



Formulation And Evaluation of Moisturizing Cream Containing *Hibiscus Rosa-Sinensis* Extract

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ABSTRACT

Background: Herbal cosmetics are gaining popularity due to their wide range of phytochemical properties. The powerful antioxidant activity of Hibiscus rosa-sinensis (HRS) is attributed to its phenolic and flavonoid compounds. HRS is a good option for topical products meant to safeguard and rejuvenate skin because of its bioactive ingredients. **Aims:** The study aimed to formulate and evaluate herbal cosmetic creams containing Hibiscus rosa-sinensis extract, focusing on phytochemical composition, antioxidant activity, stability, and rheological characteristics. **Methodology:** Phytochemical screening, total phenolic content (TPC), and total flavonoid content (TFC) were evaluated to confirm the extract's antioxidant potential (TPC: 78.03±1.50 mg QE/g, TFC: 92.03±1.74 mg QE/g). Antioxidants capacity was assessed using DPPH and reducing power assays, showing inhibition levels up to 92.12%. Three cream formulations (F1 and F2 containing 4% of extract and F3 (placebo) were prepared and analyzed for organoleptic properties, stability under varying temperature, conductivity, and rheological behavior. Stability and parameters were recorded after storage at accelerated conditions. **Results:** Phytochemical evaluation confirmed the presence of strong antioxidant constituents. All formulations showed excellent physical stability, with no phase separation or major changes in organoleptic properties across different storage conditions. Conductivity remained consistent, and rheological analysis indicated desirable pseudo plastic, shear-thinning behavior ideal for topical applications. The optimized formulations demonstrated ease of application, good spread ability, and compliance with cosmetic quality standards. **Conclusion:** Overall, Hibiscus rosa-sinensis extract is used to make stable, smooth, and skin-beneficial cream. It is a natural and safe ingredient that works well in skincare products.

INTRODUCTION

Many people suffer from skin issues like flaking, edema, irritation, itching, and dehydration. Numerous poor countries account for 21–87% of dermatological diseases, according to the World Health Organization (WHO). About 32.4% of Indians claimed to have "sensitive" or "exceptionally hypersensitive" skin, with 27.9% of them being men and 36.7% being women. Nearly 75 percent of

young people use moisturizing products on a regular basis; several of them have soothing properties when utilized on rashes or damaged skin, and they may stop allergic reactions to the skin. Like, glycerin stimulated enzymes to be the in charge of appropriate desquamation and dimethicone is a palliative that softens and hydrates the skin (Gala et al., 2022). The plant Hibiscus rosa sinensis is used for a number of significant pharmacological activities,

including: memory enhancement, hepatoprotective, fibrinolytic, antitussive, neuroprotective, gastroprotective, immune response, antipyretic, anti-inflammatory, anti-cancer, antioxidant, antibacterial, anti-diabetic, wound and healing, antifungal, antimicrobial, activity (toxicity, Hepatoprotective effects, Antihyperlipidemic activity, Hair growth promoting activity) (Jena et al., 2023). Medicinal plants are a vital source of innovative and potent pharmaceuticals. The antibacterial and antifungal properties of the extract and probiotic-containing cream formulations varied depending on the clinically derived microorganisms that were tested (Sağlam et al., 2023). When formulated in topical preparations, like cosmetics, antioxidant effects are more effective than when administered orally. The plant hibiscus flower contains flavonoids, which are antioxidants that can moisturize, improve circulation, and shield the skin from damage caused by free radicals (Ashiilah et al., 2025).

The skin has two layers, the epidermis and dermis, with subcutaneous tissue beneath. The stratum epidermis is formed by keratinocytes, melanocytes, Langerhans cells, mastocytes and merkel cells. Dermis consists of connective tissues and is separated into two layers: reticular and papillary. It is made up of fibroblasts, those are the cells that make collagen, elastin and glycosaminoglycans (GAG), along with lots of blood vessels, nerve endings and sebaceous glands. The skin performs a complexity of functions including metabolic and homeostatic activities, material removal, selective penetration and retention. It also protects us from biological, physical and toxic agents (Michalak, 2023). The term "wound" refers to the impairment of tissue's anatomical and cellular continuity brought on by chemical, physical, thermal, microbial, or immunological damage. To restore the structural and functional integrity of the injured tissue, wound healing procedures involve interconnected cellular and biochemical cascades (Dwivedi et al., 2022).

A common medicinal plant in China and other tropical nations is *Hibiscus rosasinensis*, a member of the Malvaceae family. All its components have been used as contraceptives as well as to treat fever, inflammation, and bacterial infections. Since they are found in various extracts and are most likely in charge of their biological activities, flavonoids, tannins, terpenoids, saponins, and alkaloids are the primary phytochemicals. This plant may be eligible for use as a novel therapeutic agent due to its lower toxicity (Singh et al., 2024).

To preserve the outer skin barrier and moisture levels, moisturizers are crucial for regular skin maintenance. A moisturizer's effectiveness is influenced by things like applying it frequently and choosing the right moisturizer for your skin type. In addition to their regular use for healthy skin, creams and lotions are additionally utilized for the management of psoriasis, dermatitis, and atopic diseases. Doctors advise using moisturizing products as a supplement to treat acne because they may help to alleviate the condition's symptoms. Consequently, it's critical that a skin-care item be both effective and non-comedogenic. Cyanoacrylate biopsies are a long-standing and useful technique for evaluating skin care products non-comedogenic effectiveness and counting microcomedones. Venusia Max Cream (paraben-free) is a

herbal-based, powerful moisturizer that is non-comedogenic. Using it in a daily skincare regimen can be helpful, especially for severely dehydrated and acne-prone individuals because it increases moisture retention (Gala et al., 2021). The formulation of a herbal ointment with antibacterial, anti-inflammatory, and wound-healing qualities obtained from specific medicinal plant extracts. The plant components underwent solvent extraction, and standard phytochemical screening procedures were used to characterize the phytoconstituents that were produced. To create a stable herbal ointment, these extracts were then mixed with a semi-solid base. The formulation's important physicochemical characteristics, such as pH, homogeneity, spreadability, and viscosity, were assessed (Patel et al., 2024).

