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FORMULATION AND EVALUATION OF COSMECEUTICAL ANTI-AGING NIGHT LOTION OF *MADHUCA LONGIFOLIA* EXTRACTS

JADHAV P[1], WAROKAR A[2]*, DANAO K[3], MAHAJAN U[4]

1: Research Scholar, Department of Quality Assurance, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra-440037 India

2: Associate Professor, Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra-440037 India

3: Assistant Professor, Department of Pharmaceutical Chemistry, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra-440037 India

4: Professor, Department of Quality Assurance, Dadasaheb Balpande College of Pharmacy, Nagpur, Maharashtra-440037 India

*Corresponding Author: Dr. Amol Warokar: E Mail: amol_warokar@rediffmail.com

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ABSTRACT

The aim and objectives of the research are to formulate and evaluate the cosmeceutical anti-aging night lotion of *Madhuca longifolia* (ML) extract. To determine the efficacy of the extracts by their quantitative estimation of total polyphenolics and flavonoids content, and in vitro DPPH antioxidant potential. The fatty acid components in petroleum ether extracts were separated on the TR-FAME capillary column of gas chromatography and effluent monitored by a flame ionization detector (FID). Total phenolic and flavonoid content were determined by Folin Ciocalteu and aluminum chloride reagent respectively. Antioxidant activity is determined by the DPPH assay. The anti-aging lotion containing petroleum ether and hydroalcoholic extracts of ML seeds was formulated by o/w type of emulsion. Gas chromatography revealed the presence of oleic acid, palmitic acid, stearic acid, and linoleic acid. The total phenolic and flavonoid content was found to be 43.9 ± 0.13mg/g GAE and 41.7 ± 0.125 mg/g equivalent to naringenin. DPPH assay of the hydroalcoholic extract showed IC50 value 43.419 µg/ml. The optimized formulations batch F3 & F4 complies with evaluation parameters like viscosity, spreadability, and are devoid of any irritation, microbial growth. Anti-aging night lotion showed good hydration of the stratum corneum after

a month of application. The night lotion follows the ICH guideline of stability shown at a temperature of 40^0C±2^0C with75±5% RH for 3 months. The phytoconstituents in ML extracts elicit antioxidant activity due to high flavonoid content. ML extract loaded w/o emulsion lotion revealed its effectiveness as an anti-aging night lotion.

Keywords: ***Madhuca longifolia,*** **Cosmeceutical, Anti-aging, Night lotion, Antioxidant, DPPH assay**

INTRODUCTION

Skin is the perceptible organ that involves critical tasks such as altering body temperature and noticing pressure, temperature and pain responses. Also acting as a major barrier for the allergens present in the environment and pollution which responsible for the aging. Skin aging is itself showing with dryness of skin, thinning, flaccid and presence of age spot **[1, 2]**. Moreover, this phenomenon may progress in generation of oxygen free radicals that involves forming cross-linkage with collagen protein causing to loss of the elasticity of the skin **[1]**. Thus, the antioxidant plays a crucial role to prevent or slow down oxidative damage **[3]**. However, cosmoceutical formulation which exhibits antioxidant activity derived from the herbal extract is highly desirable. Herbs have antioxidant activity due to the redox properties of phenolic compounds which allowed to act as a reducing agent, hydrogen donors and singlet-oxidant quenchers **[4]**. The medicinal plants have containing antioxidant polyphenols which involve in the scavenging and elimination of free radical process **[5]**.

Skin aging is a multidimensional process triggered by persistent UV irradiation, which causes phenotype changes in cutaneous cells, resulting in skin damage **[2]**. Furthermore, the manufacturing of collagen and elastin is inexorably reduced as people age. Elastin calcification is increased in older skin, which leads to elastin fiber degradation **[3]**. Skin ages in both men and women as a result of parallel internal and environmental processes that contribute to a progressive loss of skin integrity, structural stability, and physiological function **[4]**. Skin aging is linked to a variety of elements, and when it comes to aging studies, reactive oxygen species (ROS) play a significant role. Solar elastosis is caused by reactive oxygen species (ROS) causing collagen breakdown and build-up in the dermis.

The antioxidant enzymes such as Superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) are antioxidant enzymes that regulate ROS levels in the skin **[5]**. This results in oxidative stress, which destroys skin cells and changes their gene expression, causing photoaging as well as cutaneous carcinogenesis **[6]**. When it comes to

combating skin aging, night moisturizers are crucial. At night, skin behaves differently than it does during the day. During the day, the skin's rhythm alters, and it plays a protective role, defending itself from environmental elements. The skin is also busy while you're sleeping, seeking to repair and regenerate damaged sustained throughout the day, so giving them a boost with food or substances that promote collagen creation is a smart idea. Oils have ability to infiltrate deeply into skin so became major key anchor in night-time moisturizers **[7, 8]**.

Madhuca longifolia (ML), often known as Mahua, is an important commercial plant that grows throughout the subtropical region of India and Pakistan. *Madhuca longifolia* is a well-known medicinal plant that has been used in traditional medicine for a variety of purposes **[9]**. Fat derived from mahua seeds has a variety of medical uses, including emulsifying, emollient heating, and astringent properties for irritation, swelling, and skin illness. Tribal societies use these plant species to address common aesthetic issues including skincare and hair maintenance **[10]**.

According to a review of the literature, no herbal cosmetic formulations including *Madhuca Longifolia* have been developed to address skin aging. *Madhuca longifolia* contains a high concentration of polyphenolic phytoconstituents that help to naturally address aging issues. As a result, the current research was conducted, as well as marketed preparations are toxic since they include numerous chemicals and harmful preservatives, causing rashes, inflammation, and burning sensations, as well as the possibility of skin cancer with continued usage.

2. MATERIALS AND METHODS

2.1 Plant material

The proposed study of plant *Madhuca Longifolia* seeds was collected from Manas Ayurved, Nagpur, Maharashtra and authenticated with Voucher specimen no. 10212 by Dr. Nitin Dongarwar, Field Botanist. University Department of Botanical Science, Nagpur, Maharashtra, India.

2.2 Preparation of extract

Dried *Madhuca longifolia* seeds (175 g) were crushed to a coarser powder. Further, it was subjected to a hot continuous extraction in a soxhlet extractor with various solvents (400 ml) n-hexane, ethyl acetate, acetone, and methanol at a temperature not above the solvent's boiling point. Moreover, the extracting material passed in the next solvent and dried in a hot air oven at temperatures below 50°C each time. Each extract was filtered through Whatman filter paper before being concentrated by distilling the solvent and drying it in a water bath. For further study, all of the extracts were kept in a desiccator **[11]**.

