Journal of Pharmaceutical and Sciences
Electronic ISSN: 2656-3088 DOI: https://doi.org/10.36490/journal-jps.com
Homepage: https://journal-jps.com
ORIGINAL ARTICLE JPS. 2026, 9(2), 1668-1675

Identification and Quantification of Total Phenolic Content of Ginger (*Zingiber officinale*) Extract as a Preliminary Step for Topical Salve Development

Identifikasi dan Kuantifikasi Kadar Fenol Total Jahe (*Zingiber officinale*) Ekstrak sebagai Tahap Pendahuluan untuk Pengembangan Salep Topikal

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Abstract
Ginger (*Zingiber officinale*) is traditionally used for inflammatory conditions and is currently being developed as a topical agent for psoriasis, with therapeutic effects largely attributed to phenolic constituents such as gingerols and shogaols. Prior to formulating ginger extract into 5% and 10% salves, qualitative confirmation and quantitative determination of phenolic content are essential to ensure adequate active marker levels. This study aimed to identify and quantify the total phenolic content of ginger extract intended for topical salve development. Qualitative identification used the ferric chloride ($FeCl_3$) 1% test. Quantitative determination employed the Folin–Ciocalteu method with gallic acid as reference standard. Approximately 0.2 g of extract was dissolved in methanol p.a., reacted with 7.5% Folin–Ciocalteu reagent and 1% NaOH, and absorbance measured at λmax in triplicate. Total phenolic content was expressed as % gallic acid equivalents (GAE). The $FeCl_3$ test produced a distinct color change confirming phenolic compounds. λmax was 733 nm with operating time 44–46 minutes. The calibration curve was linear ($Y = 0.0652 + 0.00706X$; $r = 0.9991$). Mean total phenolic content was 7.47 ± 0.36% GAE (74.70 ± 3.63 mg GAE/g extract; CV = 4.86%, meeting the < 5% precision criterion). The value measured by the Folin–Ciocalteu method reflects the total reducing capacity of the extract, which is primarily attributable to phenolic constituents such as gingerols and shogaols, although contributions from other reducing substances cannot be excluded. This batch-specific value provides a baseline reference qualifying the extract as the candidate active ingredient for subsequent 5% and 10% topical salve formulation, with confirmatory chromatographic identification recommended prior to scale-up.

***Keywords:** Zingiber officinale, total phenolic content, Folin–Ciocalteu, gallic acid equivalent, topical salve*

Abstrak
Jahe (*Zingiber officinale*) secara tradisional digunakan untuk mengatasi kondisi inflamasi dan saat ini sedang dikembangkan sebagai agen topikal untuk psoriasis, dengan efek terapeutik yang sebagian besar berasal dari konstituen fenolik seperti gingerol dan shogaol. Sebelum diformulasikan menjadi salep 5% dan 10%, konfirmasi kualitatif dan penentuan kuantitatif kandungan fenolik diperlukan untuk memastikan kadar penanda aktif yang memadai. Penelitian ini bertujuan mengidentifikasi dan mengkuantifikasi kadar fenol total ekstrak jahe yang ditujukan untuk pengembangan salep topikal. Identifikasi kualitatif menggunakan uji ferri klorida ($FeCl_3$) 1%, dengan munculnya warna hijau, biru, atau ungu sebagai indikator hasil positif. Penentuan kuantitatif menggunakan metode Folin–Ciocalteu dengan asam galat sebagai standar pembanding. Sekitar 0,2 g ekstrak dilarutkan dalam metanol p.a., direaksikan dengan reagen Folin–Ciocalteu 7,5% dan NaOH 1%, dan absorbansi diukur pada λmaks secara triplikat. Kadar fenol total dinyatakan sebagai % ekuivalen asam galat (GAE). Uji $FeCl_3$ menghasilkan perubahan warna yang khas, mengonfirmasi keberadaan senyawa fenolik. Nilai λmaks adalah 733 nm dengan operating time 44–46 menit. Kurva kalibrasi linear ($Y = 0{,}0652 + 0{,}00706X$; $r = 0{,}9991$). Kadar fenol total rata-rata sebesar 7,47 ± 0,36% GAE (74,70 ± 3,63 mg GAE/g ekstrak; CV = 4,86%, memenuhi kriteria presisi < 5%). Nilai yang diukur dengan metode Folin–Ciocalteu mencerminkan kapasitas pereduksi total ekstrak, yang terutama berasal dari konstituen fenolik seperti gingerol dan shogaol, meskipun kontribusi dari senyawa pereduksi lain tidak dapat dikesampingkan. Nilai spesifik-bets ini menyediakan referensi baseline yang memenuhi syarat menjadikan ekstrak sebagai kandidat bahan aktif untuk formulasi salep topikal 5% dan 10% pada tahap selanjutnya, dengan identifikasi kromatografi konfirmatori direkomendasikan sebelum peningkatan skala.

