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# FORMULATION, CHARACTERIZATION AND CLINICAL INVESTIGATION OF *CORDIA MYXA* EXTRACT LOADED EMULSION FOR UV PROTECTION AND ANTI-AGING POTENTIAL

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## Abstract

Phyto-constituents from *Cordia myxa* (CM) demonstrate anti-inflammatory, UV protection and anti-aging properties due to its mucilage contents. However, incorporation and stabilization of *Cordia myxa* extract into a suitable delivery system has always been a challenge. To overcome, this study aimed to formulate a topical formulation, preferably emulsion, characterize its physic-chemical properties, quantify its sun protection factor, investigate its effects on skin physical parameters, and evaluate its anti-aging potential, extraction and phyto-chemical analysis of CM extract. *Cordia myxa* (CM) extract loaded emulsion was formulated and characterized. The *Cordia myxa* plant extract exhibited anti-oxidant eventual (86.26±1.005% DPPH inhibition), Phenolic contents (214.38±0.412 mg of GAE/g extract), and Flavonoid contents (46.01±0.47 µg of QE/mg of extract) while the extract loaded emulsion demonstrated a sun protection factor of 17. Formulation showed stability in terms of organoleptic, pH, microscopy, and rheological investigations. Non-invasive in vivo investigations on 13 healthy volunteers showed significant improvements in skin elasticity and hydration while the skin erythema and melanin contents were reduced.

**Keywords:** *Cordia myxa* extract, Antioxidant, Sun Protection Factor (SPF), Skin Elasticity, Anti-inflammatory, Photo-aging

## 1. Introduction

The skin serves as a barrier against hazardous pathogens, toxins, and UV radiation. It performs functions of excretion, water retention, thermoregulation, sensibility, and vitamin D production (1). Therefore, it's crucial to keep the skin's anatomical and functional integrity. Even though aesthetic features like wrinkles, spots, and sagging represent vitality, the issue of preserving vitality extends beyond maintaining beauty. The important qualities like the skin's capacity for defence and repair tend to deteriorate with age (2). Skin thins, stiffens, relaxes, and loses flexibility, reducing its ability to protect the body from mechanical harm. Skin aging is the result of both internal and external variables. The impact of sun radiation on skin health is one of the most well-known extrinsic variables. Besides solar damage, extrinsic aging is caused by air pollutants and smoke (3).

Literature clearly demonstrates a connection between these elements by developing the wrinkles and Melanosis. Both components speed up the aging process by oxidative stress, a typical mechanism that harms cellular functions like DNA replication. Visible radiation impacts an oxidative effect via heat creation, comparable to that of infrared radiation, adding together to the ultra-violet (UV) vicinity of solar radiations which are responsible for cellular damage (3). To stop the skin damage brought on by atmospheric variables, new treatment approaches are being explored to counteract the formation of reactive oxygen species. In addition to applying sunscreens to protect skin from UV rays, some methods rely on topically applied compounds, including anti-aging, anti-oxidants and anti-inflammatory substances that permeate the cutaneous tissues to safeguard and restore it from the inside out (4).

As the majority of people in the globe experience allergic reactions, harm to the skin microbiome, and skin health problems while using synthetic formulations, acceptance of natural herbal products in the topical delivery system has significantly increased to date (5). By avoiding synthetic components and adopting the phrase "herbal cosmetics" by using plant-based items in its formulation, the cosmetics industry is vying for prominence (6). Plant extract based herbal creams not only provide anti-aging and whitening effects but also fulfil major nutritional requirements of the skin. A vanishing cream is a formulation having a low-fat moisturizer that vanishes from skin. The skin is softened leaving nothing behind. The incorporated plant extracts are a important spring of a broad variety of the secondary metabolites, possessing anti-oxidant, anti-inflammatory and skin protecting properties (7).

*Cordia myxa* (CM), locally called as “Lasoora” belongs to the family Boraginaceae, which has about 300 species spread across the globe, primarily in the tropics. Literature review revealed that many Cordia species have been used for a variety of traditional medical purposes (8). Protein, lipids, fibre, and carbohydrates are all present in the *Cordia myxa* extract. The extracts' high energy content, therapeutic worth and physiological activity are all a result of the presence of minerals like K, Ca, and Zn. The extract has astringent, anti-bacterial and cytotoxic, analgesic, gastro-protective, anti-inflammatory, wound healing, and demulcent properties (9). The phyto-chemical screening carried out on *Cordia myxa* (CM) leaves extract shown the existence of Tannins, Flavonoids, Phenolic acids, Alkaloids, Coumarins and Glycosides (10). The anti-inflammatory, astringent, skin protecting and healing, and enzyme inhibition properties make it a prospective candidate to be used as cosmetic ingredient (8).

Leaves and Ripe fruit of *Cordia myxa* Un-ripe fruit of *Cordia myxa*

**Figure 1**. Fruit of *Cordia myxa*

This study mainly aimed at:

(i) anti-oxidant and phyto-chemical analysis of *Cordia myxa* (CM) leaves extract (8, 10-12)
(ii) (ii) development of *Cordia myxa* (CM) extract loaded emulsion, its characterization, and stability studies (13, 14)
(iii) (iii) *in-vitro* sun protection analysis of *Cordia myxa* (CM) extract loaded emulsion (15)
(iv) (iv) in-vivo non-invasive evaluation of *Cordia myxa* (CM) extract loaded emulsion in terms of the skin biophysical parameters and the skin elasticity (8, 10-12, 16)

## 2. Materials and Methods

### 2.1. Extraction

Fresh leaves of *Cordia myxa* were harvested when photosynthesis was sufficient in the month of July from the Islamia University Bahawalpur, Bahawalpur, Pakistan. Authentication of the plant was confirmed by a botanical taxonomist and specimens were deposited at the herbarium for future reference. The leaves were washed and dried in shade. Dried leaves were powdered till coarse powder obtained. Direct extraction was performed on 300g of the powder with 1 L Methanol, macerated for about 3 days at a temperature of 25°C. The mother liquor was concentrated by a rotary evaporator. Crude extract was stored in closed container at 2-8 °C for further use.

