Formulation and Characterization of Anti-aging Herbal cream

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ABSTRACT

The study involved formulating creams based on the antioxidant potential of herbal extracts and evaluating their effectiveness. Linseed and lemongrass leaves were dried in the shade and then extracted using the Soxhlet method with various solvents such as n-hexane, benzene, and alcohol, to isolate different metabolites. The creams were created using ingredients like hard paraffin, soft paraffin, cetyl alcohol, and glyceryl monostearate, with varying concentrations (F1 to F15). Stability tests were conducted following ICH guidelines at 30 ± 2 °C/ 65 ± 5 % RH and 40 ± 2 °C / 75 ± 5 % RH over two months. Additionally, real-time stability studies were carried out for 12 months. It was concluded that these herbal creams, which possess antioxidant properties and are free from side effects, can serve as a protective barrier for the skin and help prevent signs of aging.

KEYWORDS: Anti-aging, Herbal Cream, Linseed oil, Lemon grass oil, Cosmetics, Antioxidant

INTRODUCTION

The Demand of herbal cosmetics due to the availability of new ingredients the financial. The increasing demand for herbal cosmetics is attributed to the availability of new ingredients, the financial benefits of developing successful products, and the importance of maintaining quality standards. Cosmetics is applied to the body and face. Creams are used commonly for softening and cleansing properties. The Ayurvedic system of medicine is one of the oldest systems, relying on herbs and plant extracts for the treatment and management of various diseases [1].

Lemongrass, also referred to as Cochin grass due to 90% of its global export being managed through Cochin port in India, holds significant potential as both food and fodder. It is packed with vital nutrients, including vitamins A, C, and E, folate, niacin, riboflavin, protein, antioxidants, and various minerals such as nitrogen (0.74%), phosphorus (0.07%), potassium (2.12%), sulfur (0.19%), magnesium (0.15%), calcium (0.36%), zinc (35.51 ppm), manganese (155.82 ppm), iron (126.73 ppm), and copper (56.64 ppm) [2, 3].

Lemongrass cultivation is rapidly expanding due to its industrial value in the pharmaceutical, food, and cosmetics sectors. Globally, it is grown over 16,000 hectares, producing around 1,000 tons of

lemongrass essential oil (LEO) annually [4]. India contributes to about one-fourth of the total production and cultivated area worldwide. Lemongrass farming provides a net profit of approximately 300 USD per hectare each year [5].

Flaxseed (or linseed) oil is derived from flaxseed and is gaining popularity in nutrition. It contains 9.0 g of saturated fatty acids (SFA) per 100 g, 18.4 g of monounsaturated fatty acids (MUFA) per 100 g, and 67.8 g of polyunsaturated fatty acids (PUFA) per 100 g, of which 53.4 g is alpha-linolenic acid (ALA). This high ALA content makes flaxseed oil highly susceptible to oxidation, which is why it is not typically sold as pure oil but combined with a suitable antioxidant. Additionally, flaxseed oil is rich in alpha- and gamma-tocopherols, vitamin K, and phytosterols [6].

Skin aging results from a continuous process of cellular DNA and protein damage. It can be categorized into two distinct types: "intrinsic aging" (also called sequential aging) and "extrinsic aging" (commonly known as photoaging). Each type has its own clinical and historical characteristics.

Intrinsic skin aging is a universal, natural, and predictable process marked by physiological changes in skin function. During this process, keratinocytes lose the ability to form a fully functional stratum corneum, and the formation of neutral lipids slows down, leading to dry, pale skin with wrinkles. In contrast, photoaging is the result of prolonged exposure to ultraviolet (UV) rays from sunlight. It manifests as dry, pale skin with shallow fine lines and deep wrinkles due to the breakdown of both epidermal and dermal structures, often accompanied by elastosis and heliodermatitis. Herbs and plants have shown their value in complementary medicine as tools to combat these effects.

