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Formulation and Evaluation of Anti-Aging Cream Using Grape Seed Extract by Microencasulation Method

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ABSTRACT

In the present study an attempt was made to prepare and evaluate herbal microencapsulation by using the grape seed oil extract. Grape seed oil is good for skin care and effective anti-oxidant that protects our skin from free radical damage and slows down the skin's aging. Different formulations were prepared by high-energy emulsification method, where microencapsulation incorporated into cream base. Microencapsulation formulated in four variations of grape seed oil concentration 3%, 3.5%, 3.8% and 4%. The characterization of microencapsulation was carried out for particle size and SEM analysis. Particle size analysis indicated mean particle sizes ranging from 34.5 ± 0.70µm to 42.25 ± 2.29µm. As the concentration of grape seed oil increases particle size also increases. Scanning electron microscopy of optimized formulation F3 confirmed smooth, stable morphology and spherical in shape. Hence formulation F3 showed better result in all characterization so considered as optimized formulation and incorporated into cream base. Cream formulated in 5 variations of microencapsulation concentration 1g, 1.5g, 2g, 2.5g, and 3g. The cream formulations were evaluated for organoleptic properties, dye test, pH, viscosity, spreadability, *in-vitro* anti-oxidant activity, and stability study. Results demonstrated a favorable pH range suitable for skin application. Formulation F4 exhibiting the good viscosity and resistance to spread. Formulation F4 showed maximum *in-vitro* anti-oxidant activity. Stability studies over two months showed good physical integrity with minor changes in pH and viscosity indicating stable formulations. Thus, the prepared cream as promising candidate for anti-oxidant and protective skincare formulations, with applications in cosmeceuticals and therapeutics.

Keyword: Grape seed oil, Microencapsule, Cream, Anti- Oxidant activity, Particle Size, Stability.

INTRODUCTION

Skin is one of the most essential organs in our body. Generally, women, as well as men, wish to have clean and bright skin. As a result, along with the increasing demand of the cosmetic market, there is a growing need of producing clean and bright skin and anti-aging. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Cosmetics are a category of health and beauty products that are used to care for the face and body, or used to accentuate or change a person's appearance. Cosmetics are not only employed to modify appearance of an individual, but are also used for care of skin and body, besides to add fragrance to that person. Although, cosmetics are known for skin and body care, there are various types of cosmetics with specific and significant purpose. Many distinct races and cultures employ cosmetics in the day-to-day life.[1]

1.1 Anatomy and Function of the Skin

The skin, is also known as integumetary system, it is the largest organ in humans body, measuring 2 m^2 on the surface and 3.6 kg weight in adults. It functions as an insulating, waterproof barrier to insulate the body from the shocks of the environment. In addition, it generates antimicrobial peptides that inhibit infections as well as hormones, neuropeptides, and cytokines that have biological effects on the skin and body as a whole.

The underlying mesenchyme and the surface ectodermal layer give rise to the integumentary system. It is made up of the skin, appendages, and their derived structures, such as the sebaceous and sweat glands, nails, and hair follicles. Dermis, hypodermis, and epidermis are the three layers that make up the skin. It acts as a vital link between the body and the outside world, maintaining vital functions including temperature control, hydration and fat storage, protection, and the preservation of water and electrolytes. It also plays a significant part in the immunological and endocrine systems.[2]

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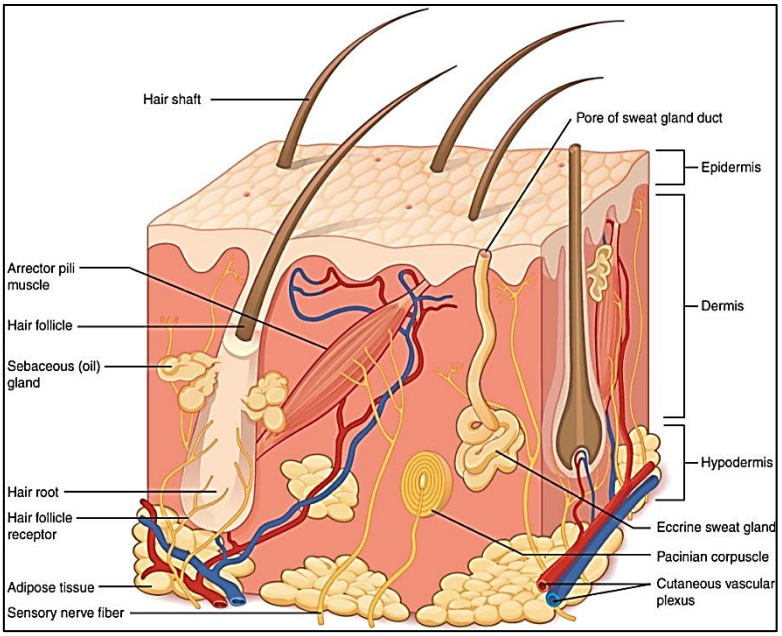

Fig 1. Structure of the skin.

1.1.1 Epidermis[3,4,5]

The epidermis is divided into four main layers based on the morphology and function of the keratinocytes:

1. Basal layer (Stratum Basale)
2. Prickle call layer (Stratum Spinosum)
3. Granular cell layer (Stratum Granulosum)
4. Cornified layer (Corneum)

1.1.2 Dermis

In contrast to the densely packed keratinocytes of the epidermis, the dermis is characterized by a more complex and dispersed arrangement of cells and extracellular matrix components. Here's a closer look at the dermis and its components:

1. Cells in the Dermis

a) Fibroblasts

b) Immune and inflammatory cells

c) Nervous tissue

d) Blood vessels

2. Extracellular Matrix Components

a) Collagen

b) Elastin

3. Aging and damage

1.1.3 Hypodermis

The subcutaneous layer underneath the dermis is called the hypodermis, and most of it is made up of fat. It shields the body from the cold, helps absorb trauma, and serves as the primary structural support for the skin. Nerves and blood vessels are entwined inside it.

