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RESEARCH ARTICLE

Development and Evaluation of Ginger Oil-Enriched Medicated Ointment

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Abstract: The aim of this study is to develop and evaluate a novel medicated ointment formulation enriched with ginger (*Zingiber officinale*) oil. Fresh ginger rhizomes were processed and subjected to hydrodistillation, yielding 2.34% essential oil. Five different ointment formulations (F1-F5) were prepared with varying ratios of ginger to virgin coconut oil (5:95 to 25:75) using a systematic formulation approach. The optimized base contained white soft paraffin, beeswax, and cetyl alcohol as stabilizing agents. The formulations were evaluated for physicochemical properties including spreadability, hardness, viscosity, pH, and particle size distribution. Results indicated that F3 (15% ginger oil) exhibited optimal characteristics with spreadability of 7.5 ± 0.2 cm, hardness of 189 ± 4.8 g-force, and viscosity of 3680 ± 142 cP. Phytochemical screening revealed significant presence of bioactive compounds, with F3 showing optimal concentrations of gingerols (4.78 ± 0.25 mg/g) and paradols (3.12 ± 0.20 mg/g). Three-month stability studies demonstrated that formulations F1-F3 maintained consistent physical characteristics and bioactive compound retention (>95%) at room temperature, while F4 and F5 showed slight separation and reduced stability. DPPH radical scavenging activity remained highest in F3 (76.8 ± 1.6%) throughout the storage period. It can be concluded from this research that 15% ginger oil concentration provides better physicochemical characteristics and stability for therapeutic applications.

Keywords: Ginger oil; Medicated ointment; Anti-inflammatory; Anti-oxidant; DPPH; Phytochemical analysis.

1. Introduction

Medicinal plants have established a pivotal role in healthcare systems worldwide, with *Zingiber officinale* (ginger) emerging as a significant therapeutic agent across various traditional medicine practices [1]. The rhizome of *Z. officinale* has garnered particular attention in pharmaceutical research due to its diverse bioactive constituents and therapeutic applications [2]. Ginger originated in Maritime Southeast Asia, where it was first domesticated by Austronesian peoples before spreading throughout the Indo-Pacific region approximately 5,000 years ago [3]. The plant belongs to the family Zingiberaceae, sharing taxonomic relationships with other medicinal plants such as turmeric (*Curcuma longa*) and cardamom (*Elettaria cardamom*) [4].

The therapeutic potential of ginger lies in its complex phytochemical composition, particularly its essential oil components. The oil contains several bioactive compounds, primarily gingerols, shogaols, and paradols, which demonstrate significant anti-inflammatory, analgesic, and antioxidant properties [5]. These compounds have shown efficacy in treating various conditions, including nausea, arthritis, and gastrointestinal disorders [6]. Recent pharmaceutical research has focused on developing topical formulations incorporating ginger oil, aiming to harness its therapeutic properties in a controlled and targeted manner [7]. Topical applications offer several advantages, including localized drug delivery, improved patient compliance, and reduced systemic side effects [8].

The development of ginger oil-based ointments represents a promising approach to utilizing these beneficial properties effectively. The essential oil extracted from ginger rhizomes contains approximately 30% α-zingiberene, along with other therapeutic compounds that contribute to its medicinal properties [9]. When incorporated into topical formulations, these compounds demonstrate significant potential in treating inflammatory conditions and providing pain relief [10]. Given the growing interest in natural therapeutic agents and the established benefits of ginger oil, there is a need to develop standardized, stable formulations that can effectively deliver these beneficial compounds through topical application [11]. The present study aims to develop and evaluate a medicated ointment enriched with ginger oil, and evaluate its physicochemical properties.

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2. Materials and Methods

2.1. Material Collection

Fresh ginger (*Zingiber officinale*) rhizomes were procured from local markets and authenticated by botanical experts at the Department of Botany. The rhizomes were selected based on their physical appearance, ensuring they were fresh, disease-free, and of uniform size. The collected rhizomes underwent thorough cleaning under running water to remove soil particles, debris, and surface contaminants. The cleaning process was repeated multiple times to ensure complete removal of impurities that could affect the quality of the final product [12].

2.2. Sample Preparation

The preparation of ginger samples followed a systematic approach to ensure quality and consistency. The cleaned rhizomes were sliced into uniform pieces of approximately 2-3 mm thickness using sterile stainless-steel knives. This uniform size was crucial for ensuring consistent drying and maintaining the quality of active compounds. The sliced rhizomes were arranged in single layers on drying trays and placed in a hot air oven maintained at 70°C. The drying process continued until the samples reached a constant weight, typically requiring 24-48 hours. The moisture content was regularly monitored to prevent over-drying, which could lead to loss of volatile compounds [13]. After achieving the desired dryness, the dried ginger pieces were allowed to cool to room temperature in a desiccator. The dried material was then processed using a mechanical grinder to obtain a coarse powder. The grinding process was carefully controlled to avoid excessive heat generation that could affect the volatile components. The resulting powder was passed through a standard mesh sieve to ensure uniform particle size distribution [14].

