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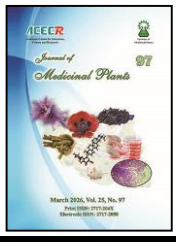

Research Article

Design, formulation, and content determination of an anti-aging herbal eye cream enriched with green tea (*Camellia sinensis* (L.) kuntze) extract

Shima Hashemi[1], Soliman Mohammadi Samani[2], Ardalan Pasdaran[3,4], Mohammad Mehdi Zarshenas[4,5,*]

[1] *Department of Pharmacognosy, Faculty of Pharmacy, Ramsar Campus, Mazandaran University of Medical Sciences, Ramsar, Iran*
[2] *Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*
[3] *Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*
[4] *Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[5] *Department of Phytopharmaceuticals (Traditional Pharmacy), School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*

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ABSTRACT

Background: The use of herbs and natural compounds in cosmetics is one of the most important issues for the treatment of skin disorders. In most cultures and societies, plants are traditionally used to treat skin diseases. Green tea or *Camellia sinensis* (L.) Kuntze, which belongs to the Theaceae family is a medeicinal herb which can be very practical in cosmetic formulations. **Objective:** In this study, hydroalcoholic green tea extract (2.5 %) has been applied for the preparation of herbal eye cream and then pharmaceutical and phytochemical properties of the product were evaluated. **Methods:** High-Performance Thin Layer Chromatography (HPTLC) and also spectrophotometric procedures were used for quantification of Epigallocatechin (as the main component in the extract), total phenol and flavonoid contents, respectively. On the other side, pharmaceutical evaluation tests including color and odor test, emulsion stability test, pH measurement, rheology and the total microbial count were investigated. **Results:** Epigallocatechin gallate, total phenolic compounds, and total flavonoid amounts of the final product have been calculated to be 13.02 ± 0.05, 198.35 ± 2.79, and 19.56 ± 0.76 mg / 30 g product respectively. After 6 months no changes in color, stability and amount of ingredients were observed. Regarding the microbial limit test, no signs of pathogen presence were observed. **Conclusion:** Based on the results of current investigation, this herbal formulation, with evaluated parameters and controlled items, could be considered as a perfect candidate for clinical evaluation in the field of periorbital cosmetics and anti-aging herbal eye cream.

Abbreviations: HPTLC, High-performance Thin Layer Chromatography; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; O/W, Oil in water; TLC, Thin Layer Chromatography; UV, Ultraviolet; ATS4, automatic TLC sample 4; ADC2, automatic developing chamber; FCR, Folin–Ciocalteu reagent; RSD, Relative Standard Deviation

*Corresponding author: zarm@sums.ac.ir

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1. Introduction

Periorbital skin plays a pivotal part in aesthetic dermatology. The anatomy of this region is extraordinary and unique, and drug delivery to this area is challenging and demands special considerations [1]. The skin around the eyes is susceptible, and due to the specific anatomy, it is vulnerable to the aging process and injury. The dermis and epidermal layers of this area are thin, and the hypodermis layer is more superficial than that in other parts of the body. As a result, the skin of this area is prone to various kinds of problems such as darkness, eyelid edema, wrinkles, skin elasticity loss, dryness of the skin, and etc [2, 3]. Periorbital aging is caused by different endogenous factors such as: genetic, age, excessive vascularity, vitamin K deficiency, facial anatomy, thin skin, and infraorbital swelling. In addition, exogenous factors such as sun exposure, allergic factors, fatigue, and hormonal therapy also play an essential role. Occasionally, particular medical complications are the leading causes contributing to aging of this area, such as cardiovascular disorders, vitamin K deficiency, or some circulation conditions that cause excess fluid retention. [4]. As a result, periorbital aging is a frequent problem and many ways of treatment are designed to address it such as: sunscreen creams, makeup tools, and other invasive procedures like injections and surgery [5].

