

# Formulation and Antioxidant Activity of Liquid Soap Containing Soursop (*Annona muricata* L.) Leaf and Soybean (*Glycine max* (L.) Merr.) Seed Extracts by DPPH Assay

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## Formulation and Antioxidant Activity of Liquid Soap Containing Soursop (*Annona muricata* L.) Leaf and Soybean (*Glycine max* (L.) Merr.) Seed Extracts by DPPH Assay

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### Abstract

Skin aging is driven by intrinsic and extrinsic factors, with oxidative stress as the main mechanism of extrinsic aging. Natural antioxidants, such as soursop (*Annona muricata* L.) leaves and soybean (*Glycine max* (L.) Merr.) seed coats, may serve as alternatives to synthetic antioxidants for skin protection. This study aimed to formulate liquid soap containing extracts of soursop leaves and soybean seed coats, and to evaluate its physicochemical properties and antioxidant activity. Three formulations (F1, F2, F3) with different soybean extract concentrations (0.25%, 0.5%, 1%) were tested for organoleptic properties, pH, foam height, spreadability, homogeneity, and quercetin content using TLC-densitometry. Results showed that F1 had good physical properties, including homogeneity, spreadability (5–7 cm), and suitable pH (9–10). Antioxidant activity was weak in all formulations ( $IC_{50} > 250 \mu\text{g/mL}$ ), likely due to low extract levels and soap matrix interactions. TLC-densitometry confirmed the presence of quercetin with an average content of 55.73 ppm (0.189% w/w). In conclusion, the formulated liquid soap demonstrated good physicochemical quality but limited antioxidant activity, indicating the need for further optimization while showing the potential of local herbal ingredients for functional cosmetic development.

Keywords: liquid soap, DPPH, antioxidant, *Annona muricata* L, *Glycine max* L.

### Introduction

<sup>4</sup> The skin is the largest and outermost organ of the human body, functioning as a protective barrier against environmental influences and reflecting an individual's overall health condition (Brodell & Rosenthal, 2008). Proper care and maintenance are essential to maintain healthy, elastic, and radiant skin. However, with aging, the skin undergoes both intrinsic and extrinsic

aging processes characterized by decreased elasticity, firmness, and the appearance of wrinkles. While intrinsic aging occurs naturally due to genetic and physiological factors, extrinsic aging is mainly influenced by environmental factors such as UV radiation, pollution, and unhealthy lifestyles. A key mechanism underlying extrinsic aging is oxidative stress, which triggers excessive free radical formation, leading to structural damage of lipids, proteins, and DNA in skin cells (Allemann & Baumann, 2020).

Antioxidants play a crucial role in neutralizing free radicals through endogenous protection and exogenous supplementation (Haerani *et al.*, 2018). Synthetic antioxidants have been widely used due to their strong radical-scavenging activity, but their potential adverse effects on human health, including liver, lung, and gastrointestinal toxicity, have raised safety concerns (Hutapea *et al.*, 2021). Consequently, natural antioxidants derived from plants are increasingly explored as safer alternatives. Endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) provide defense against oxidative stress, yet their capacity declines with age, thus requiring supplementation from exogenous sources (Hidayatullah, 2022).

Natural products such as soursop (*Annona muricata* L.) leaves and leaf extract and soybean (*Glycine max* (L.) Merr.) seeds have been reported to contain potent antioxidant compounds. Soursop leaves are traditionally used as herbal medicine and contain flavonoids, tannins, alkaloids, and terpenoids with strong antioxidant activity (Ihsan, 2022). Previous studies demonstrated that soursop leaf extract exhibits high free radical scavenging activity against DPPH with IC<sub>50</sub> values categorized as strong antioxidants (Ramaswamy *et al.*, 2021). Similarly, mung beans are rich in phenolic compounds, flavonoids, and tannins (Miller, 2018). The acetone extract of mung bean seed coats has been shown to exhibit significant antioxidant activity with more than 80% inhibition of DPPH radicals (Ramaswamy *et al.*, 2021). In Indonesia, mung bean by-products such as seed coats are underutilized and often discarded as waste, despite their potential as valuable sources of natural antioxidants (Riswanto *et al.*, 2022).

