RESEARCH PAPER

Development, Standardization, and Evaluation of a Polyherbal Topical Ointment Incorporating Tea Tree and Rosemary Extracts

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Abstract:

The present study focused on the development and evaluation of a polyherbal ointment formulated using extracts of *Melaleuca alternifolia* (Tea Tree) and *Rosmarinus officinalis* (Rosemary) for topical application. The plant materials were collected, authenticated, shade-dried, and subjected to Soxhlet extraction using suitable solvents. Physicochemical standardization parameters, including ash values, moisture content, extractive values, and pH, confirmed the purity and quality of the crude drugs within acceptable limits. Preliminary phytochemical screening revealed the presence of flavonoids, phenolics, tannins, steroids, and triterpenoids in both extracts, while saponins were detected in rosemary. Five ointment bases were prepared and evaluated for color, odor, consistency, pH, and viscosity, from which three optimized bases were selected for further formulation. The final herbal ointments (HFB-1, HFB-2, and HFB-3) were assessed for physicochemical properties including pH, viscosity, moisture content, spreadability, and extrudability, all of which were found to be within acceptable ranges for dermal use. Among the formulations, HFB-3 demonstrated a balanced profile of consistency, spreadability, and stability, while HFB-2 showed superior extrudability. Overall, the developed polyherbal ointments were stable, skin-compatible, and rich in bioactive constituents, indicating their potential as effective and safe natural topical formulations.

Keywords:*Melaleuca alternifolia*, *Rosmarinus officinalis*, Polyherbal ointment, Phytochemical screening, Physicochemical evaluation

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1. Introduction

Herbal medicines have gained considerable scientific attention due to their diverse pharmacological activities and comparatively lower side effect profiles than synthetic agents, making them attractive candidates for topical therapeutic applications (Carson et al., 2006; Kairey et al., 2023). Essential oils and plant extracts are particularly studied for their antimicrobial, anti-inflammatory, antioxidant, and wound healing properties, which are critical for effective dermal therapy and skin care formulations (Carson et al., 2006; Alves et al., 2024). The growing interest in natural alternatives reflects both traditional knowledge and modern pharmacological validation, emphasizing the potential of botanicals in supporting skin health.

Melaleuca alternifolia (tea tree), native to Australia, is well known for its essential oil rich in terpinen-4-ol and other monoterpenes. These bioactive compounds have demonstrated broad-spectrum antimicrobial activity against skin pathogens and the ability to accelerate wound closure, reduce bacterial load, and support healing in infected wound models (Carson et al., 2006; Edmondson et al., 2011; Alves et al., 2024). Clinical studies indicate that tea tree oil can be comparable to standard therapies for decolonizing methicillin-resistant *Staphylococcus aureus* from wounds, highlighting its translational relevance in topical treatments (Edmondson et al., 2011; Kairey et al., 2023).

Similarly, Rosmarinus officinalis (rosemary) has long-standing applications in both traditional and modern phytotherapy, owing to bioactive constituents such as 1,8-cineole, α-pinene, camphor, carnosic acid, and rosmarinic acid (Borges et al., 2019; Li Pomi et al., 2023; Gonçalves et al., 2022). These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial effects, contributing to enhanced wound contraction, accelerated re-epithelialization, and reduced inflammatory cell infiltration in preclinical models (Abu Al Basal, 2010; Xu et al., 2025; de Oliveira et al., 2019). Rosemary also demonstrates antibiofilm activity against resistant pathogens, reinforcing its potential in topical formulations aimed at wound care and infection control (Alves et al., 2024; Rosmarinus officinalis anti-biofilm study, 2026).

The pharmacological profiles of tea tree and rosemary—covering antimicrobial, antioxidant, and anti-inflammatory actions—provide a strong rationale for developing polyherbal ointments that

combine complementary mechanisms of action (Meroni et al., 2020; Hussain et al., 2010; Kairey et al., 2023). Studies suggest that combined essential oils can synergistically enhance wound healing responses in vivo, indicating that properly standardized formulations may achieve improved therapeutic outcomes compared to individual extracts (Labib et al., 2019; Xu et al., 2025). Despite extensive evaluation of these herbs individually, research on optimized polyherbal topical bases incorporating standardized M. alternifolia and R. officinalis extracts remains limited.

Therefore, systematic development, standardization, and physicochemical evaluation of polyherbal ointments containing tea tree and rosemary are essential to ensure efficacy, stability, and skin compatibility. The combination of their antimicrobial, anti-inflammatory, and antioxidant effects offers a rational basis for enhancing wound healing and dermal repair. This study aims to formulate and evaluate such polyherbal topical systems, leveraging the complementary bioactivities of M. alternifolia and R. officinalis to achieve enhanced therapeutic outcomes in skin care and wound management applications.

2. Collection of Plant Specimens

The fresh leaves and essential oils of Melaleuca alternifolia (commonly known as Tea Tree) and Rosmarinus officinalis (Rosemary) were collected during the appropriate harvesting season to maximize the yield and potency of their active constituents. The collection was performed from a certified botanical local farm known for cultivating medicinal plants or purchased from a verified commercial supplier.

