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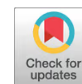

Comparison of Antioxidant Activity of Cream Preparations Combining Avocado and Green Tea Extracts Using DPPH, FRAP, and ABTS Methods

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Abstract: Oxidative stress caused by reactive oxygen species (ROS) plays a major role in skin aging and UV-induced damage. Natural plant-based antioxidants, such as those found in avocado (Persea americana) and green tea (Camellia sinensis), are increasingly studied for incorporation into topical cosmetic formulations. However, their combined use and comparative antioxidant evaluation in cream matrices remain insufficiently explored. Four cream formulations (F1–F4) with increasing concentrations of avocado extract (EDA) and green tea extract (EDT) were prepared using an oil-in-water emulsification method. Antioxidant activity was evaluated spectrophotometrically using three assays: DPPH (radical scavenging), FRAP (ferric reducing power), and ABTS (total antioxidant capacity). In the DPPH assay, formulation F2 showed the lowest IC50 value (336.34 ± 13.34 µg/mL), indicating the highest radical scavenging activity, while DPPH results did not follow a consistent concentration-dependent trend. In contrast, FRAP and ABTS assays demonstrated a clear dose-response relationship, with F4 showing the highest antioxidant capacity (522.08 ± 14.08 µmol Fe^{2+}/g and 599.64 ± 8.94 mM TE/g, respectively). The combination of avocado and green tea extracts contributes to measurable antioxidant activity in cream formulations. These preliminary findings support multi-assay evaluation as a more comprehensive approach to antioxidant characterization. Further studies including statistical analysis, single-extract controls, and stability evaluation are necessary to confirm interaction effects and optimize formulation performance.

Introduction

The skin constitutes the primary barrier between the human body and the external environment, defending against physical, chemical, and biological stressors, including ultraviolet (UV) radiation (1). Prolonged or excessive UV exposure stimulates the overproduction of reactive oxygen species (ROS), leading to oxidative stress, which has been implicated in premature skin aging, collagen degradation, hyperpigmentation, and increased risk of skin cancer (2). To counteract these effects, antioxidant compounds are frequently incorporated into cosmetic formulations to neutralize free radicals and reduce oxidative damage (3). Natural plant-derived antioxidants have gained considerable attention over the past decade owing to their favorable safety profiles, biodegradability, and broad spectrum of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (4). Among the most extensively studied plant sources are avocado *(Persea americana)* and green tea *(Camellia sinensis)*. Avocado contains vitamin E (tocopherol), phytosterols, and monounsaturated fatty acids, which contribute to its antioxidant and emollient properties (5, 7). Green tea is rich in polyphenolic catechins, particularly epigallocatechin-3-gallate (EGCG), which is recognized as one of the most potent naturally occurring antioxidants (6, 8). While the individual antioxidant properties of avocado and green tea extracts are well-documented, studies evaluating their combined use in topical cream formulations remain limited. Previous investigations have largely examined single-extract preparations, with few studies exploring potential synergistic or additive interactions between the two extracts in a complex formulated matrix. Furthermore, the comparative evaluation of antioxidant capacity using multiple analytical methods within a single cream system has not been adequately explored. Antioxidant capacity can be assessed through multiple complementary assays

that differ in their mechanistic basis. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay measures hydrogen atom or electron transfer-based radical scavenging activity (9). The FRAP (Ferric Reducing Antioxidant Power) assay evaluates the capacity of compounds to reduce Fe^{3+} to Fe^{2+} under acidic conditions, reflecting electron-donating ability (10). The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) method measures the ability to quench ABTS radical cations and is applicable in both hydrophilic and lipophilic systems (11). Using multiple assays is essential for a comprehensive characterization of antioxidant potential, as different compounds may respond differently across methods.

This study aims to compare the antioxidant activity of cream formulations containing varying concentrations of avocado and green tea extracts using DPPH, FRAP, and ABTS assays. Additionally, the study evaluates how extract concentration influences antioxidant activity across different mechanistic pathways, providing preliminary data to support the rational design of antioxidant-based cosmetic formulations.

Methodology

Materials

The materials used include: fresh avocado fruit flesh, green tea leaves, 95% ethanol solvent (for maceration), CO_2 and methanol (for supercritical extraction), DPPH solution, L-DOPA substrate, tyrosinase enzyme, vitamin C, quercetin (positive control), and additional cream formulation ingredients such as Carbopol Aqua SF-1, Promulgen D, SP Arlacel 170, phenoxyethanol, propylene glycol, and triethanolamine. The equipment used includes: a supercritical extraction unit SFT-250, a Heidolph rotary evaporator, a UV-Vis spectrophotometer, a Brookfield viscometer, a pH meter, an oven, a microscope, and other cream-making tools (12).