Herbal cosmetics are products with one or more herbal ingredients, and there is no compulsion for all of the ingredients of the product to be herbal [6, 7]. Green tea (*C. sinensis*) from Family: Theaceae is widely used in medicine and cosmetics. Green tea contains several bioactive compounds and between them polyphenols are the most important group which are free radical scavengers, and have protective properties for skin cells [8 - 10]. Green tea extract is extremely practical in the cosmetic industry and especially periorbital cosmetic owing to its multiple properties such as anti-inflammatory and antioxidant properties and are usually formulated as anti-aging products [11, 12]. Green tea extract contains catechin derivatives (a subset of polyphenols), predominantly epicatechin, epigallocatechin (EGC), epicatequinagalate, and epigallocatechin-3-gallate (EGCG), which have anti-oxidant properties, and are effective in treating inflammatory problems, skin darkness, aging, and cancers and most of the healing properties of green tea are due to the presence of these ingredients. [13 - 15]. Furthermore, green tea extract has other ingredients which are effective in skin features, such as vitamins B, C, and E that are nutritious and lightening agents. In addition, caffeine in green tea extract serves as a blood flow accelerator. Besides, proteins, amino acids, and lipids are nourishing and protective agents. Overall, green tea extract is used in cosmetic products owing to its anti-oxidant properties [16]. Although green tea-containing eye creams are commercially available, most lack comprehensive evaluation of active compounds and pharmaceutical properties. This study provides a standardized formulation with quantified Epigallocatechin, total phenol, and flavonoid contents, along with assessment of stability, rheology, and microbial safety, providing a reproducible basis for future clinical studies.

Regarding the various effectiveness and potential activities of green tea extract in eyelid skin problems and periorbital aging, this study was performed to design, formulate, and standardize a herbal eye cream from hydroalcoholic green tea extract to introduce a herbal anti-aging product.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents including: gallic acid, cetomacrogol, liquid paraffin, cetostearyl alcohol, butylated hydroxytoluene (BHT), glycerin, methylparaben, propylparaben, chloroform, acetone, and formic acid were purchased as the pharmaceutical grade from Merck (Germany). Epigallocatechin (EGC, 989-51-5) was purchased from Sigma-Aldrich (USA).and Silica gel 60F-254 plates (10×10 cm) were used for TLC/HPTLC analyses purchased from Merck, Germany.

Instruments: spectrophotometer (T80 plus, PG Instrument, UK), UV-visible spectroscopy (T80 plus, PG Instrument, UK). HPTLC system instruments (CAMAG, Switzerland).

2.2. Preparation of the extract

Fresh green tea leaves were collected in Spring from Lahijan (North of Iran), specified with a voucher number (PM1324). The leaves were air-dried in the shade, powdered and subsequently subjected to hydroalcoholic extraction using an ultrasonic bath (30°C for 15 minutes). The extract was later concentrated by speed vacuum concentrator, followed by drying procedure through a freeze dryer for 48 hours. The final dried extract was kept at 4°C for further steps.

2.3. Formulation of the cream, Physical and pharmaceutical evaluation

Herein, out of various proportions of the ingredients, the selected O/W emulsion was prepared by mixing the oily and aqueous phases at 75 °C ± 1 °C (600 rpm by the mechanical mixer for about 15 minutes). Oily phase contained Cetomacrogol (4 %), Liquid paraffin (15 %), cetostearyl alcohol (10 %), Butylene hydroxytoluene (0.145 %). The aqueous phase consisted of glycerin (5 %), methyl-paraben (0.2 %), propyl-paraben (0.05 %), and water (q.s). The green tea extract powder (2.5 %) was dissolved in the aqueous phase during the cream preparation. Later, the emulsion was cooled to room temperature. The cream was analyzed both organoleptically and physically. The pH value was determined by a digital pH-Meter in three intervals, on the production day, three months later, and lastly 6 months after the production date. Stability test were also conducted at different conditions for the cream to note the effect of these conditions on the storage of the emulsion. Concerning the stability test, 15 g of the cream was divided into three closed tubes in different situations. These tests were carried out on samples, kept at 4 ± 0.1 °C in the refrigerator, 25 ± 0.10 °C in room temperature, and 40 ± 0.1 °C in an incubator. The selected formulation was subjected to the rheology assessment test. A Brookfield cone and plate viscometer was utilized to determine the rheology of the cream. The cone used in the Brookfield cone-and-plate viscometer was specified as CPE-40 (4 cm diameter, cone angle 1°). This size is standard for semi-solid formulations and provides accurate measurement of viscosity across the selected shear rate range [17]. Finally, microbial test was also carried out on the cream sample, according to the British Pharmacopeia procedure [18].

