Evaluation and Formulation of a Natural Antioxidant Cream Using Various Antioxidant Assays Enriched with *Glycyrrhiza glabra, Hibiscus sabdariffa* extracts for Hyperpigmentation Treatment

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ABSTRACT

Hyperpigmentation, a common dermatological condition marked by excessive melanin production, is often exacerbated by oxidative stress, hormonal fluctuations, and sun exposure. In response to the limitations of conventional treatments, such as hydroquinone and isotretinoin, this study investigates the formulation of a natural antioxidant cream using aqueous extracts of Glycyrrhiza glabra (licorice) and Hibiscus sabdariffa (roselle), enriched with Aloe vera and vitamin E. The extracts were evaluated for phytochemical content (phenolics, flavonoids, catechins, anthocyanins, and ascorbic acid) and subjected to antioxidant assays (DPPH and $KMnO_4$). High-performance liquid chromatography (HPLC) confirmed the presence of bioactive compounds such as chlorogenic acid, catechin, and ellagic acid. Among the tested ratios, the 1:2 ratio of Glycyrrhiza glabra to Hibiscus sabdariffa exhibited the strongest antioxidant activity in the $KMnO_4$ assay (99.9% inhibition), while the 1:1 ratio was most effective in the DPPH assay (98.5%). The formulated cream showed promising consistency and sensory acceptability. The findings support the synergistic potential of these extracts in neutralizing free radicals and reducing melanin biosynthesis, offering a natural, effective alternative for managing hyperpigmentation in cosmetic and therapeutic applications.

Key words: *Skin oxidative stress, topical antioxidant therapy, melanin inhibitor, phytochemical analysis, plant-based formulation*

INTRODUCTION

Hyperpigmentation is a skin condition characterized by dark spots that are more pronounced than the surrounding skin, resulting from excessive melanin production. These spots can vary in size, shape, and intensity and commonly appear on the hands, neck, and face [1]. The condition may be caused by several factors, including hormonal changes, sun exposure, aging, skin injuries or acne. One of the primary causes of hyperpigmentation is sun exposure, which stimulates melanin production and leads to the formation of dark patches as a protective response against harmful UV rays [2]. Skin infections such as acne, papules, and pustules can also trigger post-inflammatory hyperpigmentation (PIH), particularly when acne lesions are picked or squeezed [1]. Pigmentary disorders are among the most common dermatologic diagnoses in individuals with skin of color, particularly in Africans and African Americans [3]. For instance, a study conducted in Durban, South Africa, found that 7.97% of 3,814 patients had pigmentary disorders, predominantly affecting black African women [3]. Similarly, in Egypt, a study at Ain Shams University Clinic assessed the prevalence and causes of facial hyperpigmentary disorders (FHP) among 125 patients with facial hyperpigmentation; of these, 44% had melasma, 16% suffered from PIH, and 15% had freckles [4]. The findings revealed that facial hyperpigmented was most common during the summer months, consistent with Egypt's hot climate. Most patients were women in their forties with Fitzpatrick skin types III and IV [4].

Treating hyperpigmentation is complex due to its multifactorial nature. Conventional approaches include topical therapies, such as hydroquinone and retinoids, which inhibit melanin synthesis, and oral therapies such as tranexamic acid (TXA) and isotretinoin [5-7]. Hydroquinone, a tyrosinase inhibitor, is effective but can cause erythema, pruritus, desquamation, and burning. Additionally, it can lead to a rare condition known as "hydroquinone halo" characterized by hypopigmentation around treated areas [8]. Prolonged or improper use of hydroquinone may also result in hyperpigmentation and exogenous ochronosis, a permanent skin darkening [9]. TXA, commonly used for melasma, inhibits UV-induced plasmin activity but carries risks such as thrombosis in predisposed individuals [7,8]. Isotretinoin, a synthetic derivative of vitamin A, is known for its immunological and anti-inflammatory properties. It is also a fat-soluble micronutrient essential for cellular differentiation, immunity and vision [10]. However, it is a well-known teratogen and is associated with side effects such as cheilitis (cracked lips), xerosis (dry skin), dyslipidemia (abnormal lipid levels), and transient transaminitis (liver enzymes elevation) [7,11]. Sunscreens, especially those containing iron oxide, are critical for effectively improving pigmentation appearance. Compared to mineral SPF 50+ sunscreen, iron oxide formulations are especially beneficial for individuals with Fitzpatrick skin types III and above, highlighting the essential role of sunscreens in managing hyperpigmentation [12].

Natural products are gaining attention as safer, biocompatible alternatives for the treatment of hyperpigmentation [13]. Compounds such as glabridin (GLA) from *Glycyrrhiza glabra* (licorice) and anthocyanins from *Hibiscus sabdariffa* (roselle) exhibit antioxidant and tyrosine inhibiting properties [14,15]. Some studies reported that glycyrrhizin does not reduce hydroxyl or superoxide anion radicals but effectively reduces 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [16]. Conversely, other studies suggest that glycyrrhizin does not act on DPPH radicals but interacts with reactive oxygen species (ROS) instead [14]. A study by Karunarathne *et al*. [17] tested the effects of anthocyanins from Pulsae (PS) and Paektanshim (PTS), extracted from purple and white Hibiscus petals, on melanin biosynthesis. The findings showed that both PS and PTS effectively reduced

melanin synthesis in extracellular and intracellular B16F10 cell lines, with only slight inhibition of the MV tyrosinase activity in vitro. Moreover, both extracts reduced the expression of microphthalmia-associated transcription factor (MITF) and the tyrosine induced by α-melanocyte-stimulating hormone (α-MSH), supporting the potential of natural agents in hyperpigmentation treatment [17].

