

DEVELOPMENT OF HERBAL FORMULATION FOR SKIN CARE

A Thesis submitted to Gujarat Technological University

for the Award of

Doctor of Philosophy

in

Pharmacy

by

Jethva Monica Jayeshbhai

Enrollment No.: 199999901510

under supervision of

Dr Mamta B. Shah

Former Professor and Head, Department of Pharmacognosy,
L M College of Pharmacy, Ahmedabad.



GUJARAT TECHNOLOGICAL UNIVERSITY
AHMEDABAD

[August-2026]

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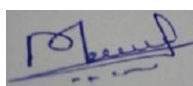
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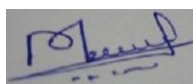
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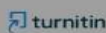
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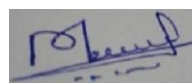
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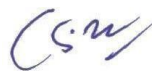
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ABSTRACT

Nano-cosmetics are formulations that incorporate nanomaterials, which possess at least one dimension between 1 and 100 nm. These nanomaterials can improve the performance, stability, and transport of active ingredients. Herbal nano-cosmetic formulations combine herbal ingredients with nanotechnology for improvement of drug delivery. The goal of our research work was to develop and evaluate herbal cream containing solid lipid nanoparticles. Incorporation of SLNs into herbal creams offers several advantages, including increased stability, improved skin penetration, prolonged release of active compounds, and enhanced hydration. In this study, an herbal cream was developed using aqueous extracts of *Nelumbo nucifera* (lotus-Flower), *Averrhoa carambola* (starfruit-Fruit), *Nigella sativa* (kalonji-Seed) and *Sphaeranthus indicus* (gorakhmundi-Whole Plant).

SLNs loaded with herbal extracts (*N. sativa*, *N. nucifera*, *A. carambola*, *S. indicus*) were synthesized using the microemulsion method, and evaluate physical and chemical properties. Particle size of SLNs was measured by using particle size analyser, while morphological structure was assessed by scanning electron microscopy (SEM). Comparison between pure extracts and SLNs-loaded extracts was evaluated using FTIR. SEM images confirmed the spherical shape of SLNs. The formulation had a mean particle size between 48-68 nanometre, depending on the extract used. FTIR results indicated no major interactions between the extracts and the excipients, confirming compatibility. Overall, SLNs prepared by the microemulsion technique exhibited suitable size, morphology, and stability for topical formulation.

A simple method was employed to prepare an oil in water cream. The oil part was made up of stearic acid (4%), glyceryl monostearate (2%), and cetyl alcohol (2%), all were melted at 70 °C. The water phase was made up of paraffin oil (8%), tween 80 (2%), triethanolamine (0.25%) and water (77.75%) and it was also boiled at 70 °C. The aqueous phase or water phase has been mixed with oil part and keep it stirred followed by cooling to room temperature. Perfume and methyl paraben (preservative) were added at the end, and 4% of the SLNs–herbal extract mixture was incorporated after cooling. The cream was evaluated for pH, homogeneity, colour, spread ability, ease of removal, type of smear, acid value, saponification value, and total phenolic content.

Keywords: Herbal cream, nano-cosmetics, solid lipid nanoparticles, ageing, acne.

Acknowledgement and / or Dedication

“Arise, awake and stop not till the goal is reached.”

I consider it a privilege to express my heartfelt gratitude to all the dignitaries who have directly or indirectly contributed to the successful completion of this dissertation.

The satisfaction that accompanies the completion of any task would be incomplete without acknowledging the people whose constant guidance, support, and encouragement made this work possible. I am deeply grateful to Almighty God, for it is He who began this work in me and carried it through to completion. He has blessed me with the presence of many wonderful individuals whom I feel honoured to acknowledge here.

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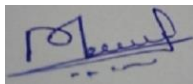
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Date: **31/08/2026**

Place: Ahmedabad.