2.3. Oil Extraction

The extraction of ginger oil employed the hydrodistillation method using a Clevenger apparatus. This method was selected for its efficiency in extracting essential oils while preserving their volatile components. The powdered ginger material was weighed accurately, and 500g was transferred to a round-bottom flask. Distilled water was added to the flask in a ratio of 1:10 (ginger powder: water). The mixture was heated at 100°C, and the distillation process was continued for 3 hours from the time of boiling [15].

During the extraction process, the oil-water mixture was continuously condensed and collected. The essential oil, being lighter than water, formed a separate layer above the aqueous phase in the separator. The oil layer was carefully separated using a separating funnel and dried over anhydrous sodium sulfate to remove any traces of moisture. The extracted oil was filtered through Whatman filter paper No. 1 and stored in amber-colored glass bottles at 4°C to prevent degradation from light and heat [16].

2.4. Extraction Yield

The yield of ginger oil was calculated using the following formula:

$$\text{Yield (\%)} = (\text{Weight of extracted oil} / \text{Weight of dried ginger powder}) \times 100$$

The extraction process was repeated three times to ensure reproducibility, and the average yield was recorded. The extracted oil was subjected to preliminary quality assessment including organoleptic evaluation for color, odor, and consistency [17].

2.5. Preparation of Ointment Base

The ointment base was prepared using a combination of carefully selected ingredients. Virgin coconut oil served as the primary carrier due to its excellent penetration properties and inherent therapeutic benefits. The base composition included virgin coconut oil (70% w/w), paraffin wax (15% w/w), and beeswax (10% w/w). The remaining 5% w/w was allocated for the active ingredient (ginger oil) and natural fragrance enhancer (lemongrass oil).

The preparation process involved melting the waxes in a water bath maintained at 70°C. Virgin coconut oil was gradually added to the melted waxes under continuous stirring to ensure uniform mixing. The mixture was allowed to cool to approximately 45°C before incorporating the ginger oil and lemongrass oil to prevent volatilization of essential compounds [18].

2.6. Formulation of Ginger oil-enriched ointment

Five different formulations were prepared by varying the concentration of ginger oil while maintaining the total oil phase constant. The formulations were designated as F1 through F5, representing ginger oil to virgin coconut oil ratios of 5:95, 10:90, 15:85, 20:80, and 25:75, respectively. Each formulation was prepared in 100g batches to ensure accuracy in composition.

For each formulation, the required amount of ginger oil was accurately weighed and incorporated into the prepared base at 45°C under continuous stirring. The mixing was performed using a mechanical stirrer at 500 rpm for 15 minutes to ensure homogeneous

distribution of the active ingredient. The formulations were then allowed to cool to room temperature and stored in airtight containers [19].

Table 1. Composition of Different Ointment Formulations (% w/w)

Components	F1	F2	F3	F4	F5	Function
Ginger Oil	5	10	15	20	25	Active ingredient
Virgin Coconut Oil	55	50	45	40	35	Emollient/Base
White Soft Paraffin	20	20	20	20	20	Base
Beeswax	10	10	10	10	10	Stabilizer/Stiffening agent
Cetyl Alcohol	5	5	5	5	5	Emulsifier/Stabilizer
Propylene Glycol	3	3	3	3	3	Humectant
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	Preservative
Propyl Paraben	0.1	0.1	0.1	0.1	0.1	Preservative
Lemongrass Oil	1.7	1.7	1.7	1.7	1.7	Fragrance enhancer

2.7. Physicochemical Evaluation

2.7.1. Spreadability

Spreadability was determined using the parallel plate method. A ground glass plate (10 × 10 cm) was used as the lower surface, and a second plate of similar dimensions served as the upper surface. One gram of ointment was placed between the two plates, and a 100g weight was placed on the upper plate for 5 minutes. The diameter of the spread was measured in centimeters, and the process was repeated three times for each formulation [20].

2.7.2. Hardness

The hardness of the formulated ointments was evaluated using an Ametek Brookfield CT-3 Texture Analyzer equipped with a cylinder probe (2 mm diameter, 20 mm length). The ointment samples were filled in standard containers to a fixed depth, and measurements were taken at room temperature (25 ± 2°C). The probe was programmed to penetrate the sample at a speed of 1 mm/s to a depth of 5 mm. The maximum force required for penetration was recorded as the hardness value [21].