Antioxidants are compounds that neutralize free radicals and ROS [18]. They could be classified as either synthetic or natural based on their sources, mode of action, or solubility [19]. Natural antioxidants, particularly those found in red, orange and purple-colored fruits and vegetables, exhibit high antioxidant activity [18]. Phenolics, flavonoids and carotenoids are well-known and well-established antioxidant groups [20]. *Glycyrrhiza glabra* contains glycyrrhizin, demonstrating significant antioxidant activity against ROS, while *Hibiscus sabdariffa* is rich in anthocyanins that reduce melanin production in cell lines [14,17].

This study aims to develop and evaluate a natural antioxidant cream formulated with aqueous extracts of *Glycyrrhiza glabra* and *Hibiscus sabdariffa*, enriched with vitamin E and *Aloe vera*. By examining their phytochemical profiles, antioxidant capacities, and compatibility in a topical formulation, we seek to explore their potential as safe, effective alternatives for the treatment of hyperpigmentation.

MATERIALS AND METHODS

Plant material collection

Hibiscus sabdariffa and *Glycyrrhiza glabra* plants were collected from a local market in Giza, Egypt, during the autumn of 2024. The freshly collected samples were air-dried and then ground into a fine powder.

Preparation of plant extracts

The dried plant powders were extracted with distilled water at a ratio of 1:10 (w/v), repeated three times, following the method described by Rosenthaler [21]. The aqueous extracts were filtered using Whatman No.1 filter paper, and the filtrates were stored in a refrigerator at approximately ±5°C until further use.

Thin layer chromatography bioautography (TLC)

Precoated silica gel plates (TLC F254) were used to separate the active components of the plant extracts using various solvent systems as mobile phases, including ethyl acetate: acetone 7:3 (v/v). Antioxidant activity was detected using potassium permanganate (KMnO4, 0.2% in methanol) as a spray reagent following the rapid TLC screening method described by Nair *et al.* [22].

Total phenolic compounds content

The total phenolic content of the plant extracts was determined using the Folin-Ciocalteu method, as described by Singleton and Rossi [23]. The concentrations were calculated using a gallic acid standard curve and expressed as mg GAE/g of extract,

Total flavonoid content

The total flavonoid content was determined using the method described by Zhishen *et al*. [24]. Briefly, 125 µl of the tangerine peel ethanol extract and 150 µl of 10% AlCl3 were placed in a test tube and left to react for five minutes. Then, 750 µl of 1M NaOH was added, and the mixture was incubated in the dark for 15 minutes. After thorough mixing, the absorbance was measured spectrophotometrically at 510 nm against a blank. Quercetin was used as the standard, and results were expressed as mg QE/g.

Total catechins content

The total catechin content was determined using the vanillin assay as described by Khlifi *et al*. [25] and Belyagoubi-benhammou *et al.* [26], with slight modifications. In this method, 50 µl of the tangerine peel ethanol extract was mixed with 1.5 mL of 4% vanillin in methanol. Then, 750 µl of concentrated HCl was added. The mixture was allowed to react for one hour at room temperature. Absorbance was measured at 550 nm against a blank. The total condensed tannin content was expressed as mg CE/g,

Determination of ascorbic acid content

Ascorbic acid content was determined using the dichlorophenol indophenol (DCPIP) titration method, as described by Highet and West [27]. One millilitre of tangerine peel ethanol extract was placed in a test tube, and DCPIP solution (0.1%) was added dropwise until a persistent blue colour appeared. The concentration of ascorbic acid in the extract was calculated and expressed as units per gram of fresh mass using the conversion: 1 mL of 0.1% DCPIP (Mwt = 290.08) = 6.071x10-4 g ascorbic acid (Mwt = 176.12).

Extraction and determination of anthocyanin content

Anthocyanins content was extracted using acidified methanol (1% HCl). The absorbance of the clear, filtered pigment solution was measured spectrophotometrically at 535 nm using the molar absorption coefficient of cyaniding 3-glycoside (29,500 M^{-1}), as described by Stickland and Sunderland [28].

High-performance liquid chromatography (HPLC) of a promising aqueous extract

HPLC analysis was performed using an Agilent 1260 series system with an Eclipse C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of water and 0.05% trifluoroacetic acid in acetonitrile, delivered at a flow rate of 0.9 mL/min. A linear gradient was applied as follows: 0 minutes (82% A), 0-5 minutes (80% A), 5-8 minutes (60% A), 8-12 minutes (60% A), 12-15 minutes (82% A), 15-16 minutes (82% A), and 16-20 minutes (82% A). Detection was performed at 280 nm using a multi-wavelength detector, with an injection volume of 5 µL. The column temperature was maintained at 40 °C following the method of Loon *et al.* [29].