1.2 Anti-aging[6]

Aging process is classified into two distinct types, i.e. "sequential skin aging" and "photo-aging". Both types have distinct clinical and historical features. Sequential skin aging is universal and predictable process characterized by physiological alteration in skin function. In the aging process keratinocytes are unable to form a functional stratum corneum and rate of formation from neutral lipids slows down, resulting in dry pale skin with wrinkle. In contrast, photo aging is caused by over exposure to UV rays from sunlight. It is characterized by dry, pale and shallow skin, displaying fine wrinkles as well as deep furrows caused by the disorganization of epidermal and dermal components associated with elastosis and Helio dermatitis. Herbs and plants have already proved useful as a tool in complementary medicine. Here's a brief overview of the categories mentioned;

1. Biological factors
2. Environmental factors
3. Mechanical factors
4. Metabolism and Physiological factors

1.2.1 Causes of aging[7.8]

Aging can be caused by various factors including metabolic damage, cellular senescence, cellular death, and toxic and non-toxic garbage accumulation. Metabolic damage involves glycation, mitochondrial damage, and respiratory chain dysfunction, which generate free radicals and contribute to aging. Respiratory chain dysfunction accumulates various ageing phenotypes.

Generation, leading to tumor proliferation. Most causes of senescence are due to induction of tumor suppression genes p53 and $p19^{Arf}$, while Skp2 E3-ubiquitin ligase can induce cancer. Inflammation can induce cellular senescence and death, including DNA damage, telomerase shortening, inadequate anti-oxidant systems, DNA repair and autophagy, defective cell cycle control, and defective proteasomes, lysosomes and shock proteins. Advanced glycation end products, cortisol and lipofuscin.

Solar radiation, primarily from the ultraviolet (UV) region, can cause harmful effects on the skin. UVA, UVB, and UVC are the three UV regions, with UVC being filtered out by the atmosphere. UVB, not completely filtered by the ozone layer, causes sunburn and pyrimidine dimers damage.

UVA radiation penetrates deeper skin layers, causing premature skin aging and free radical generation. UVB radiation causes 65% of skin damage. Exposure to UV radiation can lead to photo aging, sunburn, and skin cancer, highlighting the importance of avoiding sun exposure.

1.3 MICROENCAPSULATION[9.10]

Microencapsulation is the process in which small droplets or particles of liquid or solid material are surrounded or coated by a continuous film of polymeric materials. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating or embedded in a homogeneous or heterogeneous matrix, to give small capsules with many useful properties. Microcapsules is a modern dosage form which has been widely used to improve the stability of active substances or for any other medical purposes. Microencapsulation can provide a physical barrier between the core compound and the other components of the product. It is a technique by which liquid droplets, solid particles or gas compounds are entrapped into thin films of a food grade microencapsulating agent.

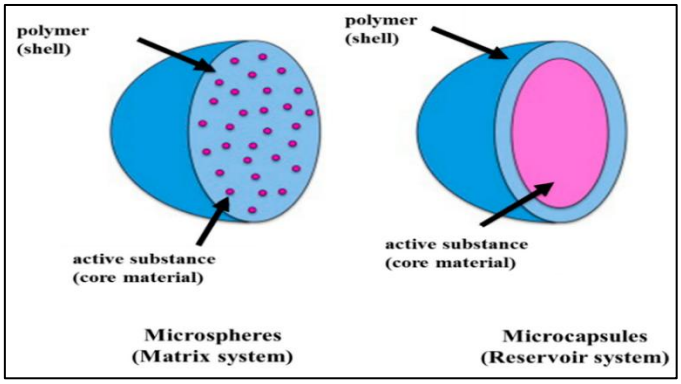

Fig 2. Difference between the Microspheres and Microcapsules

1.3.1 Advantages

1. High production rates and efficiency
2. Easy handling product powders
3. Reproducibility
4. Low operation cost
5. Short time process
6. Heat-resistant
7. Different core compounds can be used
8. Stable products Solubility and stability of hydrophobic actives

1.3.2 Disadvantages

1. Rapid release of actives
2. Specific for hydrophobic compounds
3. Long processing times
4. High energy, and high production cost
5. Highly sensitive to pH and ionic strength

1.3.3 Techniques of microencapsulation[11,12]

1. **Physical**

These rely primarily on mechanical or physical processes for encapsulation.

- Spray drying

A solution or suspension containing the core material is sprayed into hot air, rapidly drying the droplets and forming microcapsules.

- Solvent evaporation

The core material is dissolved or dispersed in a volatile solvent along with a polymer. The solvent is then evaporated, leaving behind solid microcapsules.

2. **Chemical**

These involve chemical reactions to form the capsule wall around the core material.

- In situ polymerization

Polymer forms directly on the surface of the core material via chemical reaction in the same phase.

- Interfacial polymerization

Polymer forms at the interface of two immiscible phases (usually oil and water), encapsulating the core material in the process.

- Suspension polymerization

Monomer droplets containing the core are suspended in a continuous phase, and polymerization occurs within those droplets.

- Emulsion polymerization

A fine emulsion of monomer and core material is polymerized, usually in an aqueous phase, to form microcapsules.

3. **Physical – chemical**

These methods combine physical and chemical processes.

- Coacervation

A polymer-rich phase separates from a solution and deposits around the core, forming a capsule upon solidification.

- Sol-gel method

Involves the transition of a solution (sol) into a solid (gel), entrapping the core material within the formed matrix.

1.3.4 APPLICATION

1. Pharmaceutical Industry
2. Food Industry
3. Cosmetics and Personal care
4. Agrochemical Industry
5. Textile Industry
6. Paints and Coatings
7. Printing and Paper Industry

Aim: The aim of present study is to formulate and evaluate of antiaging cream using grape seed extract by microencapsulation method.

Objective:

1. Extraction of grape seed.

2. Phytochemical analysis of grape seed extract.
3. Formulation of microcapsules.
4. Pre formulation studies of extract.
5. Incorporation of microcapsules into cream.
6. Evaluation of cream.
7. Determination of Antiaging factor.
8. Stability studies as per ICH guidelines

METHODOLOGY

1. Authentication of *Vitis vinifera* seed (Grape seed).

Grapes was collected from local market, Bengaluru. Seeds was separated, dried under sunlight and authenticated by Central Ayurveda Research Institute Bengaluru.

2. Extraction of Grape seeds.

Grape seeds was washed and dried under sunlight. For oil extraction cold pressing method was used. This method involves the pressing the grape seeds with a mechanical press to squeeze out the oil and extracted grape seed oil stored in a cool and dark place.

Fig 3. Extraction of Grape seeds.

3. Phytochemical analysis of extracts.[13,14,15]

a) Qualitative analysis:

1. **Steroids:** An aliquot of the seed extract 1ml was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns to red colour and sulphuric layer showed yellow with green fluorescence. This indicated the presence of steroids.