***Kata Kunci:** Zingiber officinale, kadar fenol total, Folin–Ciocalteu, ekuivalen asam galat, salep topikal*

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Article History:

Received: 29/02/2026,
Revised: 31/05/2026,
Accepted: 09/06/2026,
Available Online: 11/06/2026.

QR access this Article

https://doi.org/10.36490/journal-jps.com.v9i2.1597

Introduction

Plant-derived phenolic compounds represent one of the most extensively studied classes of secondary metabolites because of their broad pharmacological profile, particularly their antioxidant, anti-inflammatory, and immunomodulatory activities [1–3]. Among medicinal plants of pharmaceutical interest, ginger (*Zingiber officinale*) has received sustained attention for its therapeutic potential in inflammatory disorders. Its rhizome contains more than 200 identified compounds, of which the phenolic fraction—including gingerols, shogaols, paradols, and zingerone—is regarded as primarily responsible for the observed biological effects [1,4].

Mechanistically, ginger phenolics, particularly 6-gingerol and 6-shogaol, exert anti-inflammatory effects through inhibition of nuclear factor kappa-B (NF-κB) signaling, suppression of pro-inflammatory cytokine release such as TNF-α and IL-1β, and downregulation of inducible nitric oxide synthase [4–6]. These pathways are highly relevant to chronic inflammatory dermatoses, including psoriasis, in which sustained NF-κB activation drives keratinocyte hyperproliferation and inflammatory infiltration. Preclinical evidence indicates that ginger administration can attenuate these processes [1].

Translating ginger extract into a topical dosage form requires that the active ingredient be properly characterized before incorporation into the vehicle. In particular, two preformulation requirements must be addressed: confirmation of the chemical class responsible for the intended activity, and quantification of marker compounds. For phenolic-bearing extracts, the ferric chloride ($FeCl_3$) test remains a routine qualitative method based on the formation of a colored ferric–phenolate complex with phenolic hydroxyl groups [7]. Meanwhile, the Folin–Ciocalteu spectrophotometric assay using gallic acid as the reference standard is the most widely used method for quantifying total phenolic content [8,9].

Several recent studies have quantified phenolic content in ginger extracts, with reported values varying considerably depending on extraction solvent, plant variety, and analytical conditions, ranging from approximately 13.8 mg GAE/g in ginger ethanol extracts to over 100 mg GAE/g in solvent-fractionated or methanol-rich preparations [11–13]. This variability underscores the necessity of characterizing each batch of extract prior to its use in formulation development. In the present study, a ginger extract intended for incorporation into 5% and 10% topical salve formulations was subjected to phenolic identification using the $FeCl_3$ test and to quantitative determination of total phenolic content using the validated Folin–Ciocalteu method, with the aim of confirming the chemical identity of the active marker class and providing batch-specific quantitative data to support subsequent stability and pharmacological studies of the salve formulations.

Material and Apparatus

Materials

Materials used in this study consisted of ginger (*Zingiber officinale*) rhizome extract, gallic acid (analytical grade), Folin–Ciocalteu reagent 7.5%, sodium hydroxide (NaOH) 1%, methanol pro analysis, ethanol 96%, ferric chloride ($FeCl_3$) 1%, and distilled water. Equipment included an analytical balance, UV-Vis spectrophotometer, volumetric flasks, micropipettes, beaker glasses, magnetic stirrer, filter paper, and standard laboratory glassware.

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Preparation of Ginger Extract

Fresh rhizomes of ginger (*Zingiber officinale*) were obtained from the Testing Laboratory of the Tawangmangu Medicinal Plants Functional Unit, where the plant material was botanically authenticated. The rhizomes were cleaned, sliced, and dried in a drying oven at 50 °C until a constant weight was obtained, then ground into a coarse powder. The dried powder (500 g) was extracted by maceration using 96% ethanol at a sample-to-solvent ratio of 1:10 (w/v). Extraction was carried out at room temperature for 3 × 24 hours with occasional stirring, the solvent being renewed every 24 hours. The combined filtrate was concentrated using a rotary evaporator at 40–50 °C under reduced pressure to yield a thick (concentrated) extract, which was stored at 4 °C until analysis. The percentage yield of the extract was approximately 10% (w/w).