Cosmetic products are formulated to protect the skin from harmful exogenous and endogenous factors, enhancing both skin health and appearance. Their use not only promotes an attractive external look but also helps maintain skin health by preventing disorders. Ingredients, whether synthetic or natural, in skincare formulations play key roles in moisturizing the skin, preserving its elasticity, reducing type I collagen degradation, and offering photoprotection. These benefits are primarily due to the presence of antioxidants, which help reduce free radical production, preserving the skin’s properties for longer durations.

Cosmetic products are increasingly preferred for treating skin conditions like hyperpigmentation, aging, wrinkles, and rough texture. The growing demand for herbal cosmetics is notable. Vitamin A, for example, acts as a powerful antioxidant, slowing the aging process, while Vitamin C helps produce collagen, a critical protein responsible for skin elasticity and wrinkle prevention. Studies show that antioxidants work together in a "protection chain," providing a synergistic effect by protecting one another from destruction during the neutralization of free radicals and other reactive species [7, 8, 9, 10, 11].

MATERIALS AND METHODS

PREPARATION OF LINSEED AND LEMONGRASS OIL EXTRACT

Lemongrass was sourced from a local market in Mumbai, while linseed powder was obtained from Vishal Chemicals. Additionally, lemongrass oil was procured from the Institute of Chemical Technology (ICT) in Mumbai. The plant materials were thoroughly cleaned before proceeding with the extraction process. Linseed oil was extracted using cold maceration of the powdered seeds.

For lemongrass oil, two extraction methods were employed:
1) Cold maceration
2) Soxhlet extraction

In the cold maceration method, lemongrass was cut into small pieces using scissors, with 100g of the leaves being placed in a 1-litre iodometric flask along with 500 ml of petroleum ether. The flask was shaken at regular intervals to ensure proper mixing of the solvent with the lemongrass. The extraction process was completed after 72 hours.

For the Soxhlet extraction method, 100g of chopped lemongrass leaves were extracted using 500 ml of distilled water over the course of 8 hours.

Cream formulation

A cream is a semisolid emulsion made by blending two different phases: an oily phase and an aqueous phase. The oily phase consists of ingredients such as hard paraffin, soft paraffin, cetyl alcohol, and glyceryl monostearate, while the aqueous phase contains distilled water. To prepare an oil-in-water (o/w) cream, the ingredients for both phases are placed in separate beakers. Both beakers are then heated in a water bath to a temperature between 70-80°C. Once both phases reach a temperature above 70°C, the oily phase is gradually poured into the aqueous phase with continuous stirring at high speed using a homogenizer. The mixture is stirred until it achieves a creamy consistency.

EVALUATION OF CREAM

ORGANOLEPTIC EVALUATION [12]

The prepared cream was assessed for its organoleptic properties, including color, odor, and texture. The appearance was evaluated based on its color and smoothness, and each attribute was rated accordingly. The results of this evaluation are presented in Table 2.

pH of the Cream [13]

The pH meter was calibrated with a standard buffer solution. Approximately 0.5 g of the cream was weighed and then dissolved in 50 ml of distilled water, after which the pH of the solution was measured.

Stability studies

Stability testing of drug products starts during the drug discovery phase and continues until the product is discontinued or removed from the market. To evaluate the stability of both the drug and the formulation, stability studies were conducted following ICH guidelines. The cream was placed in bottles and stored in a humidity chamber set at 30 ± 2°C / 65 ± 5% RH and 40 ± 2°C / 75 ± 5% RH for a duration of two months. At the conclusion of the study, the samples were analyzed for their physical properties and viscosity.

Spreadability studies [14]

A key criterion for semisolid formulations is their spreadability, which refers to how easily the cream spreads when applied to the skin. The effectiveness of a formulation often depends on this spreadability. A specialized apparatus is used to measure this property. Spreadability is quantified by the time in seconds it takes for two glass slides, separated by the formulation, to slip apart under a specified load. A shorter separation time indicates better spreadability.

To measure spreadability, two glass slides of standard dimensions are used. The formulation to be tested is applied to one slide, and the second slide is placed on top, creating a sandwich with a 5 cm length of formulation between them. A 100 g weight is then placed on the upper slide to evenly press the formulation into a thin layer. After removing the weight, any excess formulation adhering to the slides is scraped off.