The application of Venusia CeraPlus moisturizers (lotion and cream) as occluded patches reduced the quantity of microcomedones as determined by light microscopy. Compared to the test goods, the positive control, coconut oil, had a noticeably higher comedogenicity. Since no negative effects were noted, the items have been shown to be safe for their intended usage (Aich et al., 2025).

The three primary layers of human skin are the dermis, epidermis, and subcutaneous. The epidermis is crucial for dermatology and cosmetics because it provides the skin's hydration and textural characteristics. Numerous skin issues arise when the skin barrier is compromised. Roughness, scaling, fissures, redness, and loss of water content are the most prevalent conditions, which can cause skin to feel tight and occasionally dry, causing itching and stinging. By making sure the patient looks healthy, the cream treatment seeks to maintain the integrity and health of the skin. All moisturizers have the same basic humectant, occlusive, and emollient qualities, even though they are categorized under the terms natural, safe, organic, and herbal. The base of most current moisturizers is made of synthetic adhesives, emulsifiers, thickeners, pigments, fragrance compounds, and surfactants. The regular function of epithelium depends on pantothenic acid. Dexpanthenol, the stable alcoholic analogue of pantothenic acid, is applied topically to preserve skin suppleness and elasticity, increase stratum corneum hydration, and decrease trans-epidermal water loss. Nowadays, many topical dexpanthenol formulations are made to meet specific needs. (Barbar and others, 2023).

A semi-solid product for improving the complexion acts as a moisture cream. Plant-based creams offer significant advantages over synthetic ones. Additionally, there are serious downsides to chemical-based creams, such as hypersensitivity and discomfort. Botanical creams do not have these detrimental consequences. Through the gradual transpiration of moisture from the skin's surface, the hydrating lotion containing herbal extract provides a calming and lubricating sensation (Chaudhari et al., 2025). A cream is a heterogeneous combination of two immiscible liquids (typically oil and water), one of which is dispersed evenly as tiny droplets throughout the other (the continuous phase), and stabilized through a third ingredient, the emulsifying agent. Pharmaceutical creams often include polydispersions with sizes between 0.1 and 50 μm (Rai et al., 2019). Creams are semisolid products

that are generally accepted by the public. According to I.P. requirements, the formulations should undergo a stability study for at least one month. Creams are designed for broader uses on the skin, such as maintenance, therapy, regeneration, and beauty. The type and use of cream are determined by the individual's needs. Herbal creams are becoming more widely accepted than commercially sold synthetic ones. Creams with a herbal base will eventually take over the market (Sahu et al., 2016).

Numerous factors contribute to the complexity of wound healing, which is a multi-stage process that necessitates an appropriate wound closure environment. However, several circumstances, such as infection and the existence of specific disorders, can cause delays in the healing process. Unlucky situations may result in amputation due to a poor wound healing procedure. Although it tends to dry out the wound bed, gauze is the standard bandage used to cover wounds. To create a favorable environment, avoid skin dryness, and promote angiogenesis in the wound region, moist dressing may be beneficial. Applying topical cream instead of a moist dressing may speed up wound healing since the former promotes healing by preserving the hydrated state of the afflicted skin (Gwarzo et al., 2022).

People of every demographic and age are affected by skin diseases, which are considered the most prevalent health problems in the entire globe. Artificial substances are frequently used in conventional skin care procedures, which can have adverse reactions and pose potential health hazards. The organic nature, reliability, and possible medicinal uses of botanical products have drawn interest in the field of skin care. This study investigates the effectiveness of herbal treatments for a range of skin conditions, such as microbial infections, psoriasis, eczema, and acne. It looks at how biologically active substances that are present in herbal products, like terpenes, alkaloids, and flavonoids, provide anti-oxidative, anti-inflammatory, and antimicrobial qualities (Purnachander et al., 2025).

Cannabinoids, bioactive lipids, microbiome modulators, and antioxidant enzymes are among the components found in newly produced moisturizers. In addition to having all the benefits of conventional moisturizers, these products claim to have extra biological outcomes that enhance skin functionality, which include increasing lipid synthesis, reducing neurosensory propagation of itching sensations, preventing oxidative damage, lowering allergic activity in cells and production of cytokines, and altering skin microbial community (Chandan et al., 2021). So, the recent moisturizers are very different from the traditional moisturizers (Table 1).

Table 1

Characteristics of traditional moisturizer in contrast to bioactive moisturizer

Traditional Moisturizer	Bioactive Moisturizer
Replenish intercellular lipid lamella	Replenish intercellular lipid lamella
↓ trans epidermal water loss (TEWL)	↓ trans epidermal water loss (TEWL)
	Up regulate lipid synthesis
	↓ neurosensory transmission of itching signals.
	Preventing oxidative damage

↓ inflammation
Alter skin microbial community

The Chinese rose, or *Hibiscus rosa sinensis*, is a member of the Malvaceae family. In many tropical nations, the herb is utilized medicinally for managing bruises, inflammation, hay fever, congestion, diabetes, bacterial and fungal infections, thinning hair, and stomach ulcers. According to phytochemical studies, flavonoids, tannins, terpenoids, saponins, and alkaloids are the primary bioactive substances that give it its therapeutic effects. Recent research revealed that a variety of compounds derived from every part of *H. rosa sinensis* demonstrated a broad range of positive impacts, including low blood pressure, anti-pyretic, anti-allergic, anti-cancer, antioxidant, anti-bacterial, anti-diabetic, wound recovery, and abortifacient properties (Missoum, 2018). The growing prevalence of illnesses has led to widespread use of natural plant products in recent years. The plant *Hibiscus rosa sinensis* Linn. (Family Malvaceae) is found all over the world. The Indian traditional medical system uses its leaves, bark, roots, and flowers to cure a variety of illnesses. Numerous investigations have demonstrated that the various sections of *Hibiscus rosa sinensis* plants have anti-inflammatory, anti-microbial, antidiabetic, anti-ulcer, hepatoprotective, antifertility, and antigenotoxic qualities that aid in the treatment of numerous illnesses (Khristi et al., 2016).