2.4. High-performance thin-layer chromatography (HPTLC) determination of epigallocatechin

Preliminarily, detection of various phytochemical components was performed via the Thin Layer Chromatography (TLC) technique in the fresh green tea extract and in the final cream. To achieve the best resolution of the components, the solvents mixed in these ratios (Chloroform/ Acetone/ Formic acid, 100: 16: 8.5). 30 mg of the cream-yielded extract was dissolved in pure methanol (1 ml) and loaded on a TLC plate

using a capillary tube parallel with an Epigallocatechin as control. The derivatization of the constituents was done under under normal and Ultra Violet (UV) light (254 nm). The HPTLC assessment was carried out using the CAMAG TLC system equipped with ATS4 (automatic TLC sample 4) and an automatic developing chamber (ADC2). The related software was WinCATS Planar Chromatography Manager. Silica gel plate 60F-254 (10 × 10 cm) was employed as the stationary phase while (Chloroform/ Acetone/ Formic acid, 100: 16: 8.5) was considered as the used mobile phase.

Stock solutions of Epigallocatechin (5, 2.5, 1.25, and 0.625 mg/ml) were prepared in methanol. For HPTLC analysis, 25 µl of each solution was applied per band, resulting in actual amounts of 125, 62.5, 31.25, and 15.62 µg per band, respectively. These values were used to construct the calibration curve. On the other side, a series of 20, 10, 5, and 2.5 mg of the solution from the prepared cream was dissolved in 1 ml of methanol and considered as unknown. The loading volumes for the unknown samples were 25 µl per band. The mobile phase volume was 10 ml. The drying time was 2 minutes. Cream samples were prepared similarly, and the amounts per band were calculated based on the loaded volume and extract concentration. Following the development of the TLC, the chromatographic profile was observed with ultraviolet lamps emitting at 254 nm [19].

2.5. Determination of total phenol and total flavonoid content in the cream

The amount of total phenols' content in cream was determined by the modified FCR method with a gallic acid standard [20, 21]. In this survey, Gallic acid was applied as the standard, and the total phenol content was reported in terms of mg of Gallic acid per 30 g of cream. Gallic acid-methanol solution was prepared at concentrations of 200, 100, 50, 25, 12.5, and 6.25 mg/l. 0.5 ml of various concentrations of Gallic acid was diluted with 2.5 ml of Folin-Ciocalteu and mixed with 2 ml of sodium carbonate (75 g/l), remaining at 20 °C for one hour in the dark. The stock solution of the extract was at 400 mg/l. The absorbance was subsequently read by a spectrophotometer at 765 nm. It was also considered to read the methanol absorption as blank. This test was carried out for each concentration of Gallic acid-methanol solution three times. The standard curve was plotted by the curve expert software, and the line equation was calculated.

The total flavonoid content in the cream was determined by the modified colorimetric method with quercetin as standard [22]. Briefly, 2 mg of quercetin was dissolved in 10 ml methanol. The serial dilution technique was used to prepare different concentrations (50, 25, 12.5, 6.25, 3.125 mg/l) by adding methanol. A stock solution of the extract (800 mg/l) was mixed with 2 ml of 2 % Aluminium chloride and kept in dark for 10 minutes at 25 °C, and was investigated for determination of the content. Finally, the absorbance against the blank was recorded at 415 nm wavelength by UV-visible spectroscopy. All determinations were performed in triplicate This test was carried out for each concentration of quercetin -methanol solution. The standard curve was plotted by the curve expert software, and the line equation was calculated.

3. Results

3.1. The yield of extraction

213 g of dried green tea leaves were subjected to the extraction process and finally, 31.6 g hydroalcoholic extract was obtained (The yield was 13.7 % W/W).

3.2. Evaluation of the prepared cream

3.2.1. Assessment of organoleptic properties, stability, rheology and microbial count tests

The final cream color was shiny light green. The texture was soft with no roughness. In addition, no signs of instability were observed in the final formulation. The pH value was 6.83 on the first day whereas the value declined to 6.78 and 6.75 after three and six months from the first day, respectively. After 6 months no changes in color, stability and amount of ingredients were observed.