DPPH radical scavenging activity

The radical scavenging activity of the aqueous plant extracts was assessed using the DPPH method described by Yen and Chen [30]. The absorbance of all the sample solutions was measured at 517 nm. The percentage of scavenging activity was calculated using the following equation:

% Antioxidant Activity = [(Control-Sample) x 100] / Control,

where the control was a DPPH solution (0.16 mM).

All assays were performed in triplicate, and results were expressed as mean ± standard error (SE).

$KMnO_4$ non-radical scavenging activity

The non-radical scavenging activity was evaluated using KMnO4 assay, following the method of Gaber *et al.* [31]. The absorbance of all sample solutions was measured at 514 nm. The percentage of scavenging activity was calculated as follows: % Antioxidant Activity = [(Control-Sample) x 100] / Control,

where the control was potassium permanganate solution (0.16 mM).

Antioxidant Cream Formulation

The antioxidant cream was formulated using a three-phase method: oil phase, aqueous phase, and final mixing with preservatives. The procedure was as follows:

1- Oil Phase Preparation:

A base cream (containing emulsifying wax, stearic acid, and paraffin) was weighed according to batch specifications (see Table 1) and melted in a water bath at 70 ± 2 °C. Once fully melted,

vitamin E (DL-α-tocopherol acetate, 4–8 g depending on batch) was added gradually under continuous stirring using a magnetic stirrer at 300 rpm until uniformly dispersed.

2- Aqueous Phase Preparation:

In a separate container, the aqueous phase was prepared by dissolving Aloe vera gel (30–75 g) and mixing it with 250–450 mL of distilled water. Then, the appropriate amounts of *Hibiscus sabdariffa* and *Glycyrrhiza glabra* aqueous extracts (15–45 mL and 25–35 mL, respectively) were added based on the targeted formulation ratio. This mixture was heated to 70 ± 2 °C to match the temperature of the oil phase.

3- Emulsification and Final Mixing:

While maintaining both phases at 70 °C, the aqueous phase was slowly added to the oil phase under constant stirring at 500 rpm for 15 minutes to ensure complete emulsification. The mixture was then allowed to cool gradually to room temperature while stirring was continued for an additional 30 minutes to improve cream stability and texture. Once cooled to 35 °C, 5 mL of a commercial cosmetic-grade preservative (e.g., phenoxyethanol) was added to each batch and stirred thoroughly to ensure uniform distribution.

The final product was dispensed into sterilized containers under hygienic conditions for further use. Table 1 presents the composition of oil and aqueous phases in different antioxidant cream formulations (batch specifications).

Table 1: Composition of oil and aqueous phases in different antioxidant cream formulations

Oil phase	**1st Batch**	**2nd Batch**	**3rd Batch**
Vit E	4 grams	6 grams	8 grams
Base cream	150 grams	100 grams	75 grams
Aqueous phase			
Distilled water	450 mL	350 mL	250 mL
Hibiscus Extract	25 mL	30 mL	45 mL
Glycyrrhiza Extract	15 mL	20 mL	35 mL
Aloe Vera gel	30 grams	50 grams	75 grams

Statistical analysis

All the data are expressed as mean ± standard deviation (SD). Statistical comparison was performed via one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT), [32]. Differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Bioautography for antioxidant activity using Thin Layer Chromatography (TLC)

The TLC bioautographic assay for identifying antioxidant compounds was performed using the DPPH free radical scavenging method. Antioxidant activity was observed as yellow-colored spots appearing against a dark purple background, indicating the presence of antioxidant compounds. Fig. 1 illustrates a TLC test using potassium permanganate ($KMnO_4$) as a staining agent to detect antioxidant components in the extracts. $KMnO_4$ reacts with phenols and flavonoids, producing yellowish-brown spots against a pink background. Therefore, discolored or faded areas indicate the presence of phenolics or flavonoid compounds, confirming the antioxidant potential of the extracts.

The TLC assay was conducted to evaluate the $KMnO_4$–reducing capability of the aqueous extracts *Hibiscus sabdariffa* and *Glycyrrhiza glabra*. Two distinct spots with varying intensities appeared against the $KMnO_4$ background, indicating antioxidant activity. This assay served as a preliminary step for further analyses, including HPLC, to quantify specific active compounds. *Hibiscus sabdariffa* demonstrated the highest background reduction, supporting its strong antioxidant activity. These findings align with prior research [33].

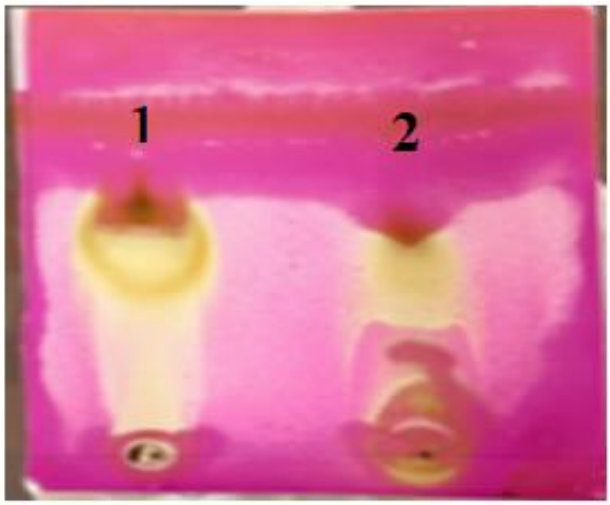


Figure 1. TLC-bioautography results of aqueous extracts of *Hibiscus sabdariff*a (1) and *Glycyrrhiza glabra* (2) using potassium permanganate as the oxidizing agent.

