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FORMULATION AND EVALUATION OF GLUTATHIONE SOAP FROM *FOMITOPSIS PINICOLA* FOR SKIN WHITENING, MOISTURIZING, AND ANTI-WRINKLE EFFECTS

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ABSTRACT

Many commercial soaps contain chemicals that can be harmful to the skin. Therefore, soaps made from fungal extracts offer an alternative choice for achieving healthy, radiant, wrinkle-free skin. In this study, glutathione was extracted from a local fungal isolate *Fomitopsis pinicola*, obtained from Al-Nimrud region. The extracted glutathione was purified using DEAE-cellulose ion exchange chromatography followed by gel filtration using Sephadex G-150. The soap base, consisted of glycerin, coconut oil, and palm oil, was prepared, and the active ingredient, the purified glutathione extract, was added to the base. The formulated soap exhibited a purple appearance, solid texture, distinctive aromatic scent, pH of 6.4, stable dense foam, ability to disperse dirt, moisturizing duration of 92 seconds, moisture content of 11.2%, cleaning ability of 88%, and stability at 45°C. Additionally, it showed no skin irritation and exhibited antibacterial activity. So, this soap can be used for skin whitening and moisturizing, combating signs of aging, and treating skin diseases, as it has the ability to inhibit the growth of bacteria and fungi as a promising natural medical soap that can be alternative to the commercial soap that contains harmful chemicals.

Keywords: Anti-aging, Anti-bacterial, *Fomitopsis pinicola*,. Glutathione extract, Moisturizing, Whitening

INTRODUCTION

The human skin is the primary barrier against environmental aggressors, including air pollution, ultraviolet (UV) radiation, and microbial exposure, all of which contribute to oxidative stress and skin disorders such as premature aging, hyperpigmentation, and inflammatory conditions **(Calvo *et al*., 2024; Lee *et al*., 2023)**. Oxidative stress depletes the skin's natural antioxidants, such as glutathione, leading to increased susceptibility to damage **(Mistry, 2017)**. However, conventional skincare products often contain synthetic detergents and preservatives that disrupt the skin's lipid barrier and exacerbate inflammation **(Mourelle *et al*., 2024)**.

Recent research highlights the significance of glutathione, a tripeptide antioxidant, in neutralizing reactive oxygen species (ROS), modulating melanogenesis, and enhancing collagen synthesis, making it a valuable dermatological agent **(Gunadi *et al*., 2024; Baharara *et al*., 2023)**. Despite its promising benefits, the low bioavailability of glutathione in commercial formulations has driven the exploration of sustainable sources **(Nowruzi and Zakerfirouzabad, 2024)**. Fungal secondary metabolites, particularly from *Fomitopsis pinicola*, have emerged as potent sources of glutathione, exhibiting strong antioxidative and antimicrobial properties suitable for skincare applications **(Shah and Chew, 2018).**

The shift towards eco-conscious skincare has prompted interest in fungal-derived antioxidants due to their scalable and consistent production compared to plant-based extracts (**Alnuqaydan *et al*., 2023)**. Fungal extracts have been shown to enhance the functionality of traditional soap ingredients, improving hydration, foam stability, and antibacterial properties **(Burke, 2005)**. However, the application of *F. pinicola*-derived glutathione in soap formulations remains largely unexplored.

This study aims to bridge this research gap by developing and evaluating a soap enriched with glutathione extracted from *F. pinicola*. By assessing its physicochemical properties, safety, and dermatological efficacy, this study contributes to the development of sustainable skincare alternatives in alignment with the United Nations Sustainable Development Goals (SDGs), particularly Goal 12 on responsible consumption and production.

MATERIAL AND METHODS

Collecting Fungal Samples

During a field survey conducted in the Al-Nimrud region, macrofungal fruiting bodies were systematically collected for analysis. To ensure the removal of dust and external contaminants, the specimens were thoroughly washed multiple times with running water. A portion of the collected fruiting bodies was preserved by immersion in 70% ethanol. Additionally, selected fragments were subjected to a sterilization protocol, involving immersion in ethanol for five minutes, followed by a transfer to sterile distilled water with continuous agitation for three minutes. These fragments were subsequently placed on sterile filter papers to remove residual moisture. To obtain pure fungal colonies, the prepared specimens were inoculated onto the surface of Petri dishes containing potato dextrose agar (PDA) medium under sterile conditions.

Extraction of Glutathione

To extract glutathione, 10 grams of dried macrofungal fruiting body powder were placed in a glass flask, and 100 mL of distilled water was added. The mixture was incubated in a shaking water bath at 78°C for two hours to facilitate extraction. Following incubation, the flask was cooled by placing it in a container filled with ice cubes. The extract was then filtered using Whatman No. 1 filter paper to remove solid residues. Subsequently, the filtrate underwent centrifugation at 10,000 rpm for 20 minutes to separate any remaining particulates. The resulting supernatant was collected, while the precipitate was discarded. To concentrate the extract, the supernatant was evaporated using a rotary evaporator at 40°C, yielding a dried glutathione-rich powder. The final product was stored under refrigeration until further use **(Xiong *et al*., 2009)**.

Determination of Glutathione

To determine glutathione concentration, the method described by **Wen *et al.* (2006)** was employed. One milliliter of the extract was placed in a clean test tube, to which 1 mL of alloxan solution (1000 mg/L) was added, followed by thorough mixing. Subsequently, 3.5 mL of phosphate buffer solution (pH 7.5) and 0.5 mL of glycerin were incorporated into the mixture, ensuring homogeneity via an electric mixer. The reaction mixture was allowed to stand for 20 minutes at room temperature. Absorbance measurements were then conducted using an ultraviolet

(UV) spectrophotometer at a wavelength of 305 nm. The glutathione concentration in each sample was determined based on a standard calibration curve for glutathione.

Purification of Glutathione

Following 12 days of incubation in potato dextrose broth (PDB), the fungal biomass was separated from the crude glutathione filtrate. For the purification process, 20 grams of Diethylaminoethyl (DEAE)-cellulose ion exchange granules were suspended in 500 mL of distilled water and heated in a water bath at 80–90°C with continuous stirring for three hours. The suspension was then allowed to cool to room temperature. The activated gel was subsequently washed multiple times with sodium phosphate buffer solution (pH 7) and degassed before being carefully poured into a chromatography column (3 × 20 cm) to ensure uniform packing.
The glutathione extract was applied onto the upper surface of the column using a sterile 10 mL medical syringe, ensuring a slow and controlled introduction. The column was equilibrated with the same phosphate buffer solution, and elution was carried out using a gradient of sodium chloride (0.1–1.0 M) at a flow rate of 30 mL per hour, with 3 mL collected per fraction. The recovered glutathione fractions were collected in test tubes, and their concentrations were determined as per the method outlined by **Whitaker and Bernard (1972)**.
For further purification, Sephadex G-150 gel filtration chromatography was employed. A 20-gram quantity of Sephadex G-150 gel was suspended in 500 mL of potassium phosphate buffer (pH 7) and activated in a water bath at 90°C for five hours to facilitate swelling. The gel was then cooled to room temperature, subjected to degassing, and washed several times with the same buffer solution to remove residual impurities. The prepared gel was transferred into a glass purification column (2 × 40 cm), ensuring even packing. Subsequently, 10 mL of partially purified glutathione obtained from the ion exchange chromatography was carefully applied to the upper section of the column. The elution process was carried out using the phosphate buffer solution at a flow rate of 30 mL per hour, with 3 mL fractions collected per elution.
The recovered glutathione fractions were collected in test tubes and quantified. To ensure the scientific and industrial applicability of the purified glutathione, a lyophilization technique was employed to obtain it in a powdered form, allowing for further biochemical characterization and potential application in soap formulation. The purity of the glutathione was verified using high-performance liquid chromatography (HPLC), wherein the retention time of the purified sample was compared with that of a standard glutathione sample **(Hamdan and Jasim, 2018).**

Formulation of Glutathione-Enriched Soap Using *F. pinicola*

The transparent glycerin-based soap formulation was prepared following the methodology of **Yuswita (2011)**, with specific modifications to enhance its stability and efficacy. The formulation components and their respective concentrations are presented in **Table 1**.