One promising application of these natural antioxidant sources is in the development of liquid soap formulations. Liquid soap offers advantages over traditional solid soap, such as practicality, ease of storage, aesthetic packaging, and potential incorporation of bioactive ingredients (Munteanu, 2021). Moreover, the addition of scrub particles can enhance exfoliating effects, improve cleansing efficacy, and provide a refreshing sensation.

Incorporating antioxidant-rich plant extracts into liquid soap formulations may provide dual benefits as both a cleansing and protective skincare product.

Antioxidant activity is commonly evaluated using several in vitro assays, including ABTS, FRAP, CUPRAC, and DPPH. Among these, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method is widely used due to its simplicity, rapidity, sensitivity, and suitability for small sample volumes (Riswanto *et al.*, 2022). This method measures the ability of antioxidants to scavenge stable free radicals, expressed as IC50 values, where lower values indicate stronger antioxidant capacity (Nguyen *et al.*, 2020). Based on this background, the present study aims to formulate and evaluate the antioxidant activity of liquid soap containing a combination of soursop leaf extract and mung bean seed coat powder using the DPPH method. This research is expected to provide scientific evidence for the potential utilization of local natural resources in the development of herbal-based cosmetic products with antioxidant benefits.

## Material and Methods

### Materials

The materials used in this study included soursop (*Annona muricata* L.) leaf extract, soybean extract (*Glycine max* (L.) Merr) seed coat powder, oleum olivae (olive oil), citric acid, potassium hydroxide (KOH), carboxymethyl cellulose (CMC), glycerin, sodium chloride (NaCl), soursop flavoring, sodium lauryl sulfate (SLS), distilled water (aquadest), ethanol 96%, and methanol 96%. The equipment used in this study consisted of an analytical balance, blender, sieve, test tubes, test tube rack, glass rod, volumetric flasks, porcelain crucible, water bath, beaker glasses, dropper pipettes, volumetric pipettes, filter paper, funnel, vial bottles, tissue paper, cuvettes, watch glasses, vortex mixer, spatula, aluminum foil, gloves, and a UV-Vis spectrophotometer (Shimadzu UV-1800).

### Soursop Extractions

The preparation of soursop (*Annona muricata* L.) leaf extract was carried out by weighing 100 g of soursop leaf simplicia powder, which was macerated using 96% ethanol at a ratio of 1:10 (sample:solvent). During the maceration process, 75 parts of 96% ethanol were initially added, and the powder was soaked for 3 × 24 hours with occasional stirring. Remaceration was performed by replacing the solvent with 25 parts of 96% ethanol every 24 hours, also with occasional stirring, followed by filtration. The combined filtrates were then evaporated using a water bath at 50 °C until a thick extract was obtained (Harningsih & Wimpy, 2018).

### Soybean Extractions

The preparation of soybean (*Glycine max* (L.) Merr.) seed extract was carried out by weighing the dried soybean seeds, which were then ground into powder. A total of 100 g of the powdered material was macerated with 96% ethanol at a ratio of 1:10 (sample:solvent). The maceration process was conducted for 3 × 24 hours with occasional stirring, and the solvent was replaced every 24 hours with fresh ethanol (remaceration). The filtrates were collected and then concentrated using a water bath at 50 °C until a thick extract was obtained.

### Preparation of Liquid Soap Scrub

The preparation of liquid soap from soursop leaf extract was carried out by first preparing all equipment and materials, followed by weighing the required ingredients. Distilled water was heated, and carboxymethyl cellulose (CMC) was added with continuous stirring until a mucilage was formed. Oleum olivae and potassium hydroxide (KOH) were then added and mixed until homogeneous. Subsequently, soursop (*Annona muricata* L.) leaf extract was incorporated and stirred until homogeneous. Sodium lauryl sulfate (SLS) was then added, followed by soybean extract (*Glycine max* (L.) Merr.) seed coat powder, with continuous stirring until homogeneous. Finally, soursop flavoring and the remaining distilled water were added, and the resulting liquid soap was transferred into prepared containers or bottles.