Total phenolic, flavonoids and catechins compounds content

Phenolic compounds are a class of secondary plant metabolites, including flavonoids, phenolic acids, tannins, lignans, and coumarins. These are naturally present compounds in fruits, vegetables, cereals, roots, and leaves. Table 2 highlights the contents of total phenolics, flavonoids, and catechins in various aqueous extract ratios of *Hibiscus sabdariffa* and *Glycyrrhiza glabra*. The 2:1 ratio of *Glycyrrhiza glabra* to *Hibiscus sabdariffa* exhibited the highest phenolic (88.12 µg/mL) and flavonoid (64.35 µg/mL) contents, suggesting stronger antioxidant potential. In contrast, the 1:2 ratio showed the highest catechins content (59.61 µg/mL), supporting its potent antioxidant profile. In contrast, the 1:1 ratio yielded the lowest values across all compounds.

Table 2: Quantitative Analysis of Total Phenolic, Flavonoid, and Catechin Contents (µg/mL) in Aqueous Extract Ratios of *Glycyrrhiza glabra* and *Hibiscus sabdariffa*

Sample ratio (*Glycyrrhiza glabra: Hibiscus sabdariffa)*	Phenolic compounds	Flavonoids	Catechins
1:1	68.99 [c] ±1.89	40.35 [b] ±0.89	34.04 [b] ±1.00
2:1	88.12[a]±1.40	64.35 [a] ±0.91	36.84 [b] ±1.20
1:2	81.59 [b] ±2.11	63.09 [a] ±1.67	59.61 [a] ±2.05

The 2:1 ratio yielded the highest phenolic and flavonoid content, whereas the 1:2 ratio excelled in catechin content. These results confirm the potent antioxidant capability of both extracts. Zeb [34] reported that phenolic compounds have strong antioxidant properties and are considered safer than synthetic antioxidants. This is further supported by Amin *et al.* [35]. The observed synergism is significant, as oxidative stress is a key factor in hyperpigmentation and neutralizing free radicals is essential for its treatment [6,36].

Ascorbic acid and anthocyanin content

The Hibiscus extract was particularly rich in anthocyanins (121.36 ± 4.09 µM) and ascorbic acid (32.45 ± 1.06 mg/100g) (Table 3), consistent with its known antioxidant efficacy. These compounds contribute to its free radical scavenging ability and skin-lightening effects through tyrosinase inhibition.

Table 3: Quantification of Anthocyanin and Ascorbic Acid Content in Aqueous Extract of Hibiscus sabdariffa

Anthocyanin (µM)	Ascorbic acid (mg/100g)
121.36±4.09	32.45±1.06

The extract`s high anthocyanin and ascorbic acid content confirms its antioxidant potential. Anthocyanins reduce oxidative damage from free radicals and effectively manage pigmentary disorders, including hyperpigmentation [17,36]. Ascorbic acid not only scavenges free radicals but also inhibits tyrosinase enzymes, thereby reducing melanin production [8,37]. This combination makes the extract suitable for long-term, non-toxic skin applications [6,38].

High-performance liquid chromatography (HPLC)

Fig. 2 presents the HPLC chromatogram of standard polyphenols. According to the HPLC results presented in Fig. 3 and Table 4, the 2:1 aqueous extract of *Glycyrrhiza glabra* to *Hibiscus sabdariffa* contains a diverse range of bioactive compounds. Fifteen compounds were identified, notable for their antioxidant and anticancer properties. Chlorogenic acid (102.98 µg/mL) and catechin (58.48 µg/mL) were found in the highest concentrations. Other significant constituents include ellagic acid (32.6 µg/mL), syringic acid (11.5 µg/mL), and gallic acid (7.83 µg/mL). Minor components such as coumaric (2.22 µg/mL), naringenin (3.52 µg/mL), rutin (1.05 µg/mL), and quercetin (0.78 µg/mL) were also

detected. The diversity and contents of these compounds reinforce the extract`s biological potential as a natural antioxidant and anticancer agent.