The main aim of research project is to prepare the moisturizing cream of *Hibiscus rosa sinensis*. The part which is used to prepare the cream is flower. The formulated moisturizing cream is evaluated by the various parameters including pH, odor, texture, color, and stability under various storage conditions. Further cream Stability, changes in appearance, texture, and chemical composition are also assessed over time.

MATERIALS AND METHODS

Tools and Materials

The instruments used in this research are hot incubator, homogenizer, PH meter, water bath, beakers, measuring cylinders, centrifuge machine, conductivity meter, funnel, test tubes, UV spectrophotometer, weighing balance, pipette, refrigerator, conical flasks, and DV-111 ultra rheometer. The materials used in this study were glycerin, Hibiscus extract, phenoxyethanol, cetyl alcohol, bees wax, paraffin oil, hard paraffin, ethanol, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and distilled water.

Plant Material Collection and Crude Extract Preparation

Flowers were collected from my home lawn Multan, Punjab Pakistan. The procedures used to prepare plant extracts are described as follows. The plant material (flowers) was cleaned off adulterants; shade dried and coarsely grounded. The powdered material (1 kg) was soaked in 80% aqueous ethanol for 3 days with occasional shaking (Agarwal & Prakash, 2014). It was filtered through a muslin cloth and then through filter paper. This procedure was repeated thrice, and the combined filtrate was evaporated on a rotary evaporation under reduced pressure to obtain the undiluted extract.

Phytochemical Screening

Phytochemical examination, comprising multiple chemical tests, was performed on the testing extracts to discover secondary metabolic chemicals (flavonoids, and phenolic compounds).

Phenols Test

The extracts were mixed with three to four drizzles of the ferric chloride test. The presence of phenols is indicated if a blue-black color develops (Sirag et al., 2014).

Flavonoids Test

Lead acetate test, some drops of lead acetate solution were added to the extracts. The presence of flavonoids would be indicated by the production of yellow precipitate (Sirag et al., 2014).

Total Phenolic Content (TPC) Determination

Following some modest modifications to (Sirag et al., 2014),s protocol, the all (TPC) of plant extract were assessed using the Reagent Folin-Ciocalteu. The analysis employed an extract with a methanolic solution concentration of 5 mg/ml. In a 96-well microplate, the sample extracts (20 µl) and the Reagent Folin-Ciocalteu (90 µl that had been diluted 10 times with distilled water) were combined.

Na₂Co₃ (90 µl, 6% w/v) was added to the mixture after it had been held at 25 °C for 5 minutes to create the final volume 200 µl. After 30 minutes, phenols were measured at 725 nm. Three experiments' mean values were found.

The calibration line was built using the same procedure and a standard gallic acid (µg of GAE/mg of extract) solution. After reading the concentration of phenolics (µg/ml) from the calibration line and calculating the phenolic contents in extract using

$$C = cV/m$$

The phenolic content of the extract was expressed as gallic acid equivalents. (g of GAE/mg of extract). Where volume is denoted by V of the extract (ml), C denotes the total phenolic compound concentration Dry extract in GAE/mg g, c is gallic acid concentration determined from the calibration curve (µg/ml), and m is the weight (mg) of the extract.

Total Flavonoid Content (TFC) Determination

The method of (Sirag et al., 2014) was used to determine the total flavonoid content (TFC) of crude plant extract. 80 ml of deionized water was poured to the 96 well microplate previously had 20 µl of an extract (5 mg/ml). Following this, the mixture was treated with a 5% NaNO₂ solution using 15 µl, it was then incubated for 6 minutes at 25 °C. 16 ml of 10% AlCl₃ and 68 µl of 4% NaOH solution were added to the microplate wells after 6 minutes, bringing the total volume to 200 µl (Agarwal & Prakash, 2014). After allowing the samples to sit at room temperature for 20 minutes, their absorbance was measured at 510 nm.

Three experiments' means were recorded. Using the same procedure, the calibration line was built using a standard quercetin solution Following reading the calibration line's flavonoid content (µg/ml), the flavonoid content in the extract was quantified according to the quercetin equivalent (µg of QE/mg of extract).

Antioxidant Activity of the Plant Extract Reducing Power Activity (RPA)

With a few minor modifications, the development of extracts with a lowering power activity was made by (Mak et al., 2013) . 24 µl of the test sample and 24 µl of the phosphate buffer (pH 7.2) were combined in the 96-well microplate. This mixture was maintained at 50 °C for 10 minutes after the addition of 50 µl of 1% potassium ferricyanide solution. Then, 24 µl of a 10% trichloroacetic acid (w/v) solution was added, and 100 µl of distilled water was added after that. The mixture's absorbance was then measured at 700 nm using a microplate reader after being added to 24 µl of freshly prepared 0.2% ferric chloride (FeCl₃) solution. As a standard, quercetin. To find IC₅₀ value, test solution at various concentrations was used.

DPPH Assay

The antioxidant activity of *Hibiscus rosa-sinensis* extracts was determined by evaluating the free radical scavenging ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The DPPH test was carried out exactly as specified by (Mak et al., 2013). The samples were combined with 10l of a 90µl DPPH solution and filled to a final volume of 100 ml with 95% methanol. The components were well combined and then kept at 37 °C for 30 minutes. Using a microplate reader (Synergy HT Biotech@ USA), At 517 nm, the absorbance was measured. Quercetin was used as the standard antioxidant in this investigation. A variety of dilutions, including 0.5, 0.25, 0.125, 0.0625, 0.0313, and 0.015 mM, were used to get the IC₅₀ values. Reduced absorbance indicates more antiradical activity.