One slide is fixed in place while the second slide, which is movable, is placed on top. This movable slide is connected to a string, which is used to apply a load through a simple pulley system with a pan. A 30 g weight is placed on the pan, and the time taken for the upper slide to travel 5 cm and separate from the lower slide under the applied load is recorded.

The spreadability was then calculated from the following
formula:
Spreadability = m ×l/ T
m = weight tied to the upper slide (30g)
l =length of glass slide (5cm)
t =time taken in seconds

Viscosity [15]

The viscosity of the formulation was measured using a Brookfield Viscometer. Specifically, the Brookfield DV-II + viscometer with an LV-4 spindle was employed. The formulation was poured into the viscometer's adaptor, and the angular velocity was gradually increased from 0.5 to 20 rpm to obtain the viscosity readings.

Particle size and zeta potential

Particle size and zeta potential measurement of cream were done in Malvern zeta sizer. The dilution media selected is water as our cream is o/w cream. The dilution was done by procedure explain as per USP. 5% w/v of dilution media is prepared and is further diluted more to get clear solution

In-vitro drug release [16]

In vitro dissolution testing of the cream was performed using a Franz Diffusion Apparatus. In this setup, the donor compartment contained 1 gram of cream, which was placed on a nylon membrane (semi-permeable membrane). The receptor compartment was filled with 22 ml of phosphate buffer at pH 5.5. The temperature in the receptor compartment was maintained at 32 ± 0.5°C, and the magnetic stirrer (REMI MS-500) was set to a speed of 150 rpm. At various time points, 1 ml aliquots were withdrawn from the receptor compartment, with the volume being replaced by fresh buffer to ensure sink conditions. The withdrawn samples were then filtered through Whatman filter paper (no. 1) and analyzed using UV-Visible spectroscopy.

Anti-oxidant studies [17,18,19,20,21]

Reactions of DPPH (free radical) to DPPH (Non radical)

Preparation of DPPH Solution:

A DPPH solution is prepared by dissolving DPPH in methanol within an amber-colored volumetric flask.

Preparation of Test Solution:

Lemongrass and linseed oils are dissolved in a chloroform solution. Concentrations of 10, 20, 30, 40, and 50 ppm of the oils are used to assess antioxidant activity.

Preparation of Standard Solution:

Ascorbic acid is used as the standard reagent. Solutions with concentrations of 10, 20, 30, 40, and 50 ppm of ascorbic acid are prepared in an amber-colored volumetric flask using methanol.

Stability [22-26]

Three samples of the formulation were exposed to conditions of 40°C ± 2°C / 75% ± 5% RH and 25°C ± 2°C / 60% ± 5% RH. Samples were collected at intervals of 0, 30, 60, and 90 days. During each interval, the samples were examined for changes in organoleptic properties and phase separation.

Results and discussion

Spreadability

The spreadability of placebo cream and final formulation was found out 19.3 cm/sec and 17.92 cm/sec respectively.

Particle size and zeta potential

The average particle size was measured 513.6 nm and the zeta potential was found out to be –1.57. From the results we conclude that the cream may penetrate into skin and the cream is stable.

Ex-vivo permeation study

From the ex-vivo permeation data, it shows only a fraction of oil reaches to blood circulation and remaining is there in the skin. From all of this result we may conclude that the cream is having a good permeability to the dermal layer and less permeable to reach blood circulation. Hence, we can say that the cream is topically acting only.

From the data we may conclude that oils as well as cream were showing antioxidant activity. The synergistic effect is seen in formulation as comparing with oils individually.

The cream was analysed at different time period to check whether there is change in physical appearance, pH, colour, odour, texture.

The stability studies were conducted at two different conditions at accelerated stability conditions and real time stability conditions and the results were found to be satisfactory. There is no change in color, texture of cream, there is slight variation of pH observed, no phase separation observed in normal and accelerated condition. No skin irritation was observed after application on skin.