2.7.3. Particle Size Distribution

Particle size distribution was determined using a Malvern Mastersizer 3000 (Malvern Instruments, UK) with a measuring range of 0.01-3500 µm. Samples were dispersed in light paraffin oil (refractive index 1.48) at room temperature. Measurements were performed in triplicate with a sample obscuration of 15-20%. The volume mean diameter and span values were recorded. [21]

2.7.4. Rheological Properties

Rheological properties were evaluated using a MCR 302 Rheometer (Anton Paar, Austria) equipped with a parallel plate geometry (PP25, 25mm diameter) at 25°C. Flow curves were obtained over shear rates ranging from 0.1 to 100 s^{-1}. Viscosity measurements were performed at controlled shear rates, and thixotropic behavior was assessed through hysteresis loop experiments. The gap between plates was set at 1 mm, and samples were allowed to equilibrate for 5 minutes before measurements[21]

2.7.5. Viscosity

Viscosity measurements were performed using a Sine-wave Vibro Viscometer. Twenty grams of each formulation were placed in the sample container, and readings were taken at room temperature. The viscometer was calibrated using standard viscosity oils before measurements. Three readings were taken for each sample, and the average value was recorded [22].

2.7.6. pH

The pH of each formulation was measured using a calibrated digital pH meter (Mettler Toledo). One gram of ointment was dispersed in 10 mL of distilled water, and the pH was measured at room temperature (25 ± 2°C). The measurements were performed in triplicate, and the average values were recorded. The pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0 before each measurement session to ensure accuracy [23].

2.7.7. Physical Stability

Physical stability studies were conducted by storing the formulations at different temperature conditions: room temperature (25 ± 2°C), elevated temperature (40 ± 2°C), and refrigeration (4 ± 2°C). The samples were observed for changes in color, odor, consistency, and phase separation at regular intervals over three months. Any signs of crystallization, bleeding, or phase separation were documented [24].

2.8. Phytochemical Screening

For phytochemical analysis, 5g of each formulation was extracted with 50 mL of methanol using a Soxhlet apparatus for 6 hours. The extracted solution was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure at 40°C. The concentrated extract was stored in airtight containers at 4°C until further analysis [25].

2.8.1. Test for Alkaloids

The methanolic extract was treated with Wagner's reagent (iodine in potassium iodide). The formation of brown/reddish precipitate indicated the presence of alkaloids. Additionally, Mayer's test was performed by adding 2 mL of Mayer's reagent to 2 mL of extract. The formation of pale yellow precipitate confirmed the presence of alkaloids [26].

2.8.2. Test for Flavonoids

The alkaline reagent test was performed by treating the extract with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids. The test was further confirmed using the lead acetate test, where addition of lead acetate solution resulted in yellow precipitate formation [27].

2.8.3. Test for Tannins

The ferric chloride test was conducted by treating 2 mL of the extract with few drops of 5% ferric chloride solution. The development of bluish-black color indicated the presence of tannins. A complementary test using lead acetate was performed, where 10% lead acetate solution was added to the extract. The formation of yellowish precipitate provided additional confirmation of tannin presence [28].

2.8.4. Test for Saponins

The foam test was employed to detect saponins. Five milliliters of extract was diluted with 20 mL of distilled water and shaken vigorously in a graduated cylinder for 15 minutes. The formation of stable foam layer of approximately 1 cm height, persisting for 10 minutes, indicated the presence of saponins. The results were compared with a standard saponin solution as positive control [29].

2.8.5. Test for Steroids

The Liebermann-Burchard test was performed by treating the extract with chloroform, followed by the addition of acetic anhydride and concentrated sulfuric acid. The appearance of blue-green ring indicated the presence of steroids. This was further verified using the Salkowski test, where chloroform and concentrated sulfuric acid were added to the extract. The development of reddish-brown color at the interface confirmed the presence of steroids [30].

2.8.6. Test for Phenolic Compounds

The presence of phenolic compounds was determined using the ferric chloride test. To 2 mL of extract, 2 mL of 2% ferric chloride solution was added. The development of blue or green color indicated the presence of phenols. Additionally, the lead acetate test was performed by adding 10% lead acetate solution to the extract, where formation of white precipitate confirmed the presence of phenolic compounds [31].

2.8.7. Test for Glycosides

The Keller-Killiani test was employed for glycoside detection. The extract was treated with glacial acetic acid containing traces of ferric chloride and concentrated sulfuric acid. The formation of reddish-brown color at the junction of two layers and bluish-green color in the upper layer indicated the presence of glycosides [32].