Preparation of Plant Extract Cream

Weigh and label all the components separately in separate beakers. The homogenization technique was used to make creams of the w/o variety (Mohiuddin, 2019). The emulsifier and oil phase concentrations were adjusted to create the creams, which were then tested for stability. The emulsifier (Franken Chemical Germany), bees wax and paraffin oil (Merck, Germany) made up the cream's oil phase, while distilled water made up the cream's watery phase.

The temperature of the oil and aqueous phases was raised to 75±3°C. When this temperature was attained, the plant extract (4%), used in formulation, was added to the aqueous phase. The base, or placebo, was made the same way but lacked the plant extracts. In the homogenizer Drop by drop, the aqueous phase was introduced to the oil phase while being continually agitated at a speed of 2000 rpm. The entire aqueous phase must be introduced to the oil phase for 15 minutes. After the homogenizer's speed was reduced, the homogenization was continued for approximately 5 minutes at a speed of 1000 rpm. The speed of the homogenizer was then reduced to 500 rpm, and homogenization continued for another 25 minutes at this rate to bring the emulsion's temperature up to room temperature.

By adjusting the amounts of Paraffin oil and emulsifier used in the manufacturing of the creams, several formulations were created. These creams were then kept at 25°C for a month in an incubator (Sanyo MIR-153, Japan). By storing the creams at different storage temperatures for three months in incubator. Thorough in

vitro, studies were then conducted on this cream. The centrifugation test and the creams' organoleptic qualities (color, liquefaction, and phase separation) were the variables that were tracked.

Creams F1, F2, and F3 Formulation

One placebo cream (F3) without plant extract and two formulations (F1 and F2) with plant extract were created (Table 2).

Table 2

Ingredients of the creams used in the research

	Oleaginous phase	Aqueous phase
For Base	Liq. Paraffin 14% Cetyl alcohol 2% Bees wax 3% Hard paraffin 1%	Distilled water q.s
For Formulation	Liq. Paraffin 14% Cetyl alcohol 3% Bees wax 3%	Herbal extract 4% Glycerin 3% Distilled water q.s

Evaluation of Organoleptic and Physical Stability Characteristics

Physical evaluations were done on the phase separation, creaming, and sedimentation of made base and cream. Additionally, the method of administration and how it appears on the skin are evaluated for organoleptic qualities (odor, color).

Centrifuge Testing

Centrifugation experiments were carried out on both prepared bases and freshly developed formulations and performed again. Over the course of three months at intervals of 15, 30, 45, 60, 75, & 90 days. To carry out the test, almost 4-5 g of test sample taken in centrifuge cannulas wrap them with foil, and 10 minutes of spinning at 3000 rpm. A quick and easy test to determine whether an emulsion is stable is centrifugation.

Figure 1

Centrifugation test



Studies on Accelerated Stability

To study how the four various situations might affect the creams, accelerated stability tests were conducted under each circumstance. These four distinct storage conditions were as follows: 8°C +/- 0.1°C, 25°C +/- 0.1°C, 40°C +/- 0.1°C, and 40°C +/- 0.1°C with 75% relative humidity (RH). The physical characteristics of the creams were then assessed at the start of the trial.

Test for Spreadability

Following preparation, the spreadability of the formulation was evaluated, and the test was repeated for the emulsions maintained at 8°C, 25°C, 40°C, 40°/ RH 75%

and for a variety of periods of time. Readings were taken every 15 days to 90 days for a period of three months using freshly made formulation.

To perform the spreadability test, a 1 cm circle was drawn on a glass slide and 0.5g of the test sample was put inside. Enter text here by clicking or tapping. Another glass slide was positioned on top of it for five minutes at once, a 500g weight was placed on top of the upper slide. After a few minutes, the weight was removed, and the sample's new diameter was carefully measured after spreading.

PH Analysis

Using a PH meter, the active formulations and base PH, which were kept constant through 8°C, 25°C, 40°C and 40°C/RH 75%, and for varying lengths of time, was measured. 15 to 90 days intervals of base also fresh prepared formulation, readings were taken for three months.

Conductivity Test

A digital conductivity meter was used to make observations throughout the entire research period to find any formulation changes. At intervals of 15, 30, 45, 60, 75, and 90 days of base as well as freshly made formulation, readings were obtained daily for three months.

Rheological Studies

Freshly manufactured creams and those stored at various conditions were subjected to Rheological testing for creams with plant extracts and a control group to observe any changes induced by these circumstances. This was accomplished using a programmable cone plate (Brookfield) rheometer. Each test required the usage of about 0.2 g of sample, and a constant temperature of 25 °C was maintained throughout the testing. Each test consisted of 12 shear rate values and was conducted three times. The flowing curves matched the appropriate mathematical model after the flow was classified. Changes in cream viscosities and shear rates were detected after applying increasing shear force at regular intervals.

Assessment Compatibility

The skin compatibility assessment was completed using the scratch test, which is intended toward detecting any possible irritant caused Using cream formulations throughout research. Each participant had their forearms treated with the formulations as part of the test procedure before 0-time readings. For this aim, the zero-time values of the erythema were measured using a mexameter on the forearms specified above, in which the blotch has to be applied on the right side of the forearm, the patch in the shape of a disc that had been saturated with 1.0 g of placebo was placed.

On left poster of the forearm, a patch soaked in 1.0 g of a formulation comprising plant extract was applied. Specified locations on the forearms were covered by the patches. To keep the patches in place for the whole 48-hour test period, these sites were first patched and then wrapped with surgical bandages. The applied patches were taken off later 48 hours, then the forearms were cleansed with physiological saline, The erythema measurements were redone and compared to the earlier readings to spot any appreciable change in the results.

Cream Type

The type of cream was identified by emulsifying it with water and oil, and w/o cream was found.

In vitro study of Emulsion

To determine whether the made cream had the desired qualities, such as consistency and stability, certain characteristics for the base and formulation were mentioned.