2. **Tannins:** An aliquot of the seed extract 2ml was added to few drops of 1% lead acetate and the yellowish precipitate indicate the presence of tannins.

3. **Saponins:** An aliquot of the seed extract 5ml was mixed with 20 ml of distilled water in a graduated cylinder and then agitated for 15 minutes. Formation of foam indicates the presence of saponins.

4. **Anthocyanins:** An aliquot of the seed extract 2ml was added to 2 ml of 2N HCl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins.

5. **Glycosides:** 2ml glacial acetic acid one drop of 5% $FeCl_3$ and concentrated H_2SO_4 was added in to 5ml of extract the appearance of brown ring indicates the presence of glycosides.

6. **Alkaloids:** A Mayer's test: - To the acidic solution, Mayer's reagent (Potassium mercuric iodide solution) was added. Cream coloured precipitate indicates presence of alkaloids.

7. **Phenol:** ½ ml of FeCl3 solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenol.

8. **Flavonoids:** An aliquot of the seed extract 2-3ml and few drops of sodium hydroxide solution were added into a test-tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

b) Quantitative analysis

i. Determination of Total Phenolic Content.

Preparation of Standard solution:

Approximately 10 mg of Gallic acid was weighed and transferred in to a 10 ml volumetric flask. Gallic acid dissolved in small amount of methanol in a volumetric flask and made up the volume up to 10 ml by using the methanol to prepare 1000 µg/ml solution.

From the above stock solution, 1ml was pipetted in to a volumetric flask of 10 ml capacity and volume was made by using distilled water to 10ml (100 µg/ml). Pipette out 1, 2, 3, 4 and 5 ml of 2nd stock solution and transfer in to the 10 ml volumetric flask. The volume was made to 10 ml to get the concentration 10 µg/ml to 50 µg/ml. Then 0.2ml of Folin-Ciocalteu phenol reagent, 0.5 ml of saturated sodium carbonate was added and volume was made up to 10ml with distilled water to get the concentrations of 10, 20, 30, 40, 50 µg/ml respectively. The standard solution was placed in a dark place for 1 hour and absorbance was measured at 636 nm using Shimadzu UV-visible spectrophotometer.

Preparation of Sample solution:

10mg of extract was transferred into 10ml of volumetric flask. Extract was dissolved with small amount of methanol and volume was made up to 10ml by using methanol to prepare 1000 µg/ml solution.

From the above stock solution, 1ml was pipetted out to a volumetric flask of 10ml capacity and volume was made up by using distilled water to prepare 100 µg/ml solution. 1 ml of above stock solution was pipette out and transferred into 10ml volumetric flask, 0.2 ml of Folin- Ciocalteu phenol reagent was added and 0.5ml saturated sodium carbonate was also added and volume was made up to 10 ml with distilled water. Sample solutions was placed in a dark place for 1h and absorbance was measured at 636 nm using Shimadzu UV-visible spectrophotometer.

ii. Determination of Total Flavonoid Content.

5 gm of extract was transferred into beaker; 50 ml of distilled water was added and mixed well. Then 2 ml of Hcl was added to a beaker and boiled on water bath for 30 minutes and 10 ml of ethyl acetate was added. Empty filter paper was weighed called as W_1. Then extract was filtered and filter paper will absorb the Flavonoids. The above residue was discarded and filter paper was dried completely and weighed; the dried filter paper called as W_2.

$$\textbf{Total Flavonoid Content} = \frac{W2-W1}{Weight\ of\ the\ sample} \times 100$$

4. Compatibility Study[16]

The compatibility study was conducted using a Bruker FTIR spectrophotometer aimed to detect any possible chemical interactions between the drug and excipients, such as oil, surfactant, and co-surfactant. A physical mixture of the drug, oil, surfactant, and co-surfactant was prepared and mixed with a suitable quantity of potassium bromide. Approximately 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons of pressure. This pellet was then subjected to IR radiation in the range of 4000 to 400 cm^{-1} in the FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those

of the pure drug, oil, surfactant, and co- surfactant, and matching was performed to detect any appearance or disappearance of peaks.

5 Preparation of Microencapsulation[17,18]

Microencapsulation preparations were made using an Ionic gelation method.

5.1 Preparation of Microencapsulation:

Oil phase

Sodium alginate was dissolved in water, with continuous magnetic stirring at 1700 rpm for 20 minutes. Then essential oil (Grape seed oil) was added in the solution with continuous stirring for 10 minutes. The oil phase was prepared separately.

Aqueous phase

Calcium chloride was dissolved with water, then it was filled into the flask with a volume of 100 ml and made up the final volume with deionized water.

Formation of microcapsule

The prepared oil phase was added dropwise by the syringe into the aqueous phase with continuous stirring. The microcapsules were formed. Further, it was stirred continuously at 1700 rpm for 15 minutes. After that, the prepared microcapsules were filtered by using Whatman filter paper 1 (125 mm) washed 2 to 3 times with deionized water and dried at RT.

Table 1. Formulation of Microencapsulation.[17]

Ingredients	F1	F2	F3	F4
Grape seed oil	3ml	3.5ml	3.8ml	4ml
Sodium alginate	1g	1.5g	1.8g	2g
Calcium chloride	16g	16g	16g	16g
Distilled water	Q. S	Q. S	Q. S	Q. S

6. Preparation of Cream base:

The oil phase in the form of stearic acid, cetyl alcohol and liquid paraffin was taken in a beaker and melted over a water bath 75°C. In another beaker glycerin, methyl paraben, propylene glycol dissolved in distilled water, heated on a water bath until it dissolves completely. The oil phase was poured in to water phase with continuous tituration. Homogeneous cream mass was formed and add a few drops of rose oil as a perfume and stirred homogeneously. Finally good particle size of micro range of microencapsulation is incorporated into cream base.

Table 2. Formulation of cream base.[19]

Ingredients	F1	F2	F3	F4	F5
Microencapsulation	1g	1.5g	2g	2.5g	3g
Stearic acid	1.5g	2g	2.5g	3g	3.5g
Cetyl alcohol	0.8g	1g	1.2g	1.4g	1.6g
Liquid paraffin	1.16ml	1.16ml	1.16ml	1.16ml	1.16ml
Glycerin	1.26ml	1.26ml	1.26ml	1.26ml	1.26ml
Methyl paraben	0.05g	0.05g	0.05g	0.05g	0.05g
Propylene glycol	6.2ml	6.2ml	6.2ml	6.2ml	6.2ml
Perfume	2 drops	2 drops	2 drops	2 drops	2 drops
Distilled water	Q. S	Q. S	Q. S	Q. S	Q. S

7. Evaluation of microencapsulation

7.1 Particle size determination[20]

Measurement of the particle size distribution and mean diameter of microencapsulation was carried out with an optical microscope. Stage micrometer was used to calculate the eye piece micrometer. 10 deviations of stage micrometer was matched with the deviation of eye piece micrometer and calibration factor was calculated. The particle size was calculated by multiplying the number of the deviation of the eye piece micrometer occupied by the particle with calibration factor. 30 randomly chosen microencapsulation taken to measure their individual size.