2.1 Preparation of Extract

The plant material was locally procured and authenticated by a Botany professor. It was washed, shade-dried for 7–12 days, and powdered. Soxhlet extraction was performed separately using methanol, ethanol, water, and hydroalcoholic (50:50) solvents at 50–60°C for 16–20 hours until the siphon solvent was colorless. Extracts were concentrated under reduced pressure below 40°C, lyophilized, weighed for yield, and stored in airtight containers at –4°C until use.

2.2 Principle editing

The Soxhlet extractor works on the principle of continuous solvent reflux and siphoning for efficient extraction of soluble constituents from solid materials. The solvent is heated, vaporized, condensed, and allowed to percolate through the sample, dissolving the extractable compounds. When the solvent reaches the siphon level, it returns to the flask carrying the dissolved constituents, and the cycle repeats, ensuring exhaustive extraction. As organic solvents may also extract fatty substances, the method generally yields a crude extract.

3. Determination of Extractive Values

Extractive values assess the quality, purity, and potential adulteration of crude drugs by measuring the amount of constituents soluble in specific solvents. For alcohol-soluble extractive, 5 g of seed powder was macerated with 100 ml ethanol for 24 hours, shaken intermittently for the first 6 hours, and then left to stand. The extract was filtered, and a portion was evaporated to dryness and dried at 105°C to a constant weight to determine the percentage of alcohol-soluble constituents.

4. Standardization parameters

The different standardization/physicochemical methods were carried out in dried powder of different Extracts of *Melaleuca alternifolia and Rosmarinus officinalis*.

4.1 Foreign Matter

Foreign matter includes any extraneous material in a crude drug that does not belong to the authentic plant, such as other plant parts, sand, stones, insects, animal excreta, or molds. To determine it, 100 g of powdered drug was spread on a white surface, examined visually and with a magnifying lens, and the foreign particles were separated and weighed to calculate their percentage in the sample.

4.2 Total Ash value

Ash is the inorganic residue left after incineration of crude drugs and reflects their quality and purity. It mainly contains salts like phosphates, carbonates, and silicates of sodium, potassium, calcium, and magnesium. Total ash is determined by burning 2–3 g of powdered drug in a crucible at 450–500 °C until carbon-free, then cooling and weighing. It can be further assessed as water-soluble ash or acid-insoluble ash.

4.3 Water soluble Ash value

Water-soluble ash represents the portion of total ash that dissolves in water and is determined by subtracting the weight of the insoluble residue from the total ash. The total ash was boiled with 25 ml of distilled water for 5 minutes and filtered through Whatman filter paper (Grade 589/3). The insoluble residue was washed with hot water, incinerated at about 450°C until free from carbon, cooled in a desiccator, and weighed. The difference in weight was used to calculate the percentage of water-soluble ash with reference to the crude drug sample.

4.4 Acid insoluble Ash value

Acid-insoluble ash (AIA) measures the inorganic residue, mainly silica, remaining after a crude drug is incinerated and treated with dilute hydrochloric acid, providing an indication of purity and mineral content. To determine AIA, the total ash is boiled with 25 ml of 2 M HCl, and the insoluble matter is collected on ashless filter paper, washed with hot water, and ignited in a crucible at temperatures not exceeding 450 °C until carbon-free. The crucible is cooled in a desiccator, and the weight of the residue is used to calculate the percentage of acid-insoluble ash relative to the original drug sample.

4.5 Loss on drying (Moisture content)

Loss on drying (LOD) measures the water and volatile content of a sample, expressed as a percentage of weight loss. It is used to determine moisture, solvents, and other volatile impurities by drying the sample under specified conditions. For testing, 5 g of powdered drug is weighed in a pre-weighed Petri dish, dried in a hot air oven at 110 °C for 120 minutes, and then cooled in a desiccator. The weight loss after drying reflects the amount of water and volatiles present in the sample.

4.6 Water soluble extractive value

Extractive values indicate the amount of active constituents, such as alkaloids, glycosides, and tannins, that can be extracted from a crude drug using a solvent. They are important for assessing the pharmacognostical and pharmacological potency of plant material—the higher the extractive value, the more potent the drug. Water-soluble extractive values measure the percentage of water-soluble constituents and are used for quality control, stability assessment, and detection of exhausted or improperly processed material. For determination, 5 g of the crude drug is macerated in 100 ml distilled water for 6 hours with stirring, allowed to stand for 18 hours, and 25 ml of the filtrate is evaporated to dryness at 110 °C. The weight of the residue is used to calculate the extractive value.

4.7 Alcohol soluble extractive value

Alcohol-soluble extractive value measures the amount of alcohol-soluble constituents present in a crude drug and reflects the potency and quality of the material. It is determined similarly to the water-soluble extractive, but using alcohol as the solvent instead of water, with the resulting extract weighed to calculate the percentage of alcohol-soluble matter in the sample.