Table 1 Composition of the Base Soap Formulation

Sr. No.	Ingredient	Added Amount (%)	Purpose of Addition
1	Coconut Oil (g)	35	Anti-fungal
2	Palm Oil (g)	35	Anti-fungal
3	Sodium Hydroxide (g)	13	Saponification agent
4	Ethanol (g)	30	Solvent
5	Glycerin (g)	20	Moisturizing agent
6	Alkyl Polyglycosides (APG) (g)	9	Cleaning agent
7	Stearic Acid (g)	25	Thickener
8	Propylene Glycol (g)	0.15	Preservative
9	Sorbitol (g)	20	Humectant (retains moisture)
10	Distilled Water (g)	50	Solvent

This formulation provides a balanced composition to ensure antifungal properties, effective cleansing, moisture retention, and structural stability of the soap base. Let me know if you need further modifications!
Purification of Glutathione and Preparation of Glutathione-Enriched Soap

Preparation of the Soap Base

To prepare the soap base, 13 grams of sodium hydroxide was dissolved in 50 mL of distilled water in a glass beaker. The solution was then placed in a water bath at 50°C and maintained for 30 minutes until a clear solution was obtained. It was subsequently allowed to cool to room temperature.
In a separate glass beaker, the required oils were combined with stearic acid and heated gently over a low-temperature surface with continuous stirring. Once the oils were completely melted and homogenized, the sodium hydroxide solution was gradually added to the oil mixture, ensuring thorough blending. The combined mixture was left undisturbed for 3 to 5 hours until it became transparent.
Following this, ethanol, alkyl polyglycosides (APG), and glycerin were incorporated into the mixture with continuous stirring, and the formulation was maintained over low heat for an additional 30 minutes. Sorbitol and preservatives were subsequently added, ensuring uniform dispersion. Finally, the prepared soap mixture was poured into molds and left to solidify at room temperature for 24 hours.
The glutathione-enriched soap was formulated by incorporating a transparent soap base with glutathione extract, vitamin E, coloring agents, and fragrance, as outlined in **Table 2**.

Table 2 Formulation of Glutathione-Enriched Soap

Sr. No.	Ingredient	Added Amount (%)	Purpose of Addition
1	Soap base	80	Cleaning agent
2	Glutathione extract (***F. pinicola***)	2	Whitening, moisturizing, anti-aging
3	Vitamin E	0.01	Antioxidant
4	Citric acid	1.0	pH adjustment
5	Rose oil	As desired	Fragrance
6	Mica color	As desired	Colorant
7	Phenoxyethanol	0.1	Preservative

Procedure for Glutathione-Enriched Soap Formulation

Eighty grams of the previously prepared transparent soap base was placed in a glass beaker and heated in a water bath at 60°C with continuous stirring until it was fully liquefied. Once the soap base reached a homogeneous liquid state, the glutathione extract and other active ingredients were sequentially added, ensuring consistent mixing. The prepared mixture was then poured into silicone molds and allowed to solidify at room temperature for 24 hours **(Almukashir *et al*., 2023).**

Evaluation of Glutathione-Enriched Soap Formulated from *F. pinicola*

Organoleptic Evaluation

The organoleptic evaluation was conducted to assess the sensory attributes of the formulated soap, including color, texture, transparency, and general appearance. The soap's fragrance was evaluated by applying a small amount to the hand and assessing its olfactory properties **(Sowmya *et al.*, 2025).**

Determination of pH

The pH of the formulated soap was determined using the method described by **Selvamani et al. (2022)**. One gram of each soap sample (formulated soap, commercial soap, and base soap) was dissolved separately in 100 mL of distilled water. The pH measurement was conducted using a calibrated pH meter.

Foam Stability Test

Foam stability was assessed following the protocol outlined by **Diwakar *et al*. (2024).** One gram of soap powder was dissolved in 100 mL of distilled water and transferred into a 250 mL graduated cylinder. The cylinder's opening was sealed with the palm, and the mixture was vigorously shaken. The initial foam height was recorded, and after 8 minutes, the final foam height was measured. Foam stability was calculated using the following equation:

$$\text{Foam Stability}(\%) = \frac{\text{Initial Foam Height}}{\text{Final Foam Height}} \times 100$$

Dirt Dispersion Test

To assess the soap's cleaning efficacy, a 1% soap solution was prepared in a glass flask, and two drops of Indian ink were added. The solution was then transferred into a graduated cylinder and shaken vigorously. The dispersion of the ink within the foam was observed to determine whether the soap effectively lifted dirt particles **(Dyagatwar *et al*., 2023).**

Wetting Time Test

To evaluate the wetting ability of the soap, a 1% soap solution was prepared and poured into a glass dish. A circular disc of linen fabric, 1 inch in diameter, was placed on the surface of the solution and allowed to float freely. The time required for the disc to completely submerge was recorded, indicating the wetting efficiency of the formulated soap **(Kumar *et al*., 2024).**

Moisture Content Determination

The moisture content of the formulated soap was assessed by weighing a 10-gram sample and placing it in an electric oven at 100°C for one hour. After heating, the sample was removed and allowed to cool to room temperature before being reweighed. The moisture content was calculated using the following equation:

$$\text{Moisture Content}(\%) = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100$$

This method ensures accurate assessment of the soap's moisture retention properties, which are critical for maintaining product stability and usability **(Sharma *et al*., 2022).**

Cleansing Ability Test

The cleansing ability of the formulated soap was determined by comparing its effectiveness against both base soap and commercially available soap. A 1-gram sample of each soap type was dissolved in 100 mL of distilled water in a glass flask. Circular pieces of linen cloth (6 × 5 cm) were immersed in the solution, and a small quantity of animal fat was added to simulate real-world cleansing conditions. The mixture was left undisturbed for one hour, after which the treated cloth samples were removed and air-dried. The fat removal efficiency was calculated using the following equation:

$$\text{Cleansing Efficiency}(\%) = \frac{\text{Treated Sample Weight}}{\text{Untreated Sample Weight}} \times 10$$

This test provided insight into the comparative cleaning performance of the formulated soap **(Tyowua et al., 2019).**

Soap Stability at High Temperatures

To evaluate the soap's structural stability under elevated temperatures, a sample of the formulated soap was placed in an electric oven at 45°C for a duration of seven days. Observations were made regarding the physical integrity and hardness of the soap over this period. This test ensured that the soap remained structurally stable and resistant to deformation under high-temperature storage conditions **(Devi *et al*., 2021)**.