### 2.2. Antioxidant Activity

Measurement of Free-radical scavenging was done by using Prieto's method made specifically for micro-plate (17). 0.2 mM of DPPH solution was solubilised in ethanol. Analysis was run on the mixture of 100 mL that contained 90 mL of solutions of DPPH and with10 mL of the sample solution. Seven different dilutions of each sample (12.5, 25, 50, 100, 200, 400, and 800 g/mL) in Methanol were made. The Micro-plate Reader was used to measure any drop in 517 nm absorbance. By the following equation anti-oxidant activity was calculated by using Ascorbic acid as a positive control;

$$\%\ Inhibition = \left[\frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ control}\right] \times 100$$

### 2.3. Estimation of Total Phenolic Content

Folin-Ciocalteu Reagent colorimetric test technique was adopted to assess total Phenolic contents (TPC), with a few minor adjustments made for Micro-plate (18). 1 mg of the test sample concentrate was diluted in one millilitre of solvent (1000 g/mL) to create the sample. Standard Gallic acid solution was diluted repeatedly between 0 and 100 L. In a 96 well Micro-plate with the sample and reference Gallic acid dilutions, 10 L of FCR solution (10 percent) was added. After 10 minutes of incubation, a 15 percent solution of Sodium carbonate was put to each of the wells. To evaluate the absorbance at 750 nm Micro-plate reader was used after the mixture was once more incubated for an additional hour at room temperature. As a negative control/blank, all solvents were combined in the identical proportions. TPC was determined using a calibration curve as mg of the Gallic Acid equivalents (GAE)/gram of extract.

### 2.4. Estimation of Total Flavonoid Content

Total Flavonoid content (TFC) in samples was estimated by Park's method (19). 0.3 ml of sample extract with 0.5 mol/L of $NaNO_2$ was added to 0.1 ml of 0.3 mol/L of the $AlCl_3.6H_2O$. The aforesaid combination was then set 3.4 ml of 30 percent the Methanol and absorbance was checked at 506 nm. The dry weight of the plant sample's total Flavonoid content (TFC) of the dry weight of the plant sample was concluded as µg Quercetin equivalents (QE)/mg of extract.

### 2.5. Preparation of CM extract loaded Emulsion and Placebo

An O/W emulsion is typically created by thoroughly dispersing the oily phase into tiny globules, encasing each globule in an Emulsifier's Envelope, and then suspending the globules in the aqueous phase. Stearic acid, Cetyl alcohol and Glycerine were the oil phase used whereas KOH and distilled

$H_2O$ was aqueous phase (Table 1). Both phases were heated at 75 ºC on the water bath. Then the oil phase was added to aqueous phase gradually, stirring continuously for 15 minutes at 2000 rpm in a homogenizer. Then homogenize at a speed of 1500 rpm by Homogenizer for 5 minutes. For further 5 minutes the Homogenizer was set at 500 rpm. Placebo formulation was prepared in the same way without incorporating extracts.

**Table 1.** Composition of CM extract loaded Emulsion (for 200 g).

| Phase | Material name | Quantity (g) |
|---|---|---|
| Oil Phase | Stearic acid | 20 |
| | Cetyl alcohol | 4 |
| | Glycerine | 28 |
| Aqueous Phase | Potassium hydroxide | 0.8 |
| | CM extract* | 8 |
| | Distilled Water | 147.2 |

*CM extract=*Cordia myxa* extract (4%)

### 2.5.1. Determination of Sun protection Factor

Sun Protection (*In-vitro)* Factor was estimated by following the previously described method (20). 1 g of the sample was diluted with methanol and made up 100 ml and then it was sonicated for 5 minutes. The first 10 ml of the resulting solution is leftover after the filtration with cotton plug. Then, 5 ml of this the dilution was again diluted with methanol and made up volume of 50 ml. 5 ml of this dilution was further diluted in methanol and made up a volume of 25 ml. Absorption was noted at 290-320 nm(20). SPF was calculated by following the Mansur's equation mechanisms (21, 22).

### 2.5.2. Characterization of the CM extract loaded Emulsion

The developed formulation and respective placebo were stored for 90 days under various conditions. The temperatures employed were 8, 25, 40°C, and 40°C and with the relative humidity (RH) of 75 percent. The formulations' physical stability and organoleptic evaluation were monitored during this time in order to detect any changes. In addition, *in-vitro* characteristics like pH, phase separation on centrifugation, globule size determination on microscopic examination, and rheology were evaluated for all of the formulations (13, 14).

### 2.5.3. Organoleptic evaluations, Centrifugation test and pH

The generated formulations evaluated organoleptically for change in their physical stability under temperature and humidity conditions at different temperature conditions (colour, odour, liquefaction, and phase separation on centrifugation). These evaluations were performed immediately following preparation and then at 24 hours, 7, 15, 30, 45, 60, and 90 days. Centrifugation test was performed on approximately 2 g sample in a 15ml centrifuge tube at 5000 rpm for 10 minutes. Each tube observed for any possible phase separation. The pH was estimated in triplicate analysis by a digital pH meter (13, 14).