Qualitative Identification of Phenolic Compounds

Qualitative identification was performed using the $FeCl_3$ test as a confirmatory assay for phenolic functional groups. Approximately 50 mg of ginger extract was weighed and placed into a test tube. One mL of 96% ethanol was added and the mixture was homogenized. Several drops of $FeCl_3$ 1% solution were then added. The appearance of a green, blue, or purple coloration was interpreted as a positive result, indicating the formation of a ferric–phenolate complex. A 0.1% (w/v) solution of gallic acid in methanol was used as the positive control, and the solvent alone (methanol) served as the negative control.

Preparation of Gallic Acid Standard Stock Solution

Gallic acid was weighed accurately and dissolved in methanol in a beaker glass, then transferred quantitatively into a 25 mL volumetric flask and made up to volume with methanol, yielding a stock solution of approximately 400 ppm. The actual stock concentration, calculated from the net weight of gallic acid (9.8 mg dissolved in 25 mL), was 320 ppm.

Determination of Maximum Wavelength and Operating Time

One mL of the stock solution was transferred to a 10 mL volumetric flask and diluted with methanol to obtain a 40 ppm working solution. From this, 1 mL was pipetted into a 10 mL volumetric flask, and 7.5% Folin–Ciocalteu reagent was added followed by 4 mL of 1% NaOH. The absorbance was measured across the wavelength range of 600–800 nm, and the wavelength giving the highest absorbance was selected as the maximum wavelength (λmax). The same reaction mixture was monitored at the determined λmax over 60 minutes, and the time interval over which absorbance reached a stable plateau was selected as the operating time for subsequent assays.

Calibration Curve

A series of working concentrations was prepared by pipetting aliquots of the gallic acid stock solution into separate 10 mL volumetric flasks, each diluted to volume with methanol, yielding nominal concentrations of 30, 40, 50, 60, and 70 ppm. From each concentration, 1 mL was transferred to a 10 mL volumetric flask, mixed with 7.5% Folin–Ciocalteu reagent and 4 mL of 1% NaOH, and absorbance was measured at λmax during the operating time interval. The linear regression equation, correlation coefficient (r), and coefficient of determination (r^2) were calculated. The 30–70 ppm range was selected to bracket the gallic-acid-equivalent concentrations expected for the diluted sample working solutions (approximately 56–62 ppm), so that all sample readings fell within the calibrated linear region rather than requiring extrapolation. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the calibration data using LOD = 3.3 × ($S_{y/x}$/b) and LOQ = 10 × ($S_{y/x}$/b), where $S_{y/x}$ is the residual standard deviation of the regression and b is the slope.

Sample Preparation and Total Phenolic Content Determination

Ginger extract, approximately 0.2 g, was weighed accurately and transferred to a beaker glass. Twenty-five mL of methanol were added and the mixture was stirred with a magnetic stirrer for 30 minutes. The solution was filtered, and the filtrate was transferred to a 25 mL volumetric flask and made up to volume. From this filtrate, 1 mL was pipetted into a 10 mL volumetric flask and reacted with 7.5% Folin–Ciocalteu reagent and 4 mL of 1% NaOH. Absorbance was measured at the established λmax during the operating time. The procedure was performed in triplicate.

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Sample concentrations were calculated by substituting the measured absorbance into the linear regression equation of the calibration curve. Total phenolic content (TPC) was expressed as gallic acid equivalents (GAE), calculated using Equation (1):

$$TPC\ (\%\ w/w) = [(Creg \times V \times FP) / W] \times 100\% \quad (1)$$

where Creg is the regression-derived concentration (mg/L), V is the volume of sample solution (L), FP is the dilution factor, and W is the sample weight (mg). Results are expressed both as % GAE and as mg GAE per gram of extract. Descriptive statistics were calculated across the three replications. Acceptance criteria for the analytical procedure were a calibration curve correlation coefficient $r \geq 0.99$ and intra-assay precision CV < 5% [5].

Results and Discussion

This study comprised two complementary analytical components: a qualitative confirmation of phenolic functional groups in the ginger extract and a quantitative determination of total phenolic content using a validated spectrophotometric procedure. Results from each component are presented below together with their interpretation in the context of subsequent topical salve development.