(Ms. Monica J. Jethva)

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List of Abbreviation

Abbreviation	Full Form
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AFM	Atomic Force Microscopy
ANOVA	Analysis of Variance
AP-1	Activator Protein 1
API	Active Pharmaceutical Ingredient
ATCC	American Type Culture Collection
AV	<i>Acne Vulgaris</i>
BBB	Blood-Brain Barrier
BSI	Botanical Survey of India
C1P	Ceramide 1-Phosphate
CFU/mL	Colony-Forming Units per Milli Litre
CLSI	Clinical and Laboratory Standards Institute
cm⁻¹	Reciprocal Centimetres / Wavenumbers
CNS	Central Nervous System
CO₂	Carbon Dioxide
CRH	Corticotropin-Releasing Hormone
DLS	Dynamic Light Scattering
DMSO	Dimethyl Sulphoxide
DNA	Deoxyribonucleic Acid

DOI	Digital Object Identifier
DPI	Department of Public Instruction
DPPH	2,2-diphenyl-1-picrylhydrazyl
DR	Dietary Restriction
DSC	Differential Scanning Calorimetry / Dynamic Scanning Calorimetry
E	Emollient
ECMp	Extracellular Matrix Proteins
Exp. Date	Expiration Date
FFVP	Fresh Fruit and Vegetable Program
FTIR	Fourier-Transform Infrared (spectroscopy)
G	Good
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GFP	Green Fluorescent Protein
GI	Gastro Intestinal
GN	Gorakhmundi Extract
HA	Hyaluronic Acid
HPH	High Pressure Homogenization
IGF-1	Insulin-like Growth Factor-1
IJNRD	<i>International Journal of Novel Research and Development</i>
IJPPR	<i>International Journal of Pharmacy and Pharmaceutical Research</i>

IRR	Irritants / Irritation
ISSN	International Standard Serial Number
IUDs	Intrauterine Devices
K	Kaempferol / Kamrakh extract
KI	Kalonji extract
KOH	Potassium Hydroxide
kV	Kilovolts
L4	Larval Stage 4 (<i>Caenorhabditis elegans</i> lifecycle stage)
LD	Laser Diffraction
LED	Lower Electron Detector
LN	Lotus extract
MAPK	Mitogen-Activated Protein Kinase
MBC	Minimum Bactericidal Concentration
Mfg.	Manufacturing
MHB	Mueller-Hinton broth
MIC	Minimum Inhibitory Concentration
MMP	Matrix Metalloproteinase (including MMP-1, MMP-2, MMP-9)
N2	Wild-type reference strain of <i>Caenorhabditis elegans</i>
NC	Negative Control
NCC	Not Change in Colour
NF-κB	Nuclear Factor kappa B
NGM	Nematode Growth Medium

nm	Nanometres
O/W	Oil-in-Water (cream/emulsion)
OD₆₀₀	Optical Density measured at a wavelength of 600 nanometres
P. acnes	<i>Propionibacterium acnes</i>
PC	Positive Control
PDI	Polydispersity Index
PEG	Polyethylene Glycol
PQ	Paraquat
PS	Particle Size
Q	Quercetin
QC	Quality Control
RESS	Rapid Expansion of Supercritical Fluid
Rf	Retention Factor
RH	Relative Humidity
ROS	Reactive Oxygen Species
RPM	Revolutions Per Minute
RT	Room Temperature
S. epidermidis	<i>Staphylococcus epidermidis</i>
SCF	Super Critical Fluid
SD	Standard Deviation
SEM	Scanning Electron Microscopy / Standard Error of the Mean
SLN	Solid Lipid Nanoparticle (including SLNs)

SN	Serial Number
Spd	Spermidine
SPF	Sun Protection Factor
T	Thymoquinone
TEM	Transmission Electron Microscopy
TLC	Thin Layer Chromatography
TPA	Texture Profile Analysis
TPB	Three-Point Bend
URL	Uniform Resource Locator
USA	United States of America
UV	Ultraviolet (including UVB and UVR)
Vit. B9	Vitamin B9 (folic acid)
Vit. C	Vitamin C
W/O	Water-in-Oil (cream/emulsion)
w/o/w	Water-in-Oil-in-Water
XRD	X-Ray Diffractometry
ZP	Zeta Potential
α-MSH	Alpha-Melanocyte-Stimulating Hormone
μg/mL	Micrograms per millilitre

List of Symbols

%	Percentage
μ	Micro
μL	Microliter
A	Alpha
B	Beta
°C	Degree Celsius
Gm	Gram
H	Hour
kg	Kilogram
Mg	Milligram
Min	Minute
mL	Milliliter
Nm	Nanometer
w/v	Weight by volume
pH	Potential of hydrogen
Sec	Second
Cm	Centimeter
SD	Standard deviation
Vs	Versus
TLC	Thin Layer Chromatography
SLNs	Solid Lipid Nanoparticles
<	Less than
>	Greater than
Γ	Gamma

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DEDICATED
TO
GOD AND MY
BELOVED PARENTS

CHAPTER 1. INTRODUCTION



AIM: Development of Herbal Formulation for Skin Care.

