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# Formulation and evaluation of herbal antioxidant cream from selected plants collected from the Nepalese Himalayan region

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**Abstract**

Due to the strong tyrosinase inhibition and antioxidant effects, green tea and licorice are valuable herbal sources in the cosmetic industry for their beneficial effects on the skin. However, data on the addition of essential oils, green tea and licorice, to cream formulations to examine antioxidant activities is limited. Herbal antioxidants fight against radicals, UV rays, and unstable oxygen molecules that affect skin cells and produce wrinkles. The purpose of this study was to develop and assess herbal creams' antioxidant and tyrosinase-inhibitory characteristics using crude aqueous extracts of green tea and licorice loaded with essential oils. To load the best concentration on cream formulations, plant aqueous extracts were designed, evaluated, and correlated in terms of total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Moreover, *Ocimum tenuiflorum* and *O. basilicum* essential oils were extracted and added to a cream formulation. The spreadability profile, water washability, centrifugation test, and organoleptic characteristics of the formulated oil-in-water cream were all satisfactory. The cream exhibited a non-Newtonian rheological profile and a pH range of 6.353±0.065 to 6.467±0.050 over successive 0, 1, 2, and 3 months at normal room temperature. The 50% inhibition concentrations shown by the herbal cream were 13.764±0.153 µg/mL, 301.445±1.709 µg/mL and 8.082±0.055 µg/mL respectively, for 2,2 -diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, ferric ($Fe^{3+}$) reducing antioxidant power (FRAP) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, and that of standard ascorbic acid were 6.716±0.077 µg/mL, 171.604±1.551 µg/mL and 5.645±0.034 µg/mL, which showed the formulated cream had strong antioxidant characteristics. The formulated herbal cream with a 50% tyrosinase inhibition concentration of 22.254±0.369 µg/mL compared to standard Kojic acid 12.535±0.098 µg/mL demonstrated a satisfactory tyrosinase inhibition profile for skin whitening properties. Herbal cream was reportedly stable in physical and chemical parameters for successive 0, 1, 2, and 3 months at both real and accelerated time study zones, according to the results of the stability study. The results provided evidence that formulated creams using plant-based natural ingredients are antioxidant-enriched products for skin protection.

## 1. Introduction

Skin, a large complex barrier organ of the body, structurally and functionally, is important for body immunologic and homeostasis regulation (Khavkin and Ellis, 2011). Being the most voluminous organ of the body, skin's multilayer composition is responsible for several major bodily functions like temperature regulation, nervous system polarization, water system regulation, and being involved in body defensive roles, for instance, mechanical concussions and body protection against foreign chemicals, radiations, and

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microorganisms (Dąbrowska *et al*., 2018; Zhang and Duan, 2018). Skin permeability, a key aspect of maintaining good skin hygiene, varies depending on individual age, ethnicity, food, gender, and body mass index (BMI), which mostly influence cosmetic beauty (Khavkin and Ellis, 2011; Dąbrowska *et al*., 2018). However, physiological and environmental factors like inherited skin conditions, metabolic and hormonal disorders (MHD), prolonged sun exposure, oxidative stress, indoor pollution, an unbalanced lifestyle, and synthetic cosmeceutical heavy metals worsen skin hygiene, hastening the onset of premature skin aging (Bonte *et al*., 2019). Reactive oxygen species (ROS) in the body interfere with the skin turnover cycle, causing dry, sagging skin, degradation of cutaneous and epidermal processes, a decline in skin elasticity and defensive function, and dermal atrophy, which is a sign of aging (Yeh *et al*., 2021). Most commonly, ultraviolet (UV) radiation accumulates free radicals on skin cells, accelerating both chronological and photo-aging phenomena and producing matrix metalloproteinases (MMPs), which inhibit hyaluronic acid (HA) synthesis, depigment skin, disarrange skin collagen and elastin, and cause the dermal extracellular matrix to degenerate over time (Cao *et al*., 2020; Yeh *et al*., 2021). Aging mostly results from bodily oxidative stress exceeding anti-oxidant properties, so in order to prevent early aging, skin care routines today include cosmetic antioxidant supplements, either herbal or synthetic. Antioxidants strengthen the skin's natural defense system, counteracting oxidative free radicals and reducing skin aging. In addition to antioxidant theory, important ongoing study areas for anti-aging techniques include microRNA, cellular senescence, and advanced glycation end products (AGEs) (Eckhart *et al*., 2019; Cao *et al*., 2020). Daily modern cosmetics like gentle surfactant cleansers or anti-pore clogging lotions, skin toners, moisturizer, and cosmeceuticals unblock have significantly slowed down the signs of aging; however, synthetic cosmetics containing parabens preservatives, formaldehyde, diethylhexylphthalates, phenylenediamine, mercury-like heavy metals, sodium lauryl sulphate (SLS) surfactant, and methylene glycol are equally harmful to skin as they cause skin irritation, dryness, hormonal imbalances, and are carcinogenic (Bonte *et al*., 2019; Draelos, 2019). Due to negative effects like redness, stinging, and itching, hydroquinone, the gold standard for skin whitening, is a contentious cosmetic chemical in the Food and Drug Administration (FDA) realm (Draelos, 2019). Additionally, loading broad-spectrum UV filter nano-metal oxides into cosmetics and personal care items like sunscreen, soap, and shampoo demonstrates both dose-dependent toxicity and eco-unfriendliness (Subramaniam *et al*., 2019). Synthetic antioxidants like retinoid derivatives, α-hydroxy acids (AHA), β-hydroxyl-butanoic acid (BHB), idebenone, or polyfluoroalkyl substances (PFAS) are cytotoxic, carcinogenic, and skin irritants, inducing premature senescence. Also, long-term usage of cosmeceutical fragrances has been linked to allergic contact dermatitis (ACD), but combining them with biological sources like crude plant extracts, essential oils, and probiotic cosmetics has demonstrated synergistic benefit, limiting any potential possible skin side effects (Alani *et al*., 2013; Carocho and Ferreira, 2013; Bonte *et al*., 2019; Subramaniam *et al*., 2019). For example, adding complex natural plant extracts or essential oils to synthetic cosmetics can stimulate beneficial skin effects (Jadoon *et al*., 2015).