Skin Irritation Test

A skin irritation test was conducted to determine the dermatological compatibility of the formulated soap. Twelve healthy volunteers, aged between 25 and 35 years, were selected for the study. A small amount of the soap was applied to clean, dry skin, and participants were monitored for any signs of irritation, redness, or allergic reactions over a specified period. This assessment ensured that the soap was safe for human use and did not induce adverse skin reactions **(Kumar *et al*., 2024).**

Antimicrobial Activity Test

The antimicrobial efficacy of the formulated soap was evaluated using the agar diffusion method. Three common skin pathogens were selected for testing: were obtained from the Central Laboratory at the College of Agriculture and Forestry University of Tikrit.

- **Pseudomonas aeruginosa** (Gram-negative bacterium)
- **Staphylococcus aureus** (Gram-positive bacterium)
- **Candida albicans** (fungus)

The bacterial strains were cultured in nutrient broth, while the fungal strain was grown in Sabouraud dextrose broth. Sterile Petri dishes containing nutrient agar (for bacterial cultures) and Sabouraud dextrose agar (for fungal cultures) were prepared. Using a sterile cork borer (6 mm in diameter), five wells were created in each plate.
The following test solutions (40 µL each) were introduced into separate wells:

1. Glycerin soap solution
2. Glutathione soap solution
3. Beesline soap solution (commercial reference)
4. Ciprofloxacin antibiotic solution (50 mg/mL, positive control)
5. Distilled water (negative control)

The plates were incubated at 37°C for 24 hours for bacterial cultures and 48 hours for fungal cultures. Following incubation, inhibition zones around each well were measured to determine the antimicrobial efficacy of the formulated soap **(Sidiq and Fauzi, 2023)**.

User Satisfaction Test

The user satisfaction assessment was conducted following the methodology described by **Badwar *et al.* (2019)**, utilizing a randomized trial involving 12 volunteers. Each participant was instructed to apply the base soap to their left hand and the glutathione-enriched soap to their right hand twice daily over a period of one month.
To quantitatively evaluate user experience, a five-point Likert scale was employed, categorizing satisfaction levels as follows:

1. **4.26–5.10** – Very high satisfaction
2. **3.46–4.25** – High satisfaction
3. **2.66–3.45** – Moderate satisfaction
4. **1.86–2.65** – Low satisfaction
5. **1.05–1.85** – Very low satisfaction

This structured evaluation enabled an objective comparison of user perception regarding the effectiveness, texture, hydration, fragrance, and overall usability of the formulated glutathione soap relative to the base soap. The collected data provided valuable insights into consumer acceptance and the potential for commercial application.

RESULTS AND DISCUSSION

Purification of Glutathione

To purify glutathione from the *F. pinicola* fungal isolate, chromatographic separation techniques were employed as an initial step, given their well-documented efficacy in protein and metabolite purification. Ion-exchange chromatography using a DEAE-Cellulose column was chosen due to its high selectivity for charged biomolecules.
The crude glutathione extract was carefully introduced onto the upper nozzle of the DEAE-Cellulose separation column using a sterile medical syringe. As illustrated in **Figure 1**, a protein peak was observed in the fractions collected during the washing process with the phosphate buffer solution, specifically in the fraction range of 10–30. However, these fractions exhibited no significant glutathione activity. In contrast, the presence of glutathione was confirmed in the recovered fractions between 55 and 70, as indicated by a single peak that corresponded with the protein peak.
To further refine the purification process and achieve a high degree of homogeneity, gel filtration chromatography was utilized. This method effectively eliminated residual impurities and provided a distinct separation of glutathione. As depicted in **Figure 2**, a single glutathione peak was observed in the recovered fractions between 16 and 28, aligning precisely with the protein peak. These findings confirm the successful purification of glutathione, ensuring its suitability for further biochemical applications.

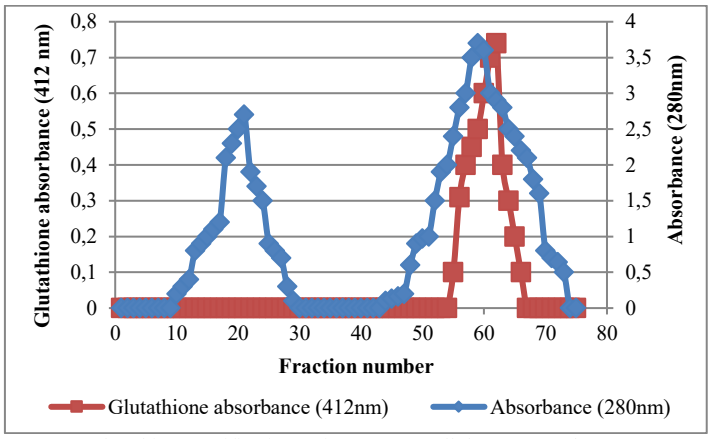


Figure 1 Glutathione Purification Using DEAE-Cellulose Ion Exchange Chromatography

The purification of glutathione was performed using a DEAE-Cellulose ion exchange column with dimensions of 2.5 × 20 cm. Equilibration and elution were carried out using a 10 mM sodium acetate buffer solution, ensuring optimal ionic conditions for selective binding and release of glutathione. The purification process was conducted at a flow rate of 30 mL per hour, with fraction collection at a rate of 3 mL per sample. This method facilitated the efficient separation of glutathione from other cellular components, yielding purified fractions suitable for further biochemical characterization.

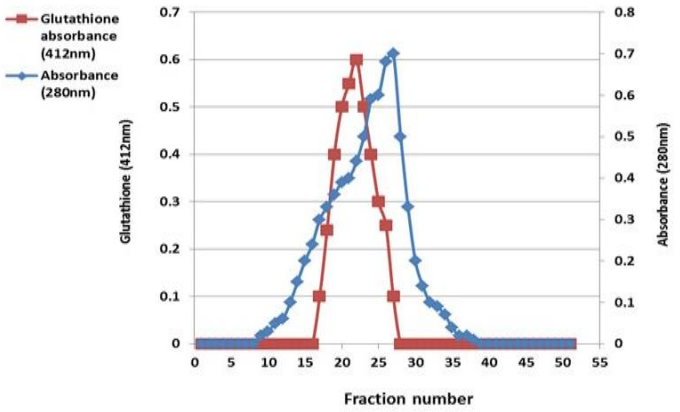


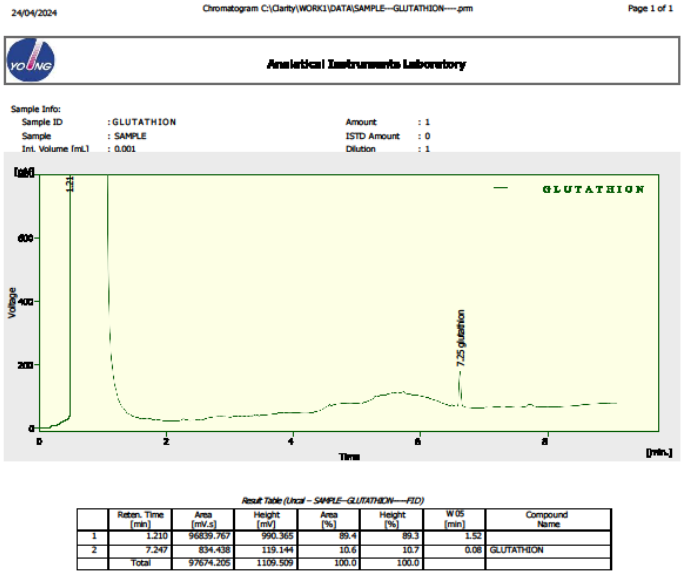
Figure 2 Glutathione Purification Using Gel Filtration Chromatography

Glutathione purification was further refined using gel filtration chromatography in a column with dimensions of 1.5 × 40 cm. The equilibration and elution processes were performed using a 10 mM sodium acetate buffer solution, ensuring optimal separation conditions. The elution was conducted at a flow rate of 30 mL per hour, with fractions collected at a rate of 3 mL per sample. This method facilitated the removal of residual impurities and provided highly purified glutathione fractions.