OBJECTIVE OF WORK:

The present study aims to develop and evaluate polyherbal skincare formulations by combining well-known medicinal plants with established anti-aging and anti-acne properties. The study focuses on the incorporation of selected herbal extracts to achieve a possible synergistic effect, thereby enhancing their therapeutic potential. The herbal extracts will be incorporated into Solid Lipid Nanoparticles (SLNs) based cream [oil in water (O/W)] is prepared that is expected to have higher skin penetration, controlled drug release, bioavailability, stability and overall efficacy of the active phytoconstituents as compared to the normal cream prepared using herbal extracts as such.

The formulated products will be evaluated for their physicochemical characteristics, quality, stability, safety, and therapeutic efficacy. The study also aims to investigate whether the incorporation of herbal extracts into SLNs and formulate a cream (O/W) that would improve the anti-aging and anti-acne effects compared with conventional herbal formulations. Additionally, the research seeks to assess product stability, user acceptability, and the release of active constituents while preserving the natural benefits of herbal ingredients.

The developed SLN-based polyherbal skincare cream is expected to provide a safe, natural, economical, and user-friendly alternative to conventional synthetic skincare products, minimizing the adverse effects commonly associated with the long-term use of chemical-based treatments. Thus, the study intends to promote the use of herbal ingredients in advanced cosmetic formulations and contribute to the development of safer, more effective, and sustainable skincare products.

RATIONALE OF WORK:

Nowadays, the incidence of skin disorders has increased considerably due to environmental pollution, hormonal imbalances, nutritional deficiencies, lifestyle changes, ultraviolet (UV) radiation, and other contributing factors. These conditions affect the physical appearance of the skin but also have an impact on an individual's self-esteem, psychological well-being, and overall quality of life. Environmental and physiological stressors disrupt the skin barrier, increase oxidative stress, and contribute to the development of common skin

problems such as acne, hyperpigmentation, inflammation, and premature dermal degeneration.

Although a wide range of conventional treatments and cosmetic products are available for managing acne and skin aging, but are expensive and may produce adverse effects, including irritation, dryness, erythema, allergic reactions, and increased skin sensitivity on prolong use.

Herbal extracts are rich sources of bioactive constituents such as flavonoids, polyphenols, antioxidants, vitamins, and other phytochemicals that possess antioxidant, anti-inflammatory, antimicrobial, and skin-protective properties. These bioactive compounds help combat oxidative stress, inhibit the growth of acne-causing microorganisms, reduce inflammation, promote collagen protection, and delay the visible signs of skin aging.

In the present investigation, the antimicrobial activity of the selected herbal extract mixture was evaluated against *Staphylococcus epidermidis* and *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and the results demonstrated significant antibacterial activity. Furthermore, the anti-aging potential of the extract mixture was assessed using *Caenorhabditis elegans* as an experimental model, where it exhibited promising anti-aging activity.

Based on these findings, the present study is undertaken to formulate and evaluate polyherbal anti-aging and anti-acne skincare product using selected herbal extracts. To further enhance the therapeutic performance of the formulation cream (O/W), the herbal extracts incorporated into Solid Lipid Nanoparticles (SLNs), to improve the stability, skin penetration, controlled release, bioavailability, and overall efficacy of the active phytoconstituents.

The formulated product cream prepared using SLNs will be evaluated for the physicochemical characteristics, quality, stability, safety, and therapeutic efficacy.

The developed SLN-based polyherbal cream is expected to provide a safe, effective and user-friendly alternative to conventional synthetic skincare products while minimizing the adverse effects associated with long-term use of chemical-based treatments. Moreover, this

study aims to promote the application of herbal ingredients in advanced cosmetic formulations and contribute to the development of safer, more effective, and sustainable skincare products with improved patient compliance and consumer acceptance.