4.8 Determination of pH Values

pH measures the acidity or alkalinity of a solution on a scale of 0–14, with 7 being neutral, values below 7 acidic, and above 7 basic. To determine the pH of the crude drug, 10 g of powder was mixed with 100 ml distilled water to make a 10% solution, stirred for 45 minutes, and filtered. The pH was then measured using a calibrated pH meter.

5. Phytochemical of medicinal plant extracts

Preliminary phytochemical screening of the extracts has shown the presence of alkaloids, tannins, saponins, steroids and flavanoids

5.1 Test for Carbohydrate

Carbohydrates provide energy, act as building blocks for complex biomolecules, and serve as storage in plants and animals. Common examples include glucose, fructose, sucrose, ribose, starch, and cellulose. The extract was dissolved in ethanol and hydrolyzed with 2N HCl to prepare a stock solution. Molisch's test detects all carbohydrates, forming a purplish-violet ring with α-naphthol and concentrated H_2SO_4. Barfoed's test distinguishes monosaccharides from disaccharides by forming a brick-red precipitate with cuprous oxide. Benedict's test identifies reducing sugars through color changes of cuprous oxide precipitate in alkaline solution. Fehling's test similarly detects reducing sugars and aldehydes by producing a red Cu_2O precipitate.

5.2 Test for Proteins

Proteins were detected by preparing a stock solution of the extract in ethanol. Biuret test identifies peptide bonds, forming a violet complex when mixed with sodium hydroxide and copper sulfate. Million's test detects phenolic amino acids like tyrosine, producing a white precipitate that turns brick red on heating with Million's reagent. Xanthoproteic test identifies aromatic amino acids, giving a yellow precipitate with concentrated nitric acid that turns orange after adding alkali.

5.3 Test for Steroids

Extracts were saponified with ethanolic potassium hydroxide, and the unsaponifiable matter was extracted with diethyl ether, evaporated, and dissolved in chloroform for testing. Salkowski test detects cholesterol, sterols, and indoles, giving a red color in the chloroform layer and greenish-yellow fluorescence in the acid layer. Liebermann–Burchard test identifies cholesterol, steroids, and triterpenoids, producing a green or green-blue color for cholesterol and deep red in the lower layer for steroids/triterpenoids.

5.4 Test for Amino Acids

The Ninhydrin test detects amino acids by reacting free amino groups with ninhydrin, producing a blue-purple color. Cysteine is specifically identified by adding 40% NaOH and 10% lead acetate to the extract; a black lead sulfide precipitate upon boiling confirms its presence. The Biuret test identifies proteins, where peptide bonds react with copper (II) ions in a basic solution to form a deep violet complex, indicating protein content.

5.5 Test for Glycosides

Stock solutions were prepared by dissolving plant extracts in ethanol. The Keller–Kiliani test detects cardiac glycosides: 2–3 ml of extract is treated with a few drops of 5% $FeCl_3$ and glacial acetic acid, followed by concentrated H_2SO_4. A reddish-brown upper layer and a blue-green lower layer indicate the presence of glycosides. The Bromine water test identifies alkenes or aldehyde groups in glycosides. The extract is dissolved in bromine water; a color change from yellow to colorless or formation of a yellow precipitate confirms glycoside presence.

5.6 Test for Flavonoids

Flavonoids in plant extracts were detected using multiple qualitative tests. In the Shinoda test, a few pieces of magnesium ribbon and concentrated HCl were added to the ethanolic extract; development of pink, scarlet, crimson, or green-blue color within a few minutes indicated flavonoids. The Alkaline Reagent Test involved treating the aqueous extract with 10% NH_4OH, producing yellow fluorescence as a positive result. In the Zinc-Hydrochloride Reduction Test, adding zinc dust and concentrated HCl to the extract gave a red color, confirming the presence of flavonoids.

5.7 Test for Vitamins

Equal quantity of 12-16 units in chloroform 1 ml and was mixed 8-10 ml antimony trichloride solution was added a moving blue color is obtained directly which shows the presence of vitamin A.

5.8 Test for Ascorbic acid

For ascorbic acid, 1 ml of 2% w/v sample solution was dissolved in 5 ml distilled water. Sodium nitroprusside (5% w/v) was added, followed by 2 ml NaOH. Upon gradual addition of 0.5–0.6 ml HCl with stirring, a color change from yellow to blue indicated the presence of ascorbic acid. For Vitamin D, an equal quantity of 100 units of Vitamin D was dissolved in chloroform and mixed with 10 ml antimony trichloride. The immediate appearance of a pinkish-red color confirmed the presence of Vitamin D.