Determination of Rheological Parameter

At 25 0.5 °C, the parameters of the formulations' shear stress, shear rate, and viscosity were Rheometer RV-DVIII Ultra by Brookfield Engineering Labs, Inc., which has been configured, was used to measure the adhesion. With CP 41, a spindle number. Rheoscalc 2.6 is utilized as a supplementary program. Each test sample's weight was between 0.5 and 0.1 grams. The samples' viscosity values were determined at 250.5C after 0 to 90 days, with CP41 spindle speeds between 5 and 50 rpm (in increments), and shear loads ranging from 10 to 1000 for each sample. Shear loads were applied to the creams at shear rates to ensure their quality.

Three times the data were analyzed. The measurements' results consider.

$$\tau = K \gamma^n$$

τ = Shear stress, γ = Shear rate, K = consistency index, n = Flow index.

Degree of non-Newtonian behavior is indicated by index flow (n), which has a range of 0 to 1. If the constant n has a lower value, it means that the formulation is more likely to exhibit non-Newtonian behavior. Pseudo plastic flows occur where n is less than 1, while dilatant flows occur where n is more than 1. Thixotropy is calculated using the hysteresis loop region located between the renogram's ascending and falling curves. The consistency index (K) designates the product's viscosity.

RESULTS AND DISCUSSION

The phytochemical analysis outcomes demonstrated the positive presence of flavonoids, phenols, and antioxidant substances in hibiscus flowers.

Total Phenolic Contents (TPC)

The extract of HRS had a significant amount of total phenolics, which was easily found using a spectrophotometer. Antioxidant activity 78.03±1.50 and TPC have a direct relationship. **Table 3** presents the total phenolic contents in mg GAE/g of extracts.

Total Flavonoid Contents (TFC)

The spectrophotometer was used to measure the total flavonoid content, and the results showed that the HRS extracts had an appropriate amount of these compounds. The extracts gain antioxidant capabilities thanks to the total flavonoid concentrations (TFC). **Table 3** shows the total flavonoid content, given in mg QE/g of extracts. Results of recent study have indicated the total flavonoid content (TFC) in the flowers extract (92.03±1.74 mg QE/g) are demonstrated in table 3. The presence of flavonoid content in HRS extract is also confirmed by previous literature (Missoum, 2018).

Table 3

Flavonoid and phenolic content overall in extracts

Plant	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
<i>Hibiscus rosa-sinensis</i>	78.03±1.50	92.03±1.74

The gallic acid equivalent abbreviation (GAE), QE is short for quercetin equivalent

Figure 2 (a)

Standard curve of Gallic acid

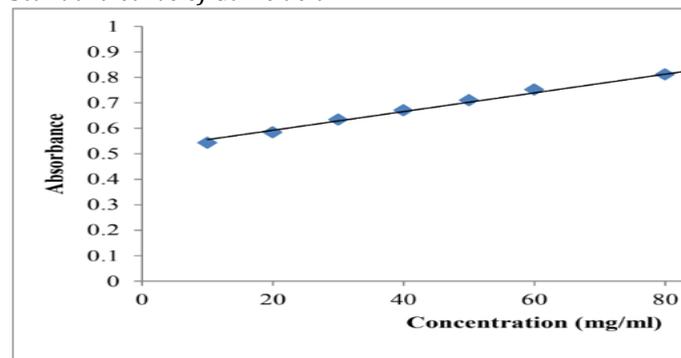
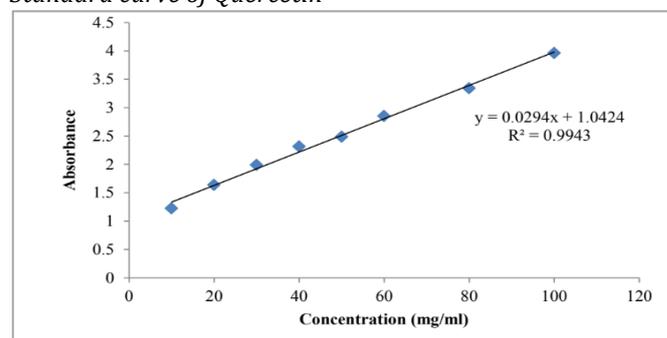


Figure 2 (b)

Standard curve of Quercetin



Antioxidant Activity

Regarding the extraction of *Hibiscus rosa sinensis* extract, results for antioxidant components and antioxidant tests are reported on a dry weight basis (d.w). Plant-based substances offer strong antioxidant activity, as demonstrated by DPPH radical foraging activity. The group includes a large variety of substances, primarily flavonoids, phenols, and pro anthocyanidins (Mandade et al., 2011)

Table 4

Antioxidant Activity of Extract

Method	Inhibition %	Inhibition %
DPPH	78.03 ± 1.50	92.12 ± 0.49
RPA	92.06 ± 2.05	0.89

Antioxidant Activity of Formulations

4% of plant extracts are present in the formulations (F1, F2) showing the potential for inhibition, according to analysis of the formulations using the DPPH technique. A control placebo formulation that contained no plant extract showed no limiting effects. Outcomes of formulations' antioxidant activity as determined by the DPPH technique displayed in **Table 5**.

Table 5

DPPH-measured creams' antioxidant activity

Formulations	Inhibition
F1	72.9 ± 1.88

F2	80.7 ± 1.76
F3	0.5±0.0

The Creams' Physical Stability and Organoleptic Evaluation

As (F1, F2, & F3) creams were all separated into four samples and stored in incubators with pre-set temperatures of 8 °C, 25 °C, 40 °C, and 40 °C +75% RH. For three months, the samples were observed for either color change, phase separation, and liquefaction, at particular time intervals.

An emulsion's stability can be investigated by storing it at various temperatures. This technique is simple to employ since thermal stress accelerates the splitting of the emulsions (Levin & Miller, 2011). While F1 was light pink, F2 was pinkish white, and all the formulations maintained their color during the 12-week research period, The new placebo formulation was white in colour.. This resistance to color change demonstrated the stability of the formulation over the course of the experiment under different storage temperatures, such as 8°C, 25°C, 40°C & 40°C/ 75% RH. According to phytochemical research (Dar et al., 2005), extracts are abundant in phenolic and flavonoid components. By limiting the growth of bacteria, these chemicals aid in the preservation of the original colour of the formulations.