PLAN OF WORK:

Phase I: Literature Review & Phytochemical Pre-Formulation

1.1 Comprehensive Literature Review

- **Phytomedicine Screening:** Comprehensive review of literature on the selected plant species, detailing its traditional use, known secondary metabolites, and therapeutic potential for dermatological conditions.
- **Nano-Carrier Rationale:** Reviewing the evolution of **Solid Lipid Nanoparticles (SLNs)** over traditional topical vectors, specifically examining how lipid matrices protect labile phytochemicals from oxidative degradation.
- **Mechanistic Targets:** Deep dive into the molecular pathways of skin aging (matrix metalloproteinases, collagen degradation) and acne pathogenesis (*Cutibacterium acnes* proliferation, sebum hypersecretion, and inflammatory cascades).

1.2 Procurement, Quality Control & Standardization of Commercial Extracts

- **Procurement & Documentation:** Acquisition of standardized, high-purity commercial extracts from a validated manufacturer, ensuring the procurement of certificates of analysis (CoA).
- **Physicochemical Verification:** Verification of the commercial extract's organoleptic properties, solubility profiles in various dermatological solvents, and moisture content.
- **Fingerprinting & Biomarker Quantification:** Quality control validation using Thin-Layer Chromatography (TLC) to confirm the presence and separation of specific active biomarkers (e.g., total polyphenols, flavonoids, or targeted alkaloids)

by comparing retention factor (R_f) values against standard reference materials and the manufacturer's qualitative specifications.

Phase II: Fabrication & Physicochemical Optimization of SLNs

2.1 Formulation and Development of SLNs

- **Excipient Solubility Screening:** Screening of solid lipids (e.g., glyceryl monostearate, Cetyl palmitate, stearic acid) based on the partition coefficient and solubility profile of the commercial extract within the molten lipid matrix.
- **Surfactant Selection:** Screening of hydrophilic and lipophilic surfactants/co-surfactants (e.g., Poloxamer 188, Tweens, Lecithin) to ensure optimal emulsification.
- **Method Optimization:** Optimization of SLN synthesis using the **microemulsion technique**. The commercial extract is incorporated into the molten lipid phase, followed by the addition of a hot aqueous surfactant solution to form a microemulsion, which is then dispersed in ice-cold water under stirring. Optimization will be driven by systematically evaluating critical processing variables, including lipid concentration, surfactant-to-co-surfactant ratios, and stirring speed, to achieve the desired particle characteristics.

2.2 Characterization of the Developed SLNs

- **Particle Size & Surface Charge Analysis:** Determination of Mean Particle Size (Z-average), Polydispersity Index (PDI), and Zeta Potential using Dynamic Light Scattering (DLS) to evaluate physical stability and size distribution.
- **Morphological Evaluation:** Verification of the nano formulation's structural morphology and surface topology via Scanning Electron Microscopy (SEM).
- **Solid-State & Interaction Studies (FTIR):** Characterization of the commercial extract, bulk lipid, and developed SLNs using Fourier-Transform Infrared Spectroscopy (FTIR) to evaluate the structural integrity of the active ingredients,

confirm successful drug entrapment, and detect any potential chemical interactions between the extract and excipients.

2.3 Stability of SLNs

- **Storage Stability Assessment:** Evaluation of the physical stability of the raw nano-dispersion stored under refrigerated conditions at 4°C over a 9-month period.
- **Physicochemical Monitoring:** Systematic tracking of the formulation at regular intervals to detect any phenomena such as phase separation, particle growth (via dynamic light scattering updates).

Phase III: Secondary Formulation Development & Stability

3.1 Development of Topical Delivery Vehicle (O/W Cream)

- **Vehicle Synthesis:** Incorporation of the optimized commercial extract-loaded SLNs into a suitable oil-in-water (O/W) cream base to achieve the optimal thickness, emolliency, and Spreadability required for topical skin use.
- **Physicochemical Characterization:** Routine evaluation of the finished cream matrix for critical quality parameters, including pH, homogeneity, Spreadability etc.
- **Total Phenolic Content (TPC) Quantification:** Determination of the total phenolic content within the final formulation to confirm the stable loading, uniform distribution, and chemical preservation of the active biomarkers within the cream matrix.