For healthy skin, phyto-oxidants like terpenes (carotenoids and essential oils) and polyphenols (flavonoids and stilbenes) are excellent green cosmeceutical substitutes and are considered nowadays as innovative beauty products (Jadoon *et al*., 2015; Bonte *et al*., 2019). Botanical creams of arbutin, aleosin, kojic acids, and licorice extract (liquirtin, glabridin, glabrene, and isoliquirtin) are effective natural tyrosinase inhibitors for skin lightening with no adverse effects (Jadoon *et al*., 2015; Draelos, 2019; Ahuja *et al*., 2021). Around 400 isolated flavonoids from licorice, such as glycyrrhizin, glycyrrhetinic acid, glabridin, quercetin, formononetin, hispaglabridin B, and methylglabridin, show potential antioxidant, anticancer, antimicrobial, and anti-inflammatory effects; however, licorice polyphenolic extracts, such as liquiritigenin, isoliquiritigenin, chalcones, and licochalchone C, are also strong skin-lightening agents (Ahuja *et al*., 2021; Wahab *et al*., 2021). Green tea (*Camellia sinensis*) aqueous or ethanolic extraction contains highly enriched green tea polyphenols (GTP) such as gallic acid (GA), gallocatechin (GC), catechin (C), epicatechingallate (ECG), and epigallocatechingallate (EGCG) that improve cell turnover by activating phase II antioxidant enzymes in the body and keep skin hydrated (Forester and Lambert, 2011; Jadoon *et al*., 2015). When green tea (*Camellia sinensis*) extract is combined with other extracts of naturally occurring antioxidant plants like *Ruscus aculeatus, Nelumbo nucifera, Calendula officinalis, Emblica officinalis, Capparis decidua, Castanea sativa, Coffea arabica, Malus domestica, Moringa oleifera, Morus alba,* and *Polygonum minus,* it demonstrates incredible skin anti-aging potential due to their multi-phytochemical synergistic interactions, which are considered more beneficial for skin than loading specific synthetic antioxidants into cosmetic creams (Jadoon *et al*., 2015; Ahuja *et al*., 2021). Topical delivery of natural phenolic acids, flavonoids, or essential oils encapsulated on synthetic lipid cream

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vesicles may potentially rejuvenate aging skin and guarantee effective green antioxidant skin care formulations with safer, eco-friendly ingredients (Ahuja *et al*., 2021; Lohani *et al*., 2021). Research has been done on probiotics found in cosmetic products like *Lactobassilus acidophilus* and V*itreoscilla filiformis* that naturally rebalance skin microbiota, restoring skin signal components, making them more beneficial to skin hygiene in addition to phyto-antioxidant therapies for treating skin aging (Bonte *et al*., 2019; Draelos, 2019; Puebla-Barragan and Reid, 2021). Cosmetic interventions on marine flora like *Sargassum myriocystum* and phytomoisturizers, supplemented with neurotransmitter peptides, are two examples of new replacement tactics in synthetic cosmetic fields that are emerging, but research study fields are still limited to this date (Bonte *et al*., 2019; Draelos, 2019; Subramaniam *et al*., 2019; Siahaan *et al*., 2022). Direct use of crude plant extracts is now a beneficial, quick approach for the commercialization of cosmetic care that provides the finest potential skincare results. Without a doubt, phytocosmetic creams are today's greatest cosmetic skin care products over synthetic ones because they are eco-friendly and give positive skin benefits with little to no side effects. Thus, this study aimed to formulate a novel phytocosmetic cream with essential oils and crude aqueous plant extracts from *Camellia sinensis* leaves and *Glycyrrhiza glabra* roots that show good tyrosinase-inhibitory and antioxidant properties. This study also aimed to provide new research and development (R and D) and secure safer alternatives to cosmeceutical skin-care formulations.

## 2. Materials and methods

### *2.1 Plant material collection*

*Ocimum tenuiflorum* and *O. basilicum* fresh plant samples were collected from Kapilvastu with latitude 27º40'7.9''N and longitude 83º10'12.9''E, and Bardiya with latitude 28º15'14.3''N and longitude 81º30'7.0''E, Nepal, in March/April 2022, respectively (as shown in Figure 1), then authorized by the National Herbarium and Plant Laboratories, Lalitpur, Nepal. Freshly verified *Glycyrrhiza glabraroots* and *Camellia sinensis* leaves were purchased from Herbs Production and Processing Co. Ltd. (HPPCL), Kathmandu, Nepal.

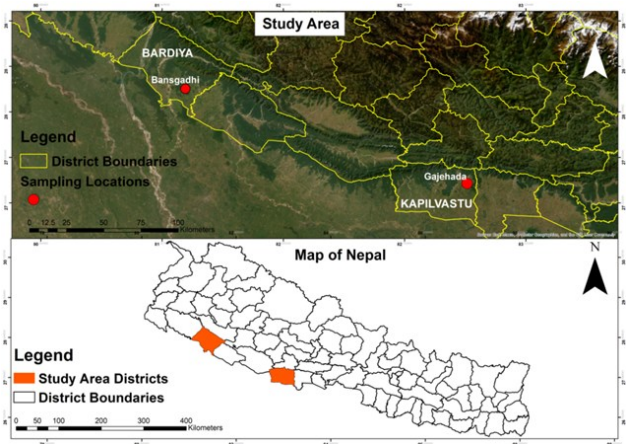


Figure 1. Geographical location of the sample collection sites.

### *2.2 Extraction of essential oils*

Each plant (100 g) was subjected to hydro distillation for 3 hrs in 500 mL of distilled water on a Clevenger apparatus (Jain Scientific Glass Work, JSGW, India) after shade-drying and chopping the aerial parts of *O. tenuiflorum* and *O. basilicum*. The extracted essential oils were dried with activated sodium sulphate, filtered, and stored at 4℃ until further analysis and cream formulation.

### *2.3 Plant extraction*

Each plant of 5 g sun-dried powder of *Glycyrrhiza glabra* roots and *Camellia sinesis* leaves was extracted separately in 50 mL of distilled water and heated in an enclosed 100 mL beaker at 70-75℃ in a heating mantle (Biobase 1000 mL capacity, Germany) for 45 mins. Such crude aqueous extracts were collected, stored, and labelled in a clean 100 mL centrifuge tube separately, after being filtered by filter paper (Whatman GE Healthcare, Cat No. 1001 125, UK) (Whangsomnuek *et al*., 2019; Kurzawa *et al*., 2022).