**Table 1.** Formulation Design of Scrub Liquid Soap

Ingredient	F1	F2	F3	Range	Function
Soursop leaf extract	0.25%	0.25%	0.25%	0.25–7%	Active compound
Soyben extract	0.25%	0.5%	1%	0.25–5%	Active compound and scrub agent
Oleum olivae (olive oil)	10%	10%	10%	4–20%	Oil phase
Citric acid	1%	1%	1%	1–2%	Buffer
KOH	3%	3%	3%	–	Alkali (saponification agent)
CMC	4%	4%	4%	3–6%	Thickening agent
Glycerin	10%	10%	10%	<30%	Humectant
NaCl	0.5%	0.5%	0.5%	0.2–0.5%	Viscosity and foam stabilizer
Soursop fragrance	1%	1%	1%	1%	Fragrance
SLS	1%	1%	1%	<10%	Surfactant and foaming agent
Distilled water (ad 100%)	100%	100%	100%	q.s.	Solvent

### Antioxidant Activity Assay

Antioxidant activity was assessed using the DPPH radical scavenging method. A 70 ppm DPPH solution was prepared in methanol and protected from light. The maximum absorption wavelength was determined by mixing the DPPH solution with methanol, followed by

incubation in the dark, and measuring absorbance at 400–800 nm. Quercetin standard solutions (2–6 ppm) were prepared from a 100 ppm stock solution in methanol. Each concentration was mixed with DPPH solution, incubated for 30 minutes in the dark, and the absorbance was measured to obtain the calibration curve. The scrub liquid soap extract was prepared as a 500 ppm stock solution in methanol and serially diluted to 50–250 ppm. Each sample was reacted with DPPH solution under the same conditions as the standard. Absorbance was measured at the maximum wavelength, and antioxidant activity was expressed as radical scavenging activity (Coscueta, 2023).

#### 1 IC<sub>50</sub> Determination

The absorbance values obtained from each sample were used to calculate the percentage of inhibition using the following formula:

$$\% \text{ Inhibition} = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100 \%$$

where Abs DPPH is the absorbance of the DPPH solution and Abs Sample is the absorbance of the test sample. The percentage inhibition values were then plotted against the sample concentrations to generate a regression equation of the form  $y = bx + a$ . This linear regression equation was subsequently used to determine the IC<sub>50</sub> value, defined as the concentration of the sample required to inhibit 50% of the DPPH radicals. The IC<sub>50</sub> value was calculated by substituting  $y = 50$  into the regression equation. The IC<sub>50</sub> value reflects the antioxidant activity of the sample; a lower IC<sub>50</sub> value indicates stronger antioxidant activity (Cahyaningsih *et al.*, 2019).

#### 3 Organoleptical Test

The organoleptic evaluation of the scrub liquid soap was carried out to assess its physical attributes, including color, odor, and overall appearance. The assessment was performed through direct visual and sensory observation to determine the consistency and acceptability of the formulation in accordance with established standards (Liu, 2024).

#### pH Test of preparations

The pH test is an essential quality requirement for liquid soap, as the product is applied directly to the skin. An unsuitable pH may cause irritation or discomfort. According to the Indonesian National Standard (SNI, 1996), the acceptable pH range for liquid soap is 8–11. The test was performed by placing 15 mL of the sample into a beaker glass, inserting a pH meter, and recording the value once stabilized.

### Foam Height Test

Foam production is one of the key attributes influencing consumer acceptance of liquid soap. According to SNI, the acceptable foam height for liquid soap ranges between 13–220 mm. The test was carried out by placing 1 mL of the soap sample into a test tube, adding 10 mL of distilled water, shaking the tube, and measuring the foam height using a caliper. After standing for 5 minutes, the foam height was measured again. Foam stability was then calculated using the formula:

$$\text{Foam Stability (\%)} = \frac{\text{Foam Height after 5 minutes}}{\text{Foam Height at 0 minutes}} \times 100$$

### Spreadability Test

Spreadability was evaluated by placing 0.5 g of the sample on a glass plate and applying a series of weights up to 50 g for 1 minute. The diameter was measured with each weight addition until a constant value was obtained. The acceptable spreadability range for liquid soap is 5–7 cm (Kim, 2021).

### Homogeneity Test

The homogeneity test was conducted to examine the presence of any undissolved particles or non-uniformity. A small amount of liquid soap was applied onto a glass slide and gently rubbed. A good homogeneous preparation should appear uniform, with no detectable coarse particles or solid residues (Maharani *et al.*, 2021).

### Determination of Quercetin Content in Liquid Soap Formulation

A sample weighing 300 mg was dissolved with ethanol in a 10 mL volumetric flask up to the calibration mark. The selected mobile phase was n-butanol : acetic acid : water (4:1:5), which was prepared and saturated with filter paper for 1 hour. The analysis was performed in triplicate, and each sample was spotted onto a silica gel F<sub>254</sub> TLC plate with a spotting volume of 4 µL. The resulting spots were observed using a densitometer, and the quercetin content in the sample was quantitatively determined.