### 2.5.4. Microscopic evaluation

Droplet size and shape of the generated formulation and respective placebo were examined under a microscope. Measurements of the internal phase's morphology and mean droplet size for formulations conducted at 8, 25, 40°C, and 40 °C and with 75 percent relative humidity. The mean droplet size of 20 droplets was estimated by using a calibrated ocular micrometre (13, 14).

### 2.5.5. Rheological investigations

Using the Brookfield DVIII Ultra Rheometer, the flow characteristics of CM extract loaded emulsion and respective placebo was evaluated. According to the Rheometer's programmed programme, the shear rate and stress were measured. A 10-rpm increment was used to gradually increase the spindle speed from 10 to 50 rpm, and a 10-rpm decrement was used to gradually decrease it from 50 to 10. Utilizing the software Rheocalc 2.6V, the data was examined. Using Ostwald-de Waele's Power Law, the rheological parameters were assessed (14, 23).

### 2.6. Non-invasive in vivo evaluation

#### 2.6.1. Study design and Ethical approval

Single-blinded, randomized and placebo controlled research was conducted for non-invasive assessment of the formulation on the human skin. The Helsinki Declaration's international standards were also followed in this investigation. All measurements of skin parameters were carried out. Thirteen volunteers, all male, between the ages of 20 and 40, in good health and non-smokers were selected. All the volunteers were educated about the significance, potential dangers, and study protocol prior to the study and consent form was signed as proof of their agreement to the study's terms and conditions. A dermatologist examined each participant for any signs of skin damage or disease, paying particular attention to the study-related skin areas. Volunteers were instructed to stick to their regular diet and rigorously avoid using any form of skin preparation in order to reduce the possibility of the product being potentiated. The temperature and humidity were kept under strict control for all skin tests (25±1 °C and 40 % RH). Placebo and active formulations were handed over to each volunteer marked as “Left” and “Right” depicting their application. Volunteers had to use formulations twice daily. The results were observed at zero-time and predetermined time intervals (13, 14, 24).

#### 2.6.2. Primary Skin Irritation Assessment

Each participant underwent a patch (Burchard) test before the trial to exclude any potential irritation caused by any constituent of the formulations. On each volunteer's forearm, a 5 x 4 cm region was designated specifically for this use. To record the skin Erythema index baseline values, Mexameter® was utilized. After that, a hypo-allergic surgical dressing was placed on the indicated the area of left and the area of right fore-arms, respectively. 1g of the active and placebo formulations were applied. Skin Erythema was once more measured after 48 hours (13, 14, 25-27).

#### 2.6.3. Melanin Evaluation and Skin Erythema

Mexameter® MPA580 was utilised to measure melanin indexes and the Erythema of the skin (21, 28). Before using the active formulation and placebo, the zero-hour reading was taken. At the 2-, 4-, 6-, 8-, 10-, and 12-week, further readings were taken. To reduce the likelihood of error, three successive readings were collected, and the mean of those readings was used as the final reading (25-27).

#### 2.6.4. Skin Capacitance Evaluation

By using Corneometer®, Capacitance level was measured. Measurements were recorded at zero time and on 2, 4, 6, 8, 10 and 12weeks. The results expressed as mean (21).

#### 2.6.5. Skin Sebum Evaluation

By using Sebumeter®, Sebum was measured. Measurements were recorded at zero time and on 2, 4, 6, 8, 10 and 12 weeks. Triplicate measurements were subjected for analysis (29, 30).

#### 2.6.6. Skin Elasticity Evaluation

In this investigation, the Elastometer® utilised to calculate elasticity of skin. Prior the administration of the active formulation and placebo, the zero-hour reading was noted. At 2, 4, 6, 8, 10 and 12 weeks, further data was collected. Results were taken thrice and represented as mean (31, 32).

### 2.7. Surface Evaluation of the Living Skin

Visioscan® VC 98 was utilised for the surface evaluation of the living skin (SELS) in this study (33). The zero time reading was taken prior to the administration of the placebo and the active formulations. Again readings were recorded after $2^{nd}$, $4^{th}$, $6^{th}$, $8^{th}$, $10^{th}$ and $12^{th}$ weeks of the administration. Measurements were taken in triplicate on tested skin and were represented as mean (34).

### 2.8. Statistical and Mathematical Analysis

By using the following formula, the percentage of changes in each skin parameters was calculated:

$$Percent\ Change = [\frac{A-B}{B}] \times 100$$

Where A represents the unique value of each parameter at the second, fourth, sixth, eighth, tenth, and twelfth weeks while B represents an initial value of parameter at the beginning. Version 8 of the Software called Graph pad Prism was used to scrutinize the data. Pair wise sample t-tests were employed to describe any changes or variations by comparing between both the formulations. ANOVA was used to evaluate any differences between various intervals. A significant difference was deemed while *p*-value came to be less than 5% ($P < 0.05$).

## 3. RESULTS AND DISCUSSIONS

### 3.1. DPPH Scavenging Anti-oxidant Activity

The hydro-alcoholic extract demonstrated strong radical scavenging action at 1 mg/ml. The percentage inhibition was found to be 86.26 ± 1.005% as illustrated in Table 2.

### 3.2. Total Phenolic Contents

The CM extract had a total phenolic content (TPC) of 214.38 ± 0.412 mg GAE/g as estimated using the conventional regression line for Gallic acid as illustrated in Table2.