Preparation of Plant Extracts

Avocado Simplisia and Extraction

Fresh avocado fruit flesh was mechanically crushed and subsequently dried using a freeze-drying lyophilization process at -46 °C for 12 h in order to preserve inherent, heat-sensitive bioactive compounds. The resulting dried material was then sieved to obtain a uniform particle size distribution prior to the extraction process. Extraction was performed using a Supercritical Fluid Extraction system (SFT-250) at the LAPTIAB-BRIN Laboratory under the following operational conditions: vessel temperature 50 °C, oven temperature 55 °C, CO_2 flow rate 6 L/min, and methanol co-solvent 1 mL/min.

Green Tea Simplisia and Extraction

Green tea leaves were washed, chopped, and dried by freeze-drying at -46 °C for 12 h. The dried material was sieved through an 80-mesh screen. Extraction was performed by maceration using 500 g of tea powder in 5 L of 95% ethanol (1: 10 ratio) for 24 h, comprising 6 h of stirring followed by 18 h of standing. The filtrate was evaporated using a rotary evaporator at 40 °C, 7 mBar for 1.5 h, yielding a concentrated extract with a yield of 32.7%.

Cream Formulation

Four cream formulations (F1–F4) were prepared with increasing concentrations of EDA and EDT, along with a negative control (F0) containing no extract. The composition of each formulation is presented in **Table 1**.

Cream formulations were prepared using an oil-in-water (O/W) emulsification method (24). The oil phase and aqueous phase were heated separately to approximately 70 ± 2 °C to ensure complete melting and homogenization of components (25). The oil phase was gradually added to the aqueous phase under continuous stirring using a homogenizer for approximately 10 min to form a stable emulsion (24). The mixture was then cooled to room temperature under gentle stirring to obtain a homogeneous cream. The extracts were incorporated during the cooling phase to minimize degradation of thermolabile compounds (25). The final formulations were stored in closed containers at room temperature prior to analysis.

Antioxidant Activity Assay

DPPH Method

The DPPH assay was performed to measure radical scavenging activity. A stock solution of 1000 µg/mL was prepared by dissolving 4 mg of each formulation in methanol. Serial dilutions were prepared to final concentrations of 10, 25, 50, and 100 µg/mL. Each concentration (1 mL) was mixed with 2 mL of methanol and 0.5 mL of 1 mM DPPH solution. After 30 min of incubation at room temperature in the dark, absorbance was measured at 517 nm using a Thermo Scientific Multiskan Sky spectrophotometer. The % inhibition was calculated as:

The IC_{50} value was determined from the concentration vs. scavenging graph using CompuSyn software. Quercetin was used as a positive control. All assays were performed in duplicate.

FRAP Method

The FRAP reagent was freshly prepared by mixing acetate buffer (pH 3.6), TPTZ solution in HCl, and $FeCl_3 \cdot 6H_2O$

Table 1. Composition of the cream formulations.

Formula	Avocado Extract (%)	Green Tea Extract (%)
F1	0.10	1.20
F2	0.25	3.01
F3	0.50	6.25
F4	1.00	12.04
F0	0	0

Table 2. IC_{50} DPPH measurement results.

Sample	IC_{50} (µg/mL)
F0 (Negative control)	Not determined
F1	592.73 ± 1.53
F2	336.34 ± 13.34
F3	872.19 ± 12.60
F4	483.69 ± 0.55

solution in a ratio of 10: 1: 1 (v/v/v). Each sample was mixed with the FRAP reagent and incubated at 37 °C for 30 min. The absorbance of the resulting dark-blue Fe^{2+}-TPTZ complex was measured at 593 nm. Antioxidant capacity was quantified from a $FeSO_4 \cdot 7H_2O$ standard calibration curve and expressed in µmol Fe^{2+} equivalent per g of sample. All measurements were performed in duplicate.

ABTS Method

The ABTS radical cation ($ABTS^{+}$•) was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and storing in the dark for 12–16 h. The working solution was diluted with methanol to an absorbance of 0.700 ± 0.020 at 734 nm. Each sample was added to the $ABTS^{+}$• working solution and incubated for 6 min at room temperature. Absorbance was measured at 734 nm, and inhibition % was calculated from the decrease in absorbance relative to the control. A Trolox calibration curve (0–37.5 µM) was used to convert inhibition values into Trolox equivalents, expressed as mM Trolox Equivalent (TE) per g of sample. All measurements were performed in duplicate.