6. Preparation of Ointment

Formulation of ointments containing using different ointment bases

List of ingredients used to formulate herbal ointment

Table 1: Herbal Ointment Base Composition (% w/w)

S.No	Ingredients	Hob-1	Hob-2	Hob-3	Hob-4	Hob-5
1	Sesame oil *(natural emollient)*	8	9	7	10	8
2	Xanthan gum *(natural gelling agent)*	1	1.5	1.2	1.3	1
3	Melaleuca alternifolia oil *(tea tree oil)*	3	2	3	2	3
4	Rosmarinus officinalis oil *(rosemary oil)*	2.5	3	2	3	2
5	Shea butter *(natural thickening agent)*	6	5	6	5	6
6	Aloe vera gel (lyophilized) *(healing aid)*	1.5	2	1.5	2	1.5
7	Lecithin *(natural emulsifier)*	2	1.5	2	1	1
8	Glycerol *(humectant, skin softener)*	4	4	4	4	4
9	Sodium benzoate *(preservative alternative)*	0.04	0.04	0.04	0.04	0.04
10	Citric acid *(for pH adjustment)*	q.s.	q.s.	q.s.	q.s.	q.s.
11	Purified Water	q.s.	q.s.	q.s.	q.s.	q.s.

7. Evaluation of ointment base

7.1 Visualization of physical changing

The physical evaluation of an ointment base assesses its quality, stability, and usability by examining attributes like color, smoothness, odor, and phase separation. Color is checked under consistent lighting for hue and any changes over time, while smoothness is evaluated by spreading the ointment on the skin or a surface to observe texture, spreadability, and residue. These observations ensure the formulation is uniform, appealing, and suitable for use.

7.2 pH measurement

Before testing, ensure the pH meter and electrodes are clean and calibrated. Prepare representative samples in suitable containers, diluting with distilled water if needed to fit the meter's range. Immerse the electrode fully in the sample without touching the container sides or bottom, and wait for the reading to stabilize. Record the pH, and rinse the electrode with distilled water between measurements to avoid cross-contamination and maintain accuracy.

7.3 Viscosity measurement

The viscosity of each formulation was measured before and after accelerated testing using a Brookfield Viscometer at 100 rpm with spindle number 7. Representative samples were prepared in clean containers, and temperature was standardized. The spindle was immersed in each sample, and readings were recorded once stabilized to establish a baseline. After subjecting the formulations to accelerated conditions, viscosity measurements were repeated under the same

parameters, allowing direct comparison to assess any changes in flow behavior or consistency.

8. Selection of a suitable ointment base

With higher percentages of lower amounts of beeswax, this formulation might result in a ointment with better cleansing properties. It could be useful for skincare products intended for oily or acne-prone skin.

8.1 Preparation of Ointment

The water phase (purified water, 70–75°C) and oil phase (coconut oil, shea butter, lecithin) were heated separately and mixed according to formulation ratios. The oil phase was slowly added to the water phase with stirring to form a stable emulsion. After cooling to 40–45°C, essential oils, Aloe vera gel, glycerol, and potassium sorbate were incorporated, and citric acid adjusted the pH to 5.5–6.5. The ointment was stirred while cooling, then transferred into clean, airtight containers for storage.

Table 2: Formulation ingredients of the ointment

S.No	Ingredients	Base Only	HFB-1	HFB-2	HFB-3
1	Hob-1	–	3.0	2.0	3.0
2	Hob-3	–	2.0	2.0	3.0
3	Hob-5	-	3.0	2.0	2.0
4	Shea butter	4.0	3.5	4.5	3.5
5	Aloe vera gel	3.0	2.0	2.0	2.0
6	Lecithin	2.0	1.5	1.5	1.5
7	Glycerol	5.0	4.0	4.5	4.0
8	Potassium sorbate	0.05	0.05	0.05	0.05
9	Citric acid (pH adjuster)	q.s.	q.s.	q.s.	q.s.
10	Purified water	q.s. to 100	q.s.	q.s.	q.s.

9. Evaluation of herbal ointment

9.2 pH measurement

The pH of each herbal ointment was determined using a calibrated pH meter. One gram of ointment was diluted with 9 mL of distilled water and mixed thoroughly for uniform dispersion. After calibrating the pH meter with standard buffers (pH 4, 7, and 10), the electrode was immersed in the sample, and the stabilized reading was recorded for each formulation.

9.2 Viscosity measurement

The viscosity of each herbal ointment formulation was measured using a Brookfield Viscometer at 100 rpm with spindle number 7. Samples were prepared in clean containers and mixed thoroughly for homogeneity. Initial viscosity readings were recorded before conducting accelerated tests to provide baseline values for comparison.

9.3 Moisture content

The moisture content of the samples was measured using an IR-30 Denver Instruments moisture analyzer with a 30 g capacity and 1 mg resolution. Accurately weighed samples were evenly spread on the sample pan and heated at 105°C using infrared elements. The analyzer continuously monitored the weight loss due to moisture evaporation, and once a stable weight was reached, it automatically calculated and displayed the percentage of moisture in the samples.