### 3.3. Total Flavonoid Contents

Total Flavonoid Content (TFC) in the CM extract was found to be 46.01 ± 0.47 µg QE/mg as shown in Table 2.

**Table 2** Phytochemical analyses of CM Leaves extract

| Parameter | CM Leaves extract | Standard |
|---|---|---|
| Radical scavenging potential (%) | 86.26 ± 1.005 | 98 ± 0.532 |
| Total Phenolic content (mg GAE/g) | 214.38 ± 0.412 | -- |
| Total Flavonoid contents (µg QE/mg) | 46.01 0.47 | -- |

### 3.4. Characterization of CM extract loaded Emulsion

#### 3.4.1. Sun Protection Factor

*Cordia myxa* (CM) extract-loaded emulsion had a very good SPF value of 17, which may be related to the phenolic and Flavonoid components found in *Cordia myxa* (CM) leaves extract.

#### 3.4.2. Organoleptic evaluations, Centrifugation Test and pH

During the trial period, there was no change in the organoleptic characteristics at any storage state and the formulation instead had an aesthetically pleasing texture and appearance as illustrated in Table 3.

**Table 3.** Organoleptic stability parameters of CM extract loaded Emulsion

| Organoleptic Parameters | Temperature | Time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 hr | 24hr | 7 | 15 | 30 | 45 | 60 | 90 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Color | 8 ºC | – | – | – | – | – | – | – | – |
| | 25 ºC | – | – | – | – | – | – | – | – |
| | 40 ºC | – | – | – | – | – | – | – | – |
| | 40 °C + 75% RH | – | – | – | – | – | – | – | – |
| Odour | 8 ºC | – | – | – | – | – | – | – | – |
| | 25 ºC | – | – | – | – | – | – | – | – |
| | 40 ºC | – | – | – | – | – | – | – | – |
| | 40 °C + 75% RH | – | – | – | – | – | – | – | – |
| Liquefaction | 8 ºC | – | – | – | – | – | – | – | – |
| | 25 ºC | – | – | – | – | – | – | – | – |
| | 40 ºC | – | – | – | – | – | – | – | – |
| | 40 °C + 75% RH | – | – | – | – | – | – | – | – |
| Phase separation (Centrifugation) | 8 ºC | – | – | – | – | – | – | – | – |
| | 25 ºC | – | – | – | – | – | – | – | – |
| | 40 ºC | – | – | – | – | – | – | – | – |
| | 40 °C + 75% RH | – | – | – | – | – | – | – | – |

* – = No change

Emulsion with *Cordia myxa* (CM) extract revealed a small drop in pH values during the study period as shown in Figure 1. Additionally, while this drop was more dramatic in samples stored at higher temperatures, statistical analysis revealed that it was not statistically significant ($p > 0.05$).

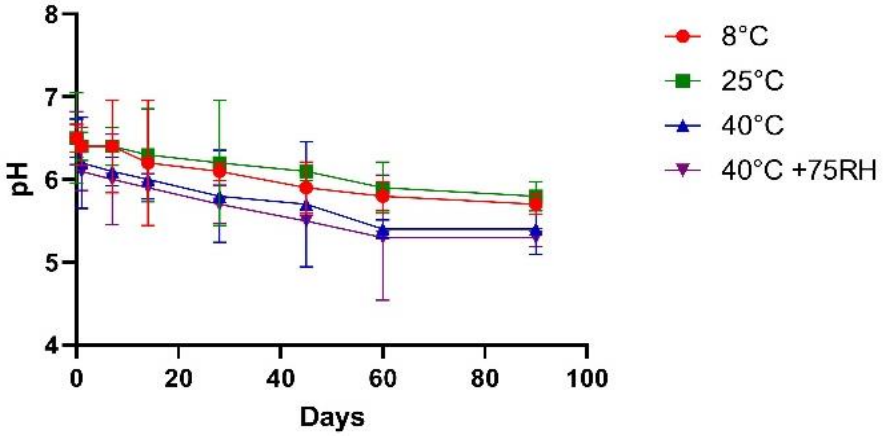


**Figure 2.** pH variations of Placebo

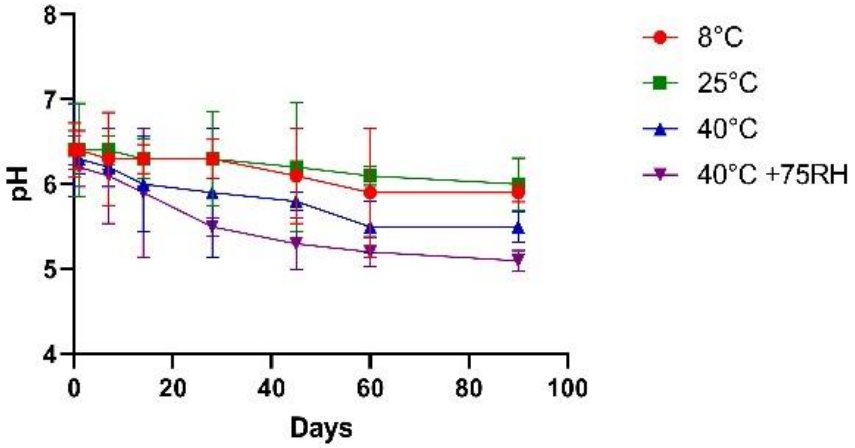


**Figure 3:** pH variations of CM extract loaded Emulsion during 90 days stability study

### 3.4.3. Microscopic Evaluation

The fresh active formulation showed 1.89 ± 0.6077 µm globules radius while after 90 days storage, the active formulation stored at 8 °C showed 1.8806 ± 0.3709 µm globules radius, 1.9857 ± 0.2367

µm globules radius at 25 °C, 2.113 ± 0.4363 µm globules radius at 40 °C, and 2.3187 ± 0.4615 µm globules radius at 40 °C + 75% RH. The globule size increased with time for accelerated stability conditions ($p < 0.05$) while the changes was insignificant for formulation kept at 8 °C and 25 °C ($p > 0.05$). This increase is due to increased movement of internal phase at higher temperatures (35). The formulation showed excellent stability at 8 °C and room temperature. The micrographs for placebo and active formulation are represented in Figure 4.

