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Evaluation of Antioxidant, Antimicrobial and Preservation Performance of a Herbal Paraben-Free Facial Cleanser

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ABSTRACT

The increasing interest in botanical cosmetics has encouraged the development of facial cleansing products that combine effective cleansing with skin compatibility and alternative preservation strategies. The present study was designed to evaluate the antioxidant, antimicrobial and preservation performance of a herbal paraben-free facial cleanser formulated using selected botanical extracts. The research framework integrates phytochemical characterization, antioxidant assessment, antimicrobial screening, microbiological quality evaluation and preservation efficacy testing to determine whether a botanical formulation can maintain acceptable quality without conventional parabens. Herbal extracts were selected on the basis of their documented phytochemical composition, traditional cosmetic relevance and reported biological activities. The formulated cleanser was evaluated for physicochemical characteristics before biological testing. Antioxidant performance was proposed to be assessed using complementary radical-scavenging assays, particularly DPPH and ABTS methods. Antimicrobial activity was designed to be evaluated against representative Gram-positive, Gram-negative and fungal test organisms using appropriate controls. Microbial quality assessment was incorporated to determine the microbiological suitability of the finished product, while preservation performance was evaluated using a controlled microbial challenge-testing approach. Particular attention was given to the distinction between intrinsic antimicrobial activity of herbal extracts and the ability of the complete formulation to remain microbiologically protected during storage and repeated use. The proposed evaluation framework recognizes that antioxidant capacity does not necessarily predict antimicrobial effectiveness and that antimicrobial activity of an individual extract cannot substitute for preservative efficacy testing of the finished cosmetic. Stability, pH, surfactant composition, water activity, packaging and interactions among botanical constituents may substantially influence preservation performance. The study therefore provides an integrated approach for assessing the biological functionality and microbiological safety of herbal paraben-free facial cleansers. Its principal significance lies in establishing a scientifically defensible basis for developing botanical cleansing systems that balance antioxidant activity, antimicrobial performance, preservation, formulation stability and consumer-oriented safety.

Keywords: Herbal facial cleanser; paraben-free cosmetics; antioxidant activity; antimicrobial activity; preservation efficacy; DPPH; ABTS; botanical extracts; cosmetic microbiology; natural preservatives.

1. INTRODUCTION

1.1 Background

Facial cleansing is an essential component of routine skin care because the skin surface is continuously exposed to sebum, perspiration, environmental particulate matter, microorganisms, cosmetic residues and other contaminants. A facial cleanser is therefore expected to remove undesirable material while minimizing disruption of the physiological skin barrier. This requirement creates a formulation challenge: increasing cleansing intensity may improve soil removal but can simultaneously increase dryness, irritation or unpleasant post-wash sensations when the surfactant system is poorly selected.

Contemporary cosmetic research has increasingly explored botanical ingredients as multifunctional components of skin-care formulations. Plants contain diverse secondary metabolites, including phenolic compounds, flavonoids, tannins, terpenoids, saponins and other constituents that may contribute antioxidant, antimicrobial, soothing or conditioning properties. However, the presence of biologically active compounds does not automatically establish the suitability of a botanical extract for a finished cosmetic product. Extract composition can vary with botanical identity, plant part, cultivation conditions, extraction solvent, processing conditions and storage.

The preservation of water-containing cosmetics represents an additional scientific challenge. Such formulations provide an environment in which microorganisms may proliferate if an adequate preservation system is not established. Consequently, a paraben-free product cannot simply be considered safe because parabens have been omitted. The complete formulation must demonstrate adequate microbiological quality and resistance to microbial proliferation under appropriately designed testing conditions.

The present research therefore focuses on three related but distinct biological dimensions: **antioxidant activity, antimicrobial activity and preservation performance**. Antioxidant assays evaluate the ability of extracts or formulations to interact with selected reactive species under defined test conditions. Antimicrobial testing evaluates inhibitory effects against selected microorganisms. Preservation testing, in contrast, examines whether the finished formulation can control deliberately introduced microorganisms over a specified testing period. Treating these endpoints separately provides greater scientific clarity.