Rheological analysis showed a shear-thinning behavior with thixotropic characteristics (indicated by a hysteresis loop between the upward and downward shear rate curves), which is desirable for topical creams to ensure easy spreading and good retention on the skin. The upper curve (increasing path) and the lower curve (decreasing path) correspond to increasing and decreasing cutting rates, respectively (Fig. 1) [17].

Microbial limit tests confirmed the absence of specified pathogens (*S. aureus*, *P. aeruginosa*, *E. coli*, *Salmonella* spp.). Total aerobic microbial count and total yeast/mold count were both below 100 CFU/g, meeting acceptable criteria for topical products [18].

3.2.2. Determination of the amount of Epigallocatechin via HPTLC method

The TLC pattern of the extract vs. Epigallocatechin at 254 nm illustrated in Fig. 2. Different concentrations of the standard sample was used to plott the calibration curve and derivatization of the spots was carried out at 254 nm (Fig. 3).

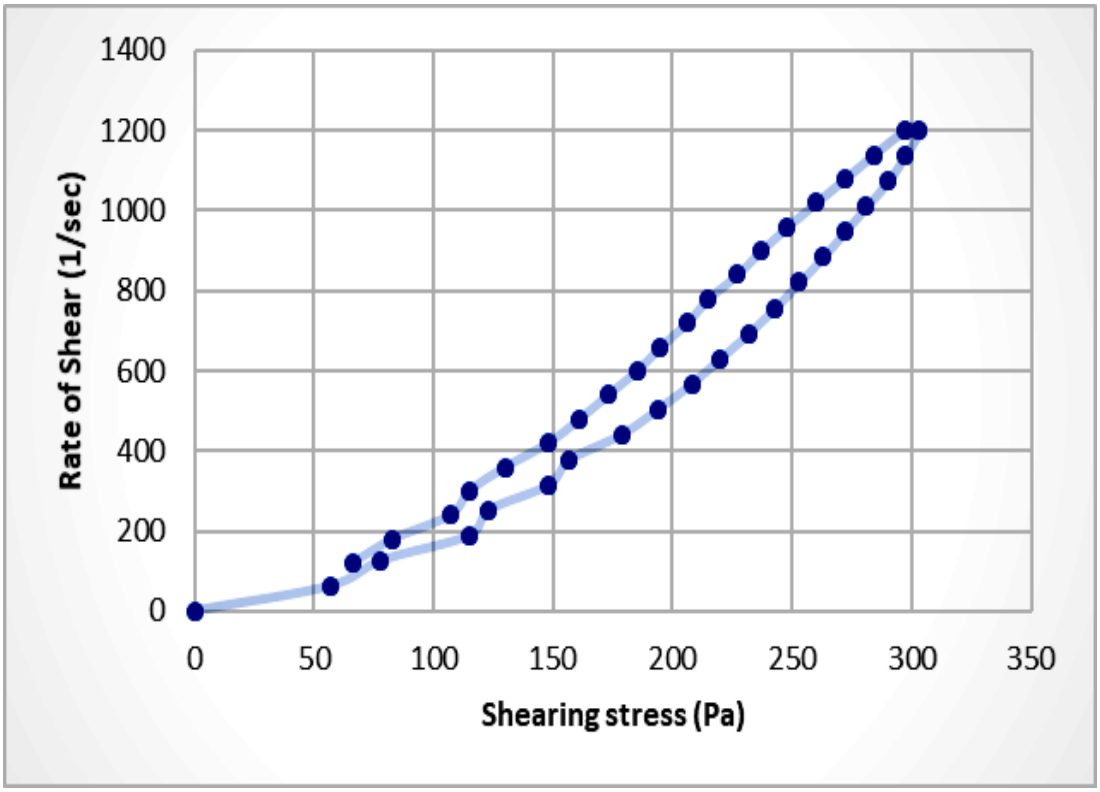

Fig. 1. Rheogram of 2.5 % *Camellia sinensis* cream formulation measured using a Brookfield cone-and-plate viscometer (CPE-40 cone, 4 cm diameter, 1° angle) over a shear rate range of 0 – 1200 s^{-1} to simulate application conditions. The Forward curve (▲) and Backward curve (▼) demonstrate the formulation's thixotropic behavior.

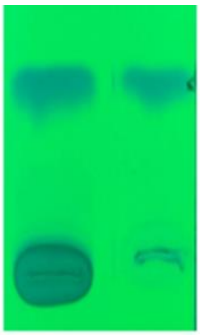

Fig. 2. TLC plate under UV 254 nm: (A) Green tea extract and (B) Epigallocatechin standard.