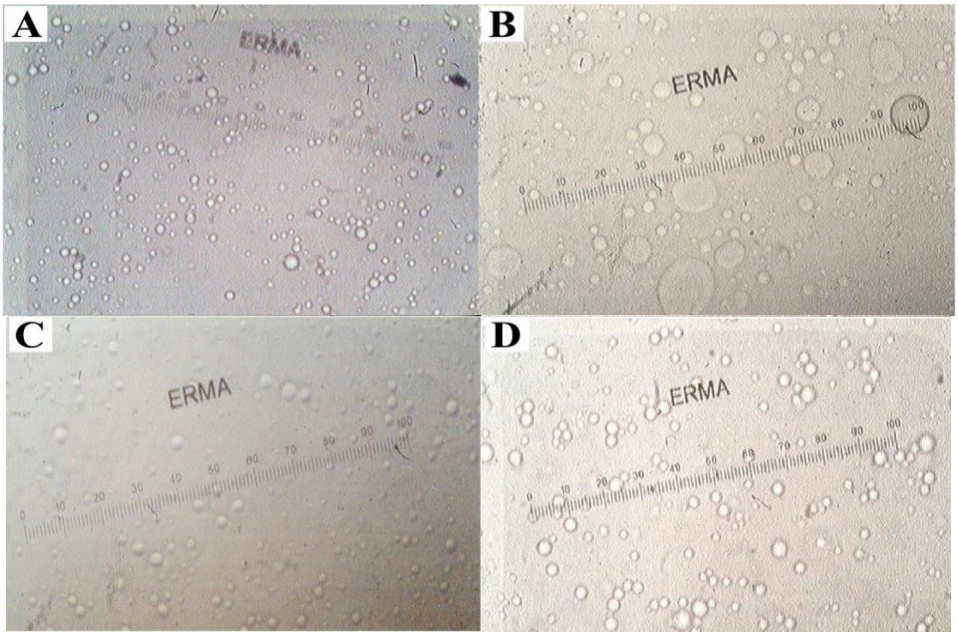


**Figure 4.** Micrographs of CM extract loaded emulsion

**(A)** Placebo (fresh), **(B)** Placebo (25 °C after 90 days storage), **(C)** CM extract loaded emulsion (fresh), **(D)** CM extract loaded emulsion (25 °C after 90 days storage)

### 3.4.4. Rheological Investigation

The formulation showed consistent flow properties stored at different temperature and (RH) relative humidity conditions as depicted in Figure 5.

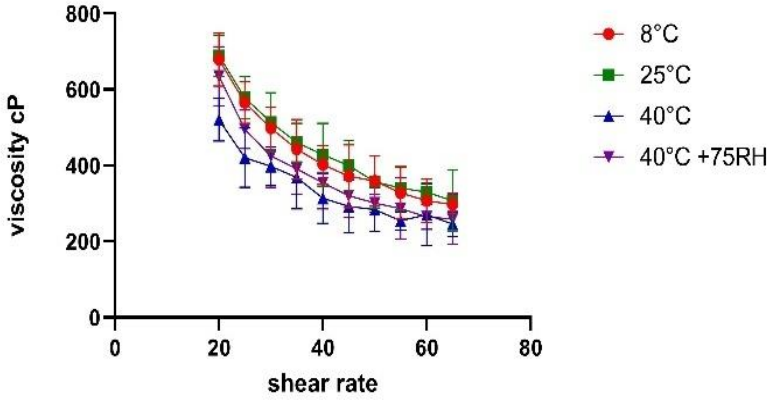


**Figure 5:** Rheological behaviour of CM extract loaded emulsion fresh

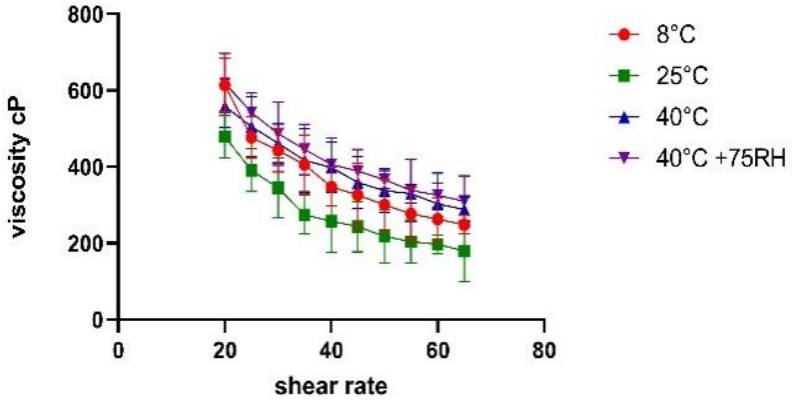


**Figure 6:** Rheological behaviour of CM extract loaded emulsion after 90 days storage conditions

### 3.5. Non-invasive in vivo evaluation

#### 3.5.1. Patch test

The results showed that neither the study formulation nor the placebo caused any volunteers to experience any sort of itchiness or allergies. After 48 hours of wearing the placebo patch and formulation, the participants' levels of Erythema decreased statistically significantly ($p$ <0.05).