1.2 Rationale

Botanical facial cleansers may provide opportunities to reduce reliance on conventional preservative systems while incorporating plant-derived functional ingredients. Nevertheless, several limitations require careful consideration. Botanical extracts may introduce colour and odour changes, interact with surfactants or polymers, alter viscosity, influence pH and potentially compromise microbial stability.

Moreover, an extract exhibiting strong activity in a laboratory antioxidant assay may have limited activity after incorporation into a complex cleanser because of dilution, extraction differences, pH effects or interactions with other ingredients. Similarly, an antimicrobial zone observed in an agar diffusion test cannot by itself establish preservative efficacy in a finished cosmetic.

Table 1. Major Biological Evaluation Domains

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Evaluation domain	Principal question	Typical assessment
Antioxidant activity	Can the formulation/extract interact with selected free radicals under assay conditions?	DPPH, ABTS
Antimicrobial activity	Does the formulation inhibit selected microorganisms?	Agar diffusion or validated quantitative method
Microbial quality	Is the finished product microbiologically acceptable?	Microbial enumeration and specified-organism testing
Preservation performance	Can the formulation control introduced microorganisms over time?	Preservative efficacy/challenge testing
Stability	Does performance remain acceptable during storage?	Physical, chemical and microbiological monitoring

1.3 Aim and Objectives

Aim

To formulate and scientifically evaluate a herbal paraben-free facial cleanser with particular emphasis on its antioxidant activity, antimicrobial properties and preservation performance.

Objectives

- To select suitable botanical extracts for incorporation into a paraben-free facial cleanser.
- To characterize the selected extracts for relevant phytochemical constituents.
- To formulate a stable herbal facial cleanser using an appropriate surfactant and preservation-support system.
- To evaluate antioxidant activity using validated or appropriately standardized DPPH and ABTS assays.
- To investigate the antimicrobial activity of the selected extracts and finished formulations.
- To assess the microbiological quality of the formulated cleanser.
- To evaluate preservation performance using an appropriate challenge-testing methodology.
- To examine the relationship between formulation composition, antimicrobial activity and microbiological stability.
- To identify the formulation demonstrating the most satisfactory overall biological and formulation performance.

1.4 Research Hypotheses

H_{01}: The herbal paraben-free facial cleanser does not demonstrate meaningful antioxidant activity under the selected experimental conditions.

H_{11}: The herbal paraben-free facial cleanser demonstrates measurable antioxidant activity under the selected experimental conditions.

H_{02}: The herbal paraben-free facial cleanser does not demonstrate antimicrobial activity against the selected test microorganisms.

H_{12}: The herbal paraben-free facial cleanser demonstrates antimicrobial activity against at least some of the selected test microorganisms.

H_{03}: The selected paraben-free preservation system does not provide adequate microbial control in the finished formulation.

H_{13}: The selected paraben-free preservation system provides adequate microbial control in the finished formulation under the specified test conditions.

H_{04}: There is no meaningful relationship between formulation composition and preservation performance.

H_{14}: Formulation composition significantly influences preservation performance.

2. REVIEW OF LITERATURE

2.1 Herbal Ingredients and Biological Activity

Botanical materials have long been incorporated into traditional skin-care preparations. Modern cosmetic research has attempted to translate this traditional knowledge into standardized formulations by identifying chemical constituents associated with particular biological effects.

Phenolic compounds and flavonoids are frequently investigated because of their electron- or hydrogen-donating properties in chemical antioxidant systems. Terpenoids and essential-oil constituents may contribute antimicrobial or fragrance-related properties. Tannins can interact with proteins and may possess antimicrobial and astringent characteristics. Saponins can influence surface activity and may therefore be relevant to cleansing preparations.

However, phytochemical complexity is both an advantage and a limitation. Multiple constituents may produce complementary effects, but the same complexity makes standardization difficult. Two extracts described by the same botanical name may differ considerably depending on extraction and raw-material quality.

2.2 Antioxidant Activity in Cosmetic Research

Oxidative processes can contribute to deterioration of cosmetic ingredients and are also relevant to biological processes occurring at the skin surface. Consequently, antioxidant-rich botanical ingredients have attracted substantial attention.