Conclusion

The study demonstrates that lemongrass and linseed oils have significant antioxidant properties, beneficial for skin regeneration. These oils, being hydrophobic and permeable, were formulated into a topical cream. The cream exhibited stability, good skin penetration, and enhanced antioxidant activity, with no observed skin irritation or sensitivity. It also functions as a penetration enhancer for the oils. Stability tests showed that the cream maintained its properties over time. Overall, the cream serves as a stable and effective topical antioxidant formulation.

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Ingredients	Hard Paraffin	Soft Paraffin	Cetyl Alcohol	Glyceryl Monostearate	Placebo	Aqueous
F1	2.2	1.1	0.2	0.2	0.6	5.7
F2	1	3	0.5	0.5	0.6	4.3
F3	0.8	3.2	0.5	0.5	0.6	4.3
F4	0.6	3.4	0.5	0.5	0.6	4.3
F5	0.4	3.6	0.5	0.5	0.6	4.3
F6	0.2	3.8	0.5	0.5	0.6	4.3
F7	0	4	0.5	0.5	0.6	4.3
F8	-	3.8	0.5	0.5	0.6	4.5
F9	-	3.6	0.5	0.5	0.6	4.7
F10	-	3.4	0.5	0.5	0.6	4.9
F11*		**3.3**	**0.5**	**0.5**	**0.6**	**5**
F12*		**3.2**	**0.5**	**0.5**	**0.6**	**5.1**
F13	-	3.1	0.5	0.5	0.6	5.2
F14	-	3	0.5	0.5	0.6	5.3
F15	-	2.9	0.5	0.5	0.6	5.4

Table no 1: Formulation of cream

Characteristic	Lemongrass Oil	Linseed Oil
Colour	Light Yellow	Pale Yellow
Physical state at RT	Liquid	Liquid
Odour	Characteristic	Peculiar
Density	0.899	0.93

Table no 2: Physical characteristics

Spindle no.	RPM	Temperature ˚C	Viscosity cP
64	50	26.7	7078
	60	26.7	5899
63	1	27.3	70785
	2	27.1	35392
62	1	28.8	17696

Table no 3: Viscosity determination

F1 = Lemongrass Oil (Cream)	F1 = Lemongrass Oil (Cream)
F2 = Linseed Oil (Cream)	F2 = Linseed Oil (Cream)

Time (min)	F1	F2	F1*	F2*
0	0	0	0	0
15	4.82±0.94	9.33±2.05	2.61±1.44	10.35±1.02
30	12.38±1.09	19.69±2.71	11.22±3.32	21.40±2.13
45	21.78±0.82	31.76±4.14	19.82±4.26	36.54±2.58
60	31.17±1.35	44.51±3.69	28.43±5.2	47.58±4.10
120	41.92±0.75	57.6±2.58	39.24±5.9	57.94±4.70
240	53.39±0.14	67.96±1.18	48.47±6.29	68.64±3.13
360	65.87±0.66	77.98±0.59	60.86±6.15	77.98±1.56
480	79.9±2.6	89.3±3.13	72.62±7.13	89.70±2.13

Table no 4: From in-vitro release data the results were showing good release of both oils as such comparing with cream formulation.

In-vitro permeation

Time (min)	F1	F2	F1*	F2*
0	0	0	0	0
15	0.544743451	1.980338072	0.223716003	1.479813065
30	2.763444404	5.603703885	0.648676088	3.351341353
45	5.824842408	10.26076265	2.807184394	6.887659338
60	9.440012761	15.64684696	6.326046512	10.96802624
120	15.89627603	23.74229491	11.16914685	16.01680023
240	26.49379339	36.01603856	16.68640484	22.96974544
360	41.00383277	50.94474095	23.16674517	30.43409663
480	63.60376385	73.89272443	30.28512755	37.73523315

Table no 5 : In-vitro permeation data shows increase in permeability of both the oils in cream formulation as comparing with oil permeation only.