## Results and Discussion

### Maceration Extraction

The extracts of soursop leaves and soybean seeds were obtained using the maceration method, followed by concentration and evaporation with a water bath at 50 °C. The

organoleptic evaluation of the soybean seed extract showed a thick consistency, brown color, and a characteristic extract odor. Similarly, the soursop leaf extract exhibited a thick consistency, brown color, and a characteristic extract odor. The concentrated extracts were then evaluated for yield percentage. The yield of soybean seed extract was 17.9%, corresponding to 17.9 g of extract, while the soursop leaf extract produced a yield of 19%, corresponding to 19 g of extract. According to the Indonesian Herbal Pharmacopoeia (2017), a good extract yield should not be less than 10%. The yield of soursop leaf and soybean seed extracts exceeding 10% indicates that the extraction process was effective in extracting bioactive compounds from the plant materials.

#### Formulation of liquid soap

The formulation of liquid soap containing *Annona muricata* L. leaf extract and *Glycine max* L. seed extract was successfully developed, with Formulation 1 (F1) selected as the optimal composition. F1 consisted of 0.25% soursop leaf extract, 0.25% mung bean powder, 10% oleum olivae, 3% KOH, 4% CMC, 10% glycerin, 0.5% NaCl, 1% soursop fragrance, 1% SLS, and distilled water up to 100%. The selection of F1 was based on its superior physical and functional characteristics compared to other formulations. At low concentrations of active ingredients, F1 maintained stability, homogeneity, and acceptable viscosity, while higher concentrations in F2 and F3 tended to reduce spreadability and consumer acceptability. This finding is consistent with Maharani *et al.* (2021), who reported that excessive use of herbal powders in semi-solid formulations can increase viscosity and impair homogeneity.



**Figure 1.** Formulation of liquid soap

The incorporation of *Annona muricata* L. extract contributed antioxidant potential to the formulation. Previous studies by Martinez *et al.* (2022) demonstrated that soursop leaves possess high antioxidant activity due to the presence of phenolic and flavonoid compounds. Likewise, *Glycine max* L. seeds are known to contain isoflavones with significant antioxidant activity (Sari, 2024). The combination of these two extracts in F1 therefore provides synergistic

antioxidant effects, which enhance the functional value of the soap beyond its cleansing properties. The presence of soursop leaf and soybean seed extracts not only provides antioxidant activity but also highlights the potential of local herbal resources to be developed into value-added functional cosmetics. The viscosity affects the texture, stability, and ease of application of the product, while homogeneity ensures that the active ingredients are evenly distributed, leading to consistent quality and performance.

#### **Organoleptic evaluation**

The organoleptic evaluation of the three formulations (F1, F2, and F3) assessed color, aroma, and physical form. All formulations showed a characteristic brown color from the soursop leaf and soybean extracts, with F1 appearing lighter and F2–F3 darker due to higher soybean concentrations. In terms of aroma, F1 exhibited a balanced scent between soursop fragrance and extract, while F2 and F3 were dominated by the stronger herbal odor.

Regarding physical form, F1 demonstrated a homogeneous and stable consistency, F2 was slightly more viscous, and F3 showed higher viscosity with fine clumping, reducing aesthetic value. These results align with previous findings (Maharani *et al.*, 2021; Maulana, 2018) that higher concentrations of herbal actives can impair homogeneity and consumer acceptance. Thus, F1 was considered the most favorable formula in terms of organoleptic properties.

#### **pH Evaluation**

The pH of all liquid soap formulations containing soursop (*Annona muricata* L.) leaf and soybean (*Glycine max* (L.) Merr.) extracts was found to be alkaline ( $\geq 9$ –10), consistent with the properties of potassium-based soaps resulting from the saponification of olive oil with KOH (Sari, 2024). The addition of citric acid slightly reduced pH but did not fully neutralize the excess base. Increasing concentrations of soybean extract contributed to a modest pH reduction, likely due to the presence of weakly acidic compounds such as isoflavones and phenolic acids (Kurokawa, 2024). Although the obtained values were acceptable for *rinse-off* soaps, they remained above physiological skin pH (4.5–6.5), which may increase the risk of irritation (Liu, 2024). Therefore, further optimization using stronger buffers or milder surfactants is recommended.