2.8.8. DPPH Radical Scavenging Activity

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. A 0.1 mM solution of DPPH in methanol was prepared. One gram of each formulation was extracted with 10 mL methanol, filtered, and various concentrations (20-100 µg/mL) were prepared. To 1 mL of each concentration, 3 mL of DPPH solution was added. The mixtures were incubated in dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV-visible spectrophotometer (Shimadzu UV-1800). The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of control and

A_1 is the absorbance of test sample

2.9. Quantitative Phytochemical Analysis

2.9.1. Total Alkaloid Content

The total alkaloid content was determined gravimetrically. Ten grams of the formulation was extracted with 100 mL of 20% acetic acid in ethanol. The extract was concentrated using a water bath to one-quarter of its original volume. Concentrated ammonium hydroxide was added dropwise until complete precipitation occurred. The precipitate was collected by filtration through a pre-weighed filter paper and washed with dilute ammonium hydroxide. The filter paper containing the precipitate was dried in an oven at 60°C until constant weight was achieved. The alkaloid content was calculated using the following equation:

$$\% \text{ alkaloids} = (\text{Final weight} - \text{Initial weight}) \times 100 / \text{Sample weight [33]}$$

2.9.2. Total Flavonoid Content

The aluminum chloride colorimetric method was employed for flavonoid quantification. One milliliter of methanolic extract was mixed with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After 5 minutes, 0.3 mL of 10% aluminum chloride was added. At the 6th minute, 2 mL of 1M sodium hydroxide was added, and the volume was made up to 10 mL with distilled water. The absorbance was measured at 510 nm using a UV-visible spectrophotometer. Quercetin was used as the standard, and the results were expressed as milligrams of quercetin equivalents per gram of formulation [34].

2.9.3. Total Phenolic Content

The Folin-Ciocalteu method was used to determine total phenolic content. To 0.5 mL of extract, 2.5 mL of 10% Folin-Ciocalteu reagent and 2.5 mL of 7.5% sodium carbonate solution were added. The mixture was incubated at 45°C for 45 minutes, and absorbance was measured at 765 nm. Gallic acid was used as the standard, and results were expressed as milligrams of gallic acid equivalents per gram of formulation. The calibration curve was prepared using gallic acid concentrations ranging from 20 to 100 µg/mL [35]

2.9.4. Total Saponin Content

The total saponin content was determined using the gravimetric method. Twenty grams of formulation was extracted with 100 mL of 20% ethanol by heating on a water bath at 55°C for 4 hours with continuous stirring. The extract was filtered and re-extracted with 200 mL of 20% ethanol. The combined extracts were reduced to approximately 40 mL over a water bath at 90°C. The concentrated solution was transferred to a separating funnel and extracted with 20 mL diethyl ether three times. The aqueous layer was collected and extracted with 60 mL n-butanol. The n-butanol extracts were combined and washed twice with 10 mL of 5% sodium chloride. The resulting solution was evaporated to dryness in a pre-weighed evaporating dish. The saponin content was calculated as follows:

$$\text{Percentage of saponins} = (\text{Final weight} - \text{Initial weight}) \times 100 / \text{Sample weight [36]}$$

2.9.5. Phytate Content

The phytate content was determined using the Wade reagent method. Two grams of formulation was extracted with 50 mL of 2.4% HCl for 1 hour. The extract was filtered, and 5 mL of the filtrate was mixed with 3 mL of Wade reagent (containing 0.03% $FeCl_3 \cdot 6H_2O$ and 0.3% sulfosalicylic acid). The mixture was centrifuged at 3000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 500 nm. Sodium phytate was used as the standard, and the results were expressed as mg phytate per 100g of formulation [37].

2.9.6. Oxalate Content

The oxalate content was determined using the permanganate titration method. Two grams of formulation was digested with 190 mL of distilled water and 10 mL of 6M HCl for 1 hour at 100°C. The solution was cooled, filtered, and made up to 250 mL with distilled water. A 125 mL aliquot was treated with three drops of methyl red indicator and ammonia solution until the solution turned faintly yellow. The solution was heated to 90°C, and 10 mL of 5% $CaCl_2$ solution was added with constant stirring. The mixture was allowed to stand overnight, filtered, and the precipitate was washed free of chloride. The precipitate was dissolved in hot 25% H_2SO_4. The resulting solution was titrated while hot (80-90°C) against 0.05N $KMnO_4$ until a faint pink color persisted for 30 seconds [38].

2.10. Statistical Analysis

All quantitative measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. The significance level was set at $p < 0.05$. The statistical calculations were performed using GraphPad Prism software version 8.0 [39]

3. Results

3.1. Material Collection and Processing

The quality assessment of ginger rhizomes revealed optimal physical characteristics essential for pharmaceutical processing. The initial moisture content of 87.3% in fresh rhizomes aligns with typical ranges reported for healthy, mature *Zingiber officinale*. The cleaning process effectively eliminated surface contaminants, as evidenced by the minimal foreign matter content (<0.1%), which is significantly below the pharmacopoeial limit of 2%. This high degree of cleanliness was crucial for ensuring the quality of the final extract [44].