2.3 Phytochemical Screening

The plant extracts were screened to evaluate the percentage yield and verified phytochemical test to identification numerous phytoconstituents viz. carbohydrate, flavonoids, tannins, saponin, and sterol.

2.4 Total phenolic content

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method. The standard gallic acid calibration curve was constructed using concentrations) *i.e.*, 20, 40, 60, 80, and 100 µg/ml (abscissa) versus absorbance (ordinate). In 200 µL of *Madhuca longifolia* seeds hydroalcoholic (MLSH) extract 1 mg/mL) and 2.0 mL of 2% Na2CO3 was mixed with 0.4 mL of 0.5 M sodium hydroxide. The mixture was incubated for 10 min. Later 0.2 mL of Folin-Ciocalteu reagent (1:1 v/v with water) was added. The solution was kept in dark for 30 min and its absorbance was recorded by UV–Vis. spectrophotometer at 765 nm. The standard solution of gallic acid was treated similarly to the sample. The total phenolic content was calculated as gallic acid equivalent (mg GAE/g of the extract by using gallic acid calibration curve **[12-14]**.

2.5 Total flavonoid content

The total flavonoid content of MLSH extract was determined by the aluminum chloride colorimetric method. A calibration curve of standard naringenin was constructed concentration 20, 40, 60, 80, 100 µg/ml versus absorbance. The test solution 100 µL of crude extract (1 mg/mL) was mixed with 1 mL ethanol followed by the addition of 4 mL of distilled water, 0.5 mL of 5% $NaNO_2$ solution. The mixture was incubated for 5 min and 0.5 mL of 10% $AlCl_3$ solution was added into it and allowed to stand for 10 min. Later, 2 mL of 1.0 M NaOH solution were added, and the volume of the resultant solution was made up to 10 mL with distilled water. The mixture was allowed to stand for 15 min, and absorbance was recorded at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was represented as milligram naringenin equivalent per gram dry weight.

2.6 Thin Layer Chromatography (TLC) Fingerprinting

TLC method was performed for the preliminary investigation of secondary metabolites in the plant extracts. MLSHE extract (1mg/ml) was applied on the TLC silica gel 60 F254. The composition of mobile phase optimized as ethyl acetate and methanol 8.4:3.6. After the development of chromatogram, the plate was dried for 15 minutes and visualizes at UV light and iodine chamber for visualization. The Rf-value of all the separated spots was calculated.

2.7 HPTLC Fingerprinting Method

HPTLC (CAMAG, TLC sample V) method was an accurate and reliable method that was

used for the separation of secondary metabolites. HPTLC fingerprinting of MLSHE (1mg/ml) was applied on 60 silica gel F254 as stationary phase. The mobile phase is optimized in mobile phase ethyl acetate: methanol (8.4: 3.6). Moreover, the appearance of the spots was observed by spraying visualizing reagents such as dragndorffs reagent and 5 % $FeCl_3$ solution. The detection of TLC spots in Lane Analyzer at visible light, 254 nm and 365 nm.

2.8 Gas Chromatography Method

GC method has an effective method for the chemical analysis which was utilized simultaneous identification and quantification of the various components of the complex mixture. The fatty acid components in *Madhuca logifolia* seeds extracts were determined on Thermo scientific, Trace 1110 MPC GC with capillary column (30 m, 0.32 mm i.d., 0.25 µm film thickness) TR FAME type stationary phase composed of 70% cyanopropyl-polysilphenylene siloxane. The effluent was monitored by a flame ionization detector. To enhance the volatility of analytes, the esterification of fatty acids was carried out by boron trifluoride method to transform into its methyl esters. The initial oven temperature program was set 160^0C for 7 min., the temperature was raised in the ramp by 5^0C/min. up to 240^0C. The injector port and detector temperature were kept at 250^0C. A carrier gas nitrogen was used at a flow rate of 1.5 ml/min within a split ration range was 60:10. The analysis time was set for 33 min. The analytes in the test sample were identified by comparing their retention time (Rt) with that of the standard authentic fatty acid.

2.9 In-vitro antioxidant potential of extract of *Madhuca Longifolia* seed by DPPH

The skin aging effect is caused due to oxidant radicals. Therefore, the antioxidant activity of MLSH extract was performed to identify the presence of antioxidant properties. In vitro, an antioxidant assay of MLSHE extracts was determined using DPPH method. A stock solution of DPPH (1 mg/mL) was prepared in diluent methanol. A series of concentrations of MLSH extract ranging from 10-100 µg/mL was prepared in the diluent. From each working solution, 1ml was transferred into 10ml volumetric flask with the addition of 100 µL freshly prepared DPPH solution (1 mg/mL) and the volume was made up to the mark. The solution was kept in dark for 30 min and absorbance was recorded at 517 nm using a spectrophotometer. IC50 was calculated from the concentration of the sample that scavenge 50% of free radical. Reference standard ascorbic acid dilutions were made similar to that of test solution. The control sample was prepared to contain the same volume without any extract. The %

scavenging of the DPPH free radical was measured using the following equation: **[16]**.

Percent inhibition = [(A control– A sample)/A control] ×100.

2.10 Formulation of the Anti-aging Lotion

Madhuca longifolia seeds hydroalcoholic extracts (MLSHE) were selected to prepare anti-aging lotion batch wise from F1-F5. The composition of the ant-aging lotion were depicted in **Table 1.**

The anti-aging lotion was formulated in o/w type of emulsion. This lotion was prepared by melting oil phase (white soft paraffin, cetosteryl alcohol, glycerin) at 60-70^{0}C with seeds oil of ML and aqueous phase (sodium lauryl sulphate, methyl paraben) heated at temperature 70-75^{0}C along with water and MHSHE. Further, both phases mixed with constant stirring making homogenous dispersion. The perfume was added at a temperature at 35°C **[17, 18]**.

2.11. Evaluation of Anti-aging Lotion

2.11.1 pH of lotion

The determination of the pH of the formulation is crucial to avoid the irritancy effect on the skin. Therefore, the pH of the formulation was determined by using a pH meter. Initially, the pH meter was calibrated with buffer pH 4 and 9. Afterward, pH of 1 % w/v solution of optimized lotion was determined at 27^{0}C **[19, 20]**. Applied statistical analysis for calculating the pH of the formulation.