3.2 Accelerated Formulation Stability Testing

- **Accelerated Stability Conditions:** Subjecting the final O/W topical cream formulation to stability chambers under accelerated parameters of 40°C±2°C and 75%±2%RH for a total duration of 3 months.
- **Critical Quality Attributes (CQAs):** Monitoring changes in key physicochemical parameters—including pH, total phenolic content, homogeneity, colour at fixed evaluation intervals (0, 1, 2, and 3 months).

Phase IV: Biological Evaluation & Therapeutic Assays

4.1 Anti-Acne Activity of the Commercial Extract

- **In Vitro Antibacterial Assays:** Evaluation of the baseline antibacterial efficacy of the raw commercial extract against key acne-associated pathogens, specifically *Staphylococcus epidermidis* and *Propionibacterium acnes* (*Cutibacterium acnes*).
- **Zone of Inhibition (ZOI) Screening:** Execution of agar well diffusion assays to visually screen and measure the zones of inhibition produced by the extract against the target microorganisms.
- **Minimum Inhibitory Concentration (MIC) & Maximum Bactericidal Concentration (MBC) Quantification:** Utilization of the tube dilution method to quantitatively determine the Minimum Inhibitory Concentration (MIC) & Maximum Bactericidal Concentration (MBC) of the commercial extract, establishing its standalone antimicrobial potency before formulation.

4.2 Anti-ageing Activity

- **Culture & Treatment:** Synchronize the N2 worms (bring them to the same age) and start giving your compound as soon as they become young adults.
- **Lifespan & Movement:** Check daily whether the worms are alive or not (Lifespan). Count their movement (Mobility).
- **Body Test:** Take photos of the worms and use ImageJ software to measure their size (Body Length) and accumulated cellular waste (Lipofuscin).
- **Stress Test:** Expose the young worms to high temperature (35°C Heat) and chemicals (Oxidative Stress), and count how many survive every hour.
- **Results (Analysis):** Analyze the data to determine whether the compound has a positive anti-aging effect or not.

CHAPTER - 1.**INTRODUCTION TO SKIN ^[1,2]****1.1 STRUCTURAL ARCHITECTURE and FUNCTIONAL LAYERS OF HUMAN SKIN:**

Our skin is the largest tissue of the human body, contributing about 15% of the total body weight. It protects the body from physical, chemical, and biological insults, to prevent excessive loss of water and to regulate body temperature. The skin, together with the mucous membrane, creates a continuous layer covering the whole body.

The integumentary system of the body is basically composed of the skin and its related structures. The skin has been divided into three main layers; epidermis, dermis, and subcutaneous tissue. The outermost part of the skin is the epidermis and it is built up primarily of keratinocytes which are responsible for producing keratin- a fibrous protein providing protection to the skin. Below this is the dermis. Dermis is mainly composed of collagen- a type of structural protein that provides strength and elasticity to the skin.

The dermis lies on the subcutaneous layer which is also known as the panniculus- contains clusters of fat cells known as lipocytes. The thickness of these 3 layers differs greatly depending on the specific region of the body. For instance, the epidermis is the thinnest on the eyelids which is less than 0.1 mm and it is thickest on the palms and soles which is up to 1.5 mm. The back of the skin contains the thickest dermis, that can be 30-40 times thicker than the epidermis above it.

Figures 1.1 and 1.2 represent the primary division of the skin into different layers. The outermost layer of the skin is the epidermis which is made up of keratinocytes. Immediately beneath the epidermis is the basement membrane known as dermo epidermal junction that is a thin multi-layered structure that deeply connects the epidermis to the underlying dermis. The deepest layer of the skin is the hypodermis which is situated below the dermis.