#### **Foam Height Test**

All formulations produced adequate and stable foam, mainly supported by SLS as a foaming agent and NaCl as a stabilizer (Myers, 2021). Increasing soybean extract concentration (F1: 0.25%; F2: 0.5%; F3: 1%) slightly reduced foam height, likely due to proteins and saponins

that increased viscosity and limited bubble expansion (Kurokawa, 2024). Nevertheless, polyphenolic compounds from the extracts may have contributed to foam stability. The foam levels obtained remained within the acceptable range for liquid soap products, ensuring consumer acceptance and cleansing performance (Lee, 20244). **Spreadability Test**

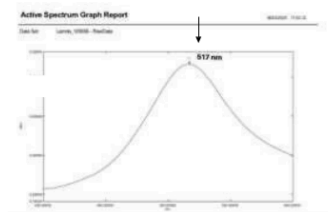
The spreadability test indicated that all formulations exhibited good spreading capacity, primarily influenced by viscosity due to the use of 4% CMC as a thickening agent. Increasing soybean extract concentration slightly reduced spreadability, likely because proteins and phenolic compounds interacted with the CMC matrix and increased viscosity (Putri Rahmani & Zulkarnain, 2023). Nevertheless, the values remained within an acceptable range for rinse-off liquid soap, ensuring practical use and even distribution of active compounds on the skin (Rubio, 2020).

#### **Homogeneity Test**

All formulations demonstrated good homogeneity, with no phase separation, sedimentation, or color differences observed. This indicates that the active extracts were well dispersed in the liquid soap matrix. The presence of 4% CMC supported uniformity by increasing viscosity and preventing particle migration, while NaCl (0.5%) contributed to system stabilization (Rahmani & Zulkarnain, 2023; Bom *et al.*, 2024). Similar findings in recent studies also highlight that proper ratios of surfactants and thickeners are critical to maintaining product homogeneity and stability in herbal-based cleansers (Esadini, 2023).

#### **Antioxidant Activity**

The maximum wavelength ( $\lambda_{max}$ ) for DPPH was determined at 517 nm, where the radical exhibits its strongest absorption. At this wavelength, the reduction of the purple DPPH solution to yellow upon reaction with antioxidants produces the most sensitive and reliable measurement of radical scavenging activity. Measuring at 517 nm ensures optimal sensitivity, linearity within the Beer–Lambert range, and consistency with recent antioxidant studies.



**Figure 1.** Result of Maximum Wavelength Measurement of DPPH

The antioxidant activity test revealed that all three liquid soap formulations containing soursop (*Annona muricata* L.) and soybean (*Glycine max* (L.) Merr.) extracts exhibited weak antioxidant capacity, as indicated by relatively high IC<sub>50</sub> values (Table 1).

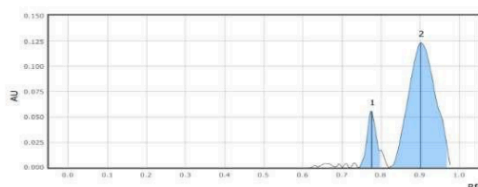
**Table 1.** IC<sub>50</sub> Values of Antioxidant Activity in Liquid Soap Formulations

Formula	Soybean Extract Concentration	IC <sub>50</sub> Value (µg/mL)	Antioxidant Activity
F1	0.25%	380.25 ± 5.12	Weak
	0.26%	385.21 ± 5.22	Weak
	0.24%	379.13 ± 4.89	Weak
F2	0.50%	352.87 ± 4.76	Weak
	0.48%	340.75 ± 4.52	Weak
	0.51%	356.24 ± 4.59	Weak
F3	1.00%	310.44 ± 6.03	Weak
	1.12%	321.25 ± 6.14	Weak
	1.08%	318.65 ± 5.92	Weak

