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Development of a Micro-sized Herbal-Based Scalp Oil Serum with Antioxidant and Anti-Inflammatory Properties

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Abstract

With the rising incidence of hair loss and scalp disorders, there is an increasing demand for hair care products that are safe, effective, and environmentally sustainable. Most commercial hair serums currently available contain synthetic chemical components, which may cause scalp irritation, long-term damage, and potential safety concerns for consumers. In contrast, this study aimed to develop a microsized natural hair growth and scalp oil serum formulated with Curcuma Xanthorizza, Curcuma longa, and Zingiber officinale, plant materials well recognised for their antioxidant and anti-inflammatory properties, which are essential for maintaining scalp health and supporting hair follicle function. Subcritical water extraction was employed to obtain bioactive-rich extracts while preserving compound stability and minimising the use of organic solvents. Formulation optimisation was performed using Design of Experiments (DoE) methodology, followed by ultrasonication to achieve microscale particle size, thereby enhancing dispersion stability and follicular targeting. Comprehensive physicochemical characterisation was conducted, including pH, viscosity, and particle size analysis, alongside functional evaluations such as antioxidant activity using DPPH and FRAP assays, and anti-inflammatory activity assessed via the bovine serum albumin (BSA) protein denaturation method. The results demonstrated that the formulated microsized serum exhibited a suitable pH range (4.5 5.0) for scalp application, a stable microscale particle size distribution (365–567 nm), and significant antioxidant activity (89–98% inhibition), together with notable anti-inflammatory activity (96–98.4%), when compared with the corresponding raw herbal extracts. The microsized delivery system enhanced bioactive retention and promoted localised activity at the scalp and hair follicle regions. Overall, this study confirms the feasibility of integrating microtechnology with herbal bioactives to develop a safe, effective, and eco-friendly hair growth and scalp serum. These findings provide valuable insights into natural cosmeceutical formulation and support the potential application of microsized delivery systems in the development of advanced hair care products.

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1. Introduction

Hair and scalp disorders are commonly associated with oxidative stress and inflammatory responses that disrupt normal hair follicle function and scalp homeostasis [1]. Excessive generation of reactive oxygen species (ROS) can damage follicular cells, accelerate hair ageing, and trigger inflammatory cascades that contribute to conditions such as dandruff, scalp irritation, and hair thinning [1]. Conventional hair care formulations frequently rely on synthetic antioxidants, preservatives, and anti-inflammatory agents, which may cause long-term irritation, environmental concerns, and reduced consumer acceptance [2].

Plant-based bioactive compounds have gained increasing attention in cosmeceutical research due to their antioxidant, anti-inflammatory, and biocompatible properties. *Curcuma longa* and *Zingiber officinale* are widely reported to contain phenolic compounds, flavonoids, and bioactive constituents capable of scavenging free radicals and modulating inflammatory pathways [3]. However, the direct incorporation of plant extracts into topical formulations often presents challenges related to poor stability, limited bioavailability, and inconsistent functional performance [3].

Recent advancements in microscale delivery systems, particularly microemulsions, offer promising solutions to these limitations. Micro-sized formulations can enhance dispersion stability, improve interaction with the scalp surface, and facilitate targeted delivery of bioactive compounds to hair follicles without the safety concerns associated with nanoscale systems [4]. Particle size plays a critical role in follicular penetration, formulation stability, and functional efficacy, making microscale optimisation an important consideration in hair serum development.

Despite growing interest in natural hair care products, comprehensive studies integrating bioactive screening, formulation optimisation, and physicochemical–functional characterisation of micro-sized plant-based hair serums remain limited. Therefore, this study aims to develop and systematically characterise a micro-sized hair and scalp oil serum formulated using selected plant-based bioactives. Emphasis is placed on evaluating antioxidant and anti-inflammatory activities, optimising formulation parameters using a Design of Experiments (DoE) approach and assessing the physicochemical stability and functional performance of the developed serum.