Table 1. Optimal concentration design for each aqueous plant extracts.

| Design | *Camellia sinesis* extraction (V1) | *Glycyrrhiza glabra* extraction (V2) |
|---|---|---|
| D1 | -1 | -1 |
| D2 | -1 | 0 |
| D3 | -1 | +1 |
| D4 | 0 | -1 |
| D5 | 0 | 0 |
| D6 | 0 | +1 |
| D7 | +1 | -1 |
| D8 | +1 | 0 |
| D9 | +1 | +1 |

### *2.4 Design for aqueous extracts*

The optimal aqueous extract load on cream formulation was designed using the Statgraphics 19 Centurion programme with three level designs, -1, 0, and +1, which corresponded to 0.5 g, 1.5 g, and 3 g of each aqueous extract, as shown in Table 1. For instance, D3 signified 0.5 g of V1 and 3 g of V2 to load on the cream formulation.

### *2.5 Total phenolic content and total flavonoid content of aqueous extract determination*

With a small modification to the previously

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described methods, Srisuksomwong *et al*. (2023) and Mungmai *et al*. (2019) were used to calculate the total phenolic content (TPC). Each 250 µL of design extract was mixed with 1250 µL of deionized water and 250 µL of ethanol (95%). Then, each mixture was subjected to 125 µL of 50% phenol reagent (Fisher Scientific, Prod. No. 35953, India), shaken for 3 mins, and 250 µL of 5% $Na_2CO_3$ added at room temperature. The absorbance of each mixture was measured at 725 nm in a UV spectrophotometer (UV-1800 Shimadzu, Japan) after only 1 hr of sample incubation at a dark room temperature. Standard gallic acid (Sigma-Aldrich, Phytolab GmbH and Co.KG, Germany) was produced in methanol at varied concentrations of 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, and 100 µg/mL, and the absorbance was measured using the same technique as for samples for developing a calibration curve and calculating TPC for samples represented as gallic acid equivalent GAE (mg/g) (Genwali *et al*., 2013; Agbor *et al*., 2014). Triplicate data were taken.

Total Flavonoid Content (TFC) for an aqueous extract was determined using the aluminium chloride colorimetric method with a small modification to the standard procedure previously described by Madaan *et al*. (2011) and Phosri *et al*. (2022). A mixture of 50 µL of 10% $AlCl_3$ and 750 µL of methanol was added to each 250 µL aqueous extract. The mixture was shaken and incubated for 30 mins at dark room temperature after the addition of 50 µL potassium acetate (1M) and 1400 µL deionized water. Absorbance was measured in triplicate for each design at 415 nm via a UV spectrophotometer (UV-1800 Shimadzu, Japan) (Srisuksomwong *et al*., 2023). Standard quercetin (Sigma-Aldrich, USA) was produced in absolute ethanol at varied concentrations of 6.25 µg/mL to 100 µg/mL by serial dilution, and the absorbance was measured three times using the same technique as for samples for developing a calibration curve and calculating TFC for samples represented as quercetin equivalent QE (mg/g).

### *2.6 Screening for DPPH activity in aqueous extract designs and essential oils*

The DPPH colorimetric method, which was slightly modified from the previously described standard method (Paudel *et al*., 2022), was used to determine antioxidant properties. Through serial dilutions, different aqueous extracts and essential oils were developed in ethanol with concentrations ranging from 15.625 µg/mL to 1000 µg/mL. Approximately 3.9 mg DPPH (Sigma-Aldrich, MO, USA) was diluted in 100 mL of ethanol for 0.1 mM DPPH stock solution preparation. Each 2 mL of different sample concentrations was mixed with 2 mL of DPPH (0.1 mM) solution, and the mixtures were allowed to stand in a dark room at room temperature for 30 mins before being measured in triplicate at 517 nm on a UV spectrophotometer (UV-1800 Shimadzu, Japan). The absorbance of control solution (2 mL DPPH stock solution and 2 mL ethanol) and blank solution (2 mL ethanol) was also measured at 517 nm, and the $IC_{50}$ value was calculated using the linearity of the DPPH scavenging effect (%) given by the authors (Hasan *et al*., 2009; Kurzawa *et al*., 2022).

DPPH scavenging effect (%) = [(Absorbance of control-Absorbance of sample)/Absorbance of control] × 100

### *2.7 Pre-formulation physical compatibility test*

Each excipient from the cream formulation was mixed in a 1:1 ratio with plant extracts and synthetic actives for 10 mins before being placed in separate vials at room temperature with direct sunlight exposure for 1 month to maximize damage effects. The physical changes observed included texture, homogeneity, odor, and any color development (Liao *et al*., 2022).

### *2.8 Antioxidant-enriched phytocosmetic cream formulation*

The oil phases were heated at 70-80°C and the water phases were heated at 50-55°C with continuous stirring. The heated oil phase was added slowly in the water phase at a maintained temperature of 50°C with continuous stirring at 2000 rpm for 25 mins, and the cold phases were added with increasing speed to 2500 rpm for 10 mins. During the mixing process of the cold phase, the optimal aqueous extract and essential oil were loaded, and the cream was cooled to room temperature (Tan *et al*., 2022). *Ocimum basilicum* essential oil was added via syringe drop as a fragrance. Carbopol-940 was soaked one night before addition to the formulation. The different compositions of formulated cream are presented in Table 2.

The design of aqueous plant extracts was loaded onto cream by best demonstrating TFC, TPC, and DPPH activity. In addition, *O. tenuiflorum* essential oil was loaded with an $IC_{50}$ value calculated from DPPH. As a fragrance, a syringe drop of *O. basilicum* essential oil was added.

### *2.9 Organoleptic characteristics*

Physical texture, homogeneity, and smoothness were evaluated by pressing a 1 g cream between the thumb and index finger. Odor and color were also accessed (Tan *et al*., 2022). Cream was inverted at 180º to observe any flow properties.

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Table 2. Herbal cream composition.

| Ingredient | Quantity for 100 g (%) |
|---|---|
| Water phase | |
| Glycerin | 9.0 |
| Sodium benzoate | 0.3 |
| Hyaluronic acid | 0.2 |
| Allantoin | 0.5 |
| Distilled water | q.s |
| Oil phase | |
| Cetyl alcohol | 3.0 |
| Arlacel | 2.0 |
| Liquid paraffin | 5.0 |
| Beeswax | 1.0 |
| Glycerylmonostearate | 2.0 |
| Cold phase | |
| Dimethicone | 3.0 |
| Triethanol amine | 0.3 |
| Cyclopentasiloxane | 5.0 |
| Potassium sorbate | 0.3 |
| Retinol | 0.2 |
| Niacinamide | 0.5 |
| Carbopol-940 | 0.2 |
| Magnesium aluminum silicate | 0.8 |
| Guar hydroxyl propyltrimonium chloride | 0.2 |
| Zincoxide | 0.1 |
| *Ocimum basilicum* | 0.1 |

*2.10 Cream type determination and water washability test*

A dilution test was carried out with a 1:10 ratio of cream to distilled water for the miscibility test with water. Oil-in-water (o/w) type cream shows good miscibility with water and vice versa (Ahmed and Nath, 2017). Also, the microscopic view (40×) for base cream and herbal loads was observed separately on the microscope (OptiCx, LABOMED, India) (Glusac *et al*., 2018).