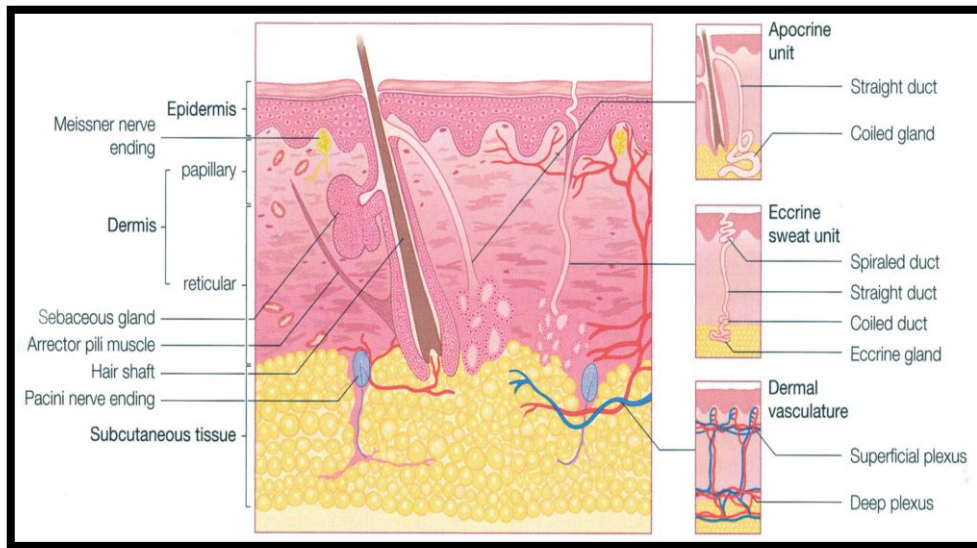


FIG 1.1 - CROSS SECTION and PANNICULUS [2]

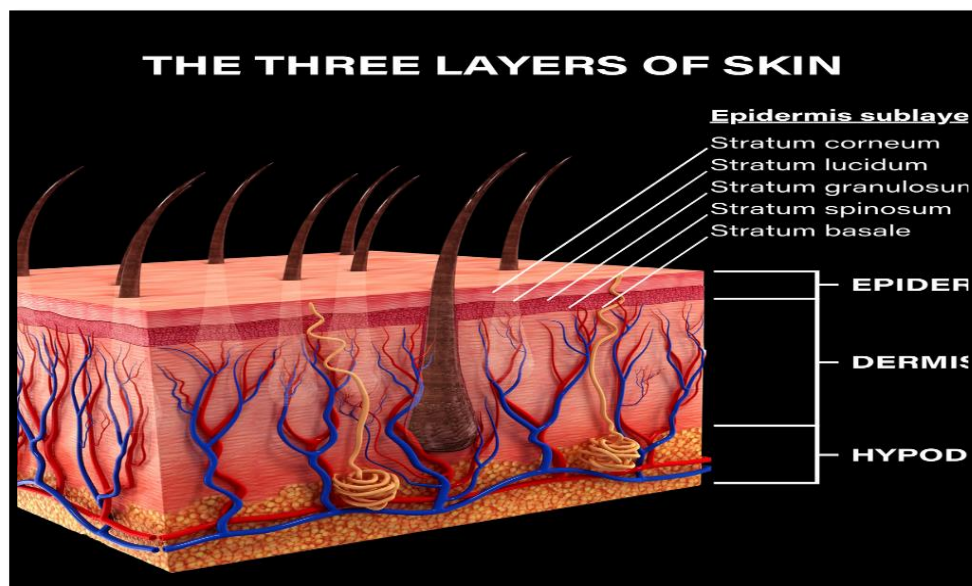


FIG 1.2 – DIFFERENT SKIN LAYERS

1.1.1 EPIDERMIS [3, 4]:

The outermost layer of the skin is the epidermis. It is defined as stratified squamous epithelium- primarily consists of keratinocytes at different phases of maturation. Keratinocytes produce keratin, a structural protein which is a vital component of the

epidermis. Since the epidermis has no blood vessels, it completely depends on the underlying dermis to provide nutrients and dispose of waste products via the basement membrane.

The primary role of epidermis is performing physical and biological barrier to the environment, blocking the entry of harmful substances like irritants and allergens. It minimizes water loss while it also promotes homeostasis.