2. Methodology

This study details focuses on the development of a herbal oil-based hair serum incorporating bioactive compounds derived from *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger). The experimental procedure are raw material preparation, bioactive extraction, serum formulation, micro-sizing, and systematic physicochemical and functional evaluations to ensure formulation stability, safety, and performance. Subcritical water extraction was employed to obtain the bioactive constituents, as this technique is effective in preserving thermolabile phytochemicals while reducing reliance on organic solvents [5]. The resulting extracts were freeze-dried to improve storage stability and maintain bioactive integrity. The formulation was optimised using DoE. Subsequently, ultrasonication was applied in the presence of varying biosurfactant concentrations to achieve microscale particle size reduction and enhance formulation stability, with particle size distribution and surface charge characterised using zeta potential analysis. The final formulations were then subjected to comprehensive physicochemical characterisation and functional assays to determine formulation quality and bioactive efficacy.

2.1 Materials

The research was carried out at the laboratories of Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Campus, with all solvents, chemicals, and reagents prepared and supplied by the Materials Laboratory and the Upstream Bioprocess Laboratory at UTHM. All three of *Curcuma longa, Curcuma xanthorizza and Zingiber officinale* utilised in this study was sourced from KIZA HERBS, Temerloh, Pahang.

2.2 Sample preparation

Samples of Curcuma longa, Curcuma xanthorrhiza, and Zingiber officinale were sourced from a local farm that practises organic fertilisation and has been verified to have soil free from detectable heavy metal contamination. The plant materials were thoroughly washed to remove surface dirt and impurities, then dried in a laboratory oven at a constant temperature of 40 °C for 24 h to reduce moisture content.

Subcritical water extraction (SWE) was employed to extract bioactive compounds from dried rhizomes of *Curcuma longa*, *Curcuma xanthorrhiza*, and *Zingiber officinale*. Distilled water was used as the extraction

solvent. The extraction system was heated to 100 °C and pressurised to 11 bar to maintain water in the liquid state under subcritical conditions. Once the target temperature and pressure were achieved, extraction was carried out for 20 minutes.

2.3 Evaluation of Antioxidant Activity of Plant Extracts

2.3.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The antioxidant activity of the plant extracts was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A 0.1 mM DPPH solution was prepared in methanol. Extract samples at 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL were mixed with the DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured using a UV–Vis spectrophotometer at 517 nm. Ascorbic acid was used as the reference standard, and the percentage of DPPH scavenging activity was calculated using the formula below to compare the antioxidant performance of the extracts.

$$\%\text{DPPH} = \frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Sample} \times 100 \ldots\ldots\ldots \text{Eqn. 1}$$

2.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted to evaluate the reducing power of the plant extracts. FRAP working reagent was freshly prepared and mixed with the extract samples. The reaction mixtures were incubated at 37 °C for 30 minutes in the dark. Absorbance was measured at 593 nm using a UV–Vis spectrophotometer. A calibration curve was constructed using ascorbic acid as the standard, and FRAP values were calculated to express the antioxidant reducing capacity of the samples.

$$\text{FRAP value } (\mu\text{mol/L}) = \frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Sample} \times 100 \ldots\ldots\ldots \text{Eqn. 1}$$

2.4 Evaluation of Anti-Inflammatory Activity of Plant Extracts

Anti-inflammatory activity was assessed using the bovine serum albumin (BSA) protein denaturation assay. A 1% (w/v) BSA solution was prepared in phosphate-buffered saline. Extract samples (0.1mg/mL, 0.2mg/mL, 0.4mg/mL, 0.8mg/mL) were mixed with the BSA solution and subjected to controlled heating conditions to induce protein denaturation. After cooling to room temperature, absorbance was measured using a UV–Vis spectrophotometer. Aspirin was used as the positive control. The percentage inhibition of protein denaturation was calculated to evaluate the anti-inflammatory potential of the extracts.