#### 3.5.2. Skin Erythema Evaluation

Results demonstrated that, in contrast to placebos, the active formulation consistently and noticeably reduced the Erythema index over the course of the 12-week research. The Erythema index was reduced with CM extract loaded emulsion by up to -11.50 percent from the starting point, according to the findings **as** shown in Figure 7. Alteration in the Erythema resulted by active formulation was significant ($p < 0.05$), while the change was negligible ($p > 0.05$) with the corresponding placebo, according to statistical analysis using ANOVA with a 5% threshold of significance. The significant suppression of cytokines and inflammatory mediators by the formulation can be linked to the presence of phenolic compounds in CM extract.

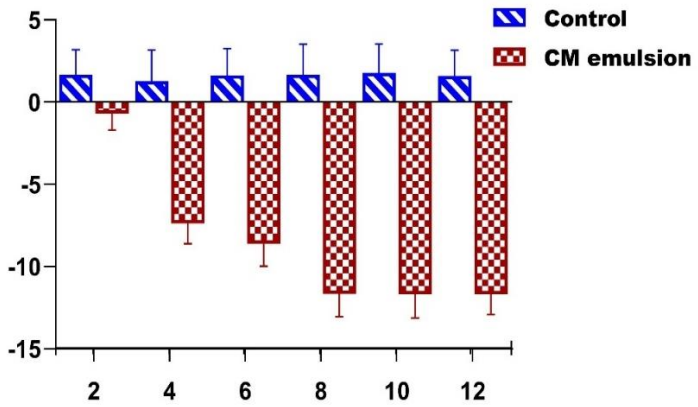


**Figure 7.** Average %change in skin Erythema at different time interval against baseline (0 hour)

#### 3.5.3. Skin Melanin Evaluation

A marked decrease in the melanin values was observed by active formulation with a percent change of -15.60 % as compared to zero-time values as shown in Figure 8, while placebo caused a negligible decrease. Two-way ANOVA analysis showed that decrease in skin melanin for test formulation was significant ($p < 0.05$) whereas the decrease caused by placebo was insignificant ($p > 0.05$).

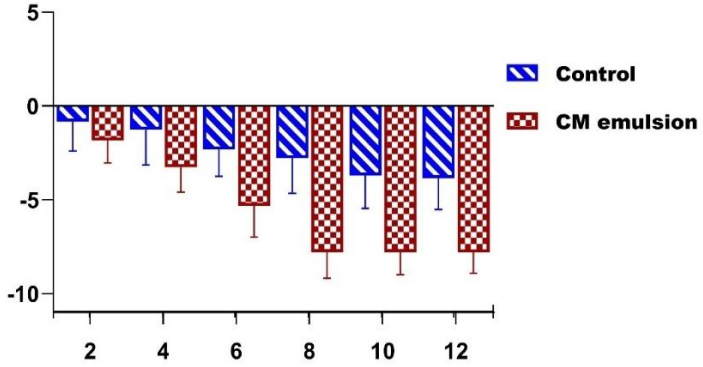


**Figure 8.** Average % in skin melanin at different time intervals against baseline (0 hour)

### 3.5.4. Skin Capacitance Evaluation

It was found that both active and placebo formulation increased ($p < 0.05$) the hydration level as depicted in Figure 9. But the formulation affect was more pronounced and 3.7 folds increased as compared to corresponding placebo.

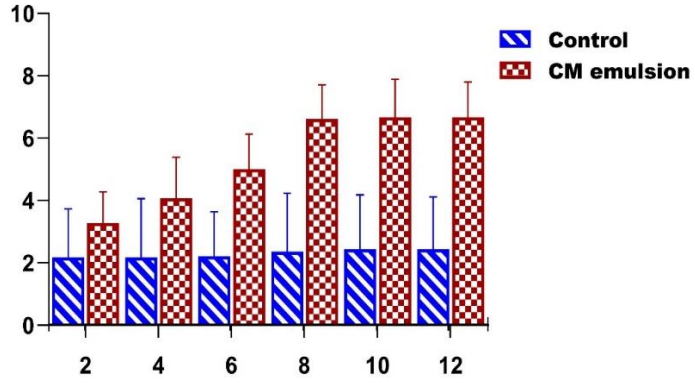


**Figure 9.** Average % change in skin hydration at different time intervals against baseline (0 hour)

### 5.5.5. Skin Sebum Evaluation

Formulation confirmed a regular decline in Sebum contents up to -12.6% as shown in Figure 10. This diminish in skin Sebum was statistically significant ($p < 0.05$). The increase in sebum by corresponding placebo was statistically insignificant.