Qualitative Identification of Phenolic Compounds

The $FeCl_3$ test produced a distinct color change in the ginger extract solution upon the addition of $FeCl_3$ 1%, consistent with the typical green-to-dark coloration range expected for the ferric–phenolate complex. The result was comparable to the positive control and clearly differentiated from the negative control, which retained the original solvent appearance. These findings confirm the presence of phenolic compounds in the ginger extract (Table 1, Figure 1).

Table 1. Qualitative phenolic identification of ginger extract using $FeCl_3$ 1% test.

Test sample	Reagent	Observation	Interpretation
Negative control (solvent)	FeCl3 1%	No color change	Negative
Ginger extract	FeCl3 1%	Green/dark coloration	Positive
Positive control (0.1% gallic acid)	FeCl3 1%	Characteristic color change	Positive

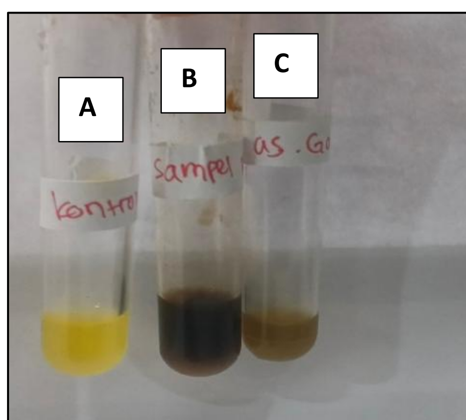

Figure 1. $FeCl_3$ test result: (a) negative control (methanol), (b) ginger extract sample, (c) positive control (0.1% gallic acid in methanol).

The qualitative reaction observed is attributable to the formation of a ferric–phenolate coordination complex, in which phenolic hydroxyl groups deprotonate and chelate Fe^{3+} ions to yield green, blue, or violet pigments depending on the substitution pattern of the phenolic ring [7]. The colour change observed in our extract is consistent with the gingerol- and shogaol-rich profile previously reported for *Z. officinale* rhizomes [4,10].

Maximum Wavelength, Operating Time, and Calibration Curve

Scanning of the gallic acid–Folin–Ciocalteu–NaOH reaction mixture across the 600–800 nm range yielded a maximum absorbance at 733 nm, which was selected as λmax for all subsequent measurements. Monitoring of absorbance at 733 nm over 60 minutes revealed that the reaction reached a stable plateau between 44 and 46 minutes, which was therefore adopted as the operating time interval for both the calibration curve and sample measurements.

The absorbance values measured at five gallic acid concentrations (30–70 ppm) are presented in Figure 2. The data demonstrated a linear relationship between concentration and absorbance, yielding the regression equation Y = 0.0652 + 0.00706X with a correlation coefficient r = 0.9991 and coefficient of determination r^2 = 0.9982, fulfilling the linearity acceptance criterion (r ≥ 0.99) for spectrophotometric quantification of plant phenolics [8]. The 30–70 ppm calibration range was chosen to encompass the gallic-acid-equivalent concentrations of the diluted sample working solutions (56–62 ppm); consequently, all sample readings were interpolated within the validated linear region rather than extrapolated beyond its upper limit. From the regression statistics, the estimated limit of detection (LOD) and limit of quantification (LOQ) were approximately 2.6 ppm and 7.8 ppm, respectively ($S_{y/x}$ = 0.0055), both well below the working concentration range and confirming that the method is sufficiently sensitive for quantifying phenolics in the ginger extract matrix.

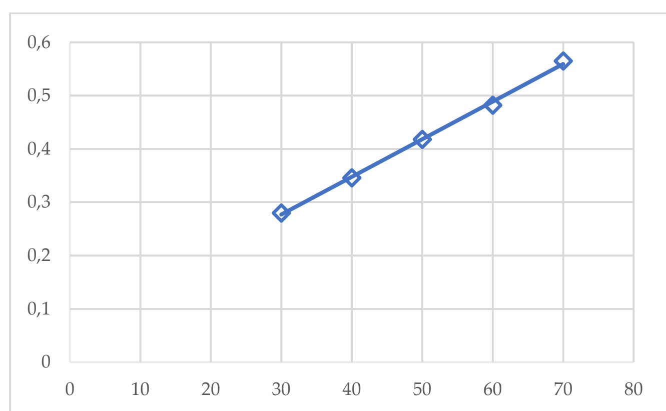

Figure 2. Gallic acid calibration curve measured at λmax 733 nm.

