QUANTITATIVE FLAVONOIDS AND ANTIOXIDANT PROFILES BY DPPH ASSAY OF *CENTELLA ASIATICA* SERUM COSMETIC PRODUCTS VERSUS ETHANOL EXTRACT

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Abstract

The cosmetic industry is currently leaning toward herbal skincare products due to strong consumer preference for natural ingredients. *Centella asiatica* (CA) is frequently used as it contains flavonoids and centelloids known for their beneficial antioxidant properties. This study aimed to assess the efficacy of CA extract when incorporated into cosmetic formulations by comparing the total flavonoid content (TFC) and antioxidant activity of five commercial serums (A-E) against a pure CA ethanol extract. The experimental study was conducted *in vitro,* utilizing the CA extract, vitamin C (as a positive control), and the five commercial serum products. TFC was determined quantitatively using UV-Visible spectrophotometry and quercetin standard. Antioxidant activity was measured using the DPPH assay to calculate the IC50 value. Pure CA extract showed a mean TFC of 37.10 ± 0.21 mg QE/g, with serum samples showing high variability; the highest being Sample C with a TFC of 216.6 (99.89 - 246.1) mg QE/g and the lowest being sample E with a TFC of 24.75 ± 1.88 mg QE/g. Crucially, the pure CA extract demonstrated strong antioxidant activity with a mean IC50 of 52.45 ± 4.96 ppm. In contrast, all serum products exhibited significantly weaker antioxidant activities. Samples C and D resulted in extremely high IC50 values of 244100 ± 116700 ppm and 319300 ± 95700 ppm respectively, categorized as lower than weak, while samples A, B, and E were unable to reach 50% inhibition, indicating an antioxidant capacity below the measurable range. In conclusion, despite the pure *Centella asiatica* ethanol extract showing robust antioxidant activity, the commercial cosmetic serums demonstrated a significantly lower range of activity and highly inconsistent TFC, suggesting that the raw extract's potency does not reliably transfer to the final product formulation.

Keywords: *Centella asiatica*, Serum, Cosmetic, Flavonoids, Antioxidant, DPPH.

Introduction

The global cosmetic industry is trending towards herbal skincare products, driven by strong consumer demand for natural ingredients. Among these, CA has gained popularity due to its active compounds, particularly flavonoids and centelloids (e.g., asiaticoside,

madecassoside). These substances have been found to exhibit significant antioxidant, anti-inflammatory, and anti-aging properties, which have led to the frequent use of CA extracts in high-concentration skincare preparations, such as facial serums.

CA is valued in dermatology and cosmetics for its active triterpenoid saponins and flavonoids, which contribute to its wound-healing, anti-inflammatory, and antioxidant properties.[1,2] The cosmetic industry has seen a surge in herbal and natural skincare products, with CA extracts frequently incorporated into high-concentration preparations like serums to address issues such as hydration and anti-aging.[3, 4]

Flavonoids are pharmacologically important secondary metabolites, and the quantitative determination of TFC serves as a standard measure of a plant's overall bioactive potential.[5,6] To assess antioxidant capacity, the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is utilized. This is a widely recognized, straightforward colorimetric method that quantifies a sample's ability to scavenge the stable DPPH radical, where results are reported as the half-maximal Inhibitory Concentration (IC50).[7, 8]

Despite the documented potency of the raw extract, commercial serums contain multiple components alongside CA in their formulations. The efficacy claims of these complex products are undermined by the inherent challenges of formulation, including ingredient interaction, chemical stability, and substantial dilution of the active extract. This raises a critical question: does the strong antioxidant activity of the crude CA extract translate effectively to the formulated serum product? This study was designed to directly address the influence of commercial formulation on the retained efficacy of CA.

This *in vitro* experimental study quantitatively analyzed five commercially available CA serum products against a crude CA ethanol extract used as a pure comparator. The primary aims were to measure TFC and determine the antioxidant activity using the DPPH assay.

Materials and Methods

This study utilized an *in vitro* experimental design involving five commercially available facial serum products (A–E) that listed CA extract in their ingredients, alongside a laboratory-prepared pure CA ethanol extract (CA) as a comparative control and vitamin C as a positive control. The CA ethanol extract was prepared by macerating 150 g of dried leaves in 150 ml of 75% ethanol. Commercial serum samples were prepared by diluting them based on their primary solvent, either water or 75% ethanol, to ensure consistency during testing. Determination of TFC was performed using UV-Visible spectrophotometry at a maximum wavelength of 376 nm, following the aluminum chloride colorimetric method with quercetin as the standard reference compound. The antioxidant activity was assessed using the DPPH radical scavenging assay, where samples were reacted with DPPH solution in a 1:1 ratio and incubated in the dark for 30 minutes before measuring absorbance at 515 nm. The IC50 values were calculated through regression analysis of the relationship between concentration and percent inhibition. All experimental measurements were performed in triplicate (n=3) on separate days. Data normality was verified using the Shapiro-Wilk test, and statistical differences between the groups were evaluated using one-way ANOVA followed by Tukey's multiple comparisons test, with significance set at $p < 0.05$.