9.4 Physical parameter

The physical characteristics of the herbal ointments were assessed at room temperature and under accelerated conditions, focusing on homogeneity and appearance. Homogeneity was evaluated visually and by touch, observing for uniform distribution, absence of clumps or separation, and consistent texture and color. Appearance was judged based on color consistency and intensity, presence of pearlescence, and surface texture, noting any roughness or irregularities to ensure uniform and aesthetically acceptable formulations.

9.5 Spreadibilty

The consistency of the moisturizer was assessed through spreadability and layer thickness using a controlled setup. One gram of the formulation was placed on a lower plate, and an upper plate weighing 42 g was applied with additional weights to exert a constant force, allowing the sample to spread evenly. The spread area was measured to determine spreadability. **After-feel** properties, including emolliency, slipperiness, and residue on the skin, were evaluated to assess comfort and absorption. The **type of smear** formed was observed for uniformity and evenness, while **removal** was tested by washing with tap water to ensure ease of cleansing without leaving significant residue.

10. Results

10.1 Macroscopic studies:

The macroscopic evaluation of Melaleuca alternifolia (tea tree) and Rosmarinus officinalis (rosemary) leaves showed clear differences in morphology and organoleptic properties. Tea tree leaves are narrow, lanceolate, 1–3 cm long, with a sharp, camphoraceous odor and bitter taste, while

rosemary leaves are linear, needle-like, 2–4 cm long, with a pine-like aroma and slightly bitter, aromatic taste. Both samples were free of foreign matter, confirming proper harvesting and processing. These characteristics serve as reliable markers for identification, authenticity, and quality of the herbal materials.

Table 3: Table comparing the macroscopic parameters of melaleuca alternifolia (tea tree) leaves and rosmarinus officinalis (rosemary) leaves:

S.No	Parameters	Observations of *Melaleuca alternifolia* Leaves	Observations of *Rosmarinus officinalis* Leaves
1.	**Shape**	Narrow, lanceolate	Linear, needle-like
2.	**Size**	1–3 cm long, ~2–5 mm wide	2–4 cm long, ~2–5 mm wide
3.	**Odour**	Strong, camphoraceous, medicinal	Strong, aromatic, pine-like
4.	**Taste**	Bitter, slightly pungent	Aromatic, slightly bitter
5.	**Colour**	Dull green	Dark green upper side, whitish underside
6.	**Foreign organic matter**	Absent	Absent

10.2 Physicochemical Standardization of Proposed Plant Drug

The standardization of Melaleuca alternifolia (tea tree) and Rosmarinus officinalis (rosemary) leaves ensures their identity, purity, and quality for therapeutic use. Physicochemical analysis showed total ash values of 6.85% (tea tree) and 9.75% (rosemary), with water-soluble ash at 2.75% and 4.75%, indicating higher soluble minerals in rosemary. Acid-insoluble ash was low (0.75% and 0.90%), confirming minimal earthy contamination. Moisture content was 7.0% (tea tree) and 9.0% (rosemary), within safe limits, though rosemary may need careful storage. Overall, both plants are of good quality, with minor differences reflecting their distinct chemical compositions.

Table 4: Standardization parameters

S.No	Parameters (% w/w)	*Melaleuca alternifolia*	*Rosmarinus officinalis*
1.	Ash value	6.85%	9.75%
2.	Foreign organic matter	1.0%	1.0%
3.	Water soluble ash	2.75%	4.75%
4.	Acid insoluble ash	0.75%	0.90%
5.	Moisture content	7.0%	9.0%

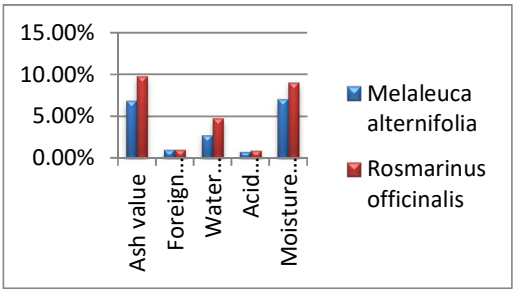

Figure 1:Standardization parameters ofMelaleuca alternifolia and Rosmarinus officinalis

10.3Prelimnary Phytochemical Analysis of Extracts

Phytochemical screening of Melaleuca alternifolia and Rosmarinus officinalis showed the presence of steroids, triterpenoids, tannins, phenolics, and flavonoids, which contribute to their anti-inflammatory, antimicrobial, and antioxidant properties. Saponins were found only in rosemary, adding emulsifying and immune-supportive effects. Proteins were absent, while reducing sugars were present in both plants. These results confirm that both herbs are rich in bioactive compounds, with rosemary offering additional therapeutic benefits due to its saponin content.

Table 5: Phytochemical Profile of Melaleuca alternifolia, Rosmarinus officinalis.