Total Phenolic Content of Ginger Extract

Three replicate measurements of the ginger extract yielded absorbance values of 0.470, 0.463, and 0.506, corresponding to gallic-acid–equivalent concentrations of 57.34, 56.35, and 62.44 mg/L, respectively (Table 2). The calculated total phenolic contents were 7.34%, 7.19%, and 7.88%, giving a mean of 7.47 ± 0.36% GAE, equivalent to 74.70 ± 3.63 mg GAE per gram of extract. The coefficient of variation across replications was 4.86%, below the 5% precision threshold and indicating acceptable intra-assay reproducibility.

The validity of the quantitative measurement is supported by three lines of evidence. First, the calibration curve demonstrated excellent linearity (r = 0.9991), exceeding the conventional acceptance threshold of r ≥ 0.99 for spectrophotometric quantification of plant phenolics [8]. Second, the absorbance values for all replicates fell within the optimal spectrophotometric range recommended for the Folin–Ciocalteu assay, where instrumental noise and stray-light effects are minimized [9]. Third, the intra-assay coefficient of variation of 4.86% is below the conventionally accepted 5% threshold, indicating adequate analytical precision for the intended preformulation purpose.

Table 2. Determination of total phenolic content of ginger extract by the Folin–Ciocalteu method.

Replication	Sample weight (mg)	Absorbance (AU)	Concentration (mg/L)	TPC (% GAE)
1	195.3	0.470	57.34	7.34
2	196.0	0.463	56.35	7.19
3	198.1	0.506	62.44	7.88
Mean ± SD	196.5 ± 1.5	0.480 ± 0.023	58.71 ± 3.27	**7.47 ± 0.36**
CV (%)	0.74	4.81	5.57	**4.86**

Note: V = 0.025 L; FP (dilution factor) = 10; calibration regression Y = 0.0652 + 0.00706X. Mean TPC = 7.47 ± 0.36% GAE (74.70 ± 3.63 mg GAE/g extract); CV = 4.86% (< 5%, meets precision criterion).

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It should be noted that the coefficient of variation differs between the intermediate and final quantities reported in Table 2. The CV of the regression-derived concentration was 5.57% (slightly above the 5% limit), whereas the CV of the final % GAE was 4.86% (below the limit). This pattern indicates that the principal source of variability lies in the spectrophotometric/concentration term rather than in sample weighing, since the CV of the sample weight was only 0.74%. Combining these contributions through standard error propagation for the relationship TPC = (C_{reg} × V × FP)/W, in which V and FP are fixed, gives an estimated propagated CV of $\sqrt{(5.57^2 + 0.74^2)} \approx 5.62\%$. The marginally lower observed CV for the final result (4.86%) arises because the highest-absorbance replicate (0.506) was also associated with the largest sample weight (198.1 mg), so that per-replicate weight normalization partially offsets the concentration spread. To verify that this third replicate was not an aberrant outlier, a Dixon Q-test was applied to the absorbance values (0.463, 0.470, 0.506): Q_{calc} = 0.036/0.043 = 0.84, which is below the critical value Q_{crit} (n = 3, 95% confidence) of 0.970. The value was therefore retained, and the small CV discrepancy is best interpreted as ordinary variability in the dominant concentration term rather than as a procedural error in weighing or dilution.

Comparison with Reported Phenolic Values for Ginger

The total phenolic content obtained in the present study (74.70 mg GAE/g) is substantially higher than several previously reported values for ginger ethanol extracts but lies within the broader range described in the literature. Amalia et al. reported a value of 21.90 mg GAE/g for red ginger ethanol extract using the Folin–Ciocalteu method [11], while Haroen et al. demonstrated that fractionation strongly influences phenolic recovery, with the ethyl acetate fraction of red ginger (*Z. officinale* var. Rubrum) yielding considerably higher phenolic concentrations than the corresponding methanol or n-hexane fractions [12]. A study on salted-egg supplementation with different ginger varieties reported total phenolic values ranging from 13.8 to 43 mg GAE/g, again with marked variation across *Z. officinale* cultivars [11]. Ghasemzadeh et al. demonstrated that drying method alone can shift phenolic recovery and shogaol formation by several-fold, illustrating the influence of post-harvest processing on the final extract composition [13]. The higher value obtained in the present study likely reflects both the specific extraction conditions used and the nature of the prepared extract concentrate, which represents a more concentrated phenolic fraction than crude ethanol macerates. Importantly, the value remains within the range of phenolic contents commonly reported for *Z. officinale* preparations and is therefore considered representative of a phenolic-rich extract suitable for further pharmaceutical development.