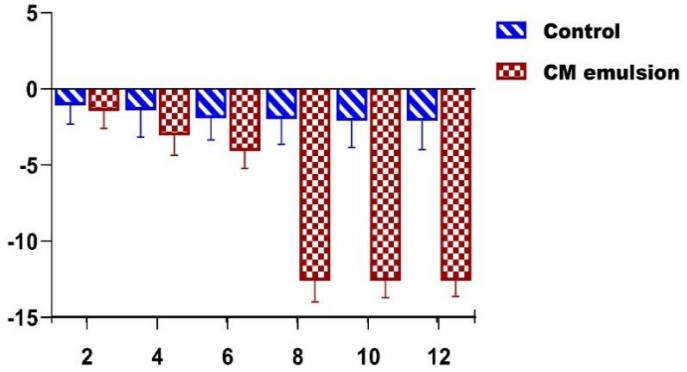


**Figure 10:** Average % change in skin Sebum at different time intervals against baseline (0 hour)

### 3.5.6. Skin Elasticity Evaluation

Elevation was noted in skin elasticity by the both test (46.20%) and placebo formulation represented graphically in Figure 11. The ANOVA analysis shows that both formulations augmented elasticity appreciably ($p < 0.05$).

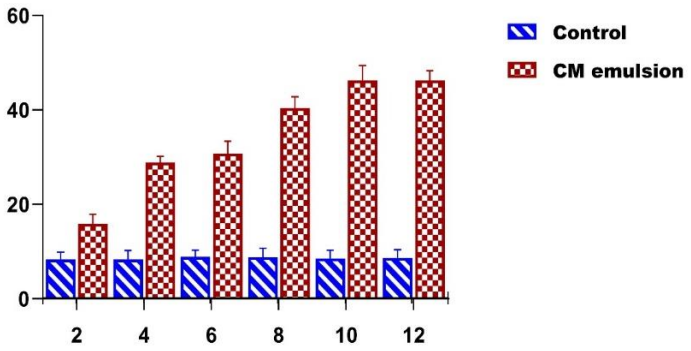


**Figure 11:** Average % change in skin elasticity at different intervals against baseline (0 hour)

### 3.6. Surface Evaluation of Living skin

In surface evaluation of living skin 4 parameters were examined as skin wrinkle index (SEw), skin smoothness index (SEsm), skin scaliness index (SEsc) and skin roughness index (SEr).

#### 3.6.1. Skin Roughness Index

According to the findings, as shown in Figure 12, the administration of the test formulation and the matching placebo during the trial resulted in a decrease in the skin roughness index. However, compared to placebo, the formulation's decrease in the skin roughness index (SEr) was additional substantial and statistically noteworthy ($p < 0.05$).

#### 3.6.2. Skin Smoothness Index

Present study exposed the consequence of the premeditated formulation on skin smoothness index (SEsm) in contrast with equivalent placebo. There was an augmented skin smoothness index caused by formulation as well as corresponding placebo as shown in Figure 12. However the raise in the skin smoothness index with the formulation was privileged than the placebo. Two ways ANOVA analysis proved that dissimilar to placebo, this raise was statistically significant ($p < 0.05$).

#### 3.6.3. Skin Scaliness Index

In recent studies, a constant decrease in the skin scaliness index was noticed. But *Cordia myxa* formulation caused 4 times more decrease in skin scaliness index in comparison with placebo shown in Figure 12. Two way ANOVA analysis exposed that decrease as significant change ($p < 0.05$).

#### 3.6.4. Skin Wrinkle Index

Figure 12 infers that both formulations as well as placebo produced a decline in the skin wrinkle index in this study. But the decrease by *Cordia myxa* formulation more potent than placebos and was statistically significant.