Validation of Purity Using High-Performance Liquid Chromatography (HPLC)

To confirm that the purified glutathione was homogeneous and free from protein contaminants, 20 microliters of the recovered fractions from the final purification step (gel filtration chromatography) were analyzed using high-performance liquid chromatography (HPLC). The chromatographic results, as presented in **Figure 3**, demonstrated a single peak for the purified glutathione, appearing at a retention time of 7.25 minutes, which closely matched the retention time of 7.52 minutes observed for the standard glutathione in **Figure 4**.

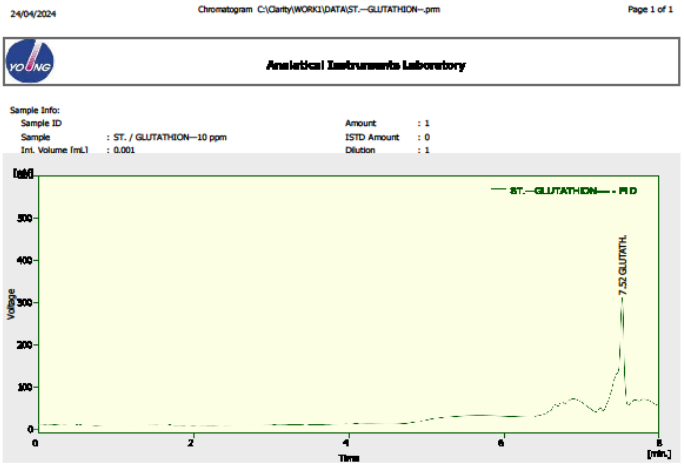
These findings provide strong evidence of the precision and efficacy of the purification process, confirming that the obtained glutathione was of high purity and devoid of significant impurities.



Result Table (Uncal – SAMPLE–GLUTATHION—FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	1.210	96839.767	990.365	89.4	89.3	1.52	
2	7.247	834.438	119.144	10.6	10.7	0.08	GLUTATHION
	Total	97674.205	1109.509	100.0	100.0		

Figure 3 Purification of Glutathione Extract from *F. pinicola* Using HPLC



Result Table (Uncal – ST.GLUTATHION— FID)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W 05 [min]	Compound Name
1	6.033	9317.804	949.869	89.0	79.2	0.26	
2	7.523	1157.462	248.750	11.0	20.8	0.06	GLUTATHION
	Total	10475.266	1198.618	100.0	100.0		

Figure 4 Standard Glutathione Chromatographic Profile Using HPLC

Physicochemical Evaluation of the Formulated Soap

To ensure the quality and effectiveness of the final soap product, various physicochemical tests must be conducted. Among these, organoleptic evaluation plays a crucial role in assessing the appearance, texture, odor, and color of the soap. These sensory characteristics provide insight into the overall aesthetic appeal and consumer acceptability of the formulated glutathione soap, transparent glycerin soap base, and commercial beesline soap, as summarized in **Table 3**.

Table 3 Organoleptic Evaluation of Different Soap Types

Soap Type	Shape	Odor	Color
Glycerin Soap (Transparent Base)	Rectangular-solid	Odorless	White-transparent
Glutathione Soap (*F. pinicola* Extract)	Oval-solid	Distinctive lavender fragrance	Dark purple
Commercial Beesline Soap	Round-solid	Odorless	Creamy

Organoleptic Evaluation

One of the most important aspects of soap formulation is its appearance, as sensory attributes directly influence consumer perception and product quality. The glutathione-enriched soap, formulated using the fungal extract of *F. pinicola*, is dark purple due to the presence of natural mica pigment **(Figure 5A)**. It also possesses a light, aromatic lavender fragrance, imparted by lavender essential oil, enhancing its sensory appeal. The soap has a smooth, cohesive texture and a solid structure, contributing to its premium quality.

In contrast, the glycerin soap is transparent, odorless, and has a smooth yet firm texture **(Figure 5B).** This clarity and consistency are characteristic of glycerin-based formulations, known for their moisturizing properties and mild cleansing action.

The commercial beesline soap, recognized for its skin-brightening effects, has a light creamy color and a fiber-enriched composition, contributing to a rougher texture **(Figure 5C)**. Unlike the glutathione soap, it lacks an aromatic fragrance, making it a more functional rather than sensory-enhanced skincare product. Glutathione is widely recognized as a key ingredient in skincare due to its skin-brightening and antioxidant properties. The glutathione soap formulated in this study is designed not only as a skin-lightening agent but also as a moisturizer, owing to its glycerin content. Additionally, the inclusion of vitamin E enhances its anti-aging benefits, providing antioxidant protection and maintaining skin elasticity.

The presence of lavender essential oil further elevates consumer appeal, adding a pleasant fragrance while contributing to skin-calming effects. These characteristics align with findings by **Joshi *et al.* (2022),** who reported that soap formulations containing extracts from *Brassica oleracea* var. capitata, *Azadirachta indica*, and *Rubia cordifolia* exhibited a firm structure, dark-green color, and a distinctive aromatic scent, emphasizing the importance of natural bioactive ingredients in enhancing both functionality and user experience.

The results of this study confirm that glutathione-enriched soap derived from *F. pinicola* combines skin benefits, sensory appeal, and effective cleansing properties, positioning it as a high-quality skincare product with strong commercial potential. The glutathione-enriched soap, formulated using the fungal extract of *F. pinicola*, exhibits a rich dark purple color, which is attributed to the incorporation of natural

mica pigment. This soap also possesses a subtle aromatic fragrance derived from lavender essential oil, imparting a refreshing sensory experience. The texture is smooth and cohesive, contributing to a luxurious feel upon application (**Figure 5A**). In contrast, the glycerin soap base is transparent, odorless, and has a smooth yet solid texture, indicative of its moisturizing composition (**Figure 5B**). The commercial beesline soap, which is recognized for its skin-brightening properties, features a light creamy color and a fiber-enriched composition, contributing to its rougher texture. Unlike the glutathione soap, it lacks an aromatic fragrance, reinforcing its functional rather than sensory appeal (**Figure 5C**).
These observations highlight the distinct characteristics of the formulated glutathione soap, demonstrating its potential superiority in both aesthetic and sensory attributes compared to conventional soap formulations.