The DPPH assay is widely used because the coloured radical provides a relatively simple spectrophotometric endpoint. ABTS-based assays offer a complementary radical-scavenging model and may respond to a broader range of antioxidant compounds. Nevertheless, both are chemical assays rather than direct measurements of clinical skin protection.

This distinction is important. A high percentage of radical scavenging in vitro should not automatically be interpreted as evidence that a cleanser prevents oxidative skin damage in vivo. The result instead provides evidence of antioxidant potential under the defined experimental conditions.

2.3 Antimicrobial Activity of Botanical Extracts

Plant-derived materials may demonstrate antimicrobial effects through several mechanisms, including disruption of microbial membranes, alteration of enzyme activity, interference with

cellular metabolism and interaction with microbial proteins. Essential oils can be particularly active because many volatile constituents interact with lipid-rich microbial membranes.

2.4 Preservation of Cosmetic Formulations

Preservation is a formulation-level property rather than merely an attribute of one ingredient. The preservative system interacts with pH, surfactants, polymers, botanical extracts, packaging and other formulation components.

A botanical extract may itself inhibit microorganisms, but its concentration in the finished product may be insufficient to protect the product throughout its shelf life. Conversely, a preservation-support system may act through multiple mechanisms, such as membrane disruption, pH reduction, metal-ion sequestration or reduction of microbial availability.

Challenge testing is therefore particularly important. It evaluates the ability of the complete formulation to control intentionally introduced microorganisms and provides information that simple antimicrobial screening cannot provide.

3. RESEARCH METHODOLOGY

3.1 Research Design

The investigation should follow an experimental formulation-and-evaluation design consisting of:

1. botanical selection;
2. extract preparation;
3. phytochemical screening;
4. formulation development;
5. antioxidant evaluation;
6. antimicrobial evaluation;
7. microbiological quality assessment;
8. preservation efficacy testing;
9. stability monitoring; and
10. statistical analysis.

The exact experimental conditions should be finalized through laboratory validation and relevant institutional procedures.

3.2 Materials

The study may use authenticated botanical materials selected according to the final formulation design. Additional materials include a mild surfactant system, humectant, viscosity modifier, pH-adjusting agents, purified water and an appropriate paraben-free preservation system.

Table 2. Experimental Material Categories

Category	Function
Botanical extracts	Functional herbal actives
Surfactant	Cleansing and soil removal
Humectant	Moisture retention and skin feel
Thickener	Viscosity and physical stability

pH modifier	pH adjustment
Preservation system	Microbial control
Chelating/preservation booster	Enhancement of preservation where appropriate
Purified water	Vehicle
Packaging	Product protection and dispensing

3.3 Preparation of Herbal Extracts

Authenticated plant material should be cleaned to remove foreign matter and dried under controlled conditions that minimize degradation of thermolabile constituents. The dried material should then be pulverized to a reasonably uniform particle size.

The extraction solvent should be selected according to the polarity and stability of the target phytoconstituents. Aqueous, hydroalcoholic or other validated extraction systems may be considered. Following extraction, the material should be filtered and concentrated under controlled conditions. The resulting extract should be stored in suitable containers under conditions that minimize moisture, light and temperature-related degradation.

3.4 Phytochemical Screening

Preliminary screening may include qualitative tests for:

- phenolics;
- flavonoids;
- tannins;
- saponins;
- alkaloids;
- terpenoids;
- glycosides; and
- other constituents relevant to the selected botanical materials.

Where resources permit, quantitative marker analysis should complement preliminary screening because qualitative tests provide only limited information regarding concentration.

3.5 Formulation Development

Several experimental batches should be prepared by varying relevant botanical and formulation variables.

Table 3. Proposed Formulation Development Matrix

Batch	Herbal active level	Preservation-support level	Purpose
F1	Low	Standard	Determine lower active level
F2	Moderate	Standard	Evaluate intermediate level
F3	Higher	Standard	Determine effect of increased active level
F4	Optimized combination	Optimized	Final candidate
Control	Without selected herbal actives	Appropriate control	Comparative reference

3.6 Antioxidant Evaluation

DPPH Assay

Appropriate concentrations of extract or formulation should be reacted with DPPH reagent under standardized conditions. Absorbance should be measured at the validated wavelength using a suitable spectrophotometer.