S.no	Chemical Tests	Melaleuca alternifolia	*Rosmarinus officinalis*
1.	Tests for		

	Steroids and Triterpenoids:		
	• Liebermann's Burchard Test	+	+
	• Salkowski Test	+	+
2.	Test for Saponins:		
	• Foam Test	-	+
3.	Tests for Alkaloids:		
	• Hager's Test	-	-
	• Mayer's Test	-	-
4.	Tests for Glycosides:		
	• Borntrager's Test	-	-
	• Keller Killiani Test	-	-
5.	Tests for Tannins and Phenolic compounds:		
	• Gelatin Test	+	+
	• Ferric Chloride Test	+	+
6.	Tests for Flavonoids:		
	• Ferric chloride Test	+	+
	• Alkaline reagent Test	+	+
7.	Tests for Proteins:		
	• Biuret Test	-	-
	• Xanthoproteic Test	-	-
8.	Test for Carbohydrates:		
	• Fehling Test	+	+

Where + is Present and – is Absent

10.4 Evaluatory parameters of Colour, Odour and Consistency in formulations

Five ointment base formulations (Hob-1 to Hob-5) were evaluated for physical characteristics including color, odor, and consistency. Hob-1 and Hob-5 were pure white, Hob-2 off-white, Hob-3 creamy white, and Hob-4 pale yellow, reflecting differences in ingredients or processing. Odor varied: Hob-1 and Hob-4 retained characteristic base scents, Hob-2 was pleasantly scented, Hob-3 mild, and Hob-5 odorless. Consistency ranged from smooth and semi-solid (Hob-1) to soft and uniform (Hob-2), slightly greasy (Hob-3), firm and homogeneous (Hob-4), and smooth, non-greasy (Hob-5), indicating differences in lipid content and spreadability.

Table 6: Results of ointment base parameters of Colour, Odour and Consistency in formulations

S. No	Evaluation parameters	Formulation % w/w				
		Hob-1	Hob -2	Hob - 3	Hob-4	Hob -5
	Colour	White	Off - white	Creamy white	Pale yellow	White
	Odour	Characteristic	Pleasant	Mild	Characteristic	Odourless
	Consistency	Smooth, semi-solid	Soft, uniform	Slightly greasy	Firm, homogeneous	Smooth, non-greasy

10.4.1 pH measurement

The pH of topical formulations is essential for ensuring skin compatibility, safety, and stability, as the natural skin pH ranges from 4.5 to 6.0. In this study, the pH of five ointment base formulations (Hob-1 to Hob-5) ranged from 6.2 to 6.6, which is close to neutral and suitable for topical application. Hob-2 showed the lowest pH (6.2), closely matching skin pH, while Hob-3 had the highest (6.6), still within acceptable limits. These slight variations may be due to differences in ingredients or processing. Overall, all formulations are appropriate for dermal use, with Hob-2 and Hob-4 being slightly more favorable due to better pH compatibility.

Table 7: pH measurement

S.No	Formulation % w/w	pH
1.	Hob-1	6.4

2.	Hob-2	6.2
3.	Hob-3	6.6
4.	Hob-4	6.3
5.	Hob-5	6.5

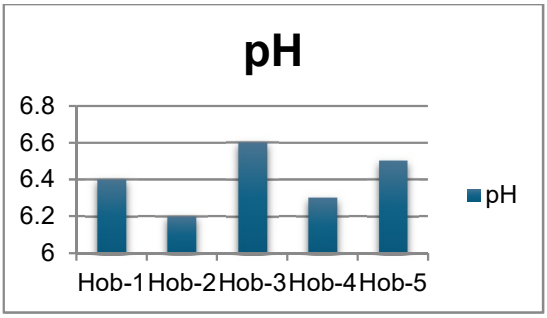

Figure 2: pH measurement

10.4.2 Viscosity measurement

Viscosity is a key physical property of topical ointments, affecting spreadability, application, and retention on the skin. In this study, the viscosity of the five formulations (Hob-1 to Hob-5) ranged from 11,500 to 15,500 cP. Hob-3 showed the highest viscosity (15,500 cP), offering better residence on the skin but potentially less ease of spreading, while Hob-2 had the lowest (11,500 cP), allowing easier application but lower adherence. Hob-1 (13,500 cP), Hob-4 (12,500 cP), and Hob-5 (14,500 cP) had intermediate viscosities, balancing spreadability and retention. Overall, all formulations are within acceptable limits, with Hob-1, Hob-4, and Hob-5 showing particularly favorable consistency for topical use.