On the upper hand's skin section, 0.2 g of cream was applied, and the area was gently washed under running water. A good washability cream can be easily removed from the application site and leaves the skin's surface without being greasy (Yadav *et al.*, 2014; Tan *et al*., 2022).

*2.11 Cream spreadability, pH determination, and centrifugation test*

A glass slide, into which 200 g of weight was placed, was used to press 0.5 g of cream placed in center of another glass slide for 2 mins at room temperature. The diameter of the cream was measured in triplicate for three months. Spreadability of 5-7 cm demonstrates that cream can be easily distributed with little effort on the site of application and shows uniform particle distribution (Yadav *et al*., 2014; Tan *et al*., 2022).

The pH of a 10% w/v cream suspension in distilled water was measured three times at 25℃ for three months using a pH meter (HI 2210 pH meter, Italy), which was previously calibrated using standard buffers with pH 4, 7, and 10, respectively. An effective cream has a skin physiological pH range of 6-8 (Pinto *et al*., 2021; Tan *et al*., 2022).

About 2 g of cream was centrifuged at 5000 rpm for 10 mins at 25℃ and emulsion instability (%) was calculated using the following equation (Restu *et al*., 2015) for three months;

Emulsion instability (%) = [Emulsion separation (cm)/ total emulsion height (cm)] × 100%

An ideal unstable cream emulsion has 100% emulsion instability (Restu *et al*., 2015; Tan *et al*., 2022). This experiment was repeated three times.

*2.12 Rheology profile study*

The formulated cream was directly immersed into the Brookfield viscometer (DV III Ultra, Italy) with spindle no. 64, initially with an adjusted rpm of 1 to 10 at room temperature and later with a shear rate of 17-102 $s^{-1}$ (Gyawali *et al*., 2020), where the shear rate was 1.7 times the adjusted spindle rpm. The final results were represented as a graph of viscosity (Pascal-second) vs. shear rate ($s^{-1}$). The thixotropic profile was also studied as a function of shear stress (Pascal) vs. shear rate ($s^{-1}$), which demonstrates that a good thixotropic formulation quickly restores to its initial position and is therefore thought to be stable (Almeida *et al*., 2015; Gyawali *et al*., 2020; Pinto *et al*., 2021). The measured dial readings were multiplied by a factor of 10 to give viscosities in centipoise (cP) or milipascal-second (mPa.s), where 1cP = 1mPa.s.

*2.13 Antioxidant assays*

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, ferric ($Fe^{3+}$) reducing antioxidant power (FRAP), and 2,2'-azinobis-3-ethylbenzothiazoline -6-sulfonic acid (ABTS) radical scavenging assay were performed to determine cream antioxidant activity.

The cream was produced on ethanol from 1.25 µg/ mL to 40 µg/mL on serial dilution after filtration using the DPPH method as mentioned above for aqueous extract designs. A standard ascorbic acid (Fischer Scientific, India) sample was also prepared in ethanol to various concentrations ranging from 1.25 µg/mL to 40 µg/mL by serial dilutions, and absorbance was measured as described above. This experiment was carried out three times (Paudel *et al.*, 2022; Phosri *et al*., 2022).

A slight modification of the previously established

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protocol was used for the FRAP assay (Park *et al.*, 2008). All chemicals for this assay were purchased from Fischer Scientific, India. By serial dilutions, standard ascorbic acid and cream formulations were developed in ethanol to various concentrations ranging from 31.25 µg/mL to 500 µg/mL. Freshly prepared solutions of 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%) were added to each 1 mL sample preparation and incubated for 20 mins at 50°C before adding 2.5 mL trichloroacetic acid (10%) and centrifuging for 10 mins at 1000 rpm. 2.5 mL of each collected supernatant was diluted to 5 mL with distilled water before adding 0.5 mL of $FeCl_3$ (0.1%) by vigorous shaking at room temperature. After 30 mins, absorbance at 700 nm was measured three times on a 96-microplate reader (BioTek, EPOCH).

A graph of mean absorbance vs. sample concentration was plotted to compare effective concentrations ($EC_{50}$-value, µg/mL) with standard ascorbic acid, where the $EC_{50}$ value represents the effective reducing concentration of the sample with a mean absorbance value of 0.5 (Paudel *et al.*, 2022; Phosri *et al*., 2022). The lower the $EC_{50}$ value, the greater the antioxidant capacity of the sample to reduce ferric ($Fe^{3+}$) ions to ferrous ($Fe^{2+}$) ions.

For the ABTS radical scavenging assay, a slightly modified version of the previously described method was used (Re *et al*., 1999). Standard ascorbic acid (Fischer Scientific, India) and cream formulations were developed in methanol using serial dilutions to concentrations ranging from 1.25 µg/mL to 40 µg/mL. A 7 mM ABTS (38.402 mg of ABTS in 10 mL distilled water) (Glentham Life Sciences, UK) was mixed with potassium per sulfate (6.600 mg) to give the final ABTS stock (10 mL, 2.45 mM) and allowed to stand at room temperature for 14-16 hrs to form a stable oxidized ABTS radical. This working ABTS stock solution was diluted with methanol (1:90 v/v) until the absorbance at 734 nm was 0.70±0.02. Now 140 µL of ABTS stock solutions were added to each 70 µL of sample preparation in a 96-well plate, incubated for 10 mins at dark room temperature, and triplicate absorbance was measured at 734 nm using a 96-microplate reader (Bio Tek, EPOCH). Using the given equation (Shah and Modi, 2015), the linear percentage inhibition concentration (IC%) was calculated, and an $IC_{50}$% value was compared with the standard.

IC % = [(mean absorbance of control-mean absorbance of sample)/mean absorbance of control] × 100

As a control sample, 70 µL of methanol was mixed with 140 µL of ABTS stock solutions.