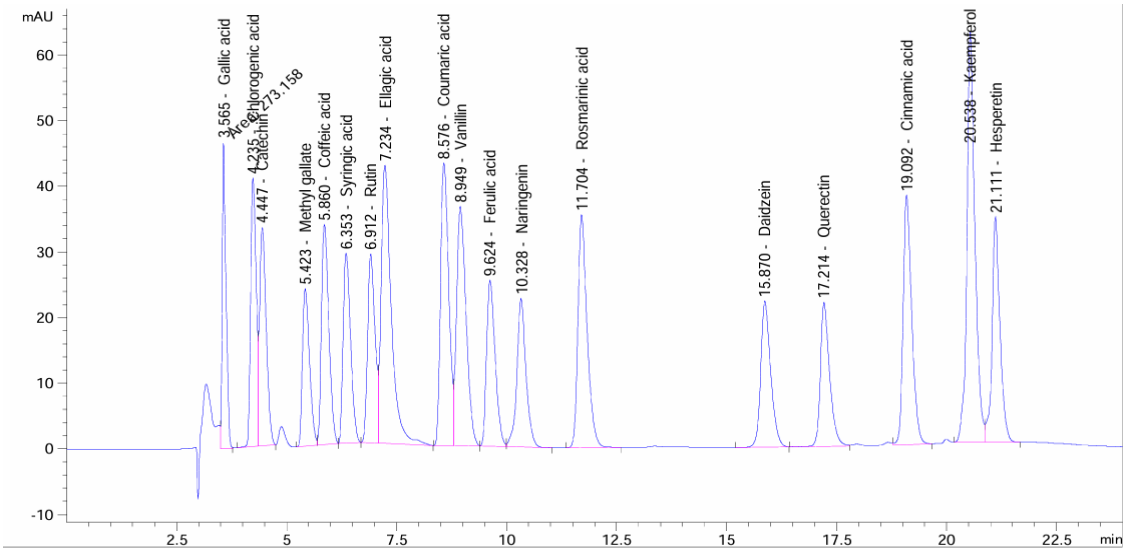


Figure 2: HPLC Chromatogram of *Hibiscus sabdariffa* Ethanolic Extract Highlighting Dominant Phenolic Peaks

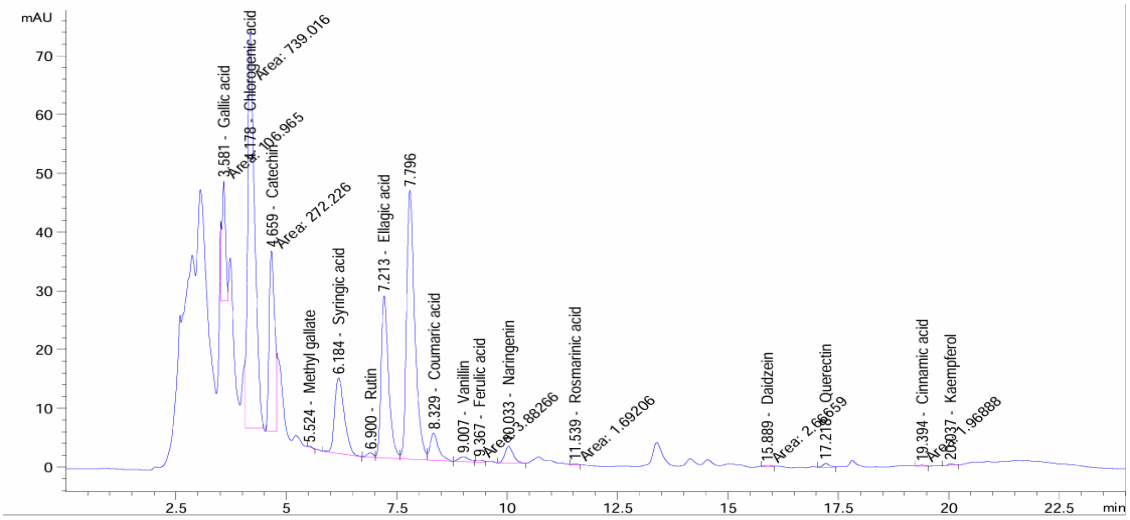
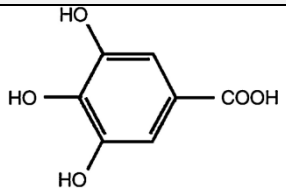
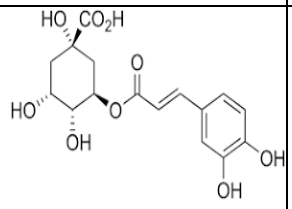
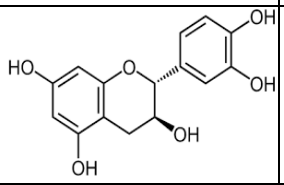
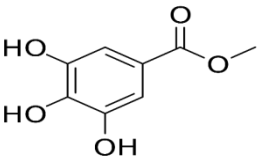
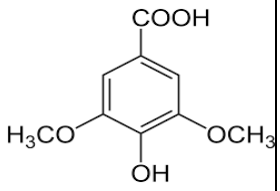
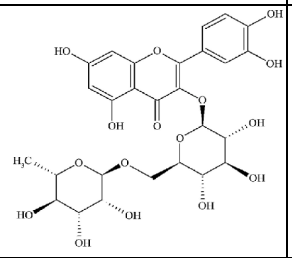
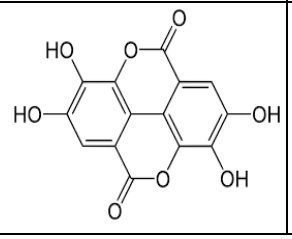
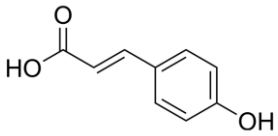
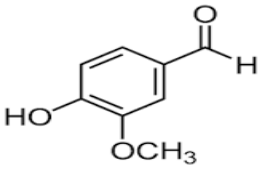
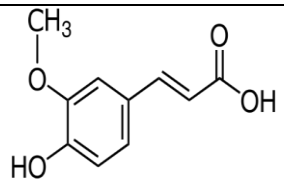
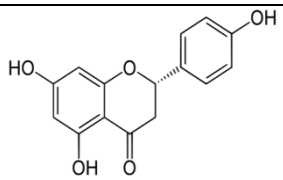
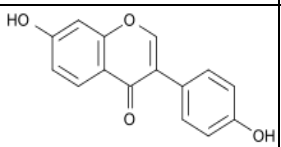
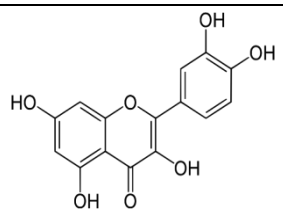
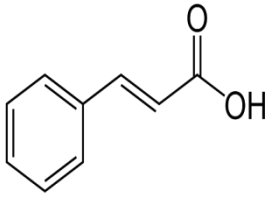
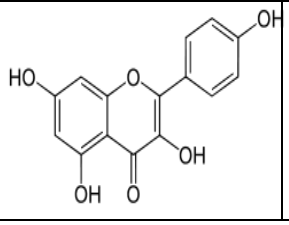