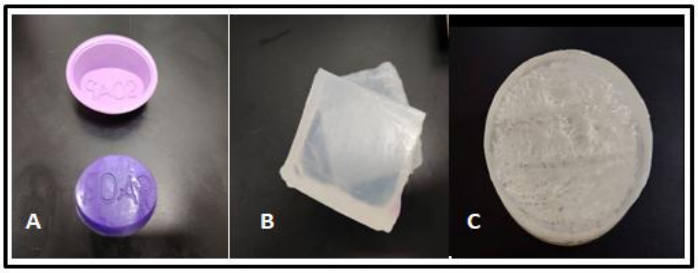


Figure 5 Visual Comparison of Soap Types (A) Transparent Glycerin Soap, (B) Glutathione-Enriched Soap Derived from *F. pinicola*, (C) Commercial Beesline Soap

pH Determination

One of the critical parameters in soap formulation is pH, as it plays a fundamental role in determining the product's safety, stability, and compatibility with the skin. The pH level serves as an indicator of the chemical reactions occurring within the soap formulation and can significantly impact skin health.
The pH analysis conducted in this study revealed the following values:

- Transparent glycerin soap: pH 7.2
- Glutathione-enriched soap: pH 6.4
- Commercial beesline soap: pH 8.0

It is well established that newly formulated soaps initially exhibit higher pH values, which gradually decrease and stabilize over a period of approximately 15 days post-production. Consequently, the pH values of both the transparent glycerin base soap and the glutathione soap were re-evaluated after 15 days to monitor this expected reduction.
The results of this study align with previous literature. For instance, **Wankhade *et al.* (2024)** reported that herbal soaps formulated from plant extracts such as Neem, Tulsi, and Aloe vera exhibited an initial pH of 8.5, while commercial herbal soaps had a slightly lower pH of 8.2. Similarly, **Selvamani *et al.* (2022)** found that soaps derived from various plant extracts maintained a pH of 6.7, highlighting the influence of natural bioactive ingredients on pH regulation. In contrast, **Awang *et al.* (2022)** demonstrated that a soap formulated from the fungal isolate *Schizophyllum commune* exhibited a notably higher pH of 10.34, emphasizing the variability in pH levels based on the origin of raw materials used in soap formulation.

Foam Stability Assessment

Foam stability is an essential attribute of soap, directly influencing its cleansing efficiency and ability to remove dirt, oils, and bacteria from the skin. The foam stability test conducted in this study assessed the rate at which foam dissipated over time for the glutathione-enriched soap, transparent glycerin soap, and commercial beesline soap.
The results indicated that:

- The foam produced by the glutathione soap and the transparent glycerin soap decreased by only 2 cm after 5 minutes, as illustrated in **Figure 6A** and **6B**, respectively.
- In contrast, the commercial beesline soap exhibited a greater foam reduction of 3 cm over the same duration, as shown in **Figure 6C**.

Foam stability is influenced by the composition of surfactants and other active ingredients in the soap. According to **Lavanya *et al.* (2025),** foam is essentially a dispersion of gas within a liquid, forming air pockets encased by a thin film of liquid. The results of this study indicate that the incorporation of fungal extracts from *F. pinicola* did not compromise foam stability, confirming its suitability as a functional skincare ingredient.
The findings of this research are consistent with previous studies. **Diwakar *et al.* (2024)** observed that soaps formulated using plant extracts such as Tulsi and Aloe vera through the cold process method produced foam levels of up to 31 cm after 5 minutes. Additionally, a study by **Nijam and Udapurkar (2023)** demonstrated that soap formulations incorporating Neem and Tulsi extracts maintained excellent foam stability, with foam height decreasing by only 2.5 cm after 5 minutes.
These results confirm that the glutathione soap formulated with *F. pinicola* extract maintains high foam stability, comparable to plant-based formulations, thereby ensuring effective cleansing performance without compromising user experience.

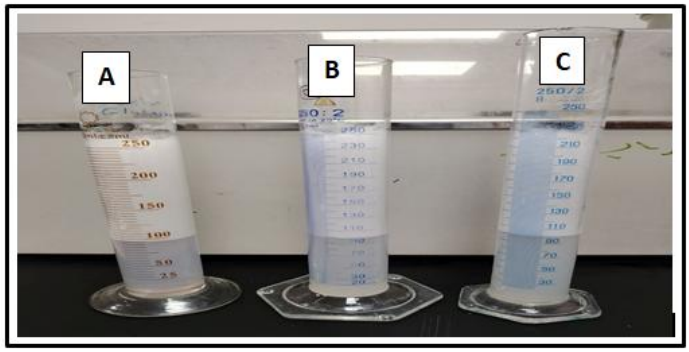


Figure 6 Foam Stability After 5 Minutes, (A) Glutathione-enriched soap (*F. pinicola* extract) (B) Transparent glycerin soap base (C) Commercial beesline soap

Dirt Dispersion Assessment

The effectiveness of the formulated soaps in dispersing dirt was assessed using Indian ink in a 1% soap solution. The results showed homogeneous ink dispersion in the liquid phase of the glutathione-enriched soap, as seen in Figures 7A, 7B, and 7C. However, no ink dispersion occurred in the foam, which remained clean. Similarly, glycerin and **Beesline** soap solutions exhibited stable foams, preventing external contaminants. This stability is attributed to surfactants, which suspend dirt in the liquid phase while maintaining foam integrity.
These findings align with studies highlighting surfactants' role in foam stabilization and dirt removal. **Samadi *et al.* (2024)** found that nonionic surfactants like saponins enhance foam stability and prevent dirt penetration, supporting this study's results. **Wallon *et al.* (2024)** examined surfactant monolayers at oil–water interfaces, showing their impact on foam resilience and dirt dispersion, particularly in anionic and amphoteric surfactant formulations. **Wang *et al.* (2025)** further demonstrated that higher surfactant concentrations improve foam integrity, reinforcing this study's observation that tested soaps effectively dispersed dirt while maintaining foam stability.

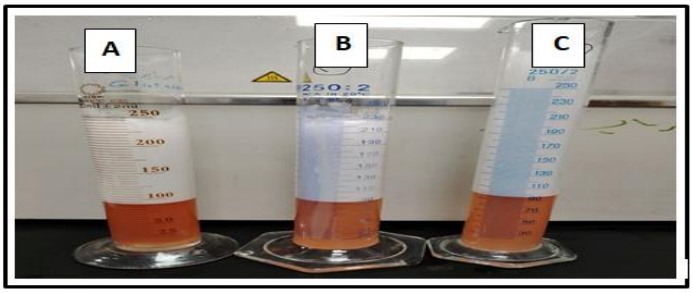


Figure 7 Ink Dispersion Test in Formulated Soap Solutions, (A) Glutathione-enriched soap (*F. pinicola* extract) (B) Transparent glycerin soap base (C) Commercial beesline soap

Wetting Time Analysis

The moisturizing effect of the formulated glutathione soap was evaluated using the cloth disc method. The results showed a **wetting time of 92 seconds**, indicating superior moisture retention. In comparison, **glycerin soap took 137 seconds,** while commercial **Beesline soap required 125 seconds.** This suggests the glutathione soap offers enhanced hydration, likely due to its **glycerin content.**
These findings align with previous studies on **glycerin and glutathione in skincare. Kushwaha and Kushwaha (2023)** confirmed that glutathione-based products improve **moisture retention,** supporting our observation of a **lower wetting time. Morganti (2023)** found that **glycerin-containing formulations** significantly **reduce wetting time**, enhancing hydration. Similarly, **Anestopoulos *et al*. (2020)** showed that **glycerin-based surfactants** improve **wetting efficiency,** reinforcing our results.

Moisture Content Analysis

The moisture content of soap plays a crucial role in determining its stability, resistance to environmental conditions, and overall quality. Excessive moisture content can lead to hydrolysis, increasing the risk of soap disintegration, cracking, and microbial growth during storage. High-moisture cosmetic products often provide a favorable environment for bacterial contamination, making moisture

regulation essential for product safety and longevity. Ideally, the recommended moisture content in soap formulations ranges between 10% and 20%.