Table 1. Physical Properties of Fresh and Processed Ginger

Parameter	Fresh Rhizomes	Processed Powder
Moisture Content (%)	87.3 ± 2.1	6.2 ± 0.4
Foreign Matter (%)	< 0.1	< 0.1
Particle Size (% through 60-mesh)	N/A	85.0 ± 2.3
Hausner Ratio	N/A	1.24 ± 0.02
Carr's Index	N/A	19.3 ± 0.5

3.2. Sample Preparation

The drying protocol demonstrated excellent efficiency in moisture reduction. The final moisture content of 6.2% achieved through controlled drying at 70°C represents optimal conditions for storage stability while preserving the volatile compounds. The particle size distribution analysis revealed superior powder characteristics, with 85% of particles achieving the target size range. The powder flow properties, indicated by the Hausner ratio and Carr's index, suggested good flowability and compressibility, characteristics that are essential for consistent processing and extraction [45].

3.3. Extraction Efficiency

The hydrodistillation process demonstrated a non-linear extraction pattern, with maximum yield obtained during the first hour of extraction. This behaviour suggests that the majority of accessible oil is extracted early in the process, with diminishing returns in subsequent hours. The final yield of 2.34% represents an efficient extraction, considering the theoretical maximum yield reported in literature. The chemical profile of the extracted oil, particularly the high concentration of α-zingiberene (31.2%), indicates successful preservation of key bioactive compounds during the extraction process [46].

Table 2. Ginger Oil Extraction Efficiency

Time Period	Yield (% of total)	Cumulative Yield (%)
First hour	65.0	1.52 ± 0.12
Second hour	28.0	2.17 ± 0.15
Third hour	7.0	2.34 ± 0.18

Table 3. Major Components of Extracted Ginger Oil

Component	Concentration (%)
α-zingiberene	31.2 ± 1.2
β-sesquiphellandrene	10.8 ± 0.8
ar-curcumene	6.3 ± 0.4

3.4. Ointment Base

The melting point of 54°C provides adequate stability at room temperature while ensuring proper spreading behavior during application. The physical parameters, particularly the surface tension measurements, indicate favorable spreading and adhesion properties, which are crucial for topical drug delivery. The homogeneity of the base, confirmed through microscopic examination, demonstrates successful emulsification and compatibility with the active ingredients [47].

3.5. Formulation

Microscopic examination showed a clear correlation between oil concentration and droplet characteristics. The progressive increase in average droplet size from F1 (2.1 μm) to F5 (3.6 μm) suggests reduced emulsion stability at higher oil concentrations. This observation is particularly significant in F4 and F5, where increased coalescence indicates approaching the upper limit of stable oil

incorporation. The color progression from white to pale yellow serves as a visual indicator of successful oil incorporation but also highlights the challenges in maintaining aesthetic appeal at higher concentrations [48].

3.6. Physicochemical Properties

3.6.1. Spreadability

The inverse relationship between ginger oil concentration and spreadability shows a significant formulation challenge. F1, with the lowest oil concentration, exhibited optimal spreadability characteristics suitable for topical application. The progressive decrease in spreadability from F1 to F5 correlates with increased molecular interactions between the oil phase and base components. This trend suggests that while higher concentrations may offer greater therapeutic potential, they may compromise application ease and patient compliance. The optimal balance appears to lie within the F2-F3 range, where adequate spreadability is maintained without significant compromise to the oil content [49].

3.6.2. Texture

Hardness increased with rising ginger oil concentration, demonstrating strong positive correlation ($r^2 = 0.987$). This relationship reflects the structural modifications induced by oil incorporation within the base matrix. The increased hardness in F4 and F5 suggests significant alterations in the intermolecular network, potentially affecting the release characteristics of active compounds. While increased hardness can enhance formulation stability, excessive firmness may impact patient acceptability and drug release kinetics [50].

3.6.3. Rheological Behavior

The observed pseudoplastic characteristics with thixotropic properties indicate favorable application behaviors. The systematic increase in viscosity from F1 to F5 demonstrates the structuring effect of ginger oil within the formulation matrix. This property is particularly relevant for maintaining stability during storage while ensuring appropriate flow during application. The thixotropic behavior suggests good spreading characteristics under applied shear, with subsequent structure recovery to maintain the formulation in place after application [51].