Figure 3: HPLC chromatogram of aqueous extract of *Glycyrrhiza glabra* and *Hibiscus sabdariffa* in the ratio of 2:1

Table 4: Phytochemical constituents (as µg/mL) of aqueous extract (2:1 ratio) of *Glycyrrhiza glabra* and *Hibiscus sabdariffa* using HPLC

Peak No.	Retention time (min)	Chemical name	Chemical structure	Cons. (µg/mL)	Biological activities	References
1	**3.58**	**Gallic acid**		7.83	Anticancer Antioxidant	**[39]**
2	**4.01**	**Chlorogenic acid**		102.98	Anticancer Antioxidant	**[40]**
3	**4.389**	**Catechin**		58.48	Anticancer Antioxidant	**[40]**
4	**5.452**	**Methyl gallate**		0.06	Anticancer Antioxidant	**[40]**
5	**6.261**	**Syringic acid**		11.52	Anticancer Antioxidant	**[40]**
6	**8.022**	**Rutin**		1.05	Anticancer Antioxidant	**[40,41]**
7	**8.659**	**Ellagic acid**		32.62	Anticancer Antioxidant	**[42]**

Peak No.	Retention time (min)	Chemical name	Chemical structure	Cons. (µg/mL)	Biological activities	References
8	**8.90**	**Coumaric**		2.22	Anticancer Antioxidant	**[43]**
9	**9.590**	**Vanillin**		0.49	Anticancer Antioxidant	**[40]**
10	**10.303**	**Ferulic acid**		0.23	Anticancer Antioxidant	**[41]**
11	**10.752**	**Naringenin**		3.52	Anticancer Antioxidant	**[41]**
12	**12.269**	**Daidzein**		0.15	Anticancer Antioxidant	**[40]**
13	**12.870**	**Querectin**		0.78	Anticancer Antioxidant	**[44]**
14	**14.194**	**Cinnamic acid**		0.04	Anticancer Antioxidant	**[42]**

Peak No.	Retention time (min)	Chemical name	Chemical structure	Cons. (µg/mL)	Biological activities	References
15	**15.098**	**Kaempferol**		0.05	Anticancer Antioxidant	**[42]**

HPLC analysis identified additional bioactive compounds in the extract, known for their antioxidant and anticancer properties, such as chlorogenic acid, catechin, and ellagic acid [39,40]. These compounds contribute to the therapeutic value of the extract, especially by enhancing antioxidant capacity and suppressing melanin synthesis. The variety of these compounds underscores the complex nature of natural extracts and their potential for multifaceted intervention in skin pigmentation pathologies. The extract's biological potential is significantly high, mainly because of the presence of chlorogenic acid and catechin, which were found in higher concentration and are recognized for their antioxidant and anticancer activity. Other bio-active phytochemicals, such as ellagic acid, syringic acid, and gallic acid, were also detected at moderate levels, thereby contributing to the extract`s overall activity. According to Tang *et al.* [45], chlorogenic acid (CGA) has demonstrated significant antioxidant properties across various validated experiments in different fields, including chronic disorders, cardiovascular diseases and cancers. Gallic acid (GA), as confirmed by Monteiro *et al.* [46], is a phenolic acid found in a wide range of plants and is known for its antioxidant activity. Moreover, a study conducted by Peng *et al.* [6] highlighted the effect of gallic acid on melanin production using the B16F10 cell line as an in vitro model; the results showed effective suppression of melanin synthesis.