ABTS Assay

The ABTS radical system should be generated according to the validated laboratory protocol and adjusted to the required absorbance range. Sample activity should be determined relative to the control and expressed according to the selected analytical protocol.

Using DPPH and ABTS together can provide complementary evidence rather than treating either assay as definitive.

3.7 Antimicrobial Evaluation

The antimicrobial investigation should include suitable reference microorganisms selected according to the study objectives and laboratory biosafety requirements. The test panel may include representative Gram-positive bacteria, Gram-negative bacteria and yeast/fungal organisms.

Appropriate positive and negative controls should be incorporated. Where agar diffusion is employed, inhibition zones should be measured using standardized procedures. Where possible, quantitative methods such as minimum inhibitory concentration or equivalent validated procedures can provide stronger comparative evidence.

3.8 Microbial Quality Assessment

The finished cleanser should be assessed for microbial quality using appropriate enumeration methods and examination for relevant specified or objectionable microorganisms. Testing should follow recognized microbiological quality procedures applicable to cosmetic products. Importantly, microbial quality testing and preservation efficacy testing should not be conflated. The former assesses contamination status of the product, whereas the latter examines its capacity to resist or control microbial challenge.

3.9 Preservation Efficacy Evaluation

A controlled challenge test should be performed using defined microbial strains and predetermined sampling intervals. The formulation should be inoculated under controlled conditions, followed by microbial recovery at specified times.

The principal outcome is the change in recoverable microbial population over the testing period. Acceptance criteria should be selected according to the applicable recognized standard or pharmacopoeial/cosmetic microbiological framework rather than invented specifically for the study.

3.10 Stability Evaluation

Samples should be stored under selected long-term, accelerated and temperature-variation conditions appropriate to the research design. Where relevant, freeze-thaw and light-exposure studies may also be conducted.

The following parameters should be monitored:

- Colour;
- Odour;

- Appearance;
- Ph;
- Viscosity;
- Phase separation;
- Antioxidant activity;
- Marker compounds;
- Microbial quality; and
- Preservation performance where appropriate.

4. RESULTS AND INTERPRETATION

Important: The following numerical tables are **illustrative examples only** and are not experimental findings. They demonstrate how the final manuscript can present results once actual laboratory data are available.

4.1 Antioxidant Activity

Table 4. Antioxidant Results

Formulation	DPPH inhibition (%)	ABTS inhibition (%)	Interpretation
F1	48.2 ± 1.6	52.4 ± 1.8	Moderate activity
F2	61.7 ± 1.4	65.1 ± 1.5	Higher activity
F3	69.3 ± 1.7	72.6 ± 1.3	Highest among preliminary batches
F4	71.5 ± 1.2	74.1 ± 1.1	Optimized formulation
Control	8.4 ± 0.9	10.1 ± 1.0	Low baseline activity

Illustrative values only; replace with actual observations.

If the actual data demonstrate an increasing antioxidant response with increasing botanical concentration, this could suggest that the botanical fraction contributes substantially to radical-scavenging activity. However, such an interpretation should be supported by appropriate statistical analysis and, where possible, phytochemical quantification.

A formulation showing lower activity than its corresponding extract would not necessarily indicate formulation failure. Surfactants, pH, dilution and other excipients can influence assay response.

4.2 Antimicrobial Activity

Table 5. Illustrative Antimicrobial Screening

Test organism	F1 inhibition zone (mm)	F2 (mm)	F3 (mm)	F4 (mm)
Organism A	11.2 ± 0.5	14.4 ± 0.6	17.1 ± 0.5	18.3 ± 0.4
Organism B	9.6 ± 0.4	12.7 ± 0.5	15.2 ± 0.4	16.1 ± 0.5
Organism C	8.4 ± 0.5	10.6 ± 0.4	13.5 ± 0.6	14.2 ± 0.5

If actual results follow such a pattern, increasing botanical concentration may be associated with stronger inhibition. Nevertheless, zone diameter should not be interpreted as a direct measure of preservative strength because diffusion properties differ among active compounds and microorganisms.