7.2 Scanning electron microscopy (SEM) analysis

The morphological study was performed by scanning electron microscopy (SEM) for Grape seed oil microcapsules. The microcapsules were coated with gold on a Nova NanoSEM (NPEP303) with an applied voltage 5.00 Kv. The photomicrographs were obtained from Image J Software.

8. Evaluation of cream

8.1 Organoleptic properties[21]

The cream thus obtained was evaluated for its organoleptic properties like color, odor and texture. The appearance of the cream was judged by its color and roughness.

8.2 Dye test[22]

The Scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide then covers it with a cover slip, and examines it under a microscope. If the disperse globules appear red the ground colourless then its w/o type. The reverse condition occurs its o/w type cream i.e. the disperse globules appear colourless and ground red.

8.3 pH determination

The pH meter was calibrated using standard buffer solution. pH electrode was dipped in to the cream and its pH was measured with the help of digital pH meter.

8.4 Viscosity determination[23]

Viscosity measurement was carried out by placing the preparation in a 100 ml glass beaker and selecting the spindle number 64 at 10 rpm and maintained at temperature at 25 °C. The measurement was carried out thrice by using a Brookfield Viscometer.

8.5 Spreadability[24]

Spreadability of formulated cream was measured by placing sample in between two slides then compressed to uniform thickness by placing a definite weight for defined time. The specified time required to separate the two slides was measured as spreadability. Spreadability was calculated by the following formula

$$\text{Spreadability} = \frac{ML}{T}$$

Were,

M = Weight tied to the upper slide

L = Length of glass slide

T = Time taken in seconds.

8.6 *In-vitro* antioxidant activity[25]

The DPPH assay is performed by A 100 µl aliquot of the different concentrations of formulations along with standards were added to 1ml of DPPH solution into test tubes. A black is methanol, while a control is set up with 1ml DPPT and 1ml methanol. All the tubes are incubated in the dark at room temperature for 30minutes. After that the absorbance of each solution was determined by using Shimadzu UV-visible spectrophotometer at 517 nm. The percentage inhibition was calculated as follows.

$$\text{Percentage inhibition} = [(\text{Abs. Control} - \text{Abs. Sample}) \times 100)/ (\text{Abs. control})]$$

Were

Abs. Control = Absorbance Control

Abs. Control = Absorbance Sample

8.7 Stability studies[26]

Stability studies were carried out as per ICH guidelines. The optimized cream formulation (F4) was placed in airtight glass containers and kept under accelerated conditions (temperature 40 ± 2 °C and RH 75 ± 5%) using stability chamber for the period of 2 months. The sample were withdrawn at 1st month, 2nd month, and evaluated for their color, odor, Ph, viscosity and phase separation.

RESULTS

5.1 Phytochemical analysis of extract

A. QUALITATIVE ANALYSIS

Table 3. Phytochemical analysis of Grape seed extract.

SL.NO.	PHYTOCHEMICAL CONSTITUTION	OIL EXTRACT OF GRAPE SEED
1.	Steroids	++
2.	Tannins	+++
3.	Saponins	++
4.	Anthocyanins	-
5.	Glycosides	++
6.	Alkaloids	++
7.	Phenols	++
8.	Flavonoids	+++

Phytochemical screening tests for constituents of grape seed extract (oil extract). (+++), (++), (+) and (-) refer to high, moderate, low and absent amount respectively.

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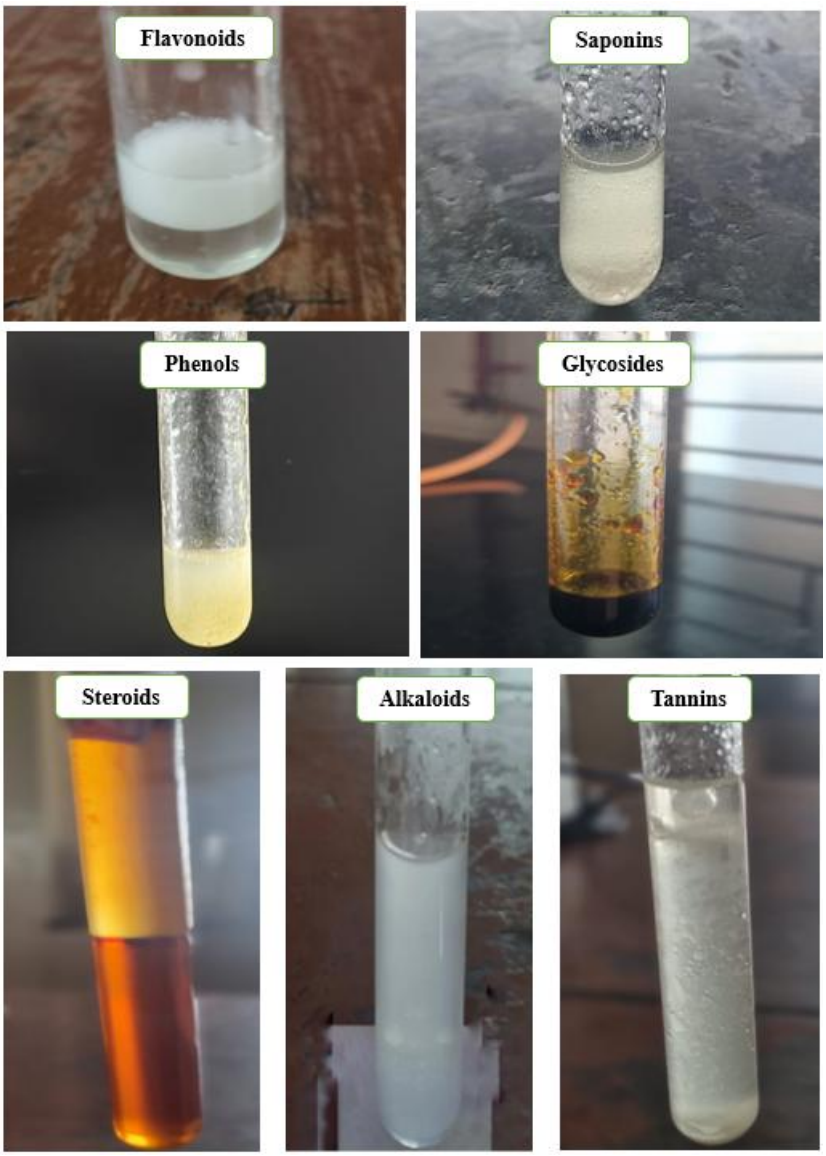

Fig 4. Phytochemical screening tests for constituents of grape seed extract.