Table 8: Viscosity measurement

S.No	Formulation % w/w	Viscosity
1.	Hob-1	13,500
2.	Hob-2	11,500
3.	Hob-3	15,500
4.	Hob-4	12,500
5.	Hob-5	14,500

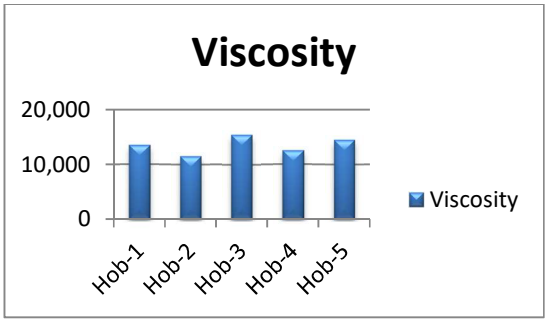

Figure 3:Viscosity Measurement

The best three formulations based on the provided results

The best ointment formulations (Hob-1 to Hob-5) were selected based on viscosity, which affects spreadability, stability, and skin retention. Hob-3 had the highest viscosity (15,500 cP), offering a firm, stable base suitable for prolonged contact but slightly harder to spread. Hob-5 (14,500 cP) provided a thick yet easily spreadable consistency, balancing stability and comfort, while Hob-1 (13,500 cP) offered moderate viscosity for easy application with adequate body. Hob-2 and Hob-4, with lower viscosities (11,500 cP and 12,500 cP), were less favorable. Overall, Hob-3, Hob-5, and Hob-1 were selected for their optimal combination of stability, retention, and ease of use.

Table 9: Viscosity-Based Ranking of Polyherbal Ointment Formulations

Rank	Formulation	Mean Viscosity (cP)	Reason for Selection
1	**Hob-3**	15,500	Highest viscosity, good for stable, firm base
2	**Hob-5**	14,500	Slightly less viscous, still thick and stable
3	**Hob-1**	13,500	Moderate viscosity, balanced spreadability

10.4.3 Physical parameter

The herbal ointments HFB-1, HFB-2, and HFB-3 were evaluated for colour, odour, and consistency. HFB-1 was off-white with mild herbal odour and smooth, soft texture; HFB-2 was creamy yellow with characteristic odour and firm, slightly greasy texture; HFB-3 was pale yellow with pleasant mild odour and smooth, non-greasy feel. All showed acceptable physical properties, with HFB-3 preferred for daily use and HFB-2 suited for longer skin retention.

Table 10: Evaluatory parameters of Physical parameter

S. No	Formulation	Evaluation Parameter		
		Colour	Odour	Consistency
1.	HFB-1	Off-white	Mild herbal	Smooth, soft
2.	HFB-2	Creamy yellow	Characteristic	Firm, slightly greasy
3.	HFB-3	Pale yellow	Pleasant mild	Smooth, non-greasy

Evaluatory parameters of pH of ointment formulation

The pH of topical formulations is key for stability, efficacy, and skin compatibility. HFB-1, HFB-2, and HFB-3 showed pH values of 6.3, 6.5, and 6.4, all within the normal skin range of 4.5–6.5. These values indicate that the formulations are safe, unlikely to cause irritation, and suitable for topical use, balancing stability with skin compatibility.

Table 11: Evaluatory parameters of pH

S.No	Formulation	Evaluation Parameter pH
1.	HFB-1	6.3
2.	HFB-2	6.5
3.	HFB-3	6.4

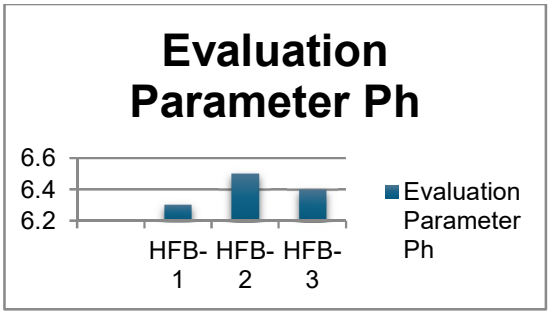

Figure 4:pH of ointment

10.4.4 Viscosity

Viscosity is an important factor in topical ointments, affecting spreadability, stability, and patient compliance. The three formulations showed viscosities between 12,500 and 14,200 cP. HFB-2 had the highest viscosity (14,200 cP), offering thicker consistency and potentially better skin retention, while HFB-1 was the least viscous (12,500 cP), allowing easier application. HFB-3 (13,800 cP) provided a balanced consistency. All formulations fall within an acceptable range, combining stability with ease of use.

Table 12: Evaluatory parameters of Viscosity

S.No	Formulation	Evaluation Parameter(Cp)
1.	HFB-1	12,500
2.	HFB-2	14,200
3.	HFB3	13,800

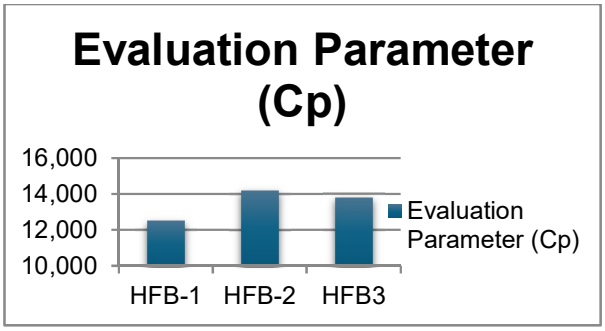

Figure 5:Evaluation Parameter (Cp)

10.4.5 Moisture content

Moisture content is a key factor in ointments, affecting stability, texture, and microbial safety. The three formulations showed moisture levels

between 3.8% and 4.2%, with HFB-2 having the lowest (3.8%), potentially enhancing shelf life and stability, while HFB-1 and HFB-3 (4.2% and 4.1%) maintained adequate softness and spreadability. All values are within acceptable limits, ensuring balanced consistency and durability across the formulations.