4.3 Preservation Performance

Table 6. Illustrative Challenge-Test Framework

Microbial group	Initial challenge	Day 7	Day 14	Day 28	Interpretation
Bacterial group	100% baseline	Reduced	Further reduced	Controlled	Satisfactory pattern*
Yeast group	100% baseline	Reduced	Reduced	Controlled	Satisfactory pattern*
Mould group	100% baseline	Reduced	Reduced	Controlled	Satisfactory pattern*

Preservation performance should be interpreted at formulation level. If F4 demonstrates effective microbial control while maintaining acceptable pH, viscosity and appearance, the result would support its selection as the optimized formulation.

5. DISCUSSION AND CONCLUSION

5.1 Discussion

The scientific evaluation of a herbal paraben-free cleanser requires interpretation across several levels. The first level concerns the biological properties of the botanical materials themselves. The second concerns whether those properties survive incorporation into the formulation. The third concerns whether the complete product remains microbiologically stable during storage.

Antioxidant activity is particularly relevant when phenolic and flavonoid-rich botanical extracts are used. If both DPPH and ABTS assays show concentration-dependent activity, confidence in the general radical-scavenging capacity of the sample is greater than if only one assay is used. However, assay results remain chemical evidence rather than clinical proof.

The antimicrobial findings require similarly cautious interpretation. Botanical extracts may inhibit selected microorganisms, but activity can vary substantially according to microbial species and extraction conditions. In addition, antimicrobial activity in an agar system does not necessarily indicate that the same concentration will preserve a complex aqueous cleanser.

Preservation performance is consequently the most formulation-specific endpoint. An effective preservation system must function in the presence of surfactants, botanical constituents, polymers and other ingredients. Product pH, packaging and repeated exposure to environmental microorganisms may also influence performance.

A successful formulation should therefore not be selected solely because it demonstrates the strongest antioxidant or antimicrobial activity. Excessive botanical concentration could negatively affect odour, colour, viscosity, stability or skin compatibility. Optimization requires a balance among biological effectiveness, physical stability, microbiological safety and user acceptability.

5.2 Relationship Between Antioxidant and Antimicrobial Properties

The two activities should not be assumed to be interchangeable. A formulation can possess strong antioxidant activity without substantial antimicrobial activity, and conversely, an antimicrobial ingredient may have limited radical-scavenging activity.

Table 7. Interpretation of Biological Endpoints

Finding	Possible implication	Limitation
High DPPH activity	Strong radical-scavenging potential	Does not establish clinical antioxidant protection
High ABTS activity	Supports antioxidant potential	Still an in-vitro chemical assay
Large inhibition zone	Microbial growth inhibition	Diffusion affects zone size
Good challenge-test performance	Supports preservation capability	Conditions must match recognized criteria
Stable antioxidant activity during storage	Suggests retention of chemical activity	Does not establish skin efficacy

5.3 Conclusion

The evaluation of antioxidant, antimicrobial and preservation performance provides a comprehensive framework for assessing herbal paraben-free facial cleansers. Botanical extracts may contribute useful biological functionality, but their incorporation into a cosmetic vehicle introduces additional variables that can modify activity and stability.

The most scientifically defensible approach is therefore to evaluate the extract, formulation and finished product separately while subsequently integrating the findings. DPPH and ABTS assays can characterize antioxidant potential; antimicrobial testing can identify inhibitory effects against selected microorganisms; and microbial quality and challenge testing can establish whether the complete formulation possesses appropriate microbiological characteristics.

A paraben-free formulation should not be regarded as inherently safer or more effective merely because it excludes parabens. Its scientific merit depends on demonstrated product quality, preservation adequacy, stability and skin compatibility. Accordingly, optimization should be based on a multidimensional assessment rather than on a single biological endpoint.

The proposed research framework can contribute to the development of herbal facial cleansers by demonstrating how botanical functionality and microbiological protection can be evaluated simultaneously. Further work should emphasize standardized botanical extracts, quantitative marker analysis, validated preservation systems, extended stability assessment and appropriately designed human skin-compatibility studies.

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