Antioxidant activity

The results in Table 5 illustrate the antioxidant activity of the aqueous extract of *Hibiscus sabdariffa* assessed using $KMnO_4$, at three different concentrations: high, moderate, and low. At the highest concentration (200 µg/mL), the extract exhibited high antioxidant activity at 98.56%. Similarly, at a moderate concentration (100 µg/mL), it maintained strong antioxidant activity at 92.35%, proving its effectiveness even at lower concentrations. At the lowest concentration (50 µg/mL), the activity decreased to 74.09%. These findings underscore the outstanding antioxidant potential of Hibiscus sabdariffa, proving its value as a promising candidate for therapeutic applications aimed at reducing oxidative stress.

Table 5 shows that the antioxidant activity of *Hibiscus sabdariffa* was particularly striking, with the highest activity observed at 200 µg/mL. This agrees with the previous studies on anthocyanins and ascorbic acid in Hibiscus species as potent antioxidants [13,17]. The presence of these compounds in *Hibiscus sabdariffa* not only confers its antioxidant capacity but also increases its potential as a natural alternative to synthetic antioxidant compounds, which often have adverse side effects Table 4 shows that the antioxidant activity of *Hibiscus sabdariffa* was particularly striking, with the highest activity observed at 200 µg/mL. This agrees with the previous studies on anthocyanins and ascorbic acid in Hibiscus species as potent antioxidants [13,17]. The presence of these compounds in *Hibiscus sabdariffa* not only confers its antioxidant capacity but also increases its potential as a natural alternative to synthetic antioxidant compounds, which often have adverse side effects [38].

Table 5: Comparative antioxidant activity (%) of different extract ratios from *Hibiscus sabdariffa* assessed by $KMnO_4$ assay

Contents (µg/mL)	Antioxidant activity (%)
200	98.56^{a}±2.89
100	92.35^{b} ±2.05
50	74.09^{c} ±1.49

The results in Table 6 compare the antioxidant activity of different ratios of *Glycyrrhiza glabra* to *Hibiscus sabdariffa* extracts using KMnO4 and DPPH assays at two different concentrations (100 µg/mL and 50 µg/mL). In the KMnO4 assay at 100 µg/mL, the 1:2 ratio (Glycyrrhiza glabra: Hibiscus sabdariffa) showed the highest antioxidant activity at 99.9%, followed by the 2:1 ratio at 88.43%, and the 1:1 ratio at 77.45%. At 50 µg/mL, the 1:2 ratio again exhibited the highest activity at 68.32%, followed by the 2:1 ratio at 63.64% and the 1:1 ratio at 46.24%. In the DPPH assay at 100 µg/mL, the 1:1 ratio showed the highest antioxidant activity at 98.5%, followed by the 2:1 ratio at 79.5% and the 1:2 ratio at 70.8%. At 50 µg/mL, the 1:1 ratio again showed the highest activity at 75.5%, followed by the 2:1 ratio at 72.48% and the 1:2 ratio at 56.5%. These findings indicate that in the KMnO4 assay, the 1:2 ratio consistently outperformed other ratios at both concentrations, suggesting that *Hibiscus sabdariffa* has superior antioxidant activity. In the DPPH assay, the 1:1 ratio was the most effective at both concentrations. This demonstrates the synergistic effect of combining *Hibiscus sabdariffa* and *Glycyrrhiza glabra* in enhancing antioxidant activity.

Table 6: Synergistic antioxidant activity (%) of different extract ratios from *Glycyrrhiza glabra* to *Hibiscus sabdariffa* against DPPH and $KMnO_4$ assays

Sample ratio *Glycyrrhiza glabra: Hibiscus sabdariffa*	Concentrations (µg/mL)	$KMnO_4$ assay (%)	DPPH assay (%)
1:1	100	77.45^{c} ±2.90	98.5^{a} ±3.54
	50	46.24^{e}±1.90	75.5^{c} ±2.45
2:1	100	88.43^{b} ±3.03	79.5^{b} ±1.90
	50	63.64±2.70	72.48^{d} ±1.69
1:2	100	99.9^{a}±3.80	70.8^{d} ±2.31
	50	68.32^{d} ±4.67	56.5^{e} ±2.90

The findings in Table 6 aim to understand the antioxidant potential of various ratios of *Glycyrrhiza glabra* to *Hibiscus sabdariffa* extracts using KMnO4 and DPPH assays. In the KMnO4 assay, the 1:2 ratio of *Glycyrrhiza glabra* to *Hibiscus sabdariffa* showed the highest antioxidant activity, suggesting that Hibiscus sabdariffa, with its high anthocyanin and ascorbic acid content (as shown in Table 1), plays a significant role in neutralizing free radicals. Conversely, the DPPH assay revealed that the 1:1 ratio exhibited the highest antioxidant activity, likely due to the DPPH assay's high sensitivity to phenolic compounds present in *Glycyrrhiza glabra* [40]. These variations highlight the complementary antioxidant mechanisms of the two extracts: *Hibiscus sabdariffa* contributes anthocyanins and ascorbic

acid, while *Glycyrrhiza glabra* provides glabridin and other phenolic compounds. Together, they synergistically enhance the overall antioxidant capacity [6,47]. These findings have significant implications for skincare and therapeutic applications, as the combination of these extracts exhibits potent, broad-spectrum antioxidant effects. Optimizing these ratios and concentrations could improve the efficacy of natural formulations for dermatological and therapeutic use.