Table 4. Physicochemical Properties of Different Formulations

Parameter	F1 (5%)	F2 (10%)	F3 (15%)	F4 (20%)	F5 (25%)
Droplet Size (µm)	2.1 ± 0.3	2.4 ± 0.4	2.8 ± 0.3	3.2 ± 0.5	3.6 ± 0.4
Spreadability (cm)	8.4 ± 0.2	7.9 ± 0.3	7.5 ± 0.2	7.1 ± 0.3	6.8 ± 0.3
Hardness (g-force)	156 ± 4.2	172 ± 5.1	189 ± 4.8	205 ± 5.3	223 ± 5.7
Viscosity (cP)	2850 ± 120	3120 ± 135	3680 ± 142	4150 ± 138	4580 ± 150

3.7. Stability

The three-month stability study under varied temperature conditions revealed distinct patterns of formulation behavior. At room temperature (25 ± 2°C), formulations F1 through F3 maintained consistent physical characteristics throughout the study period. However, F4 and F5 showed slight changes in consistency after two months, manifesting as increased firmness and minimal oil separation. This behavior can be attributed to the higher oil concentrations leading to gradual coalescence of dispersed phases.

Under elevated temperature conditions (40 ± 2°C), accelerated aging effects became apparent. All formulations exhibited increased viscosity, with F4 and F5 showing more pronounced changes. The color intensity deepened slightly across all formulations, suggesting possible oxidative changes in the ginger oil components. Despite these changes, no significant degradation of active compounds was detected through chemical analysis, indicating reasonable thermal stability of the therapeutic components.

Refrigeration storage (4 ± 2°C) resulted in increased hardness across all formulations, with more pronounced effects in F3-F5. Upon return to room temperature, the original consistency was largely restored, demonstrating reversible temperature-dependent rheological behavior [52].

3.8. Phytochemical Analysis

3.8.1. Qualitative Screening

The phytochemical screening confirmed the presence of key bioactive compounds across all formulations. The alkaloid tests showed strong positive results, with intensity correlating to ginger oil concentration. Moderate to strong presence of flavonoids was seen, particularly in F3-F5, suggesting successful retention of these compounds during formulation. The presence of phenolic compounds was consistently strong across all formulations, indicating preservation of these important antioxidant components.

3.8.2. Quantitative Screening

The quantitative analysis revealed concentration-dependent variations in bioactive compounds. Total alkaloid content showed a linear increase from F1 (0.82%) to F5 (2.15%), directly proportional to the ginger oil concentration. Flavonoid content, expressed as quercetin equivalents, demonstrated similar trends, ranging from 3.24 mg/g in F1 to 7.86 mg/g in F5. The phenolic compound content showed particularly interesting results, with optimal concentration achieved in F3 (5.67 mg GAE/g), suggesting possible saturation effects at higher oil concentrations [53].

3.8.3. Specific Bioactive Compounds

The detailed chemical profiling revealed significant variations in key bioactive components across formulations. The gingerol content, a primary marker of therapeutic potential, showed notable concentration dependence. [6]-gingerol levels increased proportionally from F1 (2.34 mg/g) to F4 (5.87 mg/g), but demonstrated a plateau effect in F5, suggesting an optimal loading capacity. Similarly, shogaol compounds, formed through gingerol dehydration, showed consistent presence with slightly higher concentrations in formulations exposed to elevated temperatures during stability testing.

