.75±0.35

SD – Standard Deviation, *Significance - $p < 0.05$

Propionibacterium acnes (P. acnes), Escherichia coli (E. coli), Aeromonas hydrophila (A. hydrophila), and *Staphylococcus aureus (S. aureus)* were the four pathogenic bacteria against which the anti-microbial activity of the tested samples, MS and PS, was assessed. The zone of inhibition (mm), which measures each sample's inhibitory effect, was measured.The inhibitory effect of each sample was determined by measuring the zone of inhibition (mm), and the results are presented as Mean ± SD. The results indicate that both MS and PS exhibited notable antimicrobial activity against all tested organisms, with varying levels of inhibition. *S. aureus* showed an inhibition zone of 13.6±0.56 mm for MS and 12.75±0.35 mm for PS, suggesting that MS had slightly higher efficacy against this Gram-positive bacterium. Similarly, against *P. acnes*, the zone of inhibition was 15.1±0.14 mm for MS, whereas PS showed a slightly lower inhibition of 12.5±0.70 mm, highlighting the effectiveness of MS in controlling *P. acnes*.

In the case of *E. coli*, PS exhibited a larger inhibition zone (15.6±0.56 mm) compared to MS (14.5±0.70 mm), indicating better antibacterial potential of PS against this Gram-negative bacterium. Conversely, for *A. hydrophila*, MS displayed a slightly higher zone of inhibition (15.5±0.70 mm) compared to PS (13.75±0.35 mm), demonstrating the superior effectiveness of MS against this pathogen.

According to the results, MS and PS both have strong antimicrobial activity, but MS is more effective against Gram-positive bacteria (like *S. aureus and P. acnes*) while PS is more effective against Gram-negative bacteria (like E. coli). Possible explanations for the variances in antimicrobial effects include variations in the samples' composition, bioactive chemicals, or modes of action. The results are statistically significant with $p<0.05$, confirming the reliability of the observed differences in antimicrobial activity.

These results support the potential application of MS and PS as effective antimicrobial agents in pharmaceutical or cosmetic formulations targeting bacterial infections. Further investigations, including mechanistic studies and in vivo evaluations, would be beneficial to confirm their therapeutic potential.

3.2 Stability study:

Table No 5: Stability Study of Ointment

Time period	Colou r	Consistency	Spread ability (sec)	PH	Washabil ity	Irritancy	Stability	Viscosity
Day1	Dark green	Smooth	5	6.0	Non- washable	Non irritant	Stable	4900 cps
After 3 months	Dark green	Smooth	5	6.1	Non- washable	Non irritant	Stable	4800 cps
After6 months	Dark green	Smooth	5	6.0	Non- washable	Non irritant	Stable	4800 cps

In accordance with ICH requirements, a stability study was carried out to assess the formulation's physicochemical characteristics over a six-month period. The parameters assessed included colour, consistency, spread ability, pH, washability, irritancy, viscosity, and overall stability.

On Day 1, the formulation exhibited a dark green color with a smooth consistency and a spread ability value of five. The pH recorded at 6.0, and the non- washable. It was found to be non- irritant and remained stable with a viscosity of 4900 cps.

After three months, no significant changes were observed. The colour, consistency, spread ability, pH, washability, and irritancy remained unchanged, indicating good stability. However, a slight decrease in viscosity was noted, reducing to 4800 cps, though this did not affect the formulation’s performance or quality.

4. Conclusion:

The present study successfully formulated and evaluated a polyherbal ointment for wound healing activity using *Tridax procumbens, Acalypha indica, and Mimosa pudica.* In accordance with ICH recommendations, the produced formulation's stability, anti-microbial activity, and physicochemical characteristics were evaluated. The ointment exhibited a dark green appearance, smooth consistency, good spread ability, and a pH of 8.0, ensuring its suitability for topical application. Stability studies confirmed that the formulation remained stable over six months without significant changes in its physicochemical properties. The polyherbal ointment's potential as an antibacterial agent was highlighted by the antimicrobial evaluation, which showed that it successfully suppressed the development of both Gram-positive and Gram-negative bacteria. Among the tested microorganisms, the formulation showed better inhibition against *Staphylococcus aureus, E.coli, A.hydrophila* and *Propionibacterium acnes,* supporting its efficacy in wound healing and skin- related infections. Overall, the study indicates that the formulated polyherbal ointment is a promising candidate for wound healing applications, with effective antimicrobial properties and long-term stability. Future studies involving in vivo wound healing models and clinical trials would further validate its therapeutic potential.

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All the authors contributed equally for the work.

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