Pharmacological Relevance and Preformulation Implications

The clinical relevance of confirming a high phenolic content in this extract relates directly to its intended therapeutic application. Ginger phenolics, particularly 6-gingerol and 6-shogaol, exert anti-inflammatory effects through inhibition of NF-κB activation, suppression of pro-inflammatory cytokine production, and downregulation of inducible nitric oxide synthase and cyclooxygenase-2 expression [4,14,15]. These pathways are central to the pathogenesis of psoriasis and other chronic inflammatory dermatoses, in which sustained NF-κB signaling drives keratinocyte hyperproliferation and inflammatory infiltration; preclinical evidence indicates that ginger administration can attenuate NF-κB activity in psoriasis models [1]. A quantified phenolic content thus provides a defensible chemical basis for the dosage selection employed in the subsequent salve formulation work.

From a preformulation standpoint, the data generated in this study fulfill two essential pharmaceutical requirements. They establish the qualitative identity of the active marker class, and they provide a batch-specific quantitative reference value that can be used to standardize the active ingredient input across batches of the salve, calculate the absolute phenolic load delivered by the 5% and 10% formulations, and serve as a baseline for stability monitoring of the active marker over time. These uses align with the broader recommendation that herbal-derived topical products be standardized against a quantifiable marker compound to ensure consistent therapeutic exposure [8].

The measured phenolic content can be translated directly into the anticipated marker load of the finished salve. Given a value of 74.70 mg GAE per gram of extract, a 5% (w/w) salve, which contains 50 mg of extract per gram, would deliver approximately 50 mg × 0.0747 = 3.74 mg GAE per gram of salve, while a 10% (w/w) salve, containing 100 mg of extract per gram, would deliver approximately 7.47 mg GAE per gram of salve. For a typical single topical application of 0.5–1 g of salve to an affected skin area, this corresponds to an applied phenolic load on the order of 1.9–3.7 mg GAE (5% salve) and 3.7–7.5 mg GAE (10% salve) per application. Because validated human effective-dose ranges for topically applied ginger phenolics in

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inflammatory dermatoses have not yet been firmly established in the literature, these figures are presented as theoretical baseline estimates rather than as confirmed therapeutic doses; the present value is therefore used as a baseline reference, and the actual anti-inflammatory efficacy of the 5% and 10% formulations will be determined in the subsequent pharmacological evaluation.

Several limitations of the present study should be noted. The Folin–Ciocalteu method quantifies total reducing capacity rather than phenolic compounds exclusively, and may overestimate the true phenolic content in the presence of non-phenolic reducing substances such as ascorbic acid or reducing sugars. Definitive identification and quantification of individual phenolic constituents would require chromatographic methods such as HPLC or LC-MS, which lie beyond the scope of the present preformulation evaluation. In addition, only a single batch of extract was analyzed in triplicate, so inter-batch variability could not be assessed; this should be addressed in future formulation development before scale-up. Consistent with this, the present work is intended as a preliminary characterization step and does not yet include formulation of the salve itself; extract–base compatibility testing, organoleptic and homogeneity evaluation, and preliminary stability assessment of the 5% and 10% salves were outside the present scope and are planned as the immediate next stage of development.

Conclusions

Qualitative ferric chloride testing confirmed the presence of phenolic compounds in the ginger (*Zingiber officinale*) extract intended for topical formulation, and the validated Folin–Ciocalteu spectrophotometric method determined a total phenolic content of 7.47 ± 0.36% gallic acid equivalents, corresponding to 74.70 ± 3.63 mg GAE per gram of extract, with intra-assay precision (CV = 4.86%) meeting the < 5% threshold. The phenolic level obtained falls within the range reported for *Z. officinale* extracts and supports its qualification as a candidate active ingredient for 5% and 10% topical salve formulations, with the understanding that the Folin–Ciocalteu value reflects total reducing capacity primarily attributable to phenolic constituents such as gingerols and shogaols rather than a specific quantification of these compounds. The batch-specific quantitative value established here provides a defensible chemical basis for subsequent stability monitoring and pharmacological evaluation of the salve as a candidate topical agent for inflammatory skin disease, and lays the analytical foundation for future inter-batch standardization and clinical translation. Confirmatory identification of individual gingerols and shogaols by HPLC is recommended prior to final formulation scale-up.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Electronic ISSN : 2656-3088
Homepage: https://www.journal-jps.com