Table 13: Evaluatory parameters of Moisture content

S.No	Formulation	Moisture content (%)
1.	HFB-1	4.2
2.	HFB-2	3.8
3.	HFB-3	4.1

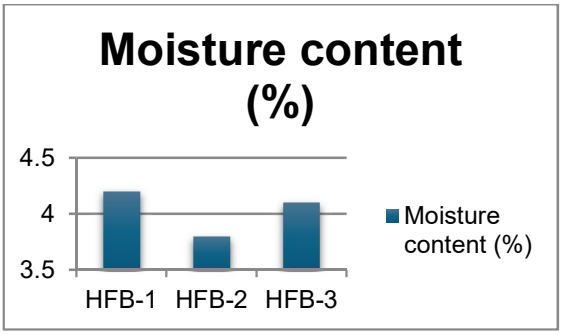

Figure 6:Moisture content (%)

10.4.6 Spreadibilty

The spreadability of the three ointment formulations reflects their ease of application on the skin. HFB-2 showed the fastest spreadability at 7.8 seconds, indicating a lighter, more easily applied consistency. HFB-3 had moderate spreadability at 8.2 seconds, balancing application ease and retention, while HFB-1 was the slowest at 9.5 seconds, suggesting a thicker base that may enhance stability. Overall, all formulations demonstrated satisfactory spreadability suitable for topical use.

Table 14: Evaluatory parameters of Spreadibilty

S.No	Formulation	Spreadibilty(sec)
1.	HFB-1	9.5
2.	HFB-2	7.8
3.	HFB-3	8.2

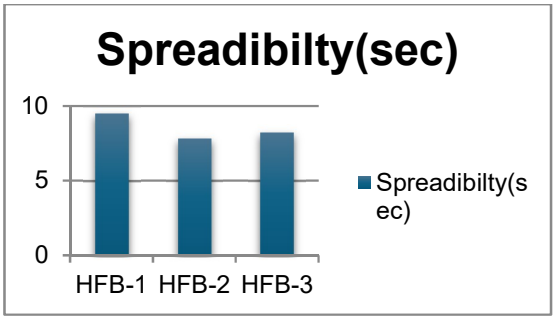

Figure 7:Spreadibilty of formulations

10.4.7 Extrudability

Extrudability reflects how easily an ointment can be dispensed from its container, impacting user convenience and dosing. Among the formulations, HFB-2 showed the highest extrudability at 18.4 g, indicating a soft, easily applied consistency. HFB-3 had moderate extrudability at 17.2 g, while HFB-1 was the firmest at 15.2 g. These differences are due to variations in base composition. All three formulations demonstrated acceptable extrudability, with HFB-2 being the most user-friendly.

Table 15: Evaluatory parameters of Extrudability (gm)

S.No	Formulation	Extrudability (gm)
1.	HFB-1	15.2
2.	HFB-2	18.4
3.	HFB-3	17.2

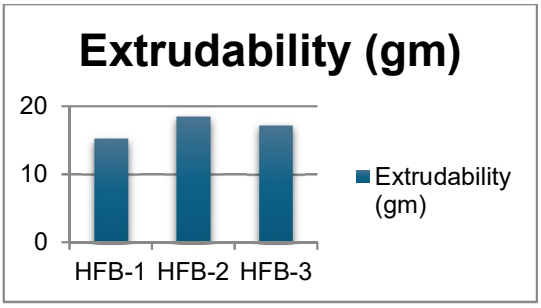

Figure 8: Extrudability (gm)

11.Conclusion

The present study successfully formulated and evaluated herbal ointments incorporating *Melaleuca alternifolia* (tea tree) and *Rosmarinus officinalis* (rosemary) extracts. Comprehensive physicochemical and phytochemical analyses confirmed the authenticity, purity, and richness of bioactive constituents, including flavonoids, phenolics, steroids, and saponins, with rosemary exhibiting additional therapeutic potential due to saponin content. Among the five ointment bases, Hob-1, Hob-3, and Hob-5 demonstrated optimal physical characteristics, pH, and viscosity, ensuring stability, spreadability, and skin compatibility. The final herbal formulations (HFB-1, HFB-2, HFB-3) exhibited acceptable color, odor, consistency, moisture content, viscosity, spreadability, and extrudability, indicating their suitability for topical application. Notably, HFB-3 balanced smooth texture, moderate viscosity, and ease of use, while HFB-2 offered enhanced skin retention and user-friendly extrudability. Overall, the study confirms that these herbal ointments are stable, effective, and safe for dermal use, highlighting their potential as natural alternatives for anti-inflammatory and skin-care applications.

12. Acknowledgements
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13. Conflict of Interest
The authors declare that they have no conflict of interest.

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