2.11.2 Acid Value

10 g lotion was mixed in blend equal quantity of alcohol and ether solvent and make up volume up to 50 ml. Further, heated the solution in reflux condesor uptil dissolve lotion completely. Add few drops of phenolphthalein, titrate using 0.1 N NaOH solution. Finally, faint pink colour appears.

Acid value = n*5.61/w

n = The number of ml of NaOH required; w = The weight of Lotion

2.11.3 Saponification Value

Weigh accurately 2 g of the lotion was refluxed with 25 ml of 0.5 N alcoholic KOH for 30 min, to this 1 ml of phenolphthalein is added and titrated immediately, with 0.5 N HCL.

Saponification value = (b-a)*28.05/w

a = The volume in ml of titrant; b = The volume in ml of titrate, w = The weight of cream

2.11.4 Determination of viscosity

The determination of viscosity of the formulation is essential for the spreadability of the skin. The viscosity of formulating lotion was measured using a Brookfield viscometer at 10 to 100 rpm, at a temperature of 25^{0}C **[21, 22]**.

2.11.5 Determination of spreadability

The smoothness effect to the skin of the formulation is determined using the parallel-plate method. The extensometer is equipped

with a sliding plate pattern which consists of a wooden block through a pulley at one end, which was used to determine the spreadability of the formulation.

2.11.6 Irritancy test

The skin irritancy test of the formulation was performed to observe sensitivity, erythema, and edema at specified areas where lotion has been applied. This test was done on five female volunteers. Further, 1 ml of lotion was applied twice a day for three days on one sq. cm. region of the left-hand dorsal side of the skin. The irritancy was tested at regular intervals up to 24 hours per three times **[25]**.

2.11.7 Microbial test

The microbial test was performed to scrutinize contagious progression in the anti-aging lotion. The Petri-plate method was done for the microbial assay. In this assay, nutrient agar media was used for the growth of different microorganisms. Poured the lotion in the well which was made using a cork borer. Further, the Petri-plate kept in an incubator for 24 hours. Subsequently, evaluate the zone of inhibition **[26]**.

2.11.8 Accelerated Stability Studies

Stability study of the anti-aging lotion can understand the degradation of the formulation. The stability of the formulation is important for using it for a long period. As per the ICH guidelines, the accelerated stability testing of the formulation was accomplished. This study was conducted at a temperature of $40^0C \pm 2^0C$ with75±5% relative humidity using REMI SC-6 plus stability chamber. Further, the testing is observed periodically. Next, evaluate the lotion formulation after three months with examined for the change in appearance, pH, spreadability, and viscosity **[27]**.

2.11.9 Assessment of skin hydration by corneometer

Skin hydration is important to avoid skin dryness which is responsible for early aging. Therefore, assessment of skin hydration was accomplished by measuring electrical capacitance with the help of a Corneometer (CM 825). Further, the anti-aging lotion was applied at the site of the anterior forearm region, previously trimmed. This study measurement was conducted under a standard condition of temperature and humidity (T° = 20-22°C, RH humidity 40-60%). The study included 5 healthy female volunteers aged 25-40 years **[28-30]**.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Analysis

Madhuca longifolia seeds (500 g) were successively extracted in soxhlet apparatus from non-polar to polar solvents. The various *Madhuca longifolia* seeds extracts were scrutinized for the presence of secondary metabolites by preliminary phytochemical test. The % yield extract revealed that n-hexane had obtained a higher yield than others extracts.

Phytochemical screening revealed the presence of phytosterols, fatty acids, and

vitamins. Ethyl acetate and acetone extracts contain phytosterols, flavonoids whereas methanolic and hydroalcoholic extracts revealed the presence of flavonoids, anthraquinones, glycosides, proteins.

3.2 Total Phenolic Content

The GAE was calculated using the linear regression equation obtained from standard gallic acid graph (r^2= 0.9981), y = 0.0033x + 0.0831. Among test extract at concentrations 100µg/ml, higher total phenolic content was found to be 43.9 ± 0.13mg/g GAE in the hydroalcoholic extract. Methanolic extract showed 25.5 ± 0.11mg/g GAE.

3.3 Total Flavonoid Content

The naringenin was calculated using the linear regression equation obtained from standard naringenin graph (r^2 = 0.9988), y= 0.0092x + 0.0139. Among test extracts at concentrations of 100 µg/ml, the total flavonoid content was found to be 41.7 ± 0.125 mg/g equivalent to naringenin in the hydroalcoholic extract. While methanolic extract showed 25.10 ± 0.81 mg/g equivalent to naringenin.

3.4 Thin Layer Chromatography (TLC) Fingerprinting

The TLC fingerprinting demonstrated the presence of various secondary metabolites in *Madhuca longifolia* seeds extracts. Detection of flavonoids on TLC plate was recognized by the appearance of yellow fluorescence under UV light at 366 nm when 1% ethanolic solution of aluminum chloride was used as a chromogenic agent. Tannins were observed blue-greenish color when sprayed with 5 % $FeCl_3$ solution. The presence of alkaloids was confirmed with orange color bands on the plate when Dragendroff's reagent was used as a visualizing agent.

3.5 HPTLC Fingerprinting

The bioactive hydroalcoholic *Madhuca Longifolia* seeds extracts were subjected for fractionation particularly for isolation of polyphenolics. The dry extract (1g) was treated with 20 ml 5 % sodium bicarbonate to form a soluble fraction of sodium salt with that of phenolate anion. The residue was filtered and the filtrate was evaporated to dryness. Then, it was subjected to HPTLC fingerprinting. The chromatography was developed in mobile phase ethyl acetate: methanol (8.4: 3.6) HPTLC was performed on Just TLC version 4.5.

Precoated TLC plate treated with ferric chloride appeared greenish indicated the presence of polyphenolics. The results of HPTLC fingerprinting observed that hydroalcoholic extract of Madhuca longifolia seeds contains polyphenolic and flavoinoids phytoconstituents prominently visualized at 254 nm 365 nm. Polyphenols and flavonoids are reported to be elicit anti-aging effect.