Experimental Design and Statistical Considerations

All measurements were performed in duplicate and expressed as mean ± standard deviation (SD). Due to the limited sample availability in this preliminary study, formal statistical significance testing (e. g., ANOVA) was not conducted, which is acknowledged as a key limitation. No single-extract control formulations (EDA alone or EDT alone) were included in this study; therefore, interaction effects between the two extracts could not be quantitatively evaluated. These limitations should be addressed in future work.

Results

This study compared the antioxidant activity of cream formulations containing combinations of avocado extract (EDA) and green tea extract (EDT) at four concentration levels using DPPH, FRAP, and ABTS assays. The results from the three methods are presented below.

DPPH Method

Antioxidant activity was spectrophotometrically measured using the stable DPPH free radical scavenging method. The calculated IC_{50} value, which is formally defined as the extract concentration required to scavenge 50% of the DPPH radicals, was used as the primary parameter for evaluation. In this assay, a lower IC_{50} value indicates a significantly greater and more efficient radical scavenging capacity. The analytical testing was conducted at the Traditional Medicine Raw Material Standardization Laboratory – BRIN. Results are presented in **Table 2**.

Formulation F2 exhibited the lowest IC_{50} value (336.34 ± 13.34 µg/mL), indicating the highest radical scavenging activity among the tested formulations. F3 showed the highest IC_{50} (872.19 ± 12.60 µg/mL), suggesting comparatively weaker scavenging activity despite containing a higher extract concentration. F4 showed moderate activity (483.69 ± 0.55 µg/mL), and F1 exhibited the highest IC_{50} among formulations with extract (592.73 ± 1.53 µg/mL). The IC_{50} of F0 was not determined as the negative control showed negligible radical scavenging, confirming that antioxidant activity is attributable to the presence of plant extracts. Notably, DPPH results did not follow a consistent concentration-dependent trend across F1 to F4.

FRAP Method

Antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) method, which measures the ability of sample components to reduce Fe^{3+} to Fe^{2+} under acidic conditions. The FRAP standard calibration curve showed a highly linear relationship ($y = 0.0095x - 0.0357$, R2 = 0.9997), confirming assay precision. Measurements were conducted at the Traditional Medicine Raw Materials Laboratory – BRIN. Results are presented in **Table 3**.

A consistent and progressive increase in FRAP values was observed from F0 to F4. The negative control (F0) showed minimal reducing activity (0.71 ± 0.04 µmol Fe^{2+}/g), confirming that the response is attributable to the plant extracts. Formulation F1 showed the lowest extract-containing activity (77.33 ± 2.19 µmol Fe^{2+}/g), followed by F2 (193.81 ± 4.01 µmol Fe^{2+}/g), F3 (322.68 ± 14.56 µmol Fe^{2+}/g), and F4, which demonstrated the highest reducing capacity (522.08 ± 14.08 µmol Fe^{2+}/g). These results indicate a clear concentration-dependent relationship between extract content and ferric-reducing antioxidant power.

Table 3. Antioxidant activity results from FRAP assay.

Cream Formula	FRAP 1	FRAP 2	Average of FRAP (mMol Fe^{2+}/g)
F0 (Negative control)	0.68	0.74	0.71 ± 0.04
F1	79.52	75.14	77.33 ± 2.19
F2	196.82	190.80	193.81 ± 4.01
F3	308.12	337.23	322.68 ± 14.56
F4	507.08	537.07	522.08 ± 14.08

Table 4. ABTS antioxidant activity test results.

Sample	ABTS (mM TE/g sample)
F0 (Negative control)	11.19 ± 4.34
F1	62.19 ± 7.43
F2	146.44 ± 5.17
F3	312.37 ± 7.40
F4	599.64 ± 8.94

ABTS Method

Antioxidant activity was measured using the ABTS radical cation scavenging assay. The Trolox standard calibration curve demonstrated excellent linearity (y = 2.5595x + 0.7999, R2 = 0.9995), allowing accurate conversion of inhibition values to Trolox equivalents. Results are presented in **Table 4**.

ABTS values increased progressively and consistently from F0 to F4. Formulation F4 demonstrated the highest antioxidant activity (599.64 ± 8.94 mM TE/g), more than 50-fold higher than the negative control (11.19 ± 4.34 mM TE/g). This linear dose-response pattern suggests a strong and stable relationship between extract concentration and total antioxidant capacity as measured by ABTS, attributable to the polyphenolic compounds in green tea extract and the flavonoids and tocopherols present in avocado extract.

Discussion

Comparative Analysis of Antioxidant Assay Methods

The three assays employed in this study – DPPH, FRAP, and ABTS – operate through distinct mechanistic principles, and the divergence in their results reflects fundamental differences in how they measure antioxidant capacity. This comparative perspective is essential for interpreting the antioxidant profile of complex cream formulations.