B. QUANTITAVE ANALYSIS

i.Determination Total Phenolic Content

The total phenolic content was determined by measuring the absorbance of the sample solution at a wavelength of 636 nm and comparing it with a calibration curve using gallic acid as a standard and calculated from the calibration curve (R^2 = 0.9991). The results shown in Table 6 & Fig 16.

Table 4. Determination of Total Phenolic Content.

SAMPLES	CONCENTARTIONS (µg/ml)	ABSORBANCE
Gallic acid (Standard)	10	0.265 ± 0.009
	20	0.481 ± 0.015
	30	0.714 ± 0.021
	40	0.997± 0.026
	50	1.178 ± 0.046
Grape seed oil extract	10	0.710 ± 0.017

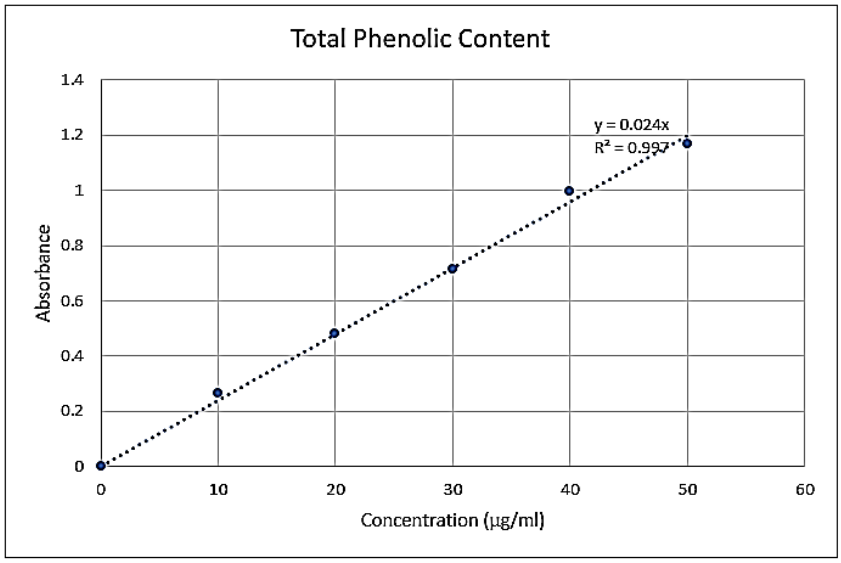

Fig 5. Standard calibration curve of Gallic acid.

ii. Determination of Total Flavonoid Content

The total flavonoid content of grape seed oil was determined by filter paper method and calculated from the formula. The total flavonoid content was found to be 24.10%.

5.2 COMPATIBILITY STUDIES

FT-IR Analysis

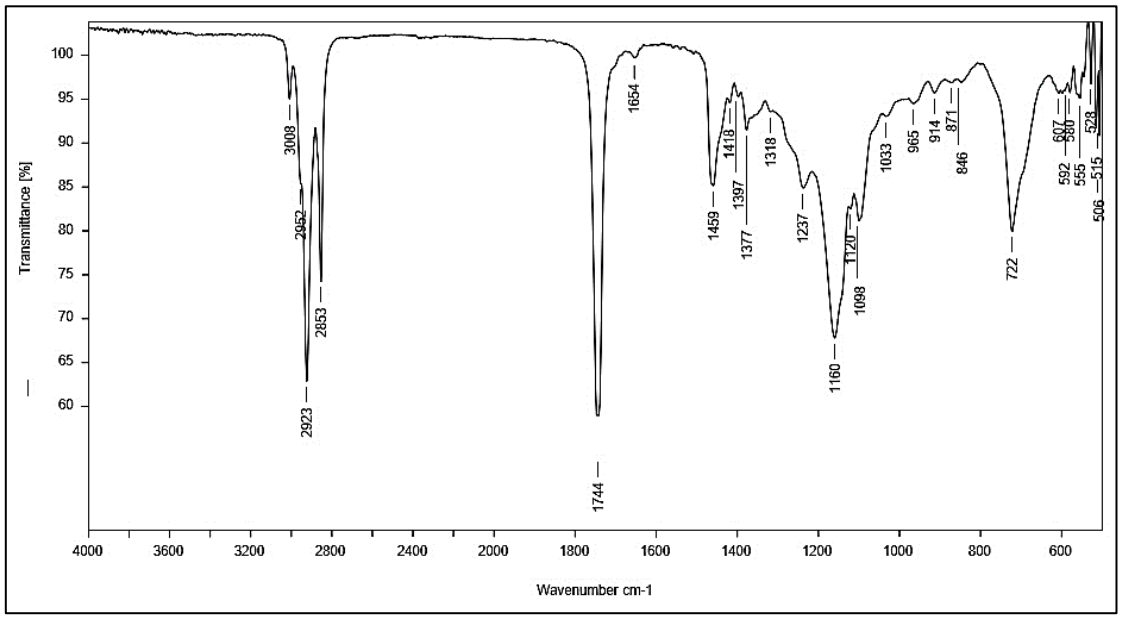

Fig 6. FTIR Spectra of Grape seed oil.

Table 5. FTIR spectral data of Grape seed oil.