*2.14 Mushroom tyrosinase inhibition study*

The inhibition activity of the tyrosinase in cream formulation was studied using the standard procedure described by Pandey et al. with minor modifications (Pandey *et al*., 2022). All chemicals were obtained from Sigma Chemical Co., USA. The substrate for this reaction was 1-4-dihydroxyphenylalanine (L-DOPA). The positive control was Kojic acid. Cream and Kojic acid were developed using serial dilutions of ethanol concentrations ranging from 1.25 µg/mL to 80 µg/mL. 685 µL of freshly prepared phosphate buffer (0.05 M, pH 6.5), 15 µL of mushroom tyrosinase (2500 units/mL), 200 µL of cream concentrations, and 100 µL of L-DOPA were allowed to react before immediately measuring the mixture reaction as dopachrome formation at 492 nm in a 96-Microplate reader (Bio Tek, EPOCH). This experiment was repeated three times. The effective concentration to inhibit 50% of mushroom tyrosinase ($IC_{50}$%) was calculated using a linearity graph of % inhibition vs. concentration, as given by equations (Di Petrillo *et al*., 2016; Vichit and Saewan, 2022):

Tyrosinase inhibition (%) = [(mean absorbance of control-mean absorbance of sample)/mean absorbance of control] ×100

In place of cream concentrations, 200 µL of ethanol was added as a control sample, and the absorbance was measured at 492 nm using the same method. The best tyrosinase inhibition property favorable for whitening impact is shown by a lower value of $IC_{50}$ (%).

*2.15 Stability study*

The formulated cream was subjected to ICH climatic zone 30±2°C and relative humidity (RH) 75±5% for 3 months. Also, the freeze-thaw cycling test for accelerated stability was carried out for 48 hrs at 5-8°C and 48 hrs at 40-42°C for three successive months. The cream's spreadability, pH, centrifugation, and antioxidant activities were determined in triplicate as previously described methods (Pinto *et al*., 2021; Plyduang *et al*., 2022).

*2.16 Statistical data analysis*

All independent experiments were run three times (n = 3), and mean ± standard deviation (SD) was used to express the data. The figures for $IC_{50}$ (%) values were computed using Microsoft Excel OriginPro 2016 64Bit (Origin version 9.3, USA). The sampling site map was made using GIS software (ArcGIS software, USA). To design the optimal aqueous extract loading concentration for analysis, Statgraphics19 Centurion (Statgraphics Technologies, Inc., USA) software was used.

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## 3. Results and discussion

### *3.1 Screening for total phenolic content, total flavonoid content, and DPPH activity in aqueous extract designs, and DPPH activity in essential oil*

The TPC, TFC, and DPPH activities for various aqueous designs and essential oils are shown in Figure 2. The aqueous design D3 had the highest TPC value of 294.029±0.227 mg GAE/g, whereas D5 had a lower value of 174.059±0.325 mg GAE/g compared to standard gallic acid, which has a linear correlation coefficient $R^2$ = 0.991, y = 0.011x+0.059. Also, D3 showed the highest TFC value of 88.504±0.315 mg QE/g, while D4 showed a lower TFC value of 10.289±0.253 mg QE/g compared to standard quercetin, which has a linear correlation coefficient $R^2$ = 0.991, y = 0.013x+0.173. Since phenolics and flavonoids are potent antioxidants, the higher the TPC and TFC values in an aqueous design, the more skin-beneficial properties are present (Kurzawa *et al*., 2022). This data showed that there were more hydrophilic polyphenolic compounds like gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), epigallocatechingallate (EGCG), and coumaroylquinic acid (CA) in crude aqueous extract than hydrophobic flavonoids (glycyrrhizin, glycyrrhetinic acid, and glabridin), as comparatively all designs had more TPC value than TFC value, since most of Licorice flavonoids are hydrophobic (Forester and Lambert, 2011; Tungmunnithum *et al*., 2018; Wahab *et al*., 2021). Secondary plant metabolites, such as phenolic and flavonoid chemicals like EGCG, EC, GA, isoliquiritigenin, and chalcones, are primarily hydrophilic and have significant UV protective benefits for sun-damaged skin (Forester and Lambert, 2011; Tungmunnithum *et al*., 2018; Wahab *et al*., 2021).

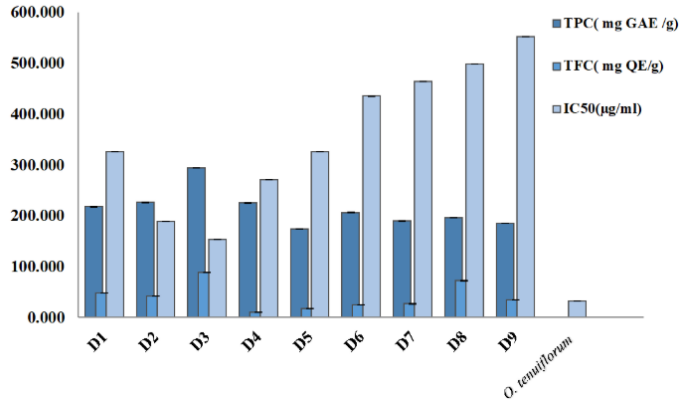


Figure 2. TPC, TFC and DPPH activities ($IC_{50}$) in aqueous extract designs and essential oil. Data presented are in mean±SD (n = 3).

The designs D2 and D3 demonstrated stronger DPPH antioxidant activities (low $IC_{50}$ values) than other crude aqueous extracts since low $IC_{50}$ values indicate that lower concentrations (µg/mL) of extracts are required to scavenge free radicals of DPPH. The D3 demonstrated strong antioxidant activity with an $IC_{50}$ value of 153.481±0.002 µg/mL, and the D9 demonstrated weaker antioxidant activity with an $IC_{50}$ value of 552.047±0.002 µg/mL, however, *O. tenuiflorum* essential oil demonstrated stronger antioxidant activity than all other crude aqueous designs with an $IC_{50}$ value of 32.073±0.020 µg/mL since *Ocimum* species essential oils show potential antioxidant properties (Awah and Verla, 2010; Chaudhary *et al*., 2020). Therefore, essential oil of *O. tenuiflorum* 33 µg/mL was loaded by syringe drop into the cream with aqueous design D3, as it showed stronger antioxidant activity than other herbal extracts during the screening process. As an initial step in the skin-anti-aging strategy, it is believed that the synergistic addition of this chosen concentration of plant extracts, when incorporated with cream bases, can strongly reduce oxidative stress on the skin by demonstrating high antioxidant capacity (Awah and Verla, 2010).