The rheology regarding creams can be used to

determine its flow characteristics, and the stability of an emulsion can be evaluated by calculating how temperature and time affect rheological properties (Bhaskar et al., 2011). No liquefaction was seen in any of the formulations held at 8 °C and 25 °C, 40°C and 40°C+75%RH (Table 6 (a) , table 6 (b) and table 6 (c)). The samples of F1, F2, and F3 held at 40 °C exhibited very little liquefaction, but samples of F3 showed on the 90th day of observation, there was liquefaction at 40°C ±75% RH. On eighth observation week, the sample of F3 held at 40 °C showed a very small liquefaction (Table 6 (c)). Some time and temperature driven mechanisms are expected to reduce the rheology of the preparation over time, resulting in liquefaction.

Due to the different densities of the two phases, the internal phase droplets travel upward or downward, creating creaming or sedimentation (Mandade et al., 2011). The droplets tend to expand during creaming or sedimentation, which causes the overall interfacial energy to decrease. The phases can sometimes separate as a result of this transition to a larger average droplet size brought on by the fusion of smaller droplets (Mandade et al., 2011) but the emulsion is still stable at low temperatures since the viscosity did not alter (Z. A. Khan et al., 2014) Phase separation is influenced by differences in the chemical components that various plant extracts possess and liquefaction of creams.

Table 6 (a)

Changes in the formulation (F1)'s physical attributes over time are stored utilizing 8 °C, 25 °C, 40 °C and 40 °C / 75% RH

Physical Characteristics	Storage Temperature	Days			
		0 week	4 th week	8 th week	12 th week
Liquefaction	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	-	+
	40°C±75% RH	-	-	-	+
Color	8°C	LP	LP	LP	LP
	25°C	LP	LP	LP	LP
	40°C	LP	LP	LP	LP
	40°C±75% RH	LP	LP	LP	LP
Odor	8°C	Same	Same	Same	Same
	25°C	Same	Same	Same	Same
	40°C	Same	Same	Same	Same
	40°C±75%RH	Same	Same	Same	Same
Phase separation	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	-	-
	40°C±75% RH	-	-	-	-

- = No change; + = slight change; LP = Light Pink.

Table 6 (b)

Changes in the formulation (F2)'s physical attributes over time are stored utilizing 8°C, 25°C, 40°C and 40°C/75% RH

Physical Characteristics	Storage Temperature	Days			
		0 week	4 th Week	8 th week	12 th week
Liquefaction	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	-	+
	40°C±75% RH	-	-	-	-
Color	8°C	LW	LW	LW	LW
	25°C	LW	LW	LW	LW
	40°C	LW	LW	LW	LW
	40°C±75% RH	LW	LW	LW	LW
Odor	8°C	Same	Same	Same	Same
	25°C	Same	Same	Same	Same
	40°C	Same	Same	Same	Same
	40°C±75%RH	Same	Same	Same	Same
Phase separation	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	-	+
	40°C±75% RH	-	-	-	+

- = No change; + = slight change; LW = lightly white

Table 6 (c)

Changes in the formulation (F3)'s physical attributes over time are stored utilizing 8°C, 25°C, 40°C and 40°C/75% RH

Physical Characteristics	Storage Temperature	Days			
		0 week	4 th week	8 th week	12 th week
Liquefaction	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	+	+
	40°C±75% RH	-	-	-	+
Color	8°C	W	w	w	W
	25°C	W	w	w	W
	40°C	W	w	w	W
	40°C±75% RH	W	w	w	W
Odor	8°C	Same	Same	Same	Same
	25°C	Same	Same	Same	Same
	40°C	Same	Same	Same	Same
	40°C±75%RH	Same	Same	Same	Same
Phase Separation	8°C	-	-	-	-
	25°C	-	-	-	-
	40°C	-	-	-	+
	40°C±75% RH	-	-	-	+

- = No change; + = slight change; W = White.

Creams type

There are various ways to determine the type of cream. Globules of the dispersed phase appeared colorless when the amaranth dye was mixed with the cream sample, as well as the red backdrop revealed it was of the formulation type that water in oil.

Rheology Studies

The flow index and consistency index for the rheological characteristics of the (F1, F2 and F3) formulations stored under various storage settings During the course of 90 days are reported as (Table 7 (a, b, c) Every value was calculated using replicas in threes (n). When viscosity and shear rate rheogram plotted, as illustrated in Figures 4.3-4.5, A non-linear relationship was discovered, which

indicated formulations with shear thinning behavior (Bhaskar, 2011). The shear thinning phenomenon demonstrated that when the shear rate rose, the rheology of formulations and its corresponding base progressively dropped. As a result, these Carbopol containing emulsion systems flow curves demonstrated shear rate dependent viscosity behavior.

The results of the current study's flow index values (shown in figure 2, 3 & 4) demonstrated that the flow index values for all formulations were less than one, placing them in the class of pseudoplastic fluids, that's the desirable feature for creams. This behavior is often seen as advantageous for disseminating and applying formulation topically to the skin surface.