FUNCTIONAL GROUP	TYPE OF VIBRATION	CHARACTERISTIC ABSORPTION (cm^{-1})
O-H	Stretching	3008
C-H	Stretching	2923
C=C-H	Stretching	2853
C=O	Stretching	1744
C=C	Stretching	1459

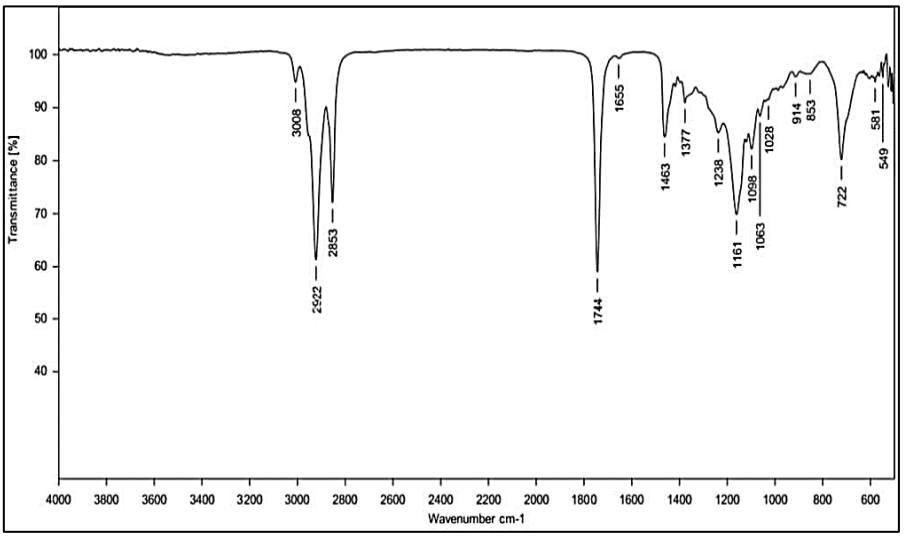

Fig 7. FTIR spectra of Grape seed oil and Cetyl alcohol.

Table 6. FTIR spectral data of Grape seed oil and Cetyl alcohol.

FUNCTIONAL GROUP	TYPE OF VIBRATION	CHARACTERISTIC ABSORPTION (cm^{-1})
O-H	Stretching	3008
C-H	Stretching	2922
C=C-H	Stretching	2853
C=O	Stretching	1744
C=C	Stretching	1463

5.3 CHARACTERIZATION OF MICROENCAPSULATION

5.3.1 PARTICLE SIZE

Table 7. Particle size of Microencapsulation (F1-F4)

SL.NO.	FORMULATION	PARTICLE SIZE(µm) (Mean ± SD)
1.	F1	**39 ± 2.47**
2.	F2	**42.25 ± 2.29**
3.	F3	**34.5 ± 0.70**
4.	F4	**39.25 ± 1.59**

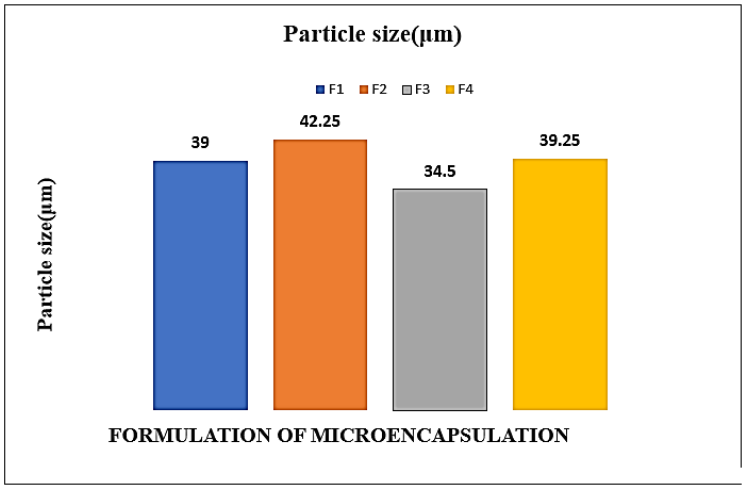

Fig 8. Particle size analysis of microencapsulation (F1-F4)

5.3.2 SEM (SCANNING ELECTRON MICROSCOPY) ANALYSIS

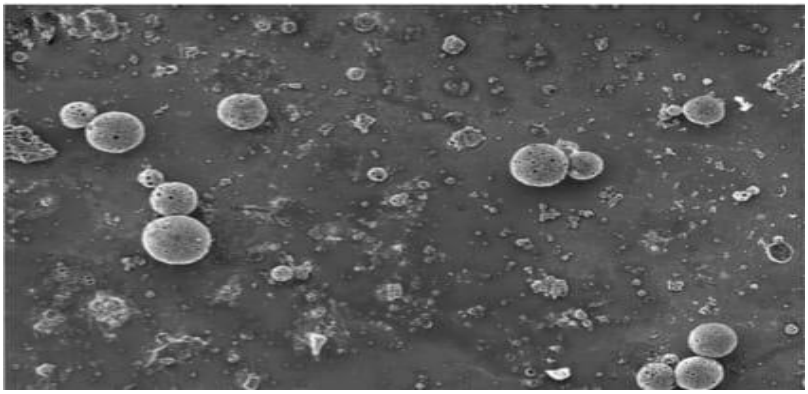

Fig 9. SEM image of optimized formulation F3 at 220KX magnification.

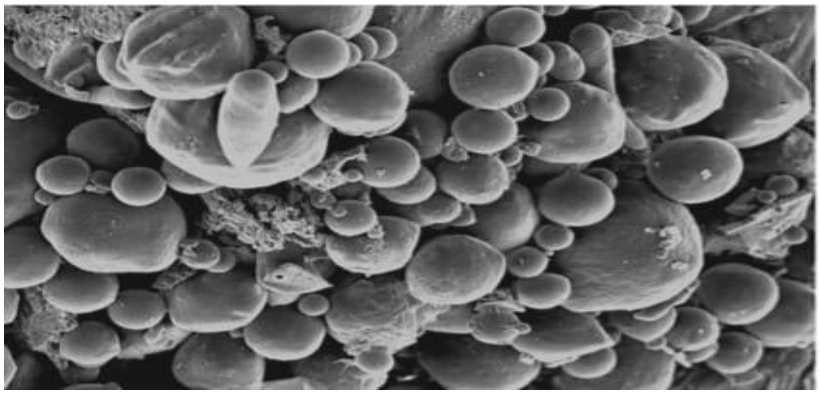

Fig 10. SEM image of optimized formulation F3 at 330KX magnification.

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5.4 EVALUATION PARAMETER OF CREAM

5.4.1 ORGANOLEPTIC PROPERTIES

Table 8. Organoleptic properties of the cream (F1-F2).