However, the correlation of DPPH activity ($IC_{50}$, µg/mL) with TPC (mg GAE/g) and TFC (mg QE/g) for aqueous designs (D1-D9) is shown in Figure 3 (a, b). The total phenolic content in aqueous extracts like EGCG, EG, GA, and chalcones contributed weakly to antioxidant activity, as indicated by the 0.562 correlation coefficient ($R^2$) between DPPH activity ($IC_{50}$, µg/mL)

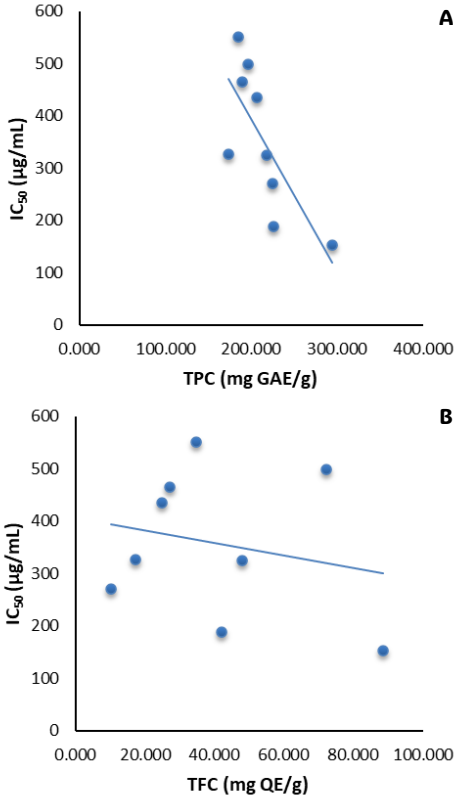


Figure 3. Correlation between DPPH activity ($IC_{50}$, µg/mL): (A) with TPC (mg GAE/g), $R^2$=0.562 demonstrated lesser correlation, (B) with TFC (mg QE/g), $R^2$=0.048 demonstrated no significant correlation. Data presented are in mean±SD (n = 3).

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and TPC (mg GAE/g), which also indicated a lower value of $IC_{50}$ (µg/mL) on a greater TPC (mg GAE/g) value. Moreover, no significant correlation ($R^2$ = 0.048) was found between total hydrophilic flavonoids and antioxidant properties in this present study, yet phenolic and flavonoid contents are both crucial for skin-beneficial properties (Kumar *et al*., 2014).

*3.2 Physical compatibility test*

The plant extracts and synthetic actives showed no characteristic change (NCC) in color, texture, homogeneity, or odor when mixed with cream-based excipients for a month in separate vials. Physical compatibility testing of excipients is required because it is important in product development and commercialization, allowing for the selection of the best excipients and maximizing shelf life (Tan *et al*., 2022).

*3.3 Organoleptic characteristics*

The organoleptic characteristics of the cream were evaluated physically. The prepared cream had a uniform texture that was smooth and calming, with no grit or greasiness at all. It had a light yellowish-brown color and a fresh *O. basilicum* aroma because it was loaded with essential oils and aqueous plant extracts. At a 180º inversion, the cream did not flow, an optimal requirement for topical application (Salehi *et al*., 2022).

*3.4 Cream-type determination and water washability test*

The cream showed good miscibility with water and easy water-washing properties. A good oil-in-water (o/w) type cream is less greasy than a water-in-oil (w/o) type cream and is mostly acceptable cosmetically (Mishra *et al*., 2014). The formulated herbal cream enriched with added phyto-antioxidants is shown in Figure 4. The microscopic view of herbal cream is also shown in Figure 5.

Figure 4. Formulated herbal cream enriched with added phyto -antioxidants.

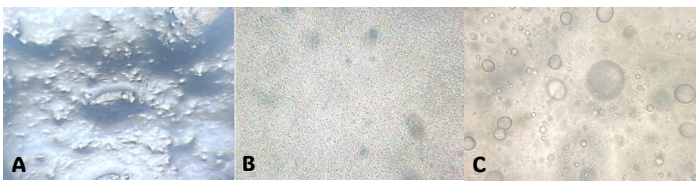


Figure 5. Microscopic profile (40×) representation, A: homogeneous distribution of cream base only, B: loaded cream base with emulsified aqueous extract design (D3), C: visible cream base with aqueous extract design (D3) and essential oil droplets seen (final herbal cream).

*3.5 Cream spreadability, pH determination, and centrifugation test*

The results for cream spreadability, pH determination, and centrifugation tests for both real-time and accelerated studies (freeze-thaw cycling) are presented in Table 3. The spreadabilities and cream pH over the course of all studies were within acceptable limits (Yadav *et al*., 2014; Pinto *et al*., 2021; Tan *et al*., 2022). The emulsion instability (%) ranged from 8.222±1.678 to 13.333±0.667, which were comparatively low and showed that the formulated cream was a good stable emulsion in both real and accelerated time studies (Restu *et al*., 2015). While pH showed no significant change, freeze-thaw cycling had little effect on cream spreadability and centrifugation parameters. This is because cream viscosity changes under accelerated conditions (Tan *et al*., 2022), whereas no significant pH change demonstrated that loaded active herbal extracts were in good condition for both long-term real and accelerated conditions. Creams with a pH close to that of the skin (pH values 5-7) irritate the application site less. Any topical cosmeceuticals having a good spreadability index (5-7 cm) show that they can be administered on the skin surface with little effort (Yadav *et al*., 2014; Tan *et al*., 2022).

*3.6 Rheology profile study*

A non-Newtonian rheology profile was shown by the herbal cream since, with increasing shear rate ($s^{-1}$), the viscosity (Pa.s) had decreased but not linearly. When the viscosity (Pa.s) of cream decreased as shear stress (Pa) was raised, this behavior was more accurately characterized as shear thinning (pseudoplastic) behavior. When this cream was applied to the skin surface, the viscosity would increase because the shear stress was initially low, but when gently rubbed, the viscosity would decrease because it behaved more like a shear thinning profile than a shear thickening profile, as shown in Figure 6. The initial apparent viscosity was highest at 101.667±2.082 Pa.s at a shear rate of 1.7 $s^{-1}$, as shear stress was low initially (56.000±2.000 Pa), but apparent viscosity decreased with increasing shear rate ($s^{-1}$). Figure 6 illustrates the thixotropic profile of the cream. The pseudoplastic profile of cosmetic formulations is advantageous because it improves cream spreadability, which in turn improves skin permeability as rubbing time increases shear stress (Korhonen *et al*., 2001). The stability of the product is also guaranteed by the rheological properties of the cream; for instance, a thermodynamically unstable cream exhibits phase separation as a result of low interfacial viscous forces between two different phases (oil/water) (Korhonen *et al*., 2001; Glusac *et al*., 2018).