As presented in **Table 4**, the moisture content of the formulated glutathione soap derived from *F. pinicola* was 11.2%, which falls within the acceptable range. The glycerin soap recorded a slightly higher moisture content of 11.6%, while the commercial beesline soap exhibited the highest moisture level at 16.9%. These values indicate that all three soaps are resistant to hydrolysis, even under conditions of high humidity, ensuring product stability and longevity.

A review of existing literature supports these findings. **Harkal and Deshmukh (2024)** reported that herbal soaps formulated with extracts of Neem, Aloe vera, Turmeric, and Tomato seed oil exhibited a significantly high moisture content of 85%, which could compromise their stability. In contrast, **Das *et al.* (2024)** observed that soap containing Ritha, Tulsi, Neem, and Aloe vera had a much lower moisture content of 1.01%, indicating enhanced durability. Furthermore, **Nijam and Udapurkar (2023)** found that a Neem and Tulsi-based soap formulation maintained a moisture content of 6.66%, further demonstrating the variability of moisture levels depending on the ingredients used.

Table 4 Moisture Content of Different Soap Types

Soap Type	Moisture Content (%)
Glycerin Soap	11.6
Glutathione Soap	11.2
Commercial Beesline	16.9

Cleaning Ability Evaluation

The cleaning efficiency of the formulated glutathione soap was evaluated and compared with glycerin soap and commercial beesline soap using a fabric-based fat removal test. In this method, circular linen cloth discs were treated with animal fat and subsequently cleansed using each soap formulation.

The results demonstrated that the glutathione soap exhibited the highest cleaning efficiency, removing 88% of fat residues. In comparison, the glycerin soap exhibited a lower efficiency of 55%, while the commercial beesline soap achieved a moderate cleansing ability of 73%, as illustrated in Figures 8A, 8B, and 8C. Based on these findings, the glutathione soap formulation is highly effective in cleansing while maintaining skin hydration, as complete removal of natural oils may lead to skin dryness and irritation. These results are further supported by **Nijam and Udapurkar (2023)**, who reported that herbal soap formulations containing Neem and Tulsi extracts effectively removed stains from fabric surfaces, demonstrating strong cleansing properties.

The findings of this study confirm that the glutathione-enriched soap derived from *F. pinicola* exhibits superior cleansing capabilities while ensuring moisture retention, making it a high-quality skincare product with strong functional and cosmetic benefits.

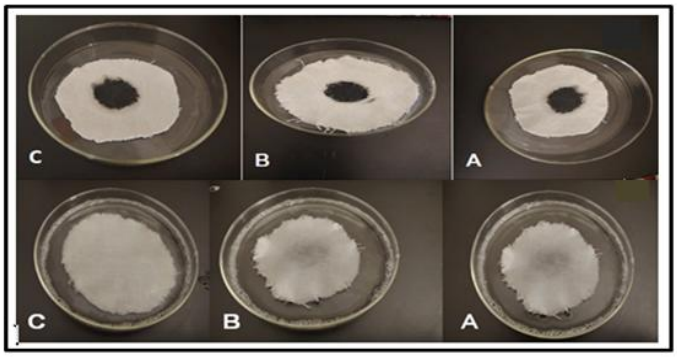


Figure 8 Soap cleaning ability: A: Formulated glutathione soap, B: Glycerine soap base, C: Commercial beesline soap.

Stability at High Temperatures

Thermal stability is a critical parameter in evaluating the quality and durability of soap formulations, particularly under high-temperature storage conditions. The results of this study demonstrated that the formulated glutathione soap, glycerin soap, and commercial beesline soap remained structurally stable when subjected to 45°C for seven days. All three soap formulations retained their solid and cohesive texture, which is a hallmark of high-quality soap products.

These findings align with previous research in the field. **Joshi *et al.* (2022)** reported that soap formulated with various plant extracts, including *Brassica oleracea var. capitata*, *Azadirachta indica*, and *Rubia cordifolia*, maintained thermal stability at 45°*C* over a seven-day period, showing no signs of degradation. Similarly, **Devi *et al.* (2021)** found that cold-processed soaps incorporating *Azadirachta indica* and *Ocimum tenuiflorum* extracts exhibited excellent stability at 45°C, further supporting the findings of this study.

Skin Irritation Test

Ensuring the safety and dermatological compatibility of a cosmetic product is essential, particularly for skincare formulations. Irritation testing is widely conducted on commercial soaps and personal care products, especially those containing chemical compounds, to assess user safety and minimize the risk of adverse skin reactions. A crucial aspect of evaluating the effectiveness and tolerability of a formulated soap is gathering consumer feedback to determine its impact on different skin types.

In this study, the skin irritation test was conducted on a group of female volunteers aged 25–35 years to assess the safety and potential dermatological effects of the glutathione soap, glycerin soap, and beesline soap. Participants applied the soap to their hands, creating a thick foam, and left it unwashed for 30 minutes to evaluate its effect on the skin. The primary observations focused on common indicators of irritation, including redness, rashes, and allergic reactions. The results revealed no adverse effects on the skin, as none of the volunteers exhibited signs of irritation, swelling, or redness following product application **(Figures 9A, B, and C).** Additionally, continuous use of the soap formulations over time did not result in any allergic reactions, further confirming their safety for daily use. Given that these soaps are formulated using natural oils and plant-derived extracts and are free from harsh chemicals, they are suitable for all skin types, including dry, sensitive, and oily skin. These characteristics make the glutathione soap an ideal choice for consumers seeking natural and skin-friendly alternatives. The findings of this study are supported by **Anzar and Nath (2024),** who reported that a soap formulated from orange peel extracts (extracted using 95% ethanol) caused no skin irritation when tested on volunteers during handwashing trials. This reinforces the gentle nature of plant-based soaps and their potential for safe and effective skincare applications.

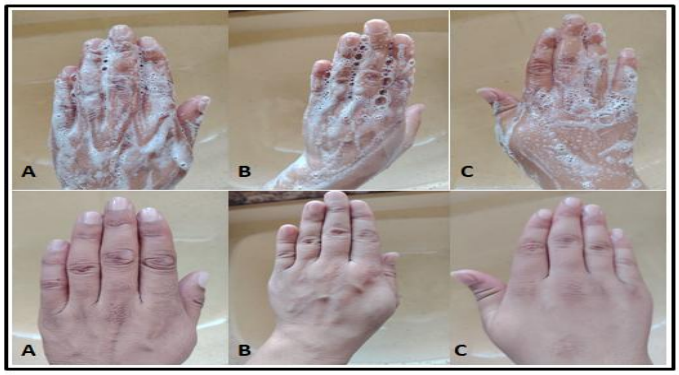


Figure 9 Skin Irritation Test Results for Soap Solutions, A: Beesline soap, B: Glycerine soap, C: Formulated glutathione soap.

Antimicrobial Activity

The effectiveness test of glutathione soap showed its ability to inhibit the growth of skin bacteria at a varying rate, in which the diameter of the inhibition zone towards *P. aeruginosa* bacteria reached 20 mm by using glycerine soap solution, and 30 mm for glutathione soap solution. Also, there was no inhibition of bacteria growth by using the beesline soap. Similarly, against *Staph. aureus* bacteria, the diameter of the inhibition zone using glycerine soap solution was 18 mm, while it reached 32 mm by using glutathione soap solution, and had no effect in regards to the beesline soap. The anti-bacterial effect for the skin fungus *C. albicans* revealed an inhibition zone diameter of 23 and 27 mm by using glycerine and glutathione soap solutions respectively, and after 48 hours of incubation, as shown in Figure 10.