SL.NO.	PARAMETER	F1	F2	F3	F4	F5
1.	Color	White	White	White	White	White
2.	Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
3.	Texture	Smooth	Smooth	Smooth	Smooth	Smooth

5.4.2 DYE TEST

The Scarlet red dye is mixed with the cream. Place a drop of the cream on a microscopic slide then covers it with a cover slip, and examines it under a microscope. The disperse globules appears red and the ground will be colorless i.e. o/w type cream.

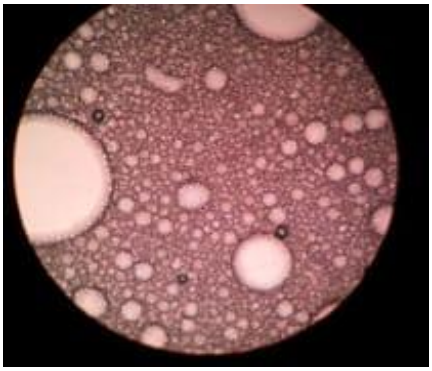

Fig 11. O/W type of cream of formulation F1-F5

5.4.3 pH DETERMINATION

Table 9. pH of creams (F1-F5).

SL.NO.	FORMULATIONS	pH*(Mean ± SD)
1.	F1	5.39 ± 0.01
2.	F2	5.41 ± 0.03
3.	F3	5.40 ± 0.05
4.	F4	5.43 ± 0.03
5.	F5	5.37 ± 0.06

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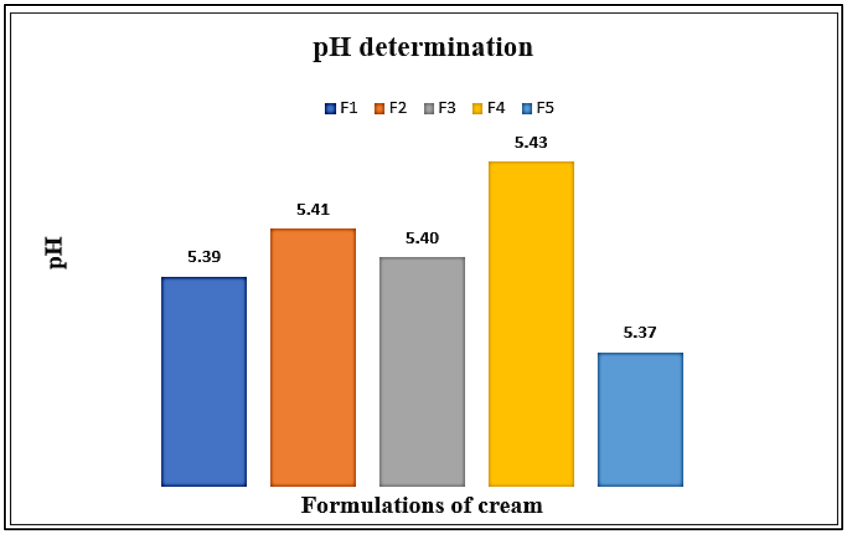

Fig 12. pH of cream (F1-F5).

5.4.4 VISCOSITY DETERMINATION

Table 10. Viscosity of creams (F1-F5).

SL.NO.	FORMULATIONS	VISCOSITY*(Cps) (Mean ± SD)
1.	F1	12433.33 ± 204.28
2.	F2	14333.33 ± 219.99
3.	F3	15500.00 ± 235.70
4.	F4	11700.00 ± 94.28
5.	F5	13333.33 ± 157.13

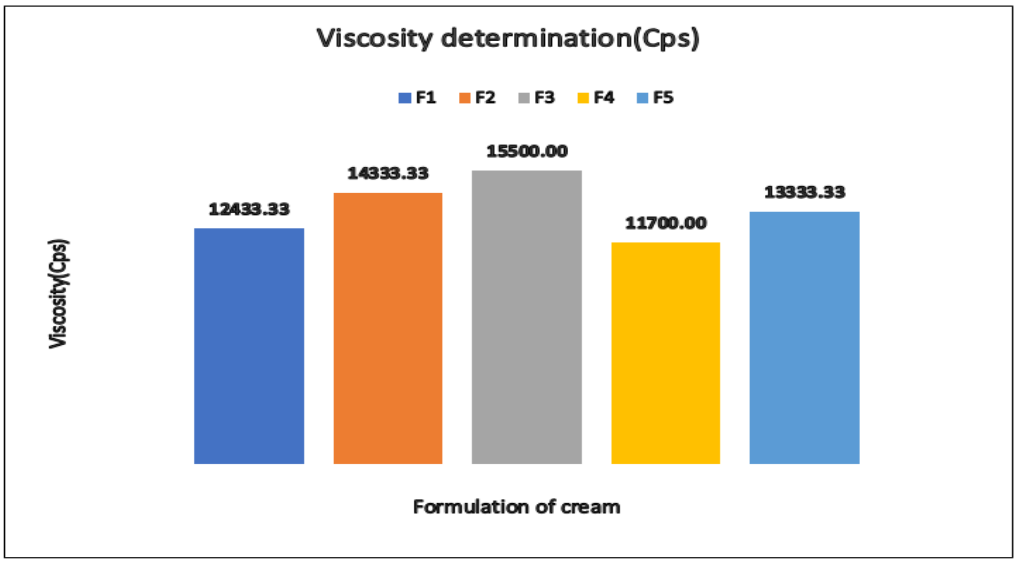

Fig 13. Viscosity of cream (F1-F5)

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5.4.5 SPREADABILITY

Table 11. Spreadability of cream (F1-F5).

SL.NO.	FORMULATIONS	SPREADABILITY* (g.cm/Sec) (Mean ± SD)
1.	F1	14.91 ± 0.17
2.	F2	12.80 ± 0.52
3.	F3	11.44 ± 0.22
4.	F4	16.23 ± 0.13
5.	F5	13.73 ± 0.11

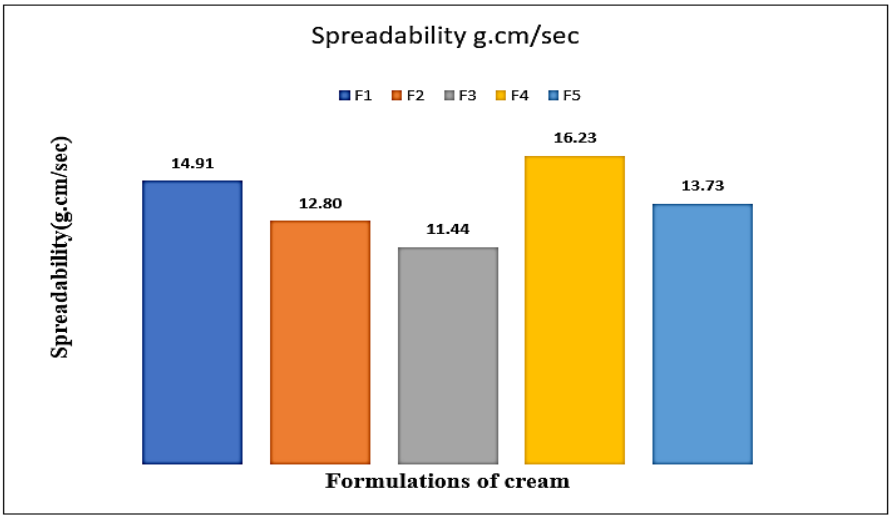

Fig 14. Spreadability of cream (F1-F5).