2.5 Formulation of Microsized Hair Serum

Based on antioxidant and anti-inflammatory activity analyses, bioactive compounds demonstrating the highest functional efficacy were chosen as the principal active constituents for the hair serum formulation and subsequently employed in further formulation steps.

2.5.1 Design of Experiments (DoE) for Formulation Optimisation

A D-optimal Design of Experiments approach was employed to optimise the formulation parameters of the microsized hair serum. For this study, there are 6 different chemicals used for the formulation, therefore six mixture component is selected. The independent variables included the volumes of castor oil and Tween 80, while the concentration of bioactive extracts was maintained constant based on pre-screening results. Both point exchange and coordinate exchange search algorithms were applied to explore the experimental design space. Point exchange was used to control candidate selection and avoid impractical combinations of formulation factors. The D-optimal design was selected to ensure efficient estimation of factor effects. The design was generated by minimising the determinant of the inverse information matrix $(X'X)^{-1}$. A quadratic Scheffé model was employed, incorporating three lack-of-fit points and no replicate runs.

2.5.2 Preparation of Microsized Serum by Ultrasonication

The selected formulation components were combined according to the DoE matrix and subjected to ultrasonication to produce a microsized microemulsion. The ultrasonic processor was operated in pulsed mode with a pulse duration of 5s at five amplitude settings for a total sonication time of 10 min. Throughout the process, the temperature of the mixtures was maintained below 35 °C to preserve the stability of the bioactive compounds.

2.6 Physicochemical Characterisation of Microsized Hair Serum

2.6.1 pH Measurement

The pH of each formulation was measured using a calibrated pH meter at room temperature. Calibration was performed using standard buffer solutions prior to measurement. The pH values were recorded to assess compatibility with scalp pH conditions.

2.6.2 Stability Testing

Physical stability of the microsized hair serum formulations was evaluated under both ambient and accelerated conditions. Samples were filled into amber glass bottles to shield from lighting, before being stored at room temperature for one week. Then to simulate a condition inside a car in the afternoon, the sample are subjected to high-temperature centrifugation at 60 °C and 3000 rpm for 10 minutes. Lastly, visual inspection was conducted to detect phase separation, sedimentation, or creaming.

2.6.3 Particle Size and Zeta Potential Analysis

To minimise multiple scattering effects and ensure accurate measurements, the serum samples were diluted 1:200 with distilled water, specifically, 20 µL of serum was diluted with 3980 µL of distilled water. Then, the solution is filled into a cuvette and placed into a zeta potential analyser. The measurements were repeated three times.

2.7 Functional Evaluation of Microsized Hair Serum

2.7.1 Total Antioxidant Activity of The Formulated Serum

The antioxidant capacity of the formulated hair serum was evaluated using DPPH and FRAP assays following similar procedures applied to the plant extracts as in section 2.3.1 and 2.32 respectively.

2.7.2 Total Anti-Inflammatory Activity

The anti-inflammatory of the formulated hair serum was evaluated using BSA protein denaturation assay following similar procedures applied to the plant extracts as in section 2.3.3.

3. Results and Discussion

3.1 Functional Screening of Plant Extracts

3.1.1 Antioxidant Activity of Plant Extracts

The DPPH scavenging activities are summarised in Table 1, showing a concentration-dependent antioxidant response for all extracts. At 1.0 mg/mL, *Curcuma longa* exhibited the highest activity (89.61%), exceeding *Zingiber officinale* (81.40%) and *Curcuma xanthorrhiza* (87.68%). This trend persisted at 0.5 mg/mL, where turmeric (81.40%) showed substantially higher inhibition than ginger (59.90%) and temulawak (62.56%). At lower concentrations, turmeric remained the most active; for example, at 0.125 mg/mL, *C. longa* (51.21%) outperformed ginger (44.69%) and temulawak (40.34%). Although all extracts showed a slight increase at 0.0625 mg/mL, turmeric retained the highest activity overall. These results confirm *C. longa* as the strongest radical scavenger across the tested range.