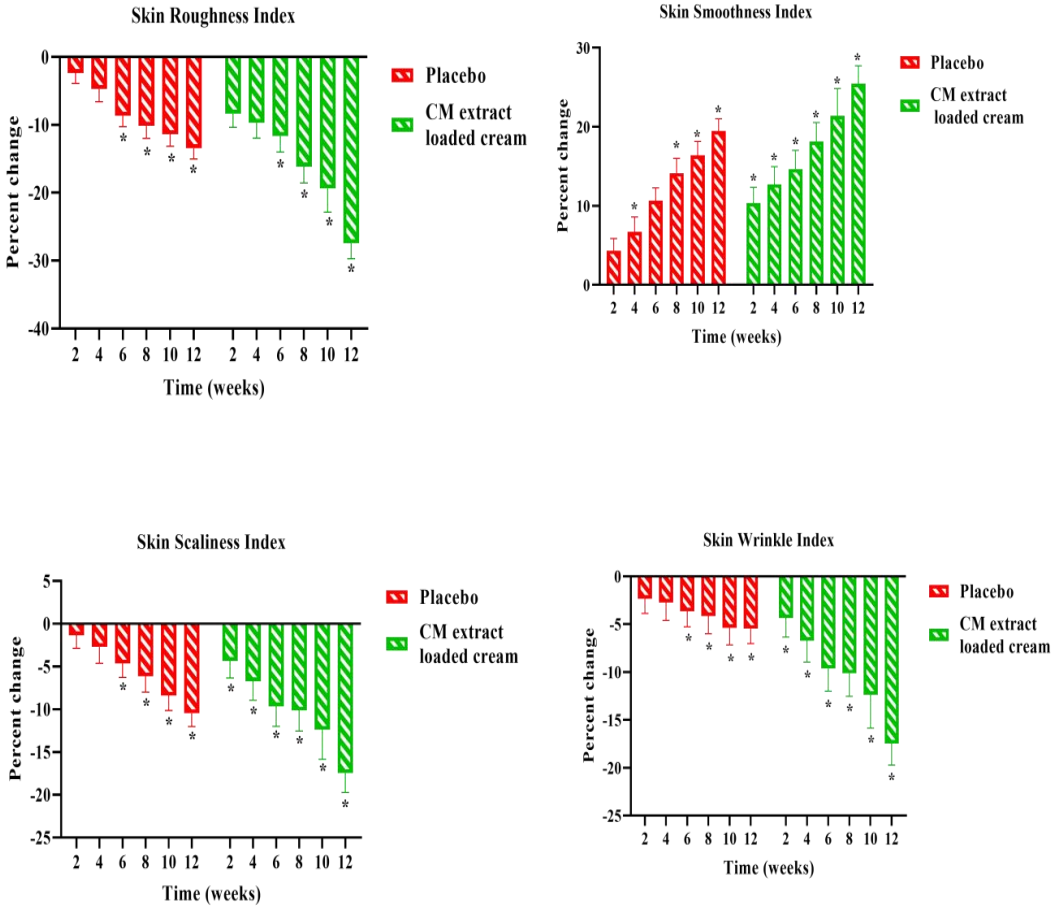


**Figure 12.** Average percentage change in surface evaluation of living skin parameters at 2nd, 4th, 6th, 8th, 10th, and 12th week with compared to baseline (0 hour) values.

## 4. Discussion

The hydro-alcoholic extract demonstrated strong radical scavenging action at 1 mg/ml. The percentage inhibition was found to be 86.26 ± 1.005%. Comparing *Cordia myxa* (CM) extract to regular ascorbic acid, it was discovered that (CM) extract has good antioxidant properties. The CM extract had a total phenolic content (TPC) of 214.38 ± 0.412 mg GAE/g as estimated. Total Flavonoid Content (TFC) in the CM extract was found to be 46.01 ± 0.47 µg QE/mg. *Cordia myxa* (CM) extract-loaded emulsion had a very good SPF value of 17, which may be related to the phenolic and Flavonoid components. During the trial period, there was no change in the organoleptic characteristics at any storage state and the formulation instead had an aesthetically pleasing texture and appearance. Emulsion revealed a small drop in pH values during the study period. The fresh active formulation showed 1.89 ± 0.6077 µm globules radius while after 90 days storage, the active formulation stored at 8 °C showed 1.8806 ± 0.3709 µm globules radius, 1.9857 ± 0.2367 µm globules radius at 25 °C, 2.113 ± 0.4363 µm globules radius at 40 °C, and 2.3187 ± 0.4615 µm globules radius at 40 °C + 75% RH. The formulation showed consistent flow properties stored at different temperature and (RH) relative humidity conditions. The Erythema index was reduced with CM extract loaded emulsion by up to -11.50 percent from the starting point. A marked decrease in the melanin values was observed by active formulation with a percent change of -15.60 % as compared to zero-time values as shown in Figure 8, while placebo caused a negligible decrease. The phenolic and Flavonoid chemicals found in *Cordia myxa* (CM) extract may be responsible for the test formulation's reduction in skin Melanin index. Studies have shown that plant-derived Phenolic and Flavonoid chemicals block the competitive Tyrosinase enzyme via a site chelation mechanism, hence lowering the formation of melanin.

Antioxidants have been shown to hydrate the skin when applied topically and systemically. So, in current study, the increased hydration can be attributed to Flavonoids present in *Cordia myxa* (CM) extract. Formulation confirmed a regular decline in Sebum contents up to -12.6%. The Stratum Corneum is waterproofed and functions as a lubricant thanks to Sebum. A brief introduction to sunlight generates ROS that could boost the activator protein-1's ability to up-regulate protein expression (AP-1). The accumulation of these un-metabolized fibres in the skin as a result of the UV-induced enzymatic breakdown of Elastin and Collagen fibres leads to intricate process of skin wrinkling. Flavonoids that are found in nature help to maintain skin suppleness by considerably inhibiting the up-regulation of the MMPs. Additionally; earlier research has proven that Flavonoids have strong anti-elastase activity. Therefore, the increased skin elasticity seen in this study may potentially be attributable to Flavonoids' anti-elastase effect. the trial resulted in a decrease in the skin roughness index. Due to the phenolic contents in the *Cordia myxa* (CM) leaves, skin elasticity significantly increased in the current study. A raise in the skin smoothness index with the formulation was privileged than the placebo. The improved elasticity and amount of moisture of the skin may be to blame for the elevated smoothness index. The fine and coarse lines are diminished when the elasticity and moisture rise, and the surface becomes smoother and less lax. *Cordia myxa* formulation caused 4 times more decrease in skin scaliness index in comparison with placebo. The skin's dryness and scaliness index are closely related. The scaliness index of skin reduces when skin moisture increases and vice versa. The formulations' phenolic ingredients, which improved skin moisture index, may be attributed for this decline. Skin's scaliness diminishes in direct proportion to increased skin moisture. Formulation produced a decline in the skin wrinkle index. Skin scaliness index and skin dryness are closely connected. Skin's scaliness index drops when the moisture index rises, and vice versa. It is inversely related to skin moisture index, as previously mentioned. The presence of phenolic chemicals in formulations, which enhanced skin moisture index, may be to blame for this drop. Scaliness of the skin lessens when moisture levels rise.

## 5. Conclusion

Sustainable use of the natural product lines is crucial for mankind. Investigations are being focused on the multifunctional bioactive constituents of plant sources worldwide. This study established promising antioxidant activity, phenolic, Flavonoid contents in *Cordia myxa*. AN established emulsion encumbered with *Cordia myxa* extract was lucratively developed, characterized, and evaluated for stability studies. The study analyzed the emerging use of *Cordia myxa* ingredients as a dermo-cosmetic product through direct measurement of skin elasticity and skin physical parameters and surface evaluation of living skin. Significant improvements were seen in skin elasticity, skin hydration and skin surface characteristics.

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