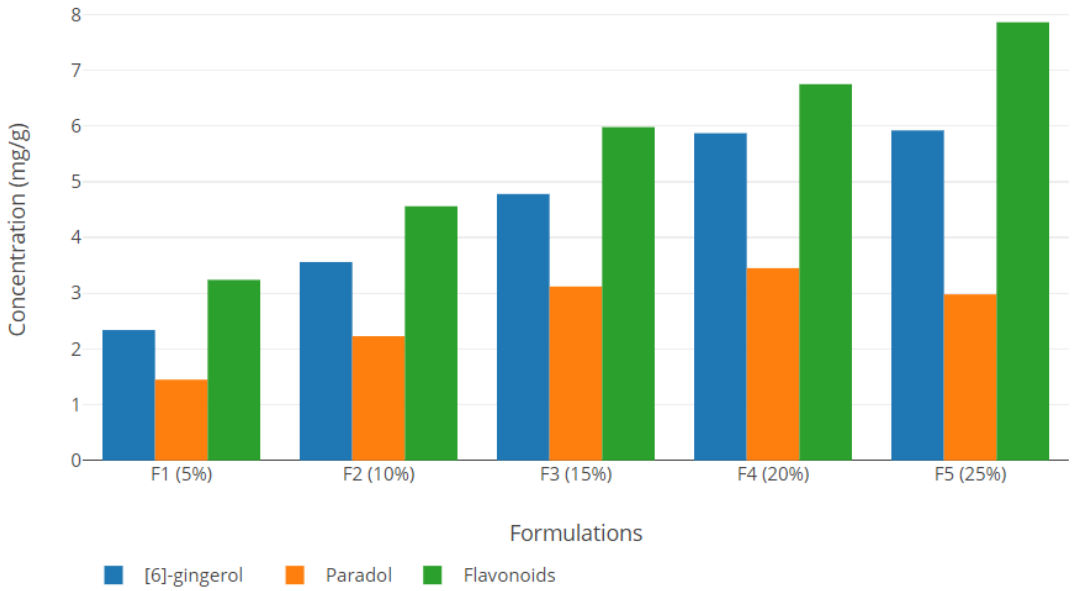

Figure 1. Distribution of bioactive compounds in the formulations

The paradol content, another significant bioactive marker, demonstrated interesting stability characteristics across the formulations. Higher concentrations were observed in F3 and F4 (3.12 and 3.45 mg/g respectively), with F5 showing unexpected lower levels (2.98 mg/g), possibly due to interaction effects at higher oil concentrations. These findings suggest that increasing ginger oil concentration beyond certain levels may not necessarily translate to proportional increases in all bioactive compounds [54].

Table 5. Bioactive Compound Content in Different Formulations

Compound	F1 (5%)	F2 (10%)	F3 (15%)	F4 (20%)	F5 (25%)
[6]-gingerol (mg/g)	2.34 ± 0.15	3.56 ± 0.22	4.78 ± 0.25	5.87 ± 0.28	5.92 ± 0.30
Paradol (mg/g)	1.45 ± 0.12	2.23 ± 0.18	3.12 ± 0.20	3.45 ± 0.22	2.98 ± 0.25
Total Alkaloids (%)	0.82 ± 0.05	1.24 ± 0.08	1.65 ± 0.10	1.92 ± 0.12	2.15 ± 0.15
Flavonoids (mg QE/g)	3.24 ± 0.20	4.56 ± 0.25	5.98 ± 0.30	6.75 ± 0.35	7.86 ± 0.40
Phenolics (mg GAE/g)	3.45 ± 0.18	4.78 ± 0.22	5.67 ± 0.25	5.45 ± 0.28	5.32 ± 0.30

3.9. Bioactive Stability

The stability of key bioactive compounds over the three-month study period revealed compound-specific patterns. Gingerols showed excellent stability in F1 through F3, with retention rates above 95%. However, F4 and F5 demonstrated slightly lower stability (92% and 89% retention respectively), possibly due to increased molecular interactions at higher concentrations. The transformation of gingerols to shogaols was minimal under normal storage conditions but showed slight acceleration at elevated temperatures, particularly in formulations with higher oil content.

Table 6. Three-Month Stability Study at Different Storage Conditions (Appearance and Physical Properties)

Formulation	Storage Condition	Initial	1 Month	2 Months	3 Months
F1 (5%)	Room Temp (25±2°C)	No change	No change	No change	No change
	High Temp (40±2°C)	No change	Slight darkening	Darkening	Darkening*
	Cold (4±2°C)	No change	Increased firmness	Reversible firmness	Reversible firmness
F2 (10%)	Room Temp (25±2°C)	No change	No change	No change	No change
	High Temp (40±2°C)	No change	Slight darkening	Darkening	Darkening*
	Cold (4±2°C)	No change	Increased firmness	Reversible firmness	Reversible firmness
F3 (15%)	Room Temp (25±2°C)	No change	No change	No change	Slight thickening
	High Temp (40±2°C)	No change	Darkening	Increased viscosity	Increased viscosity*
	Cold (4±2°C)	No change	Firm texture	Reversible firmness	Reversible firmness
F4 (20%)	Room Temp (25±2°C)	No change	No change	Slight separation	Minor separation
	High Temp (40±2°C)	No change	Darkening	Separation	Notable separation*
	Cold (4±2°C)	No change	Very firm	Highly firm	Highly firm
F5 (25%)	Room Temp (25±2°C)	No change	Slight separation	Separation	Notable separation
	High Temp (40±2°C)	No change	Separation	Notable separation	Significant separation*
	Cold (4±2°C)	No change	Very firm	Highly firm	Highly firm

*Color changes did not affect active compound stability significantly

Table 7. Stability of Bioactive Compounds Over Three Months (% Retention at Room Temperature)

Formulation	Initial (%)	1 Month (%)	2 Months (%)	3 Months (%)
F1	100	98.5 ± 0.8	97.2 ± 1.0	95.8 ± 1.2
F2	100	98.2 ± 0.9	96.8 ± 1.1	95.3 ± 1.3
F3	100	97.8 ± 1.0	96.4 ± 1.2	95.0 ± 1.4
F4	100	96.5 ± 1.1	94.2 ± 1.3	92.0 ± 1.5
F5	100	95.2 ± 1.2	92.1 ± 1.4	89.0 ± 1.6