Table 1: DPPH Scavenging Activity Across Three Extracts at Different Concentrations.

Extract Concentration (mg/mL)	DPPH Scavenging activity (%)		
	Ginger	Turmeric	Temulawak
1	81.40 ± 0.001	89.61 ± 0.003	87.68 ± 0.001
0.5	59.90 ± 0.001	81.40 ± 0.001	62.56 ± 0.001
0.25	48.07 ± 0.002	50.48 ± 0.001	56.76 ± 0.002
0.125	44.69 ± 0.001	51.21 ± 0.002	40.34 ± 0.001
0.0625	59.18 ± 0.001	64.98 ± 0.001	58.94 ± 0.002

The superior activity of *C. longa* is likely attributable to its high curcuminoid content (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), which possess strong hydrogen-donating and electron-transfer properties. In contrast, the antioxidant effects of *Z. officinale* are mainly associated with gingerols, shogaols, and zingerone, while the comparatively lower activity of *C. xanthorrhiza* may reflect reduced levels or bioavailability of xanthorrhizol and related phenolics. Overall, these findings support the selection of turmeric as the primary antioxidant source for subsequent formulation, with ginger as a complementary bioactive.

The ferric reducing antioxidant power (FRAP) values of the extracts are presented in Table 2, indicating substantial reducing capacity across all samples. At 1.0 mg/mL, *Curcuma longa* exhibited the highest FRAP value (96.60%), surpassing *Zingiber officinale* (93.45%) and *Curcuma xanthorrhiza* (91.14%) by approximately 3.4% and 6.0%, respectively. This suggests a superior electron-donating capability of turmeric extract at higher concentrations.

Table 2: Ferric Reducing Antioxidant Power (FRAP) of Three Extracts at Different Concentrations

Extract Concentration (mg/mL)	FRAP Value (%)		
	Ginger	Turmeric	Temulawak
1.0	93.45 ± 0.001	96.60 ± 0.002	91.14 ± 0.001
0.4	86.28 ± 0.001	80.43 ± 0.003	87.48 ± 0.003

At 0.4 mg/mL, temulawak demonstrated slightly higher activity (87.48%) compared with ginger (86.28%) and turmeric (80.43%), indicating that antioxidant performance varies with concentration and extract composition. Overall, turmeric showed the strongest reducing power at higher concentration, consistent with its high content of curcuminoids, which are efficient electron donors in redox-based antioxidant mechanisms. The activity of ginger is primarily attributed to phenolic compounds such as gingerols and shogaols, while the comparatively moderate activity of temulawak may reflect differences in xanthorrhizol and total phenolic content.

Collectively, the FRAP results confirm the strong reducing potential of all three extracts, with *C. longa* demonstrating superior antioxidant capacity at higher concentration, supporting its selection as a key bioactive component for formulation development.

3.1.2 Anti-Inflammatory Activity of Plant Extracts

The anti-inflammatory activity of the extracts, evaluated using the protein denaturation inhibition assay, is presented in Table 3. All samples exhibited concentration-dependent inhibition, indicating their potential to stabilise protein structures under inflammatory conditions. Among the extracts, *Curcuma longa* consistently demonstrated the highest inhibitory activity across all concentrations, achieving 88.71–99.88%, followed by *Zingiber officinale* (74.24–89.11%), while *Curcuma xanthorrhiza* showed comparatively lower activity (56.76–87.68%). At the highest concentration (0.8 mg/mL), turmeric achieved near-complete inhibition (99.88%), exceeding ginger (89.11%) and temulawak (87.68%).