In a related study, **Patidar *et al*., (2024),** reported that, the soap formulated from the glycerine and the garlic oil showed ability towards the inhibition of the growth of different types of bacteria including *Proteus vulgaris*. The inhibition zone reached 1.6 cm and 1.4 cm for *Bacillus* bacteria, and 2.7 cm for S. *aureus* bacteria.

Figure 10 Effectiveness against selected bacteria and skin fungi for commercial glycerine, glutathione, and beesline soaps.

User-satisfaction

To maximize the benefits of the soap formulated from mushroom extract, and to ensure its effectiveness, ease of application, and safety the researchers conducted a user-satisfaction test. The aim was to achieve bright, radiant skin free from defects. The test involved a group of volunteers, some of whom suffered from rough hands and visible wrinkles due to prolonged and intense sun exposure over the course of one month. The questionnaire result showed that 12 volunteers expressed very high satisfaction and high satisfaction from two volunteers, The soap demonstrated excellent absorption, whitening, moisturizing, and softening effects on the skin, and it rinsed off easily without leaving any greasy residue as **in Table (5)**. The volunteers ensured after applying the soap that it is the ideal choice for use, and that they prefer it over other alternatives from the commercial types that widely spread in local markets under different names, especially volunteers those with sensitive skin.

Additionally, a literature survey by **Badwar *et al*. (2019)** was referenced; in their study, 20 volunteers evaluated two types of soap using a 5-point Likert scale, and a high level of satisfaction was reported for the orange peel soap.

Table 5 shows the volunteers level of satisfaction using Likert scale.

Types of Soap	Very high satisfaction	High satisfaction	Medium	Low	Very low
Glycerine	0	7	4	1	0
Glutathione	10	2	0	0	0

CONCLUSION

Glutathione extract obtained from the fungal isolate *F. pinicola* was used to prepare a natural soap with multiple applications. Various physical and chemical parameters such as pH, foaming height and stability, cleaning ability, moisturizing efficacy, and irritation potential were evaluated. All tests yielded positive results and demonstrated satisfactory performance, as evidenced by its use among a group of volunteers who reported comfort and satisfy with the product. Moreover, no signs of skin irritation were observed, likely due to the absence of harsh chemical substances in the formulation. Moreover, the soap exhibited significant whitening, moisturizing, and antioxidant effects; it appears to activate skin cells and inhibit the aging process, as well as reduce wrinkles and prevent common skin disorders. Thus, this soap may be used safely for both general cleaning and as a solution for treating skin problems.

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REFERENCES

Almukashir, M., Shamsi, S. S., Alfurjany, S., Ali, E., & Zidan, H. (2023). Potent antibacterial activity of Terminalia chebula-based herbal soap formulated against Propionibacterium acnes and its cytotoxic evaluation on human skin fibroblast cells. *Journal of Applied Pharmaceutical Research, 11*(5), 1–8. https://doi.org/10.18231/j.joapr.2023.11.5.1.8

Alnuqaydan, A. M., Zainy, F. M. A., Almutary, A. G., & Sadier, N. S. (2023). Tamarix articulata extract offers protection against toxicity induced by beauty products in Hs27 human skin fibroblasts. *PLOS ONE, 18*(11), e0287071. https://doi.org/10.1371/journal.pone.0287071

Anestopoulos, I., Kiousi, D. E., Klavaris, A., & Galanis, A. (2020). Surface active agents and their health-promoting properties: Molecules of multifunctional significance. Pharmaceutics, 12(7), 688. https://doi.org/10.3390/pharmaceutics12070688

Anzar, N. F., & Nath, A. R. (2024). Formulation and evaluation of two layered soap containing poly herbal extracts. *International Journal of Pharmaceutics Research and Applications, 9*(4), 385–387. http://dx.doi.org/10.22159/ijcpr.2022v14i5.2023

Awang, Z., Awaluddin, N. A., Rodzi, N. I. S., & Hujani, M. A. F. M. (2022). The usage of waste cooking oil (WCO) in production of solid dishwashing soap with split gill mushroom (*Schizophyllum commune*) extract addition. *Politeknik and Kolej Komuniti Journal of Social Science and Humanities, 7*(1), 85–95. https://myjms.mohe.gov.my/index.php/PMJSSH/article/view/20599

Baharara, H., Rahsepar, S., & Emami, S. A. (2023). The efficacy of medicinal plant preparations in the alleviation of radiodermatitis in patients with breast cancer: A systematic review of clinical trials. *Phytotherapy Research, 37*(4), 1245–1257. https://doi.org/10.1002/ptr.7894

Badwar, M. R., Borse, S. L., Junagade, M. S., & Jadhav, A. G. (2019). Formulation and evaluation of mouth dissolving tablet of amlodipine besylate. *International Journal of Applied Pharmaceutics*, 11(4), 132–139. https://doi.org/10.22159/ijap.2019v11i4.31288

Burke, K. E. (2005). Topical nutritional antioxidants. In Draelos, Z. D. & Elsner, P. T. (Eds.), *Cosmetic formulation of skin care products* (pp. 401–421). Taylor & Francis. https://doi.org/10.3109/9781420020854-25

Calvo, M. J., Navarro, C., Durán, P., Giménez, C., González, A., & Pérez, J. (2024). Antioxidants in photoaging: From molecular insights to clinical applications. *International Journal of Molecular Sciences, 25*(4), 2403. https://doi.org/10.3390/ijms25042403

Das, S., Agarwal, S., Samanta, S., Kumari, M., & Das, R. (2024). Formulation and evaluation of herbal soap. *Journal of Pharmaceutical Phytochemistry, 13*(4), 14–19. https://doi.org/10.22271/phyto.2024.v13.i4a.14990

Devi, A. S., Sivani, D. V., Anusha, D., Sarath, G., & Sultana, S. M. (2021). Formulation and evaluation of antimicrobial herbal soap. *International Journal of Pharmaceutical Sciences, Reviews, and Research, 71*(2), 122–125. https://doi.org/10.47583/ijpsrr.2021.v71i02.019

Diwakar, A., Sharma, H., Yadav, T., Chandel, R., Sahu, G. K., Sahu, P., & Nema, R. K. (2024). Development of herpal soap for effective cleansing and skin moisturizing. *Journal of Emerging Technologies in Innovative Research, 11*(4), 2349–3162. Retrieved from https://www.jetir.org/papers/JETIR2404F72.pdf

Dyagatwar, M., Pimple, B., Kuchekar, M., Vadje, S., Gaikwad, S., Bhurkunde, V., Tare, H., & Chumbhale, D. (2023). Development and evaluation of herbal shampoo as an antifungal formulation. *International Journal of Drug Delivery & Technology, 13*(3), 913–918. https://doi.org/10.25258/ijddt.13.3.23

Gunadi, J. W., Trisnawaty, S., Dewi, P., & Raharja, A. (2024). Carotenoids in red fruit (*Pandanus conoideus* Lam.) have a potential role as an anti-pigmentation agent. *Biomedical Reports, 20*(2), 37. https://doi.org/10.3892/br.2024.1742

Hamdan, N. T., & Jasim, H. M. (2018). Purification and characterization of cellulase enzyme from *Trichoderma longibrachiatum* isolated in Iraqi soil. *Journal of Biotechnology & Biochemistry, 4*(1), 32–41. http://dx.doi.org/10.9790/264X-04013241

Harkal, V. A., & Deshmukh, S. P. (2024). Formulation and evaluation of polyherbal soap. *Journal of GSC Bio & Pharmaceutical Sciences, 27*(2), 068–079. https://doi.org/10.30574/gscbps.2024.27.2.0151

Joshi, D. P., Shah, R. R., Darji, R., & Basera, I. A. (2022). Fabrication and evaluation of polyherbal soap via utilizing a variety of herbal extracts. *International Journal of Current Research & Technology, 10*(3), 2320–2882.