Table 7 (a)

F1 flow index and consistency index values were measured at different time periods following storage at 8°C, 25°C, 40°C and 40°C / 75%RH

Time	8°			25°			40°			40°C/75% RH		
	FI	CI	CF	FI	CI	CF	FI	CI	CF	FI	CI	CF
0 week	0.54	1855	98.8	0.55	1855	99.6	0.54	1855	99.6	0.54	1855	99.6
4 weeks	0.58	1065	98.4	0.54	1293	98.4	0.58	1293	98.6	0.58	1065	98.4
8 weeks	0.60	662.2	97.2	0.54	833.2	97.6	0.61	833.5	98.2	0.62	662.2	97.2
12 weeks	0.59	740	99.3	0.58	608.9	99.3	0.59	520	99.2	0.59	360	99.3

FI= Flow index; CI= Consistency index; CF= Confidence of fit (%)

Figure 3

Rheogram Formulation of F1 kept under various circumstances A) 8°C; B) 25°C; C) 40°C; D) 40°C/75% RH

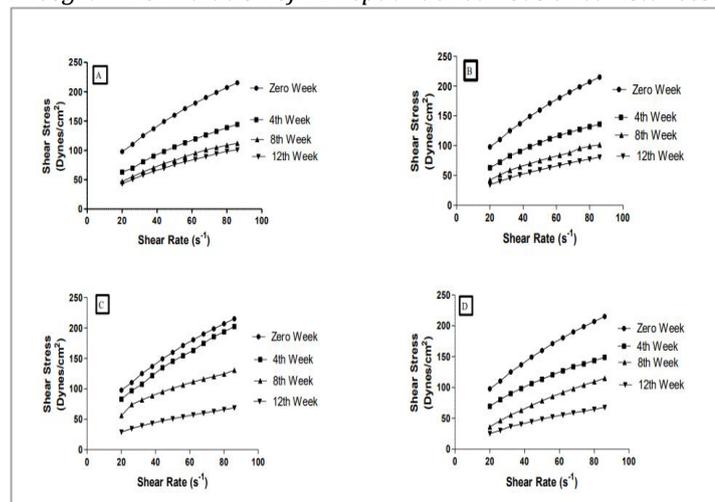


Table 7 (b)

F2 flow index and consistency index values were measured at different time periods following storage at 8°C, 25°C, 40°C and 40°C/75%RH

Time	8°			25°			40°			40°C/75% RH		
	FI	C1	CF	FI	C1	CF	FI	C1	CF	FI	C1	CF
0 week	0.6	1860	99	0.6	1860	100	0.5	1860	100	0.5	1860	100
4 weeks	0.6	1065	98	0.5	1293	98	0.6	1293	99	0.6	1065	98
8 weeks	0.6	662	97	0.5	833	98	0.6	834	98	0.6	662	97
12 weeks	0.6	740	99	0.6	609	99	0.6	520	99	0.6	360	99

CI= Consistency index; CF= Confidence of fit (%); FI= Flow index

Figure 4

Rheogram Formulation of F2 kept under various circumstances A) 8°C, B) 25°C, C) 40°C, D) 40°C/75% RH

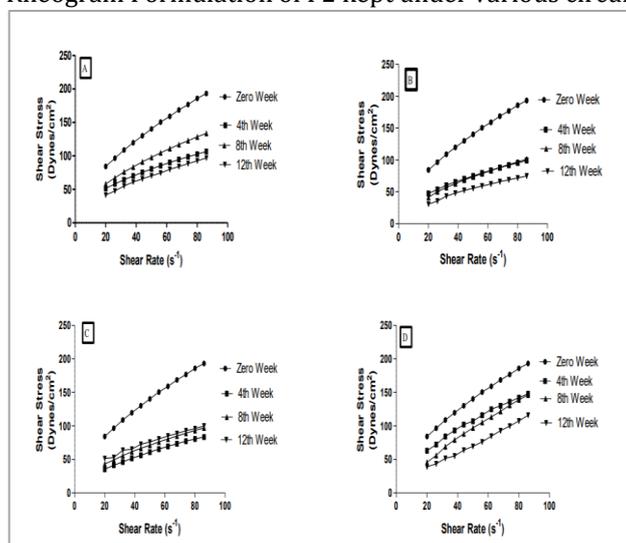


Table 7 (c)

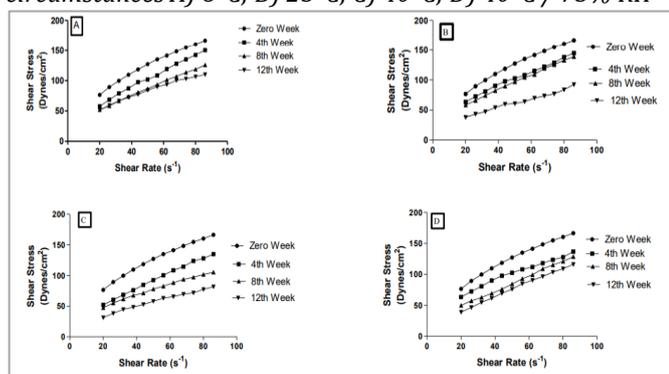
F3 flow index and consistency index values were measured at different time periods following storage at 8°C, 25°C, 40°C and 40°C/75%RH

Time	8°			25°			40°			40°C/75% RH		
	FI	CI	CF	FI	CI	CF	FI	CI	CF	FI	CI	CF
0 week	0.5	1593	99	0.6	1593	99	0.5	1593	99	0.5	1593	99
4 weeks	0.5	1075	98	0.5	1293	98	0.6	1293	99	0.6	1065	98
8 weeks	0.6	662	97	0.5	833	98	0.6	834	98	0.6	662	97
12 weeks	0.6	786	99	0.6	609	99	0.6	520	99	0.6	360	99

CI= Consistency index; CF= Confidence of fit (%); FI= Flow index

Figure 5

Rheogram Formulation of F3 kept under various circumstances A) 8°C; B) 25°C; C) 40°C; D) 40°C / 75% RH



Formulation PH values

The PH value was calculated at the storage temperature, i.e. 8°C, 25°C, 40°C and 40°C+75% RH. PH is an essential characteristic that is frequently used to determine the adequate stability and efficiency of a topical application. Furthermore, pH monitoring is essential to guarantee that the product's pH is appropriate for the formulation

elements. PH gives proof of preventing several types of patient pain at the time of product application. Most acceptable formulations for human skin care have pH values closer to skin pH (range from 4 to 6.75), seeking to reduce the risk of skin irritation. In the current investigation, we monitored the pH of the base and active formulations to assess any chemical degradation at various temperature and time interval ranges (as given by table 8).