Table 3: Comparative Protein Denaturation Inhibition of Selected Plant Extracts at Different Concentrations

Extract Concentration (mg/mL)	Protein Denaturation activity (%)		
	Ginger	Turmeric	Temulawak
1.0	74.24 ± 0.001	88.71 ± 0.001	56.76 ± 0.001
0.2	77.93 ± 0.002	94.01 ± 0.003	58.94 ± 0.001
0.4	83.69 ± 0.001	95.97 ± 0.002	62.56 ± 0.001
0.8	89.11 ± 0.003	99.88 ± 0.001	87.68 ± 0.001

The superior performance of *C. longa* is likely attributable to its high curcuminoid content, particularly curcumin and related analogues, which are known to inhibit inflammatory mediators and protect protein integrity through antioxidant and anti-denaturation mechanisms. The moderate activity of *Z. officinale* can be associated with bioactive phenolics such as gingerols and shogaols, which possess established anti-inflammatory effects. In contrast, the lower inhibitory activity observed in *C. xanthorrhiza* may reflect reduced levels or bioavailability of xanthorrhizol and related compounds.

Overall, these findings confirm that turmeric exhibits the strongest anti-inflammatory potential among the tested extracts, supporting its selection as a primary bioactive component for subsequent formulation, with ginger serving as a complementary anti-inflammatory agent.

3.2 Formulation and Optimisation of Microsized Hair Serum

Figure 1 illustrates the composition of each formulation across the six experimental runs generated using the Design of Experiments (DoE) approach. In all formulations, the concentrations of turmeric and ginger extracts were maintained at fixed levels (0.6 mL each), together with constant minor components (kaffir lime oil and Tween 20). Accordingly, variations in formulation performance were primarily governed by changes in the oil phase (castor oil) and surfactant concentration (Tween 80), which were systematically adjusted according to the experimental design.

Run	Component 1 A:Tumeric ml	Component 2 B:Ginger ml	Component 3 C:Castor Oil ml	Component 4 D:Tween 80 ml	Component 5 E:Kaffir Lime ml	Component 6 F:Tween 20 ml
1	0.6	0.6	15.6	2.2	0.6	0.4
2	0.6	0.6	13.85	3.95	0.6	0.4
3	0.6	0.6	12.975	4.825	0.6	0.4
4	0.6	0.6	12.1	5.7	0.6	0.4
5	0.6	0.6	15.6	2.2	0.6	0.4
6	0.6	0.6	14.725	3.075	0.6	0.4

Figure 1. Composition of formulation components across six experimental runs generated by the Design of Experiments (DoE) approach.

This design strategy enabled the assessment of the interactive effects of oil-to-surfactant ratios on the physicochemical behaviour of the micro-sized hair serum, while eliminating confounding variability from the bioactive extracts. The controlled modulation of castor oil and Tween 80 content is critical, as these components influence emulsion stability, droplet size, and dispersion uniformity, which are key parameters in micro-sized formulation systems. Consequently, the DoE framework provided a robust basis for identifying optimal formulation conditions with respect to stability and performance while maintaining consistent bioactive loading.

3.3 Ultrasonication and Stability Test

All formulations were processed using a sequential ultrasonication protocol to promote microemulsion formation and reduce droplet size. High-energy probe ultrasonication was conducted at a fixed amplitude of 50% for 7 minutes in pulsed mode (10 s on, 30 s off), followed by low-energy water-bath sonication for an additional 30

minutes. This two-step approach was employed to maximise energy input while minimising thermal effects, thereby facilitating efficient droplet disruption and homogeneous dispersion of the oil phase.

Post-ultrasonication, most formulations exhibited a clear to near-transparent appearance, indicating successful microemulsion formation, as shown in Figure 2. Optical clarity is generally associated with reduced droplet size in the microscale range and effective interfacial stabilisation by the surfactant system. The observed transparency suggests that the applied sonication conditions were sufficient to produce uniformly dispersed systems with enhanced physicochemical stability suitable for topical application.