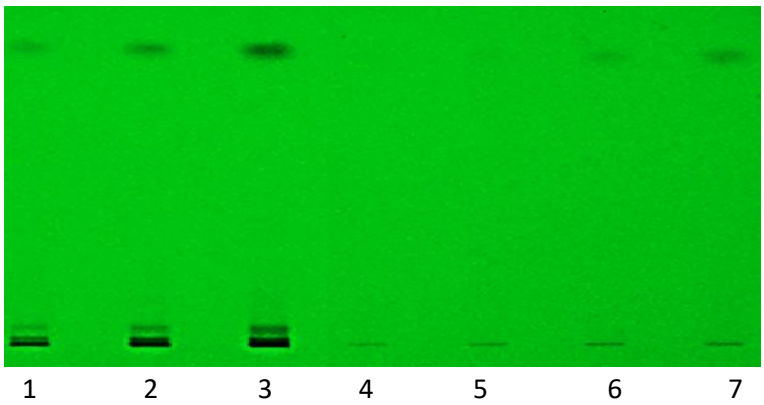

Fig. 3. HPTLC plate observed at 254 nm after derivatization: (A) Epigallocatechin standard at different concentrations (tracks 1-3), (B) corresponding spots of the cream extract (tracks 4-7)

The amount of EGC per band (µg) was plotted against the measured peak area to generate the calibration curve (Figure 4). Epigallocatechin was determined for our sample and concerning the linearity, Epigallocatechin showed a proper correlation coefficient of 0.999 (Figure 4).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by

$$\frac{3.3 \times \textit{Standard Deviation of the y intercept}}{\textit{Slope of the corresponding calibration curve}}$$

and

$$\frac{10 \times \textit{Standard Deviation of the y intercept}}{\textit{Slope of the corresponding calibration curve}},$$

respectively [23].

The results of method validation parameters for estimation of Epigallocatechin and precision studies are marked in Tables 1 and 2, respectively. The confirmation of the presence of Epigallocatechin in the extract was confirmed by comparing the R_f, absorption spectra, and derivatization at 254 nm (Figure 3) as well as overlaying with that of the respective standard using CAMAG TLC Scanner 4.

This employed method was precise as remarked by intermediate precision studies (relative standard deviation or RSD was < 5 %) for intraday variations (Table 2), confirming the method's reliability.

Regarding the determination of Epigallocatechin in the extract, the yielded hydroalcoholic extract represented a related peak in the chromatogram at an R_f value as compared to that of the Epigallocatechin standard (Figure

5). Also, all tracks of either standard samples or extract are represented in Figure 6. Eventually, after considering the dilution factor and extract percentage, the Epigallocatechine concentration was determined as 13.02 ± 0.05 mg per 30 g cream (0.04 %). According to HPTLC test results, the correlation coefficient is 0.999, the limit of detection is 1.99 µg/band and the limit of quantification is 6.05 µg/band, and also the exact amount of EGC in the cream was quantified as 0.04 % of the finished product.

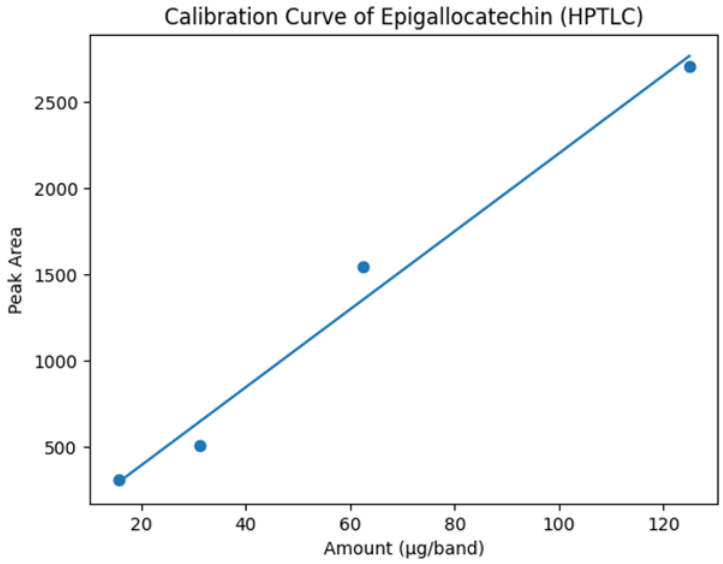

Fig. 4. Calibration curve of epigallocatechin standard (peak area vs. amount per band).