5.4.6 *IN-VITRO* ANTIOXIDANT ACTIVITY

Table 12. In-vitro antioxidant activity of the creams(F1-F5).

SL.NO.	FORMULATIONS	% INHIBITION* (Mean ± SD)
1.	F1	56.23 ± 0.35
2.	F2	60.60 ± 0.42
3.	F3	69.77 ± 0.41
4.	F4	71.50 ± 0.52
5.	F5	67.63 ± 0.53

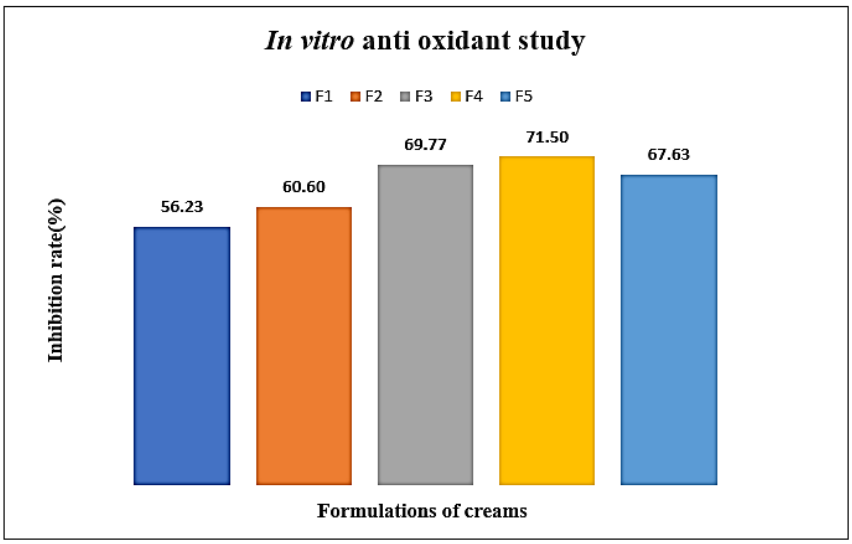

Fig 15. In-vitro antioxidant activity of the creams(F1-F5).

5.4.7 STABILITY STUDIES

Table 13. Stability studies for optimized formulation of cream.

FORMULATIONS	PARAMETER	INTIAL	1ST MONTN	2ND MONTH
F4 (40℃ ± 2℃ & RH 75% ± 5%)	Color	White	White	White
	Odor	Pleasant	Pleasant	Pleasant
	Phase separation	No separation	No separation	No separation
	pH	5.43 ± 0.03	5.39 ± 0.01	5.26 ± 0.01
	Viscosity (Cps)	11700.00 ± 94.28	10366.67 ± 78.56	9333.33 ± 219.98

DISCUSSION

In Present work, an attempt was made to formulate and evaluate antiaging cream by using grape seed oil extract. Studies were conducted using different concentrations of grape seed oil. All the prepared samples were evaluated for different properties.

The cream formulations were prepared by using an emulsification method where microencapsulation was incorporated into cream base.

The study investigated the phytochemical analysis, where qualitative analysis revealed the presence of multiple bioactive compounds and quantitative analysis of total phenolic and flavonoid content confirmed the antioxidant potential of grape seed oil, with a total phenolic content of 31.92 ± 0.01 mg/ml and flavonoid content of 24.10 %.

The compatibility study using FT-IR analysis identified key functional groups, including hydroxyl, carbonyl, and alkene groups which highlight the stability and bioactive potential of the oil. FT-IR spectrum of grape seed oil and cetyl alcohol showed no interaction.

The particle size measurements revealed good stability with particle sizes ranging from 34.5 ± 0.70µm to 42.25 ± 2.29µm. The surface morphology analysis using SEM confirmed the stability of the microencapsulation, while F3 formulation show the good particle size.

Cream formulations showed good organoleptic properties, pH, compatibility, and appropriate viscosity were observed and spreadability varied across formulations. *In-vitro* anti-oxidant activity with formulation F4 showed the highest antioxidant potential. Stability studies of optimized formulation F4 over two months indicated good physical integrity in terms of color, odor, and phase separation with minor changes in pH and viscosity within acceptable limits.

The study confirmed the potential of GSO (grape seed oil)-based cream as stable, antioxidant-rich formulations suitable for

cosmetic and therapeutic applications.

CONCLUSION

The studies included to prepare and evaluate the antiaging cream using grape seed oil by microencapsulation method. The formulation behavior was studied by varying the oil concentration and keeping the other excipients concentration constant. The prepared cream formulations were characterized for dye test, organoleptic properties, pH, viscosity, spreadability, *in-vitro* anti-oxidant activity and stability study.

The development of microencapsulation demonstrated that particle size varied with oil concentration, with smaller particle sizes observed in specific formulations. The surface morphology analysis via SEM confirmed the stability of the microencapsulation F3, which could have implications for topical applications.

The cream formulations showed organoleptic properties, pH, compatibility, and appropriate viscosity were observed and spreadability varied across formulations. The dye test confirms that F4 formulation and other are showing oil-in-water (o/w) of cream. The *in-vitro* antioxidant activity revealed that formulation F4 had the highest anti-oxidant potential. The stability study were carried out for optimized formulation F4 remained stable over two months with only minor fluctuations in pH and viscosity.

The grape seed oil extract particularly when formulated into cream by emulsification method, exhibits promising anti-oxidant properties making it a suitable candidate for use in cosmetic and pharmaceutical applications. The study confirms the oil is potential to provide multiple health benefits, especially in skin care formulations. Further research could explore its therapeutic efficacy and long-term stability in real-world applications.

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