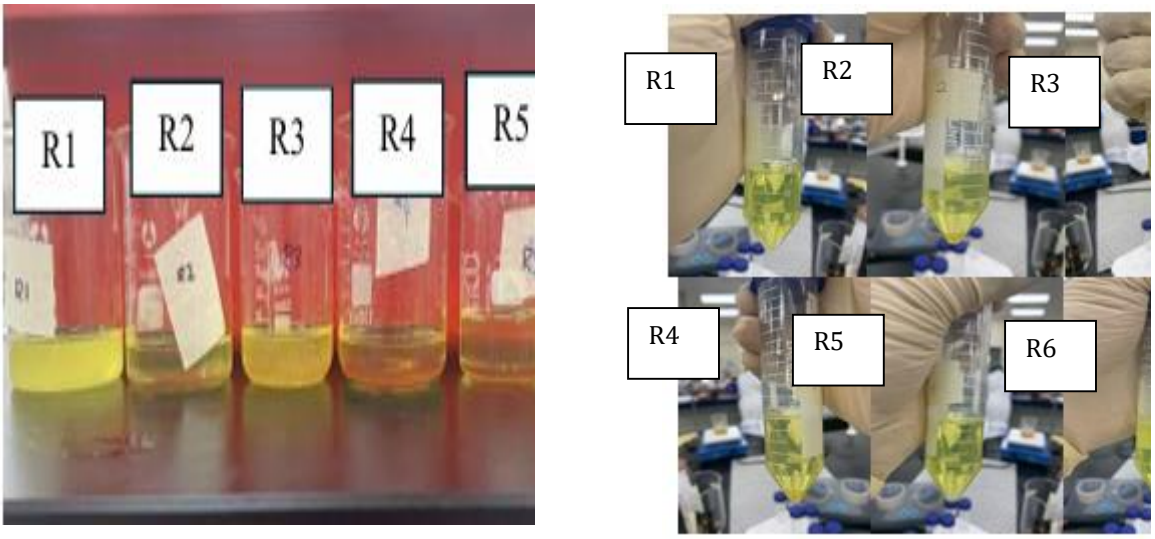

Figure 2. Visual comparison of six formulations after (a) ultrasonication and (b) stability testing

Stability evaluation further revealed that formulations R1 and R3 remained slightly turbid relative to the other samples, suggesting incomplete micro-emulsification. This reduced clarity is likely attributable to non-optimal surfactant-to-oil ratios, which may have limited interfacial coverage and hindered effective droplet stabilisation. In such cases, inadequate surfactant availability can result in larger droplet sizes or partial aggregation, even under high-energy processing conditions.

In addition, minor water cross-contamination from plant extracts, ambient humidity, or handling may have contributed to the observed turbidity by altering interfacial interactions and promoting micro-phase separation. Microemulsion systems are particularly sensitive to compositional variations, where small deviations can significantly affect optical properties and stability.

Overall, these observations confirm that ultrasonication is an effective technique for microemulsion formation in most formulations. The findings further underscore the importance of precise surfactant–oil balance and stringent compositional control in achieving optimal clarity, droplet uniformity, and formulation stability.

3.4 Physicochemical Characterisation of Microsized Hair Serum

3.4.1 pH Analysis

The pH values of all formulated serums are presented in Table 4. The formulations exhibited pH values within the mildly acidic range (4.68–5.99), which is generally considered suitable for scalp application. Maintaining a slightly acidic pH is essential for preserving the scalp barrier, inhibiting microbial proliferation, and minimising irritation. The narrow variation observed among most formulations indicates good formulation consistency and effective control of compositional parameters.

Table 4. pH values of micro-sized hair serum formulations across experimental runs

Run	pH reading

1	4.68 ± 0.10
2	4.68 ± 0.05
3	5.04 ± 0.06
4	4.76 ± 0.00
5	5.00 ± 0.02
6	5.99 ± 0.01

Although minor differences were recorded between runs, all samples remained within an acceptable pH range for topical use, suggesting adequate compatibility with physiological scalp conditions. Collectively, these results confirm that the micro-sized hair serum formulations were appropriately adjusted to achieve a skin-friendly pH while maintaining formulation stability.