MLSHE studied by HPTLC fingerprinting method was showed two lanes which was

represented by blue in lane 1 and pink in lane 2. Both the lanes visualized at visible light, 254 nm and 365 nm were identified the separate bands, retention factor value and volume of the peaks. The result obtained was pointed that the presence of polyphenols is shown in **Table 3.**

3.6.Gas chromatography (GC)

The gas chromatography was performed for the estimation of the percentile of fatty acids present in *Madhuca longifolia* seeds oil.

The GC spectra of *Madhuca longifolia* seeds shows the presence of high percentage of unsaturated fatty acids, including 16.5% polyunsaturated as linoleic acid, 25% of stearic acid, palmic acid were found to be 30% and about 28.5 % monosaturates as oleic acids indicates that the hydroalcoholic extract of *Madhuca longifolia* seeds is a potent source of antioxidant components.

3.7. *In-vitro* antioxidant potential of extract of *Madhuca Longifolia* seed by DPPH

According to DPPH method, the antioxidant activity of hydroalcoholic extract of *Madhuca longifolia* found that on increasing the concentration of extract (10 - 100 µg/ml), the percent inhibition increased from 35.93 to 82.81. The data obtained reveal that the extracts are free radical inhibitors and thus contain a potent antioxidant.

The antioxidant property (*In Vitro*) of the *Madhuca longifolia* hydroalcoholic extracts was done using DPPH Method. It was revealed IC_{50} value 43.419 µg/ml which efficient for the scavenge free radicals and promptly distorted skin aging is shown in **Table 4**. Moreover, uplift the concentration of hydroalcoholic extract, there were increased the percentage of inhibition of the oxidized DPPH radicals. This study concluded that MLSHE was superior antioxidant properties.

3.8. Evaluation of Anti-aging Lotion

The formulation of anti-aging lotion in five batches were o/w type of emulsion which was confirmed through the dye test. All batches having pH in the range of 5.5 to 6.0 which is decent and acclaimed the skin pH to avoid aging. The supported acid value and saponification value of all formulation were represented in **Table 5**, and exhibited acceptable values. The formulations F3 and F4 of anti-aging lotion were shown pH nearer to skin required.

The viscosity of lotion was in the range of 600-1000 cps which indicates that the anti-aging lotion is easily spreadable by small amounts of shear. F3 and F4 showed good spreadable property than other formulations. The irritancy test was performed on five female volunteer to evaluate the irritant, erythema and edema conditions. The formulation F3 and F4 were not showed contrary effect on the skin while performing irritancy studies. The result presented that the formulation F3 and F4 were safe to be

used on the skin which is depicted in **Table 6**.

Microbial test was done using well diffusion method to check whether formulation inhibited microbial growth or not. All the formulation were showed a prominent inhibitory effect on microbes. The result depicted that formulation F3 and F4 were high percentage of zone of inhibition and viable growth of microorganisms not exceeded than 110 colonies which measured through colony counter shown in **Table 7**

Accelerated stability studies were performed for 4 weeks at a temperature of $40^0C \pm 2^0C$ with75±5% relative humidity to evaluate all physicochemical parameters. The result revealed that the formulation F3 and F4 does not showed any alteration in appearance, spreadability, homogeneity, and odor of the anti-aging lotion which is show in **Table 7**.

3.9. Corneometer Evaluation of skin hydration

The appriasal of skin hydration was done corneometer. It had showed a very good hydration of the stratum corneum for group of volunteer studied after the application of the anti-aging night lotion, every day at night just before bedtime, for one month. The assessment of skin hydration was carried out with the base formulation containing only excipients and formulation containing *Madhuca longifolia* seeds oil and bioactive extracts. Skin hydration of antiaging lotion improved by 35-40% compared to formulation without bioactive extract and oil showed in **Figure 8**.

Scaling, itching and cracking are core characteristic of dry skin. Thus, these characteristic of dry skin leads to cause early wrinkling on the skin. All the formulation were not exhibit severe dryness to the skin. The formulation F3 and F4 showed that the absence dryness occurred in any of the volunteers. The moderate dryness was found in the formulation F2 and F5. The result were depicted in **Table 8, Figure 9 & 10.**

Table 1: Framework of Anti-aging lotion

Ingredients	Quantity (%w/w)				
Active Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
***Madhuca longifolia* seed hydroalcoholic extracts**	**1.5%**	**3%**	**6%**	**12%**	**24%**
***Madhuca longifolia* seeds oil**	**2%**	**3%**	**4%**	**5%**	**6%**
Oil Phase					
White soft paraffin	**22%**	**22%**	**22%**	**22%**	**19%**
Cetosteryl alcohol	**0%**	**5.7%**	**5.7%**	**5.7%**	**5.7%**
Propylene glycol	**10%**	**10%**	**00%**	**00%**	**00%**
Glycerine	**00%**	**00%**	**10%**	**10%**	**10%**
Aqueous Phase					
sodium lauryl sulphate	**12%**	**12%**	**12%**	**12%**	**12%**
methyl paraben	**0.05%**	**0.05%**	**0.05%**	**0.05%**	**0.05%**
Perfume	**q.s.**	**q.s.**	**q.s.**	**q.s.**	**q.s.**
Deionised water q.s.	**100%**	**100%**	**100%**	**100%**	**100%**

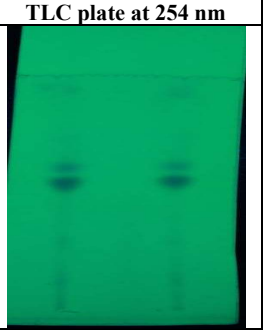
Table 2: Thin layer chromatography of *Madhuca Longifolia* seeds extracts			
TLC	**Mobile phase used**	**R_f-values**	**TLC plate at 254 nm**
Hydroalcoholic extract	**Ethyl acetate: methanol (8.4 : 3.6)**	**0.28, 0.3, 0.51, 0.61, 0.71, 0.90**	

Table 3: Lane Profile of MLSHE Studied by HPTLC Method						
Lane Profile	**LANE 1**			**LANE 2**		
	Band	**Rf-value**	**Displayed Volume**	**Band**	**Rf-value**	**Displayed Volume**
Visible Light	**1**	**Nil**	**0.079**	**1**	**Nil**	**0.052**
254 nm	**2**	**0.438, 0.362**	**0.022, 0.081**	**2**	**0.449, 0.378**	**0.024, 0.07**
365 nm	**1**	**0.362**	**0.1**	**1**	**0.38**	**0.081**