Antioxidant activity

Concentration ppm	% Radical Scavenging Activity (%RAS)			
	Ascorbic Acid	**LG oil**	**LD oil**	**Cream**
10	88.235	36.97	52.52	57.14
20	89.075	45.37	57.98	60.92
30	89.495	53.78	66.38	63.86
40	90.336	57.98	68.48	70.168
50	90.756	65.96	70.16	74.369

Table no 6: Determination of Anti-oxidant activity

Days	Color	Texture	Ph	Skin Irritation	Phase Separation
7	Off white	Smooth	5.8	No irritation	Not observed
14	Off white	Smooth	5.8	No irritation	Not observed
21	Off white	Smooth	5.8	No irritation	Not observed
30	Off white	Smooth	5.83	No irritation	Not observed
60	Off white	Smooth	5.87	No irritation	Not observed
90	Off white	Smooth	5.83	No irritation	Not observed

Table no 7 : Stability study at 25ºC ± 2ºC / 60 ± 5 % RH

Days	Color	Texture	pH	Skin Irritation	Phase Separation
7	Off white	Smooth	5.8	No irritation	Not observed
14	Off white	Smooth	5.82	No irritation	Not observed
21	Off white	Smooth	5.82	No irritation	Not observed
30	Off white	Smooth	5.83	No irritation	Not observed
60	Off white	Smooth	5.83	No irritation	Not observed
90	Off white	Smooth	5.86	No irritation	Not observed