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Table 3. Cream spreadability (cm), pH and centrifugation test (%) data at room temperature and accelerated stability study (freeze-thaw cycling).

| Duration (months) | Room temperature | | | Freeze-thaw cycling | | |
|---|---|---|---|---|---|---|
| | Spreadability (cm) | pH | Centrifugation (%) | Spreadability (cm) | pH | Centrifugation (%) |
| 0 | 6.133±0.058 | 6.467±0.050 | 8.222±1.678 | 5.633±0.153 | 6.357±0.055 | 10.000±2.000 |
| 1 | 6.000±0.173 | 6.420±0.035 | 10.667±1.155 | 5.533±0.115 | 6.260±0.017 | 11.556±0.385 |
| 2 | 5.833±0.208 | 6.420±0.092 | 8.889±0.770 | 5.467±0.115 | 6.327±0.025 | 13.333±0.667 |
| 3 | 5.567±0.208 | 6.353±0.065 | 10.000±1.333 | 5.433±0.153 | 6.230±0.062 | 12.444±0.770 |

Values are presented as mean±SD of independent triplicate (n = 3).

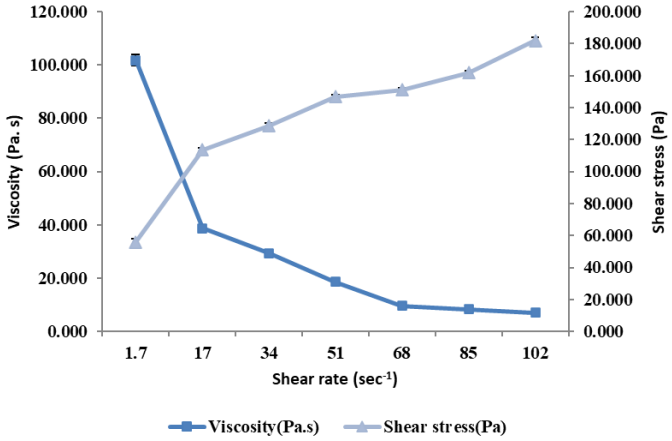


Figure 6. Rheology profile for formulated herbal cream represented graphically. At each shear rate ($s^{-1}$), the viscosity (Pa.s) and shear stress (Pa) were varied (pseudo plastic behavior). Data presented are in mean±SD (n = 3).

### *3.7 Antioxidant assays and mushroom tyrosinase inhibition study*

The antioxidant and tryosinase inhibition activities of the formulated phytocosmetic cream are given in Figure 7. Antioxidant supplements, an anti-aging marker, can help to improve skin aging caused by ROS stress (Jadoon *et al*., 2015; Bonte *et al*., 2019). A complex group of botanical extract-loaded creams have skin-protective properties; for instance, they act against skin wrinkle formation and lesions due to their antioxidant phytochemicals (Jadoon *et al*., 2015). Skin aging causes hyperpigmentation of the skin due to excessive Tryosinase, a key enzyme for melanin production that, when inhibited, reduces melanogenesis, is a key strategy for skin depigmentation and the leading anti-aging strategy. For example, synthetic tryosinase inhibitors such as hydroquinone and kojic acid are cosmetic anti-pigmenting agents (Di Petrillo *et al*., 2016; Vichit and Saewan, 2022). In this study, the DPPH activity $IC_{50}$ value shown by the herbal cream was 13.764±0.153 µg/mL, whereas the standard ascorbic acid showed 6.716±0.077 µg/mL, indicating that this cream had good antioxidant activity for skin beneficial properties. Also, the $EC_{50}$ values for cream and ascorbic acid in the FRAP assay were 301.445±1.709 µg/mL and 171.604±1.551 µg/mL, respectively. This FRAP assay also indicated that herbal cream had higher antioxidant properties than standard ascorbic acid. Furthermore, the ABTS scavenging activity of herbal cream was ($IC_{50}$ value) 8.082±0.055 µg/mL and that of standard ascorbic acid ($IC_{50}$ value) was 5.645±0.034 µg/mL, indicating cream had a potent antioxidant property. All of the antioxidant activities demonstrated by herbal creams indicated that the formulation was enriched with antioxidant chemicals such as polyphenol compounds or flavonoids, which are required for any phytocosmetic cream for skin anti-aging purposes because antioxidant properties maintain skin stress and turnover rate by interfering with any ROS in the body. The hydrophilic polyphenolic compounds from green tea, like GA, GC, C, EC, EGC, ECG, and EGCG, are more responsible for antioxidant activities in this formulated cream from other synthetically loaded actives like LMWHA (low molecular weight hyaluronic acid), Retinol, and Niacinamide (Forester and Lambert, 2011; Wahab *et al*., 2021), since the aqueous extract design D3 TPC (mg GAE/g) was significantly correlated with DPPH activity ($R^2$ value 0.562) than TFC (mg QE/g). However, loading essential oil of *O. tenuiflorum* ($IC_{50}$ value 32.073±0.020 µg/mL) in this cream demonstrated a good synergistic antioxidant effect with herbal aqueous extracts because the cream showed a low antioxidant effective concentration value as compared to loaded essential oil and aqueous designs, as it is believed that loading complex plant extracts with essential oil shows synergistic antioxidant effect in any cosmetic formulation (Jadoon *et al*., 2015).