Cream formulation

Fig.4 illustrates the variations in composition and characteristics across the three batches of cream formulations, produced from aqueous extract of *Hibiscus sabdariffa* and Glycyrrhiza glabra. These differences are critical for evaluating the formulations` suitability as natural antioxidant creams for hyperpigmentation treatment. The observed variations in color among the three batches primarily stem from the different ratios of *Hibiscus sabdariffa* and *Glycyrrhiza glabra* extract. Hibiscus sabdariffa, rich in anthocyanins, imparts a characteristic red color [48], while *Glycyrrhiza glabra* contributes a lighter, yellowish tone due to compounds like glabridin and glycyrrhizin [49]. As in the 1:2 ratio formulation, a higher proportion of Hibiscus extract may result in a deeper red color, enhancing the aesthetic appeal for consumers seeking natural skincare products. Conversely, a balanced 1:1 ratio or higher Glycyrrhiza content may produce a lighter shade, potentially affecting consumer perception of efficacy. Differences in consistency across the formulations are likely influenced by the interaction of aqueous extracts with the cream base, including emulsifiers and stabilizers such as *Aloe vera* and vitamin E [50,51]. For instance, the 1:2 formulation, which exhibited superior antioxidant activity (99.9% reduction against $KMnO_4$ and 70.8% against DPPH at 100 µg/mL), may have a thicker consistency due to the higher Hibiscus content. In contrast, the 1:1 ratio, with 98.5% antioxidant activity, may offer a smoother texture, facilitating easier application and improved skin penetration, the crucial factor for delivering active ingredients effectively in the treatment of hyperpigmentation. The third formulation, presumably with a higher proportion of Glycyrrhiza, might be less viscous, potentially due to the amphiphilic nature of glycyrrhizin, which can affect emulsion stability, as demonstrated by Ageeva *et al.* [14].

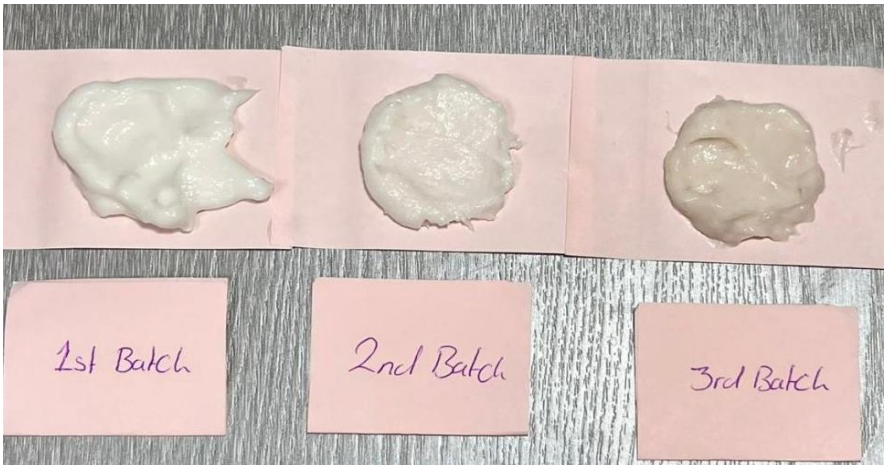


Figure 4: Skin cream produced from aqueous extracts of *Hibiscus sabdariffa* and *Glycyrrhiza glabra* in three different batches

The results of this study demonstrate the strong antioxidant capacity of extracts from *Hibiscus sabdariffa* and *Glycyrrhiza glabra* in topical formulations, especially for the treatment of hyperpigmentation. These botanicals, with their high levels of phenolic, flavonoid, and anthocyanins, provide a natural and safe substitute for the synthetic components found in dermatological products. From an industrial standpoint, this cream looks like a good option for commercialization in the markets for medicinal skincare products and natural cosmetics. Its composition satisfies the rising need for plant-based, clean-label skincare products. The formulation technique is scalable with only slight adjustments, and the active chemicals may be acquired sustainably and economically. Its market potential might be increased by collaborations with cosmeceutical businesses and incorporation into product lines targeted at dermatology. The incorporation of extracts into commercial skincare formulations would be facilitated by further standardization of their composition.

Conclusion

This study successfully developed a natural antioxidant cream using aqueous extracts of *Glycyrrhiza glabra* and *Hibiscus sabdariffa* to address hyperpigmentation. The 2:1 ratio extract demonstrated the highest total phenolic (88.12 µg/mL) and flavonoid (64.35 µg/mL) content, while the 1:2 ratio showed the strongest catechin content (59.61 µg/mL) and antioxidant activity in the $KMnO_4$ assay (99.9%). The 1:1 extract ratio exhibited the most effective DPPH radical scavenging activity (98.5%). HPLC analysis confirmed the presence of potent antioxidant compounds such as chlorogenic acid, catechin, and ellagic acid. The cream formulation containing these extracts retained significant antioxidant activity, favorable texture, and acceptable sensory properties. These findings support the potential of this plant-based cream as a safe, effective, and scalable natural treatment for hyperpigmentation.

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They are available as Supporting information.
Ethics approval and consent to participate:
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The authors declare no conflict of interest.
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