Table 4: DPPH Method Estimate Antioxidant Activity of the Extracts		
Concentration (µg/ml)	**% Inhibition**	**IC_{50} value**
10	**35.9375**	**43.419**
20	**39.4531**	
30	**42.9688**	
40	**47.6563**	
50	**50.7813**	
60	**55.0781**	
70	**60.1563**	
80	**70.7031**	
90	**73.4375**	
100	**82.8125**	
Ascorbic acid		**21.197**

Table 5: Physicochemical Parameter of Formulated Anti-aging Cream					
Formulation	**F1**	**F2**	**F3**	**F4**	**F5**
pH	**6.37 ± 0.025**	**6.25 ±0.051**	**5.7±0.033**	**5.9±0.036**	**6.12 ±0.041**
Acid Value (mg NaOH/g of lotion)	**20.31± 0.045**	**20.75±0.075**	**19.20±0.025**	**19.85±0.045**	**20.02±0.076**
Saponification Value (mg KOH/g of lotion	**44.21± 0.085**	**45.68±0.091**	**47.75±0.031**	**46.65±0.084**	**49.30±0.037**
Viscosity(cp)	**42990 ± 20.21**	**46245.41 ± 21.45**	**49821.08 ±0.46.18**	**50526 ±0.39.20**	**52341.59 ±0.13.87**
Spreadability (%)	**3.58 ±0.051**	**4.67 ±0.016**	**4.79 ±0.047**	**4.42 ±0.032**	**5.99 ±0.019**
Values expressed as Mean ± SD, n=3					

Table 6: Formulations display types of contrary effect					
Formulation	F1	F2	F3	F4	F5
Irritant	Yes	Null	Null	Null	Null
Erythema	Null	Null	Null	Null	Yes
Edema	Null	Yes	Null	Null	Null

Table 7: Physical Assessment of The Formulated Anti-aging Lotion					
Result					
Parameters	F1	F2	F3	F4	F5
Homogeneity	Decent	Decent	Decent	Decent	Decent
Appearance	Not alter colour	Alteration of colour	Not alter colour	Not alter colour	Not alter colour
Odor	Decent	Decent	Decent	Decent	Decent
Spreadability	Decent	Decent	Decent	Decent	Decent
After feel	Soothing and cageyness	Soothing and cageyness	Soothing and cageyness	Soothing and cageyness	Non Soothing and cageyness
Type of smear	oily	Non oily	Non oily	Non oily	oily
Microbial limit test	<110 clusters				
Stability	Steady for 1 months				

TABLE 8 Judgment of Formulated Anti-aging Lotion (N=15) Using Healthy Volunteers Opinion upon Corneometer Test					
Score	F1	F2	F3	F4	F5
0	7	8	13	12	7
1	8	6	2	3	7
2	0	1	0	0	1
3	0	0	0	0	0
0 = absence of dryness, 1 = mild dryness, 2 = moderate dryness and 3 = severe dryness.					