Table 1. Method validation parameters for estimation of Epigallocatechin

Parameters	Epigallocatechin
Wavelength, nm	254
Linearity range, µg/band	15.63 – 125.00
Regression equation	y=-56.43 + 22.6x
Correlation coefficient	0.99
Limit of detection, µg/band	1.99
Limit of quantification, µg/band	6.05
Specificity	Specific

Table 2. Intermediate precision studies for Epigallocatechine

Track	Concentration (µg/ml)	[a]Amount (µg)	[b]Intraday (RSD %)	Area
1	0.625	15.63 ± 0.56	3.58	309.91
2	1.25	31.25 ± 1.02	3.26	507.85
3	2.5	62.50 ± 1.21	1.93	1545.89
4	5	125.00 ± 3.74	2.99	2707.47

[a]Amount: Mean of three determinations; [b] % RSD: relative standard deviation

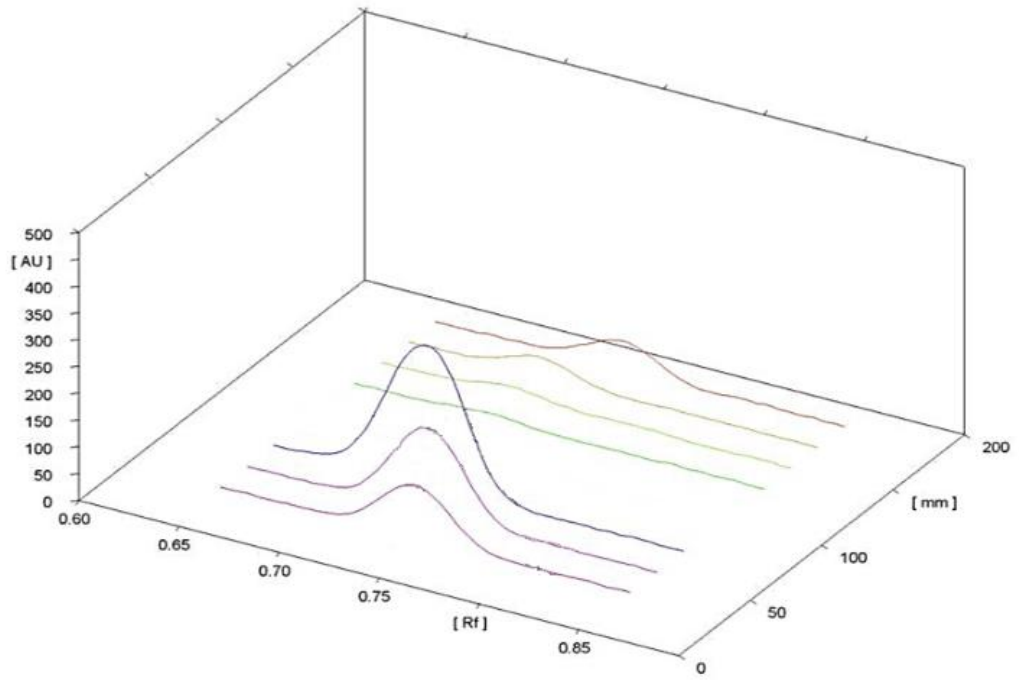

Fig. 5. Tracks of standard samples and the extract at the applied wavelength

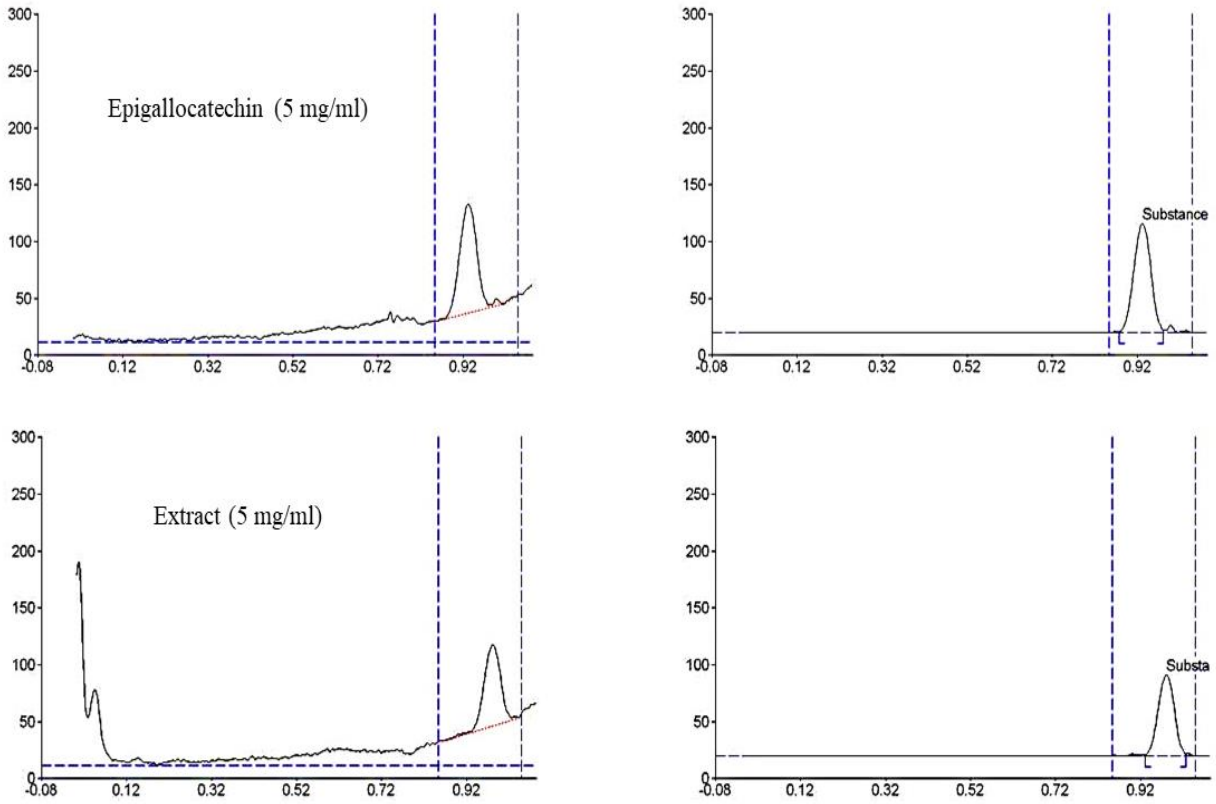

Fig. 6. Chromatogram of Epigallocatechine standard (5 mg/ml) and presence of the marker in the extract at the concentration of 5 mg/ml.

3.2.3. Determination of total phenol and flavonoid contents

The amount of total phenolic content of the formulated cream was determined by the Folin–Ciocalteu method, and gallic acid was considered as the standard (GAE). Based on the procedures explained in details in Materials and method (section 2.5), the amount of total phenolic content of the unknown sample (Hydroalcoholic green tea extract 400 mg/l) measured as indicated in table 3. From this value the total phenolic content per gram of green tea extract was derived. Because the herbal cream contains 2.5 % green tea extract, the amount of total phenolic content calculated and reported as 198.35 ± 2.79 mg of GAE/30 g of cream or 0.66 % (Table 3).

The amount of total flavonoid content of the cream was determined by the colorimetric method with quercetin as the standard. In accordance with the detailed methodology outlined in section 2.5, the flavonoid concentration was assessed in an 800 mg/l hydroalcoholic green tea extract (Table 4). This value was then used to calculate the total flavonoid content per gram of the green tea extract. Subsequently, based on the 2.5 % concentration of green tea extract in the herbal cream, the total flavonoid content of the final formulation was determined to be 19.56 ± 0.76 mg of quercetin equivalents (QE) per 30 g of cream, equivalent to 0.06 % (Table 4).