According to classification criteria, antioxidant activity is considered weak when IC<sub>50</sub> > 250 µg/mL (Devita, 2021). This finding suggests that although bioactive compounds such as polyphenols and flavonoids are present, their concentrations are insufficient to exert strong radical scavenging activity. The high IC<sub>50</sub> values may be attributed to interactions between active compounds and the soap matrix. Surfactant systems such as SLS and potassium soap derived from the saponification of olive oil with KOH may reduce the stability and bioavailability of phenolic compounds, thereby limiting their radical scavenging efficiency (Kaur, 2020). Moreover, the relatively low extract concentration in the formulations (0.25–1%) also contributes to the weak antioxidant activity observed (Cahyaningsih *et al.*, 2019). Nevertheless, the presence of even weak antioxidant activity provides an added functional value, as it may support skin protection against oxidative stress when combined with cleansing effects. Recent studies also indicate that herbal extracts with low-to-moderate antioxidant capacity can still be effectively applied in *rinse-off* cosmetic formulations (Rubio, 2020). Therefore, optimization strategies such as increasing extract concentrations, combining synergistic antioxidants, or applying encapsulation techniques may be considered for future development. The results of the antioxidant activity test are presented in Table 1, showing the IC<sub>50</sub> values of the three liquid soap formulations containing soursop (*Annona muricata* L.) leaf extract and soybean (*Glycine max* (L.) Merr.) extract.

### Determination of Quercetin Content in Liquid Soap Containing Soursop (*Annona muricata* L.) Leaf and Soybean (*Glycine max* (L.) Merr.) Using TLC-Densitometry

Based on the chromatogram analysis of the liquid soap formulation containing a combination of soursop (*Annona muricata* L.) leaf extract and soybean (*Glycine max* (L.) Merr.) seed extract using the TLC-densitometry method, two major peaks were observed with Rf values of 0.744 (sample) and 0.818 (quercetin). The peak with an Rf value of 0.744 corresponded to the quercetin standard, confirming the presence of quercetin in the liquid soap formulation. This finding indicates that quercetin is a dominant component within the flavonoid fraction detected in the preparation. The chromatogram results are presented in Figure 2.



**Figure 2.** Chromatogram of liquid soap formulation containing soursop (*Annona muricata* L.) leaves and soybean (*Glycine max* (L.) Merr.)

From the calculation, the Rs (resolution) value was determined to be 2.40. According to chromatographic criteria, an Rs value greater than 1.5 indicates baseline separation, which confirms that the quercetin peak was well resolved from the adjacent sample peak. This result demonstrates that the TLC-densitometry method applied in this study provides reliable separation and identification of quercetin within the liquid soap formulation. Adequate resolution is essential to ensure accuracy in qualitative and quantitative analysis, thereby supporting the validity of quercetin as a dominant flavonoid marker in the soursop leaf and soybean extract formulation. The determination of quercetin content in the liquid soap sample was performed in triplicate at the maximum wavelength of 284 nm. The results of the quercetin content determination in the liquid soap sample are presented in Table 2 below.

**Table 2.** Quercetin Content in Liquid Soap Formulation

Replications	Area	Concentration	Mean concentration	Concentration (%)
1	0.01208	53,8 ppm	55.73 ppm ± 3.1	0.189 %
2	0.01316	56,3 ppm		
3	0.01327	57,1 ppm		

The final test conducted was the determination of quercetin content in the liquid soap formulation using the TLC-densitometry method. The obtained peak areas were calculated using the regression equation  $y = 0.00012x + 0.00469$ , where  $y$  represents the sample area value. The mean quercetin content in the sample was found to be 55.73 ppm ± 3.1, with a total percentage content of 0.189% w/w.

### Conclusion

This study successfully formulated a liquid soap containing a combination of soursop (*Annona muricata* L.) leaf extract and soybean (*Glycine max* (L.) Merr.) seed extract and evaluated its physicochemical characteristics and antioxidant activity. The selected formulation (F1) demonstrated favorable physical properties including homogeneity, spreadability, foam stability, and acceptable pH values. Antioxidant activity testing revealed that all formulations exhibited weak activity, with  $IC_{50}$  values above 250  $\mu\text{g/mL}$ , likely due to the relatively low concentration of active extracts and interactions with the soap matrix. Furthermore, TLC-densitometry analysis confirmed the presence of quercetin as a major flavonoid component, with a mean content of 55.73 ppm or 0.189% w/w in the liquid soap formulation. Although the antioxidant capacity was classified as weak, the incorporation of these herbal extracts enhanced the functional value of the soap as a natural-based cosmetic product. Further optimization of extract concentrations or incorporation techniques is recommended to improve the antioxidant potential of future formulations.

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