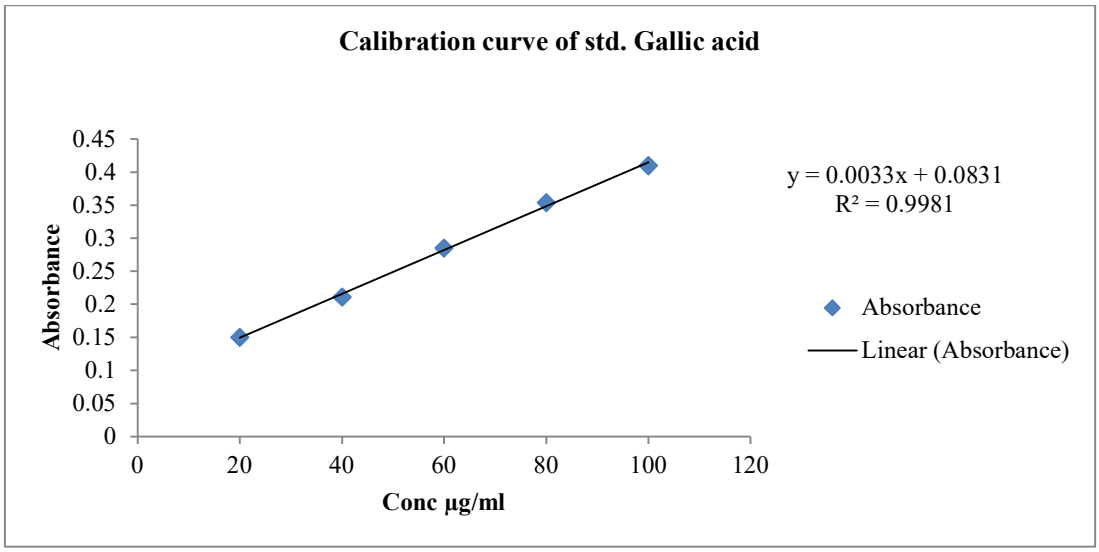


Figure 1: Standard calibration curve of Gallic acid

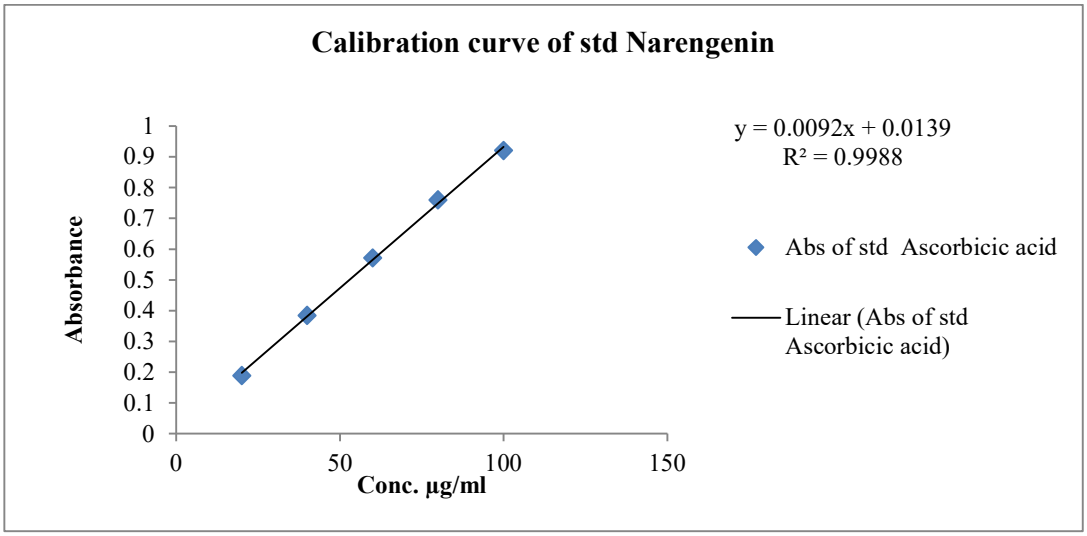


Figure 2: Standard calibration curve of Narengenin

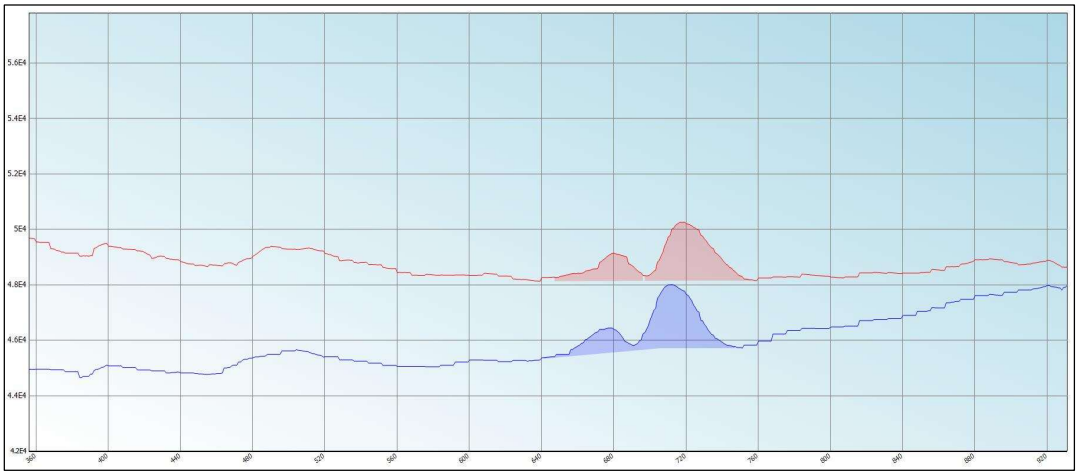


Figure 3: HPTLC Fingerprinting of Isolated Crude Phenolic constituents from Hydroalcoholic Extract of *Madhuca Longifolia* Seeds

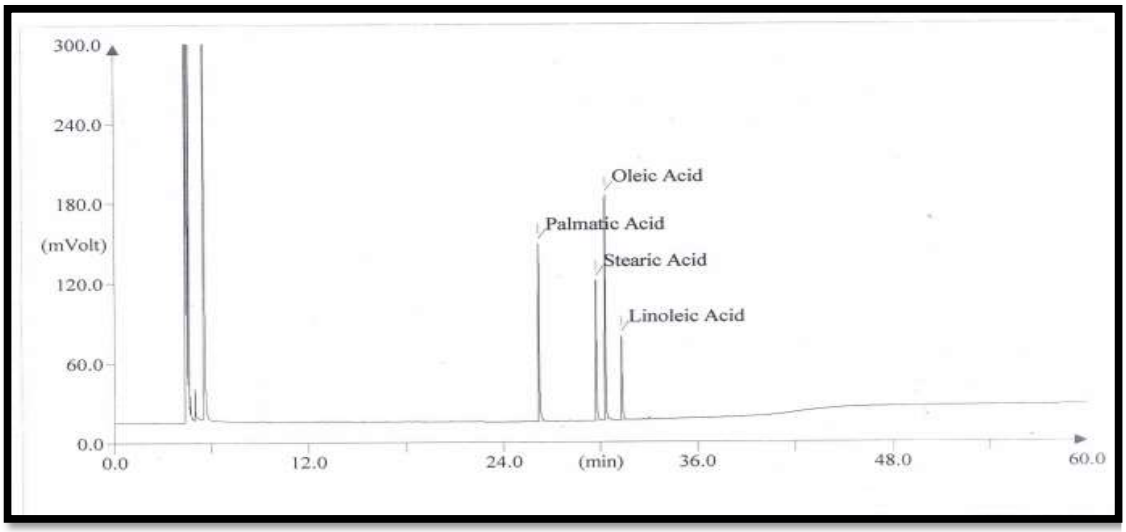


Figure 4: GC Spectrum of Petroleum ether extract of *Madhuca longifolia* Seeds

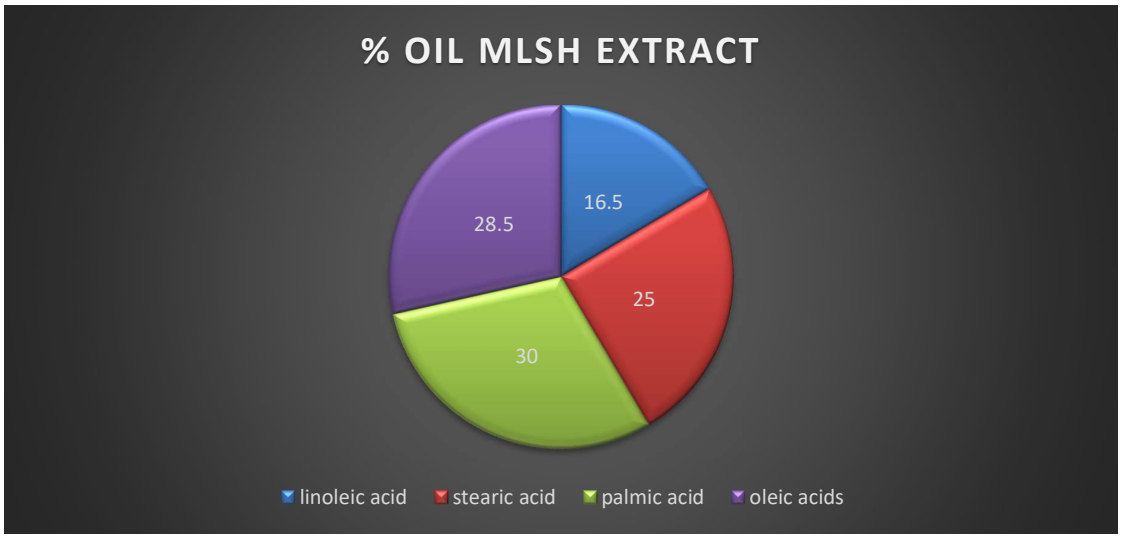
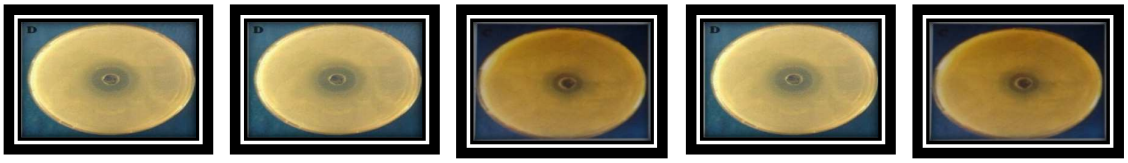