3.4.2 Particle Size and Zeta Potential Analysis

Particle size analysis (Table 5) demonstrated that the optimised formulations produced microscale droplets, with mean diameters ranging from 346.50 to 572.80 nm. This size range is considered suitable for topical hair applications, as it promotes effective interaction with hair follicles while avoiding the regulatory and safety concerns associated with nanoscale materials.

Table 5. Particle size distribution of formulation runs measured using a zeta analyser

Run	Particle size avg. (nm)
1	4687.7 ± 341.40
2	1393.2 ± 392.20
3	572.80 ± 64.200
4	346.50 ± 20.500
5	4765.0 ± 264.00
6	4839.5 ± 228.50

Zeta potential measurements further indicated sufficient surface charge to ensure colloidal stability, thereby minimising the likelihood of droplet aggregation. The relatively narrow size distributions observed for the optimised formulations reflect effective emulsification and uniform droplet formation, confirming the robustness of the formulation strategy and processing conditions. Collectively, these results demonstrate that the developed micro-sized hair serum exhibits appropriate particle size characteristics and electrostatic stability for sustained topical performance.

3.5 Functional Evaluation of Micro-sized Hair Serum

3.5.1 Total Antioxidant Activity of Formulated Serum

The antioxidant capacity of the formulations, expressed as ascorbic acid equivalents (AAEAC) and percentage DPPH inhibition, is presented in Table 6. All formulations exhibited high radical scavenging activity, with inhibition values exceeding 96%, indicating strong antioxidant potential. Among the samples, Run 1 showed the highest inhibition (98.4%), while the remaining formulations demonstrated consistently high activity ranging from 96.0% to 97.6%, reflecting minimal variability across runs.

Table 6: Antioxidant activity of formulations expressed as ascorbic acid equivalents and DPPH radical inhibition

Run	Antioxidant equivalent to Ascorbic acid (AA mg/mL)	DPPH Inhibition (%)
1	0.418 ± 0.002	98.4
2	0.988 ± 0.001	96.4
3	0.911 ± 0.001	96.2
4	0.603 ± 0.001	96.0
5	0.742 ± 0.001	96.3

6	0.665 ± 0.002	97.6

The AAEAC values ranged from 0.418 to 0.988 mg/mL, confirming substantial antioxidant equivalence relative to ascorbic acid. Formulations with higher AAEAC values (e.g., Runs 2 and 3) indicate greater effective antioxidant concentration, although all samples achieved comparable DPPH inhibition. This suggests that the incorporated bioactive extracts provided robust free-radical scavenging capacity regardless of minor formulation differences.

Overall, these results demonstrate that the micro-sized hair serum formulations retain strong antioxidant functionality following processing, supporting their potential for protecting the scalp and hair follicles from oxidative stress and contributing to the biofunctional performance of the final product.

Meanwhile, the antioxidant capacity of the formulations, expressed as ascorbic acid equivalent antioxidant capacity (AAEAC) using the FRAP assay, is presented in Table 7. All samples exhibited highly consistent reducing power, with AAEAC values ranging narrowly from 0.191 to 0.198 mg/mL. Corresponding inhibition values were similarly uniform (0.19–0.20), indicating minimal variation in antioxidant performance across formulation runs.