The DPPH assay measures the ability of compounds to donate hydrogen atoms or electrons to the stable DPPH radical (10). Its results are sensitive to the solubility and polarity of the tested compounds in the methanol medium, and the reaction kinetics can vary between fast- and slow-reacting antioxidants (22). In this study, the non-linear IC50 trend observed across F1 to F4 suggests that increasing extract concentration may introduce competing interactions among bioactive constituents or matrix effects that attenuate radical scavenging efficiency at higher doses. Such phenomena, including pro-oxidant behavior at high concentrations and compound-compound interactions within complex matrices, have been reported in previous studies (13). It is important to note that these non-linear results should be interpreted cautiously in the absence of formal statistical analysis and single-extract controls.

The FRAP assay measures the total electron-donating capacity of compounds to reduce Fe^{3+} to Fe^{2+} (15). Its linear concentration-dependent response in this study confirms that the polyphenolic compounds in green tea (e. g. , EGCG and other catechins) and the vitamin E and flavonoid content of avocado extract cumulatively contribute to reducing power in a predictable, additive manner (19). The high R^2 value of the FRAP calibration curve (0.9997) supports the reliability of these measurements. However, FRAP does not distinguish between chain-breaking and preventive antioxidants and may overestimate activity for some compound classes (14).

The ABTS assay is considered one of the most comprehensive methods because $ABTS^{+}$• radicals are compatible with both hydrophilic and lipophilic antioxidant systems, enabling the detection of a broader range of bioactive compounds (11). The strong linear dose-response observed in ABTS results aligns with the FRAP trend and reinforces the conclusion that increasing extract concentrations meaningfully enhance total antioxidant capacity. The excellent calibration linearity (R^2 = 0.9995) further supports the robustness of these measurements.

Collectively, the three methods complement each other: DPPH provides insights into specific radical scavenging kinetics, FRAP quantifies total reducing capacity, and ABTS offers a broader assessment of antioxidant activity across both aqueous and lipid phases. Using a multi-method approach, as performed in this study, is strongly recommended for comprehensive antioxidant characterization, particularly in complex heterogeneous formulations such as cosmetic creams (9).

Implications for Cosmetic Development

The positive FRAP and ABTS results across all formulations suggest that both avocado and green tea extracts contribute meaningfully to the antioxidant capacity of cream preparations, with higher extract concentrations providing greater protective potential against oxidative stress. These findings are consistent with the established antioxidant properties of catechins from green tea (6, 8) and the tocopherol and flavonoid content of avocado (5, 7).

However, the non-linear DPPH results indicate that the relationship between extract concentration and radical scavenging activity is more complex than a simple dose-response, potentially involving compound interactions within the cream matrix. These preliminary results should be interpreted cautiously, as the absence of statistical testing and single-extract controls prevents firm conclusions about synergistic or antagonistic effects between avocado and green tea extracts.

From a formulation development perspective, these data provide a useful preliminary basis for selecting extract concentration ranges to be evaluated in future, more rigorously designed studies. Importantly, the inclusion of single-extract control formulations in future work is essential to quantify the individual contribution of each extract and to determine whether their combination produces additive, synergistic, or antagonistic antioxidant effects.

Study Limitation

This study has several important limitations that affect the interpretation and generalizability of the findings. First, all measurements were performed in duplicate only, without formal statistical analysis (e. g., ANOVA with *post-hoc* testing), which limits the ability to draw statistically supported conclusions. The absence of single-extract control formulations (EDA alone and EDT alone) prevents any quantitative evaluation of extract interaction effects.

Third, no physicochemical characterization or stability evaluation of the cream formulations was conducted. Fourth, the DPPH measurements lacked clear trends that may also reflect matrix interference from the cream base. These limitations should be systematically addressed in future studies.

Conclusion

This preliminary study demonstrates that cream formulations containing combinations of avocado *(Persea americana)* and green tea *(Camellia sinensis)* extracts exhibit measurable antioxidant activity as assessed by DPPH, FRAP, and ABTS assays. FRAP and ABTS results showed consistent, concentration-dependent increases in antioxidant capacity, with formulation F4 demonstrating the highest reducing power and total antioxidant activity. In contrast, DPPH results did not follow a linear concentration-dependent trend, highlighting the complexity of radical scavenging behavior in multi-component cream matrices. These findings underscore the importance of employing multiple complementary analytical methods for comprehensive antioxidant characterization of natural cosmetic formulations. Given the preliminary nature of these data, in the absence of statistical analysis, single-extract controls, and stability testing, the results should be regarded as indicative rather than conclusive. Further studies with adequate statistical power, proper experimental controls, and stability assessments are required to confirm these findings and to support the rational development of antioxidant-based cosmetic products.

Declaration

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

Not applicable.

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