Kumar, P. P., Priyanka, N., Subrahmanyam, M. G., Kumar, K. A., Venkat, A. E., Sangeetha, V., Manjari, K., Pavani, D., & Sirisha, D. (2024). Formulation and evaluation of herbal shampoo. *Journal of Pharmaceutical Phytochemistry, 13*(2), 165–170. https://doi.org/10.22271/phyto.2024.v13.i2b.14897

Kushwaha, N., & Kushwaha, S. K. S. (2023). A comprehensive review of cosmeceutical chemistry. Current Cosmetic Science, 9(2), 101-118. https://doi.org/10.2174/2666779702666230413090319

Lee, Y., Jeong, D. Y., Jeun, Y. C., Kim, H. S., & Park, S. H. (2023). Preventive and ameliorative effects of potato exosomes on UVB-induced photodamage in keratinocyte HaCaT cells. *Molecular Medicine Reports, 27*(6), 99. https://doi.org/10.3892/mmr.2023.13054

Mistry, N. (2017). Guidelines for formulating anti-pollution products. *Cosmetics, 4*(4), 57. https://doi.org/10.3390/cosmetics4040057

Morganti, P. (2023). Skin hydration: Mechanisms and applications in cosmetic formulations. Novel Cosmetic Delivery Systems, 4(1), 45-63. https://doi.org/10.1201/9781003418078-4

Mourelle, M. L., Segura de Yebra, J., Ayats, J., Vitale, M., Rodríguez-Luna, A., & Fernández-Caballero, C. (2024). Potential benefits of a cosmetic ingredient combining thermal spring water and diatom algae extract. *Cosmetics, 11*(2), 62. https://doi.org/10.3390/cosmetics11020062

Nijam, S. N., & Udapurkar, P. (2023). Formulation and evaluation of herbal antimicrobial soap. *International Journal of Current Research & Technology, 11*(3), 2320–2882.

Nowruzi, B., & Zakerfirouzabad, M. (2024). Anti-inflammatory activities of phycoerythrin and phycocyanin on human fibroblast cells. *Phytomedicine Plus*. https://doi.org/10.1016/j.phyplu.2024.100604

Patidar, P., Goswami, P., Jaiswal, N., & Patidar, L. (2024). Formulation and in vitro characterization of herbal soap with the use of natural antifungal agent. *Journal of Emerging Technologies in Innovative Research, 11*(3), 2349–3162.

Samadi, S., Amani, H., Najafpour, G.D., Kariminezhad, H., & Banaei, A. (2024). Development of shampoo formulations using plant/microbial biosurfactants as an alternative to shampoos formulated with harsh synthetic surfactants, Journal of Surfactants and Detergents. https://doi.org/10.1002/jsde.12814

Lavanya, M., Saranya, M., Lavanya, K., Jindavali, S., Babu, V. A. & Mahesh, J. (2025). Formulation And Evaluation Of Herbal Soap, *Indo A merican Journal of Pharmaceutical Sciences*, 12(03), 160-165. https://doi.org/10.5281/zenodo.15020650

Selvamani, M., Surya, P. R., Siva, S. D., Subash, K., Siva, G. M., Vigneswaran, L. V., & Kumar, M. S. (2022). Formulation and evaluation of polyherbal soap. *World Journal of Pharmaceutical Medicine Research, 8*(2), 170–173.

Shah, S., & Chew, S. K. (2018). Efficacy and safety of a topical depigmenting agent in healthy human fair-skinned female volunteers: A single-arm study. *Journal of Cosmetic Dermatology, 17*(5), 1038–1045. https://doi.org/10.1111/jocd.12435

Sharma, S., Pradhan, S., Pandit, B., & Mohanty, J. P. (2022). Formulation and evaluation of herbal soap taking different bioactive plants by cold saponification method. *International Journal of Current Pharmaceutical Research, 14*(5), 30–35. https://doi.org/10.22159/ijcpr.2022v14i5.2023

Sidiq, F., & Fauzi, H. (2023). Learning about evaluation and antifungal activity of Simpur leaf extract shampoo (*Dillenia indica*) towards *Candida albicans* causes dandruff. *International Journal of Ethnoscience and Educational Research, 3*(1), 14–20.

Sowmya, P. S., Nandini, C., Keerthana, A. V., Rajeswari, M., Priya, P. J. & Sree, L. B. (2025).Formulation and evaluation of herbal anti- tanning soap from Potato. *World Journal of Pharmaceutical Science and Research*, 4(1), 451-456. https://doi.org/10.5281/zenodo.14787229

Tyowua, A. T., Vitalis, T. B., Terhemen, M. M., & Mbaawuaga, E. M. (2019). Aloe vera (*Aloe barbadensis Miller*) extract as a natural antimicrobial agent in hand washing liquid soap. *Journal of Nigerian Annals of Pure and Applied Sciences, 2*, 96–107. https://doi.org/10.46912/napas.113

Wallon L, Salonen A, Langevin D. (2024). Surfactant monolayers at oil–water interfaces. Behavior upon compression and relation to emulsion stability. J Surfact Deterg. https://doi.org/10.1002/jsde.12806

Wang, Jianwei, Lian, Yubo, Chen, Xiang, Yang, Haiyan, Chang, Wanjun and Zhang, Jie. (2025). "Research on a synergistic formula of anionic and betaine type surfactants for natural gas fields" Tenside Surfactants Detergents, 62(1), 19-26. https://doi.org/10.1515/tsd-2024-2608

Wankhade, S., Bhise, M., Shinde, M., Suryawanshi, J., & Tare, H. (2024). Formulation and evaluation of soap containing extracts of various ethanomedicines. *International Journal of Drug Delivery & Technology, 14*(2), 1000–1003. https://doi.org/10.25258/ijddt.14.2.60

Wen, S., Zhang, T., & Tan, T. (2006). Maximizing production of glutathione by amino acid modulation and high cell density fed-batch culture of *Saccharomyces cerevisiae*. *Journal of Process Biochemistry, 41*(12), 2424–2428. https://doi.org/10.1016/j.procbio.2006.06.030

Whitaker, J. R., & Bernard, R. A. (1972). Experiments for: An introduction for enzymology. *The Whiber Press Davis*, 2, 12–14.

Xiong, Z. Q., Guo, M. J., Guo, Y. X., Chu, J., Zhuang, Y. P., & Zhang, S. L. (2009). Efficient extraction of intracellular reduced glutathione from fermentation broth of *Saccharomyces cerevisiae* by ethanol. *Journal of Bioresource Technology, 100*(2), 1011–1014. https://doi.org/10.1016/j.biortech.2008.07.018

Yuswita, S. A. (2011). Mei pembuatan panel beton berbasis perlit dan aplikasinya sebagai insulator panas. *Journal of Ilmu Pengetahuan dan Teknologi, 29*, 29.