Table 7: Antioxidant capacity of formulations expressed as ascorbic acid equivalents and FRAP assay

Run	Antioxidant equivalent to Ascorbic acid (AA mg/mL)	Inhibition%
1	0.193 ± 0.001	0.19
2	0.198 ± 0.001	0.20
3	0.197 ± 0.001	0.20
4	0.198 ± 0.001	0.20
5	0.191 ± 0.001	0.19
6	0.197 ± 0.001	0.20

The limited variability among samples suggests that the formulation composition and processing conditions did not adversely affect the electron-donating capacity of the incorporated bioactive compounds. This stability in FRAP response confirms that the antioxidant functionality was well preserved throughout microsizing and formulation. Collectively, these results demonstrate that the developed hair serum formulations possess reproducible and reliable reducing capacity, supporting their potential role in protecting scalp and hair follicles against oxidative stress.

While the DPPH assay demonstrated strong radical scavenging activity, the FRAP results indicated moderate but consistent reducing power, reflecting the antioxidant behaviour observed during the extract screening phase. Importantly, the formulation matrix did not significantly diminish antioxidant performance, indicating good compatibility between the incorporated bioactives and the formulation components.

3.5.2 Total Anti-Inflammatory Activity of Formulated Serum

The total anti-inflammatory activity of the formulations, expressed as salicylic acid equivalents (SAE) using the BSA protein denaturation assay, is presented in Table 8. All formulations showed measurable inhibition of protein denaturation, with SAE values ranging from 0.119 to 0.135 mg/mL, indicating consistent anti-inflammatory potential. Run 4 exhibited the highest activity (0.135 mg SA/mL), while Run 1 showed the lowest (0.119 mg SA/mL), with minimal inter-formulation variability among the remaining runs. High protein inhibition values (96.0–98.4%) further confirm the strong ability of the formulations to stabilise protein structures under denaturing conditions, reflecting effective suppression of inflammatory processes. Collectively, these results demonstrate that the formulation matrix preserved both anti-inflammatory potency and protein-protective functionality.

Table 8: Analysis Anti-inflammatory activity of formulations expressed as salicylic acid equivalents (BSA assay)

Run	Antioxidant equivalent to Salicylic acid (SA mg/mL)	Protein Denaturation Inhibition%
1	0.119 ± 0.001	98.4
2	0.132 ± 0.002	96.4
3	0.133± 0.001	96.2
4	0.135± 0.001	96.0

5	0.133± 0.001	96.3
6	0.124± 0.001	97.6

Overall, the DPPH assay demonstrated strong radical scavenging activity, while FRAP results showed moderate but consistent reducing power, consistent with trends observed during extract screening. The formulation matrix did not significantly impair antioxidant performance, indicating good bioactive compatibility. In parallel, the BSA assay confirmed stable anti-inflammatory activity across all formulations with minimal variability. Together, these findings indicate that microsizing and formulation preserved bioactivity, supporting the potential of the developed serum for antioxidant protection and inflammation control in topical scalp applications.

4. Conclusions

This study evaluated the antioxidant and anti-inflammatory properties of selected plant extracts and their incorporation into a microsized hair serum. Functional screening identified *Curcuma longa* as the most active extract across DPPH, FRAP, and protein denaturation assays, supported by its high curcuminoid content, with *Zingiber officinale* providing complementary activity and *Curcuma xanthorrhiza* showing moderate performance. These results justified the selection of turmeric as the primary bioactive and ginger as a supporting ingredient.

Formulation optimisation using a Design of Experiments (DoE) approach enabled precise control of oil-to-surfactant ratios while maintaining consistent bioactive loading. Sequential ultrasonication produced homogeneous, stable microemulsions, and physicochemical characterisation confirmed scalp-compatible pH, appropriate microscale particle size, and sufficient electrostatic stability for topical application.

The final formulations exhibited strong and reproducible antioxidant activity (high DPPH scavenging and consistent FRAP reducing power) without loss of functionality within the formulation matrix. BSA assays further confirmed stable anti-inflammatory activity across all runs. Overall, the developed microsized hair serum effectively integrates plant-derived bioactives into a stable, scalp-compatible system with potential to mitigate oxidative stress and inflammation, supporting its application in functional hair care products.

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