The epidermis has been divided into different layers. In most parts of the body, it comprised of 5 layers, which consists of:

1. Horny layer or Stratum corneum
2. Stratum lucidum is found only in the thick skin of the palms, soles etc.
3. Granular layer or Stratum granulosum
4. Prickle cell layer or Stratum spinosum
5. Stratum Basale or Germinative layer

Although keratinocytes account for approximately 95% of the epidermal cell population and other specialized cells such as melanocytes and langerhans cells are also present.

1.1.2 DERMIS [5, 6]:

The epidermis, which typically thin (less than 0.1 to 1.5 mm), is much thinner than the dermis, which basically makes up the inner layer of the skin. It lies between the subcutaneous layer and the basement membrane zone. The primary function of the dermis is to support and maintain the structure of the epidermis.

The main functions of dermis are as follows:

- To protect the skin,
- Preventing mechanical damage to the deeper structures,
- To nourish the skin.
- Play a crucial role in the wound healing

Collagen fibres make up the most of the complex web of interwoven connective tissues which forms the dermis with a smaller quantity of elastin. Dispersed throughout this layer

are various specialized cells, including fibroblasts and mast cells, as well as important structures such as blood vessels, lymphatic vessels, sweat (sudoriferous) glands and nerve endings.

Despite the fact that they join the surface of the skin. The epidermal appendages are similarly situated in the subcutaneous layers or the dermis.

There are 2 basic layers of the dermis:

- The papillary dermis-more superficial
- The reticular dermis- deeper

1.1.3 HYPODERMIS:

The subcutaneous layer which is also referred to as the hypodermis. The hypodermis is basically made up of adipose tissue and it is situated below the dermis. It plays a crucial role in providing structural support to the skin, insulating the body against cold and cushioning underlying structures by absorbing mechanical shock. This layer is richly supplied with blood vessels and nerves, contributing to its functional significance.

1.2 FUNCTIONS OF SKIN [6]:

There is main three functions of skin: Thermoregulation, Sensation and Defence.

As described below, the skin performs many important and essential tasks.

DEFENCE:

The skin acts as a barrier against

- Mechanical, thermal and other physical damage
- It protects the body from harmful agents
- Prevent form the dehydration
- Protect the body from the UV radiation

THERMOREGULATION:

The skin protects the body from severe heat and maintains the body temperature. Blood flow changes regulate this within the cutaneous vascular network. This is regulated by the

alterations in the blood flow in the cutaneous vascular network. The hot weather causes blood vessels dilate and making the skin to appear red and promoting the sweating development on the skin. The blood vessels shrink in a cold state, decreasing the heat loss and flow of blood. In addition, it produces the sweat and evaporate it from the surface of the skin which has a major part in the cooling process of the body.

SENSATION:

The skin mainly works as the primary organ of touch, enabling the body to find and respond to various external stimuli, including those that may cause pain. This sensory role is particularly significant in individuals with skin conditions, as sensations like discomfort and itching can be intense and highly distressing.

Furthermore, Contact plays an important psychological and social role. Many people with visible skin disorders may experience feelings of isolation due to changes in skin appearance or social perceptions. They may be unfairly regarded as dirty or infectious, leading to decreased physical contact which can further impact their emotional well-being.

IMMUNOLOGICAL SURVIVANCE:

The skin mainly works as a vital immunological organ, comprising various specialized structures and cells that contribute to immune defence. Depending on the type of immune response, a range of cells and signalling molecules, such as cytokines, are involved.

BIOCHEMICAL FUNCTIONS:

The skin performs several important biochemical processes. Upon exposure to day light, it synthesizes vitamin D₃(cholecalciferol) from a cholesterol-derived precursor. This compound is later converted in to calcidiol in the liver and then it is further metabolized to synthesize calcitriol within the kidneys. which is physiologically active form of vitamin D. Vitamin D playing an important part in the absorption of phosphorus and calcium, which are necessary for maintaining healthy bones.

In addition, receptors for various steroidal hormones are also present in the skin, which including oestrogens, progesterone and glucocorticoids as well as vitamin A.

SOCIAL AND SEXUAL FUNCTIONS:

Appearance of an individual plays an important role in how they are perceived by others, as people often form initial judgements based on visible characteristics. Throughout the past history, individuals have been evaluated and sometimes stigmatized due to their skin if it is not because of the colour, the presence of the skin conditions and pigmentation.

Since