Table 3. Total Phenol content in the prepared cream

Sample (Extract Conc.) (mg/L)	Total phenol in sample (mg GAE /L)			Total phenol (mg GAE /g Ext.)			AV ± SD (mg GAE/g Ext.)	Total Phenol in Cream (mg GAE/30g cream.)
400	105.85	105.65	104.87	266.62	266.62	260.17	264.47 ± 3.72	198.35 ± 2.79

Table 4. Total Flavonoid content in the prepared cream

Sample. (Extract Conc) (mg/L)	Total flavonoid in sample (mg QE/L)			Total flavonoid (mg QE/g of Ext.)			AV ± SD (mg QE/g of Ext.)	Total flavonoid in Cream (mg QE/30g of cream.)
800	19.93	21.44	21.23	24.91	26.8	26.53	26.08±1.02	19.56 ± 0.76

4. Discussion

In the present investigation, we successfully formulated a herbal eye cream containing 2.5 % hydroalcoholic extract of *Camellia sinensis* and evaluated its pharmaceutical and phytochemical properties. Green tea extract is rich in polyphenolic compounds, especially catechins such as epigallocatechin gallate (EGCG) and epigallocatechin (EGC), which have been associated with antioxidant, anti-inflammatory, and potential anti-aging effects in skin applications. Recent scoping reviews on the cosmetic use of green tea underscore its broad antioxidant and anti-aging potential and indicate that catechins, particularly EGCG, play a central role in neutralizing reactive oxygen species and protecting dermal tissues from oxidative damage, which contributes to premature skin aging [24, 25].

Clinical evidence also supports the functional benefits of *C. sinensis* extracts in reducing periorbital aging features. For example, a quasi-experimental clinical study using a 5 % *C. sinensis* extract cream demonstrated statistically significant improvements in wrinkle parameters and skin elasticity after 12 weeks of application, supporting the therapeutic potential of these extracts in anti-aging eye products [26].

However, that study primarily focused on clinical endpoints and did not report detailed analytical standardization of the active constituents in the formulation or comprehensive pharmaceutical characterization over time. Our study complements this by providing quantitative data on active compound content and stability over a six-month period, thereby establishing a more rigorous standardization framework for future clinical evaluations.

While such studies provide useful baseline information on the effect of extract concentration on formulation properties, they did not quantify the biologically active compounds in the final product. In contrast, the present work employs a validated HPTLC method to quantify a specific active marker (epigallocatechin) and spectrophotometric methods to assess total phenolic and flavonoid contents in the finished cream, providing a more complete phytochemical profile of the formulation.

The antioxidant, anti-inflammatory, and free-radical-scavenging activities of green tea polyphenols are widely documented in the literature, including their ability to protect against UV-induced oxidative stress and modulate inflammatory pathways [25, 27]. Although most cosmetic applications rely on these properties, commercial formulations often list green tea extract without specifying the concentration of active catechins or providing validated analytical data to ensure batch-to-batch consistency. The present study addresses this gap by incorporating validated analytical methods, including HPTLC and spectrophotometric assays, into the evaluation scheme. By reporting the exact amounts of epigallocatechin (13.02 ± 0.05 mg/30 g cream), total phenolic compounds (198.35 ± 2.79 mg GAE/30 g cream), and total flavonoids (19.56 ± 0.76 mg QE/30 g cream), a reproducible phytochemical profile is provided that can support quality control in future research and clinical testing. Furthermore, our comprehensive pharmaceutical evaluation confirmed the cream's acceptability. The formulation showed good organoleptic properties, minimal pH variation over six months, desirable shear-thinning and thixotropic rheology for patient application [17], and passed microbial safety tests according to pharmacopeial standards [18]. The stability of both the formulation and its phytochemical content over the study period suggests satisfactory shelf-life characteristics, which is essential for clinical translation.

5. Conclusion

The results of the present study demonstrate that the hydroalcoholic *C. sinensis* eye cream is a standardized, stable, and analytically traceable formulation with quantified active components that supports its consideration as a candidate for clinical anti-aging evaluation in the field of periorbital.

Authors' contributions

S. H: Contribution to data acquisition, interpretation of data, analysis of data, Writing Original Draft and revising the work, approving the final version; S. M. S: Contribution to the design, conceptualization and data acquisition, interpretation of data, revising the work, approving the final version; A. P: Contribution to data acquisition, interpretation of data, analysis of data, drafting, and approval of the final version; M. M. Z: Contribution to the design, conceptualization and data acquisition, interpretation of data, analysis of data, Writing Original Draft and revising the work, approving the final version.

Conflicts of interest

The authors declare no financial or other conflicts of interest.

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