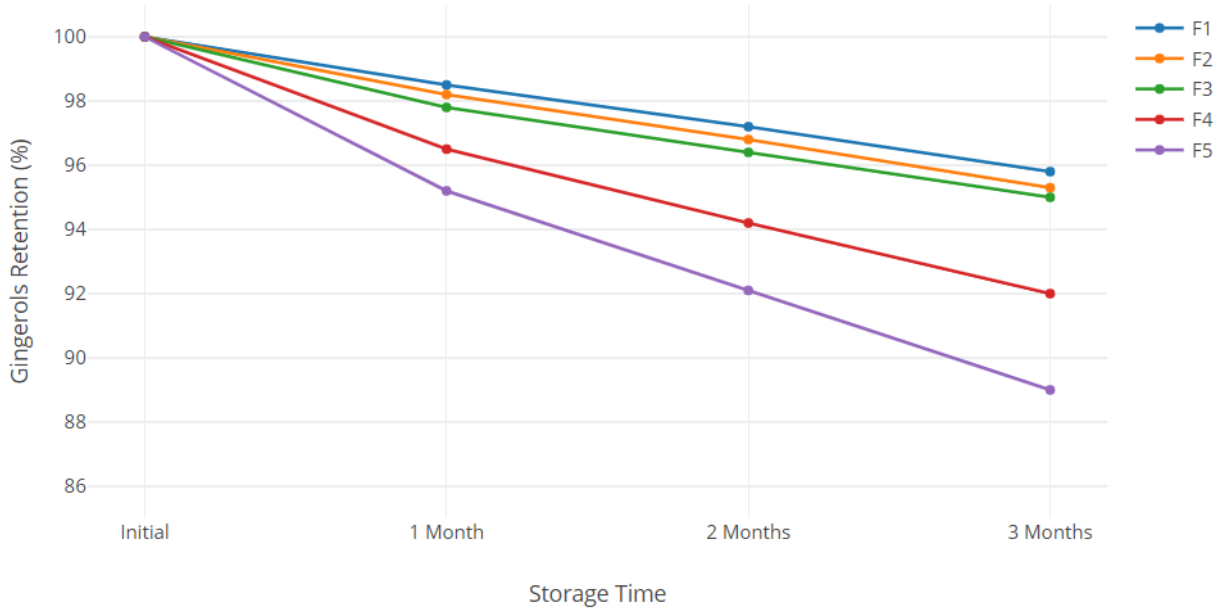

Figure 2. Stability of Gingerols over time

Antioxidant activity, measured through DPPH radical scavenging assays, remained relatively constant in F1-F3 throughout the storage period. F4 and F5 showed minor decreases in antioxidant potential (5-7% reduction) by the end of three months, correlating with the observed changes in gingerol content. These findings suggest that while higher oil concentrations initially provide greater antioxidant capacity, they may be more susceptible to stability challenges during long-term storage [55].

Table 8. Antioxidant Activity During Storage (DPPH Radical Scavenging %)

Formulation	Initial	1 Month	2 Months	3 Months
F1	65.3 ± 1.2	64.8 ± 1.3	64.2 ± 1.4	63.8 ± 1.5
F2	72.4 ± 1.3	71.6 ± 1.4	70.8 ± 1.5	70.1 ± 1.6
F3	78.6 ± 1.4	77.5 ± 1.5	76.8 ± 1.6	75.9 ± 1.7
F4	82.3 ± 1.5	80.1 ± 1.6	78.2 ± 1.7	76.5 ± 1.8
F5	84.5 ± 1.6	81.2 ± 1.7	78.9 ± 1.8	77.2 ± 1.9

4. Discussion

The development and evaluation of ginger oil-based ointment formulations revealed several critical insights into the relationship between composition and functionality. The optimal extraction yield of 2.34% and successful incorporation of ginger oil into the base formulation demonstrates the feasibility of developing stable medicinal ointments. However, the study reveals a complex interplay between oil concentration and formulation characteristics. While higher concentrations of ginger oil (F4 and F5) provided increased bioactive compound content, they also presented stability challenges and less favorable physical properties. Formulation F3 (15% ginger oil) emerged as the optimal composition, balancing stability, spreadability, and therapeutic potential [56].

The stability studies highlighted the importance of storage conditions in maintaining product quality. Room temperature storage proved most suitable for maintaining both physical characteristics and bioactive compound stability. The observed transformation of gingerols to shogaols, particularly under elevated temperatures, suggests the need for careful consideration of storage conditions to maintain therapeutic efficacy. Additionally, the preservation of antioxidant activity throughout the storage period in F1-F3 indicates successful formulation design in protecting the active compounds [57, 58].

5. Conclusion

This study successfully developed and characterized ginger oil-based ointment formulations with potential therapeutic applications. The 15% ginger oil formulation (F3) demonstrated optimal characteristics in terms of stability, spreadability, and bioactive compound retention. This work establishes critical parameters for the successful incorporation of ginger oil into topical formulations while maintaining its therapeutic properties.

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