Table no 8 : Stability study at 40°C ± 2°C / 75 ± 5% RH

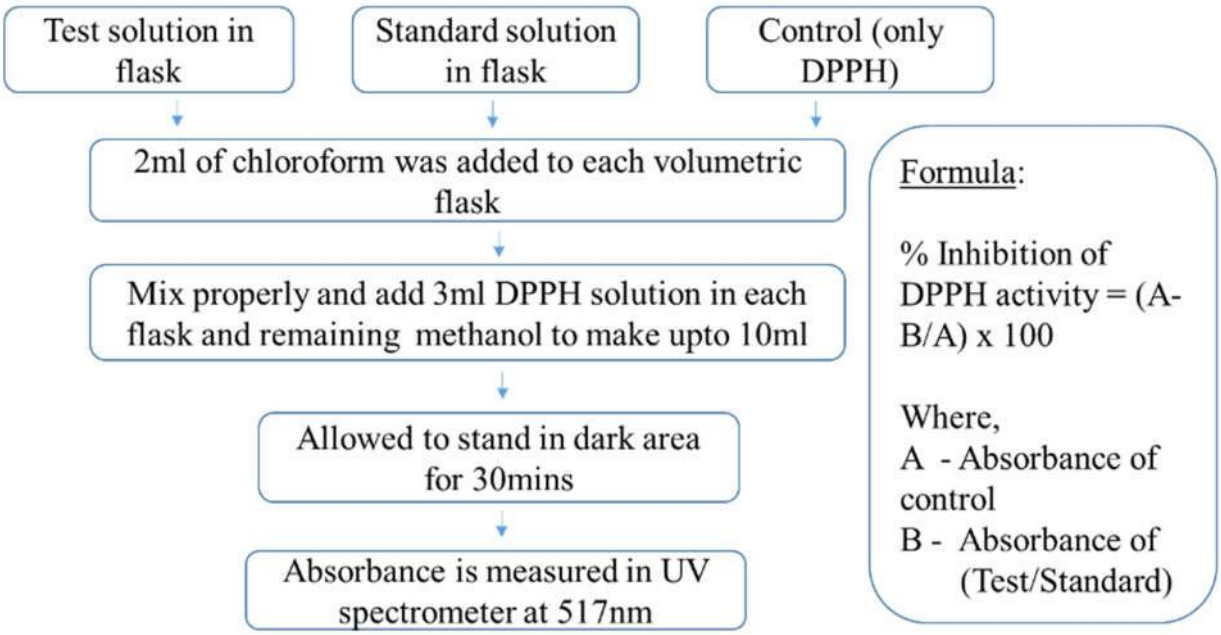

Figure no 1: Procedure of Antioxidant activity

Particle size

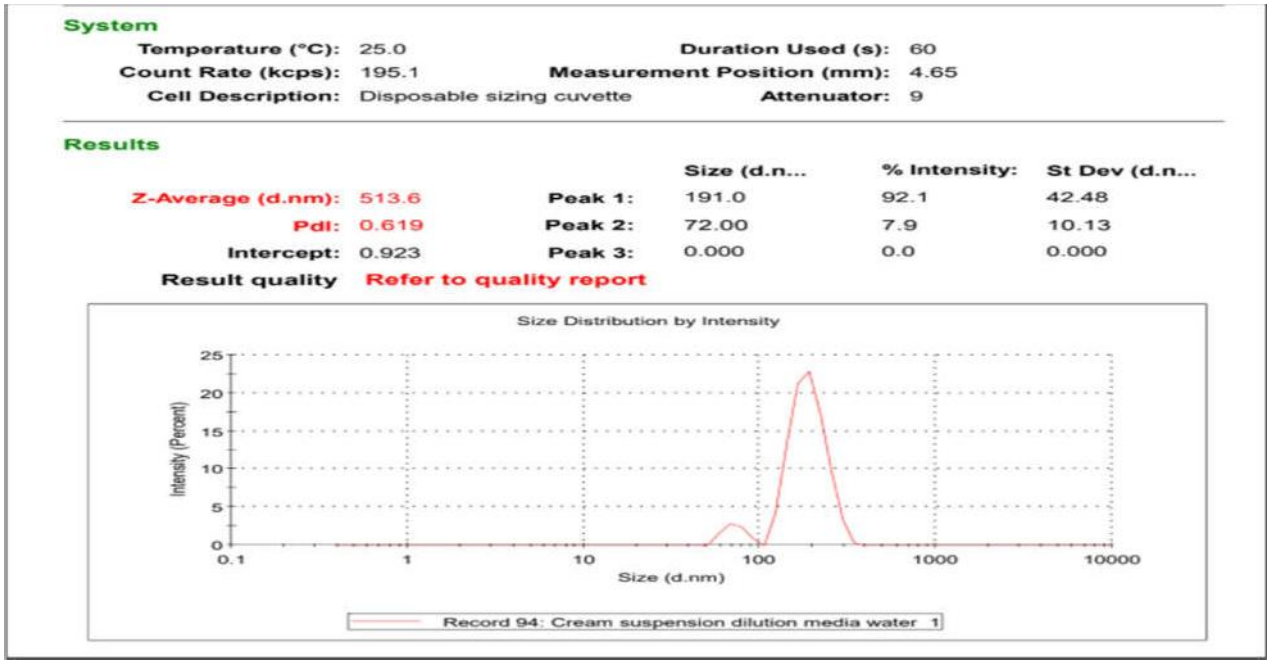