Results

CA contains flavonoid and has antioxidant activity**. Table 1** presents the differences in the TFC between the extract and the commercial products. Sample E showed significant lower TFC than the extract. Furthermore, the antioxidant capacity of the CA extract and the commercial serum products is summarized in **Table 2.** The antioxidant activity of CA extract was strong (40-100 ppm) while Samples C and D exhibited negligible antioxidant activity. It is important to note that the IC50 for Samples A and B were not measureable as even at 100% concentration they failed to achieve 50% inhibition, while Sample E showed inconsistent results.

Table 1. Results of Flavonoid Analysis

Sample (Code)	TFC (Mean ± SD)	P Value
CA	37.10 ± 0.21 mg QE/g	
A	35.78 ± 7.57 mg QE/g	
B	38.08 ± 3.59 mg QE/g	0.0062
D	28.79 ± 0.40 mg QE/g	
E	24.75 ± 1.88 mg QE/g	

Samples A-E is the label of serum cosmetic products. CA is Centella asiatica ethanol extract.

Table 2. Antioxidant Properties Measured with IC_{50}

Sample	IC50 (Mean ± SD) in PPM
Vitamin C	6.74 ± 0.39
CA	52.45 ±4.96
C	244100 ± 116700
D	319300 ± 95700

Discussion

Flavonoid content analysis and DPPH assay were conducted to evaluate the retained efficacy of CA extract when incorporated into commercial serum formulations. The pure CA extract established a baseline TFC of 37.10 ± 0.21 mg QE/g. Serum Samples A, B, and D showed TFC values (ranging from 28.79 ± 0.40 to 38.08 ± 3.59 mg QE/g) that were not statistically different from the pure extract, while Sample E 24.75 ± 1.88 mg QE/g) was significantly lower ($p = 0.0185$), directly reflecting substantial dilution in its aqueous base. Interestingly, Serum Sample B showed a TFC higher than the pure extract, likely due to interference from other reducing agents like niacinamide in the spectrophotometric assay.[9] A significant anomaly was observed with Sample C (median TFC 216.6 mg QE/g), which is almost certainly due to the inclusion of additional highly flavonoid-rich botanicals (*Peumus boldus* and dipotassium glycyrrhizate) in the formulation.[10, 11]

The antioxidant activity further clarified the impact of formulation. The pure CA extract demonstrated strong antioxidant capacity (52.45 ±4.96 ppm), confirming its known potential.[12] However, the majority of the commercial serums exhibited a profound loss of potency. Products A, B, and E failed to reach 50% inhibition (IC50) at the maximum tested concentration, indicating an inactive or very low antioxidant capacity likely due to dilution, degradation, or low solubility of the active compounds.[13] Only Samples C (244100 ± 116700 ppm) and D (319300 ± 95700 ppm) exhibited measurable activity, yet this still represented a significantly lower potency than the pure extract. This difference highlights that the strong activity of the raw extract does not reliably survive the formulation process. The superior antioxidant performance of Sample C versus Sample D suggests a powerful synergistic effect from its combined botanicals, especially *Peumus boldus* and the licorice derivative.[14] Despite Sample D containing a greater variety of extracts, its lower efficacy demonstrates that the quantity of varied ingredients does not guarantee greater potency if individual concentrations are too low.[15]

Overall, the results confirm that the strong antioxidant potency of the raw CA does not reliably translate to the finished commercial serum product due to substantial dilution and complex formulation factors. The formulation processes significantly impact the biological activity of active ingredients. Some of the samples showed very low or inactive antioxidant activity *in vitro*; therefore, further investigation is needed to determine if any residual acitivity can effectively penetrate the skin barrier to validate its skin benefits. Another study on delivery systems to protect CA from the degradation and dilution issues could also be a promising direction for optimization.

Conclusions

The TFC in the serum products was highly variable compared to the ethanol extract; most serums showed lower TFC due to dilution during formulation, while one sample exhibited a significantly higher content, likely from the inclusion of other flavonoid-rich ingredients. Crucially, while the pure CA ethanol extract showed strong antioxidant activity, all cosmetic serum products displayed a significantly lower range of activity, categorized as lower than weak. This suggests that the strong antioxidant potency of the raw extract is

not maintained in the final formulated products due to substantial dilution or interference from other formulation components.

Competing Interests

The authors declare no conflicts of interest to disclose regarding this study.

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