Kojic acid ($IC_{50}$ value 12.535±0.098) inhibited the tryosinase enzyme more strongly than herbal cream ($IC_{50}$ value 22.254±0.369). However, because both kojic acid and herbal cream showed comparable inhibition concentrations, this formulated cream also strongly showed potent tyrosinase inhibition. This high tryosinase activity was attributed to the combination of hyaluronic acid with licorice plant extracts (liquirtin, glabridin, glabrene, and isoliquirtin) (Chaiyana *et al*., 2020; Ahuja *et al*., 2021). Other actives, such as retinol, niacinamide, green tea extracts, and essential oils, also contributed to the inhibition of mushroom tyrosinase activity. This tryosinase inhibition strategy will be an excellent starting point for the skin anti-aging process because it will cure skin hyperpigmentation, which is the first sign of aging

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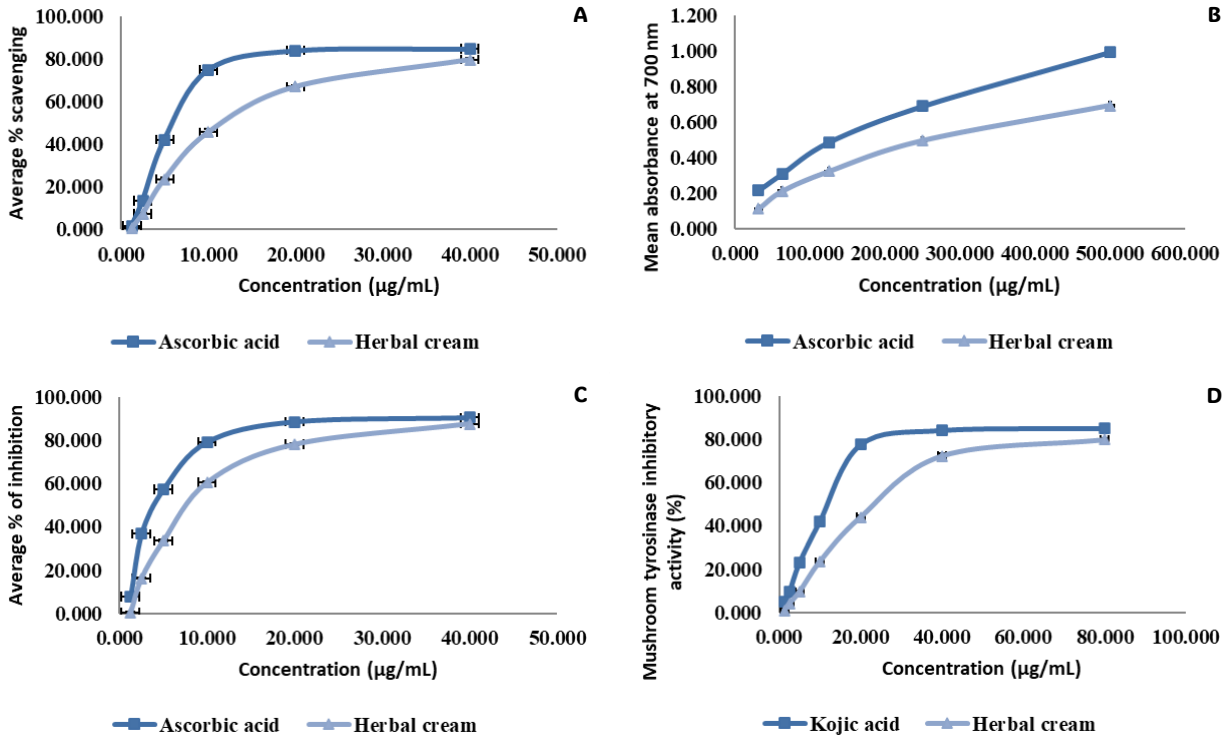


Figure 7. Antioxidant activities and mushroom tyrosinase inhibition shown by herbal cream at various concentrations, A: DPPH radical scavenging activity, B: FRAP assay, C: ABTS radical scavenging activity and D: mushroom tyrosinase inhibition activity. Data presented are in mean±SD (n = 3).

Table 4. Herbal cream antioxidant activities (DPPH scavenging activity, FRAP assay and ABTS scavenging activity) data at room temperature and accelerated stability study (freeze-thaw cycling).

| Duration (months) | Room temperature | | | Freeze-thaw cycling | | |
|---|---|---|---|---|---|---|
| | DPPH activity ($IC_{50}$, µg/mL) | FRAP assay ($EC_{50}$, µg/mL) | ABTS activity ($IC_{50}$, µg/mL) | DPPH activity ($IC_{50}$, µg/mL) | FRAP assay ($EC_{50}$, µg/mL) | ABTS activity ($IC_{50}$, µg/mL) |
| 0 | 13.764±0.153 | 301.445±1.709 | 8.082±0.055 | 12.894±0.103 | 300.871±2.001 | 7.985±0.225 |
| 1 | 13.284±0.255 | 298.542±0.564 | 8.075±1.254 | 12.845±0.158 | 300.744±0.471 | 7.994±0.364 |
| 2 | 13.294±0.345 | 301.024±0.366 | 8.014±1.244 | 12.864±0.361 | 299.687±0.334 | 7.962±1.104 |
| 3 | 13.505±0.399 | 300.014±0.214 | 8.039±0.874 | 12.860±0.247 | 300.101±0.124 | 7.914±0.941 |

Values are presented as mean±SD of independent triplicate (n = 3).

(Di Petrillo *et al*., 2016; Vichit and Saewan, 2022).

### *3.8 Stability study*

The results of cream spreadability, pH, and centrifugation tests for three months (0, 1, 2, and 3) under both normal and accelerated time conditions are shown in Table 3. The antioxidant activities of the cream in both real-time and accelerated time were not considerably impacted (Table 4), this means that all loaded herbal or synthetic actives remained stable during the analysis period without noticeably degrading (Tan *et al*., 2022). This evidenced that the formulation of phytocosmetic cream was good in terms of stability for long-term storage, which is a necessary quality for any stable cosmetic cream.

## 4. Conclusion

The crude aqueous extracts of *C. sinensis* leaves and *G. glabraroots* had good TPC and TFC properties, while GTPs like GA, GC, C, EC, EGC, ECG, and EGCG were more correlated with antioxidant properties than TFC; however, the essential oil of *O. tenuiflorum* showed stronger antioxidant properties than all. When these phytoextracts were loaded on cream emulsions containing synthetic actives like retinol, hyaluronic acid, and niacinamide, the cream demonstrated a synergistic antioxidant effect with significant mushroom tryosinase inhibition. Because the cream was made with a natural fragrance from *O. basilicum* essential oil and superiorcosmetic-grade chemicals, the physical and chemical characteristics of the cream demonstrated acceptance results in both real and accelerated time zones. This finding suggests the successful commercialization of phytocosmetic creams that contain local crude plant aqueous extracts rich with essential oils that are extremely concentrated in antioxidants and mushroom tryosinase inhibition properties, a first-step natural remedy for skin aging.

## Conflicts of interest

https://doi.org/10.26656/fr.2017.9(3).054

The authors declare no conflicts of interest.

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