Figure no 2 : Particle size determination of optimized formulation

Zeta potential

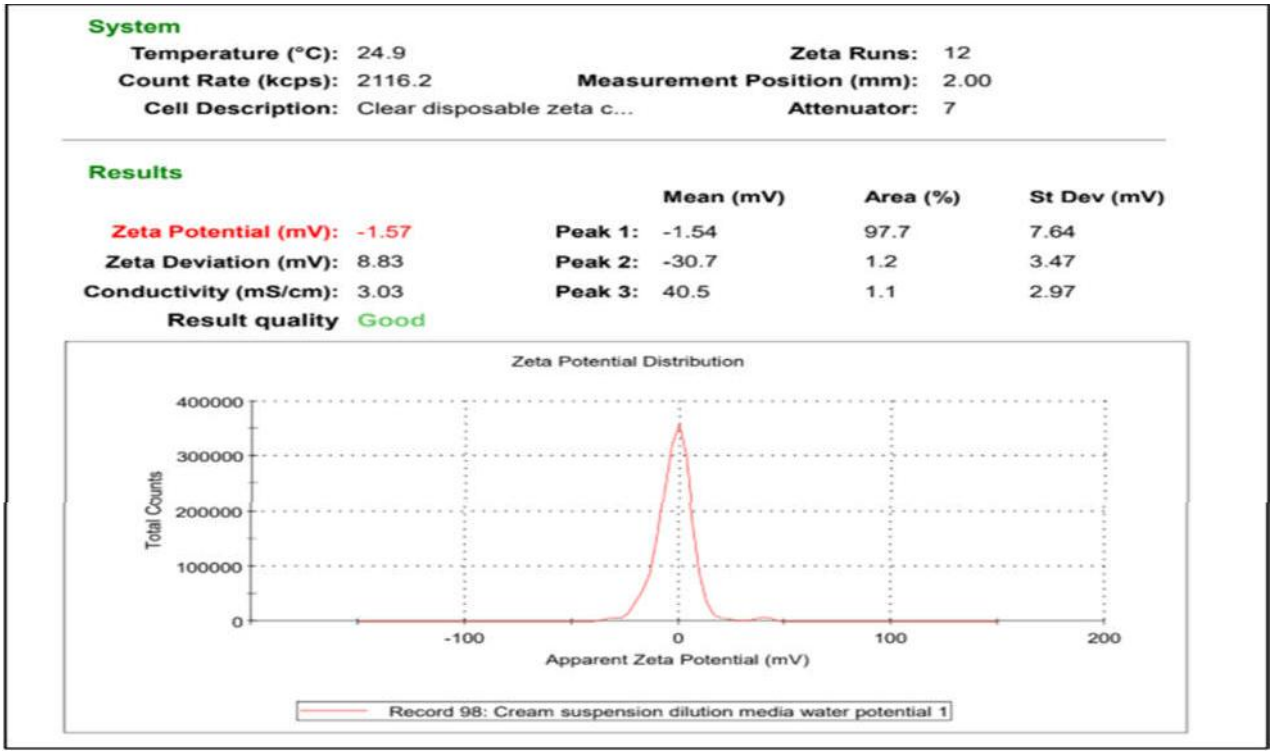

Figure no 3: Zeta potential determination of optimized formulation