Table 8

Formulation PH values

Parameter	Temp.	Fresh	15 days	30 days	45 days	60 days	75 days	90 days
PH	8°C	6.75	6.63	6.59	6.35	6.19	6.04	5.89
	25°C		6.74	6.59	6.33	6.03	5.94	5.77
	40°C		6.51	6.33	6.12	5.91	5.85	5.5
	40°C±75%		6.57	6.2	6	5.82	5.5	5.46
	RH							

Spreadability Studies

The spreadability studies of active formulations F1 and F2

were measured after every two weeks of study period. Outcomes are present in **figure 6**

Figure 6
spreadability values of active formulation F1 & F2

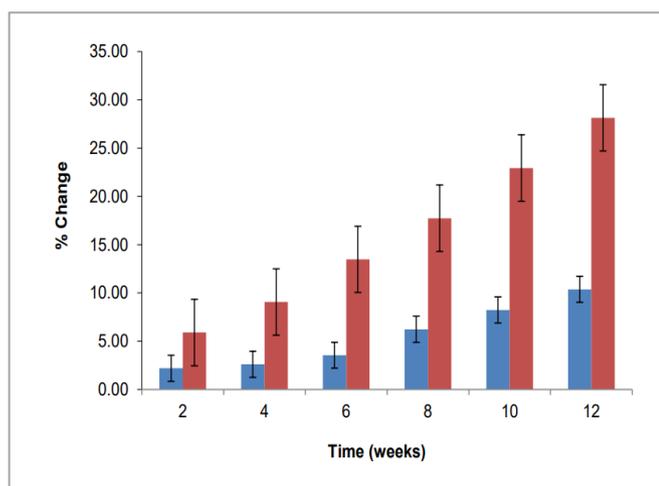


Table 9 (a)
Base centrifugation test

Parameters	Temperature	Fresh	15 days	30 days	45 days	60 days	75 days	90 days
Centrifugation	8°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	25°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	40°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	+
	40°C+75%RH	Stb.	Stb.	Stb.	Stb.	Stb.	+	+

Stb. =Stable; +=little sedimentation

Table 9 (b)
Formulation F3 centrifugation test

Parameters	Temperature	Fresh	15 days	30 days	45 days	60 days	75 days	90 days
Centrifugation	8°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	25°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	40°C	Stb.	Stb.	Stb.	Stb.	+	+	+
	40°C+75%RH	Stb.	Stb.	Stb.	Stb.	+	+	+

Stb. =stable; +=little sedimentation

Table 9 (c)
Formulation F2 centrifugation test

Parameter	Temperature	Freh	15 days	30 days	45 days	60 days	75 days	90 days
Centrifugation	8°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	25°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.
	40°C	Stb.	Stb.	Stb.	Stb.	Stb.	Stb.	+
	40°C+75%RH	Stb.	Stb.	Stb.	Stb.	+	+	+

Stb. =Stable; +=little sedimentation

Electrical Conductivity Determination

Electrical conductivity values measured for formulation are shown in the following Table 10. Specific conductance is essentially the parameter that accounts for the measurement of free ions. The assessment of conductance is thought to be a method for predicting the physical destabilization of an emulsified system before it happens. The conductive values of all formulations held at various tests storage conditions (8 °C, 25 °C, 40 °C, and 40 °C/75% RH), observed at specified time intervals over an exploratory period of 12 weeks in the current study, as shown in Figure 5. Electrical conductivity values for formulations (HRS) and their corresponding bases held at various storage conditions showed a gradual and small rising trend.

The formulations kept in accelerated storage temperatures (40°C, 40°C+75%RH) showed a greater rise

Centrifugation Test

A centrifugation test was carried out to observe the phase separation of active formulations & for base formulation, when reserved in 8°C, 25°C, 40 °C and 40 °C with RH 75%. The outcomes of centrifugation test are listed in (table 9). According to the current study's results (as seen in Tables 9 a, b, c), There was no evidence of phase separation or destabilization under the stress of centrifugal force observed in majority of the samples of base and formulations (HRS) kept at 8°C and 25°C, 40°C, 40°C+75% after centrifugation.

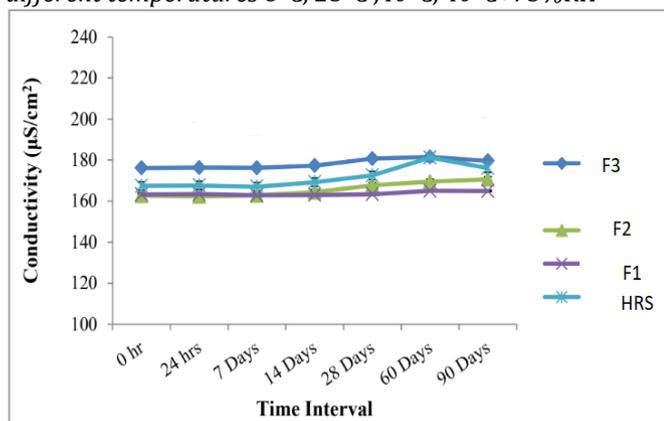
in conductivity values, However, statical analysis using two-way ANOVA revealed that all these variations in electrical conductivity values were inconsequential (p > 0.05) during the stability testing period. Because no rapid or significant rise in electrical conductivity values was seen in this study, it is reasonable to believe that all of the investigated active formulations were stable under the conditions evaluated.

The current study found that formulations including herbal extracts often have higher electrical conductivity values than bases, which is due to the acidic species created in formulations and the acidic character of extracts

Table 10
Conductivity values of formulations

Formulation	Conductivity µS/cm
F1	160.2±0.0
F2	165.3±1.2
F3	169.4±1.1

Figure 7
conductivity values of formulations kept for 90 days at different temperatures 8°C, 25°C, 40°C, 40°C+75%RH



CONCLUSION

From the current study it is concluded that HRS-rich

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