Figure 5: % oil present in MLSH extract



F1 **F2** **F3** **F4** **F5**

Figure 6: Well diffusion method for Anti-aging lotion formulation

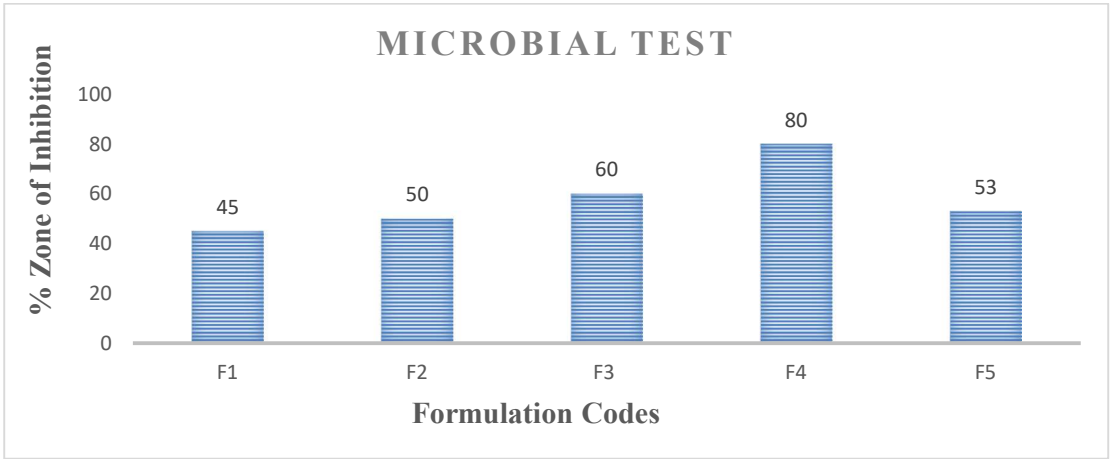


Figure 7: Microbial Limit Test of *Madhuca Longifolia* Hydroalcoholic Seeds Extract

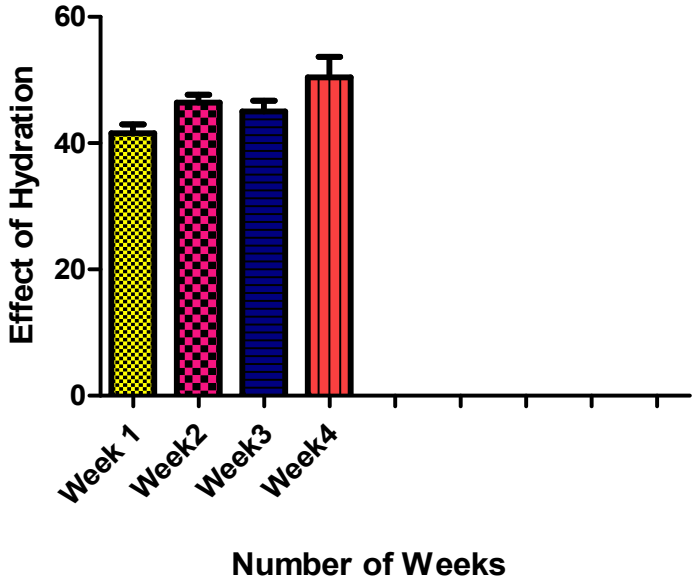
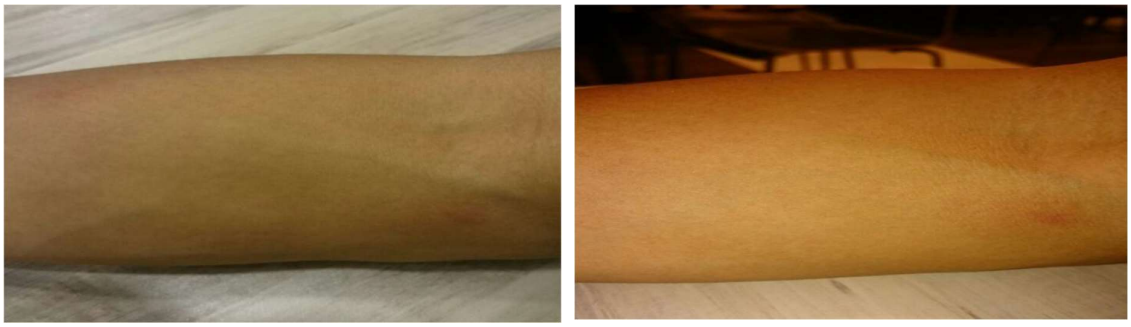
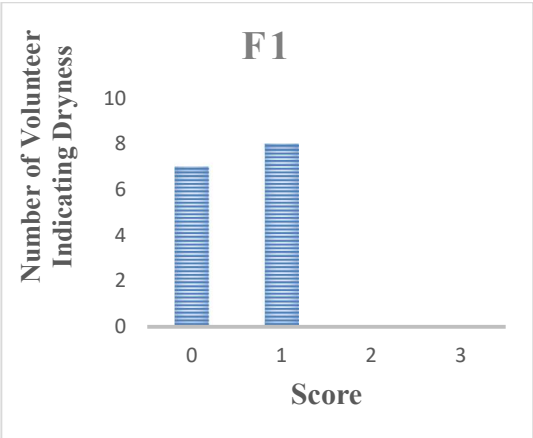


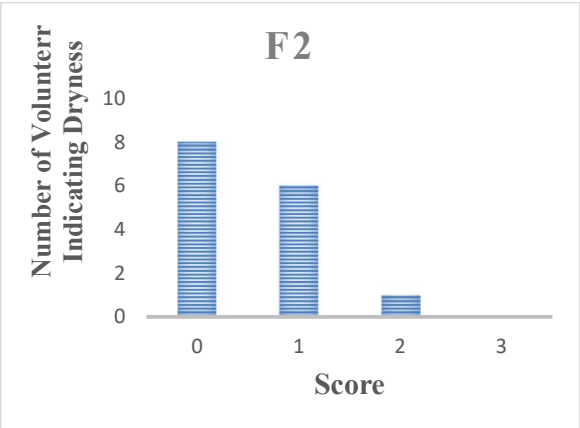
Figure 8: Hydration Effect of Anti-Aging Lotion per Week



Before **After**

Figure 9: Corneometer Test of The Formulated Anti-aging Lotion





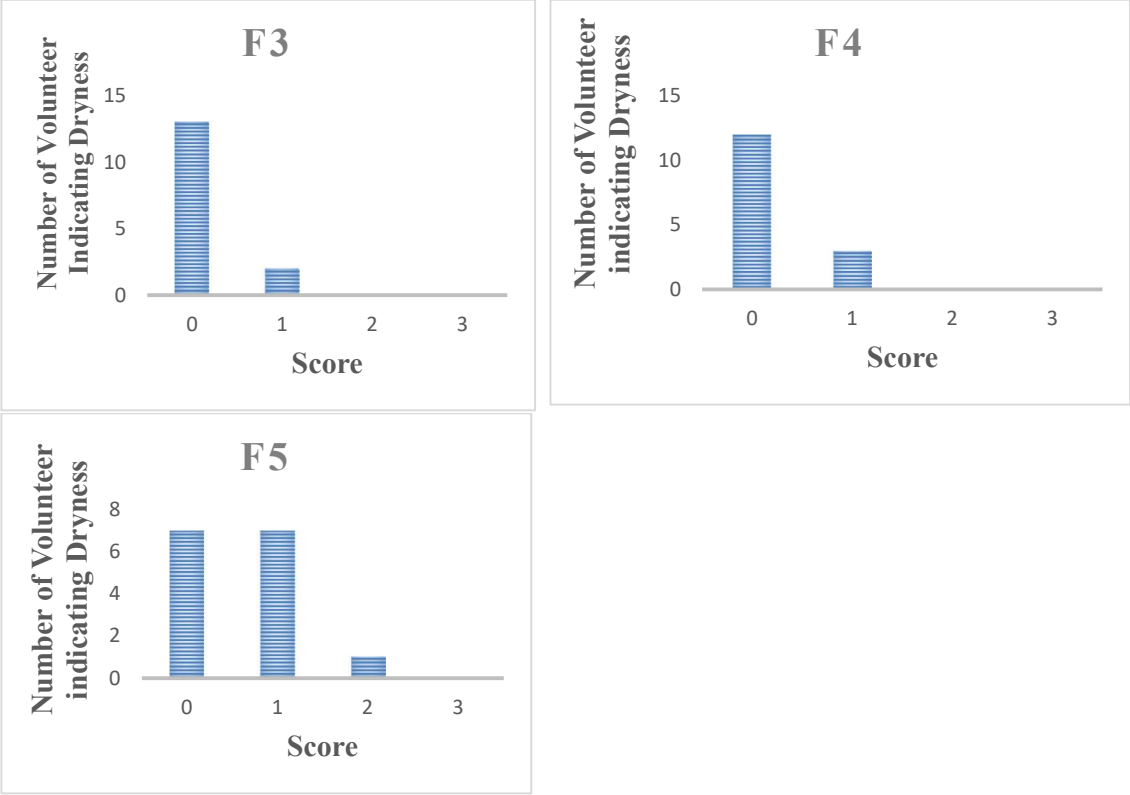


Figure 10: Opinion on the Corneometer Test After Treatment With Anti-aging Lotion Formulation (N=15)

4. DISCUSSION

The free radical scavenging property of hydroalcoholic extract of *Madhuca longifolia* was assessed by the DPPH method. DPPH radical was scavenged in the presence of hydroalcoholic extract. Subsequently, by increasing the concentration of extract (10 µg/ml to 100 µg/ml), the percent inhibition increased from 35.93 to 82.81. The results showed that the extracts are free radical inhibitors, implying that they contain a powerful antioxidant. The pH, viscosity, and spreadability of F3 and F4 formulations were found to be good during formulation development, and no irritation was reported. According to the findings of the microbiological analysis, no growth was seen. As per stability data of formulations the pH was within the range of 5.5 to 6.5. Viscosity and spreadability were found to be satisfactory and the anti-aging night lotion was easily spreadable. Formulations F3 and F4 gave acceptable results. The assessment of skin hydration by using the corneometer showed very good hydration of the stratum corneum for a group of volunteers studied after the application of the anti-aging night lotion, every day at night just before bedtime, for one month. The assessment of skin hydration was carried out with the base formulation containing only excipients and formulation containing *Madhuca longifolia* seed oil and bioactive extracts. Skin hydration of anti-aging lotion improved by

35-40% compared to the formulation without bioactive extract and oil.

5. CONCLUSION

Skin aging is itself showing with dryness of skin, thinning, flaccid, and presence of age spots. However, the presence of oxidant radicals, ultraviolet rays and dryness effect to leads early skin aging and is common in society. Therefore, we are developed of anti-aging lotion from herbal plants is safer & economical for the peoples. The unsaturated fatty acids tend to reduce harmful LDL cholesterol, heart disease risks, inflammation and increase immunity. Palmitic acid methyl esters are used as an emollient, perfuming agent, detergent making, wetting agent, stabilizer, lubricating agent and plasticizer in various cosmeceutical industries. Also, GC separate out oil from *Madhuca longifolia* seeds extracts showed higher content of oleic, linoleic and palmitic acids. The hydroalcoholic extract of *Madhuca longifolia* is an excellent source of natural flavonoids and phenols. Moreover, this plant MLSHE formulation (F3 & F4) has powerful biological activity, as well as being biocompatible with the skin and causing no signs of irritation or allergic reactions. Furthermore, the extract-loaded formulation was significantly raised skin hydration. Finally, *Madhuca longifolia* extract loaded w/o emulsion lotion is actively effective as anti-aging night lotion.

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Conflict of Interest

The authors stated No Conflict of Interest for the Publication of this research article in the Journal.

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Declaration of competing interest

The authors confirm the absence of personal and financial interests impacting the outcomes of this research article.

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