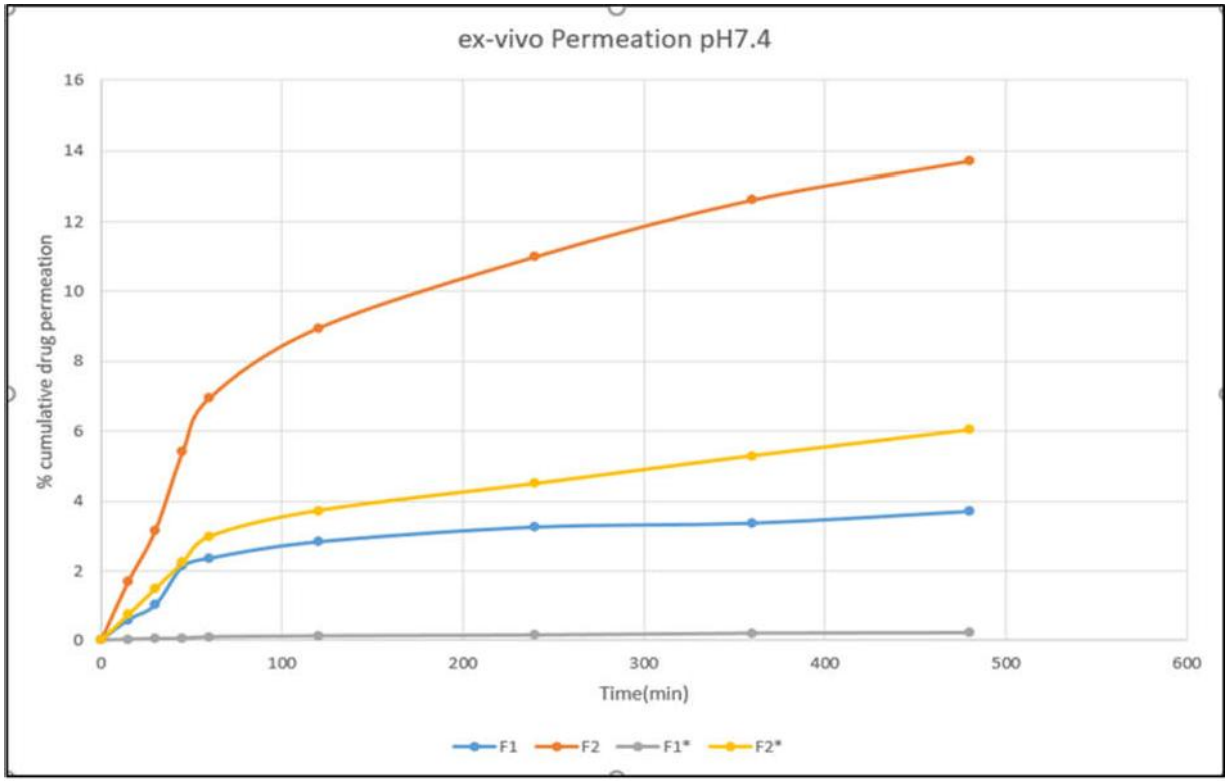

Figure no 4: Ex- vivo permeation studies

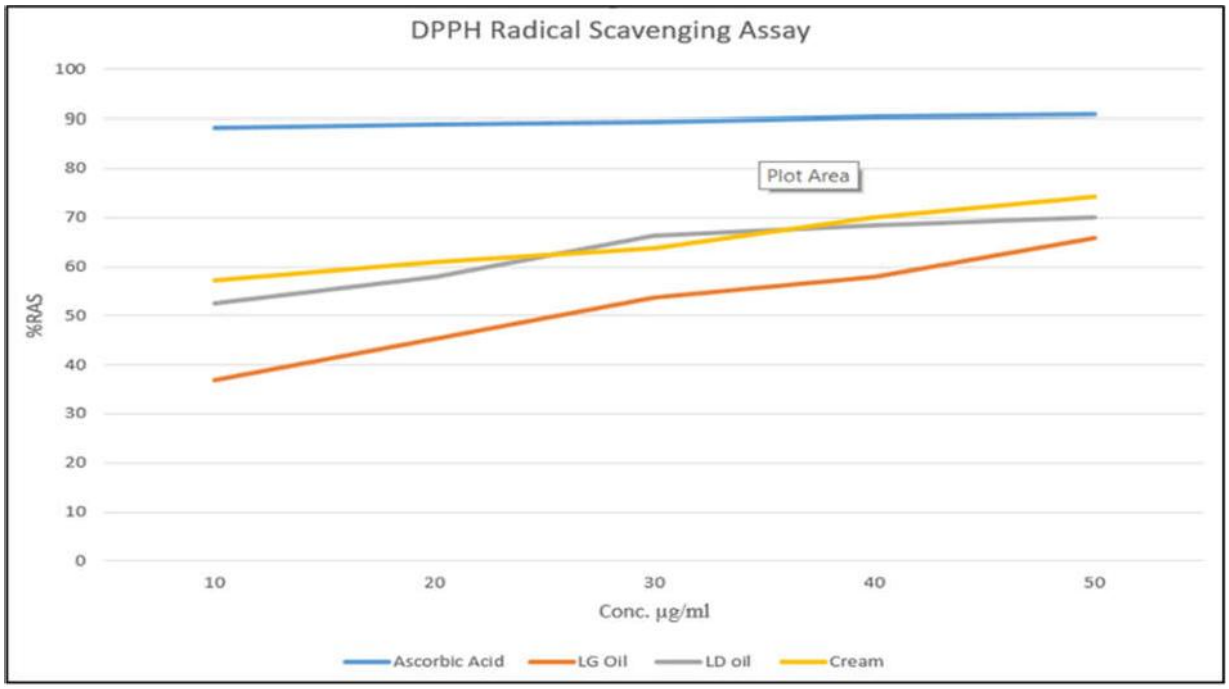

Figure no 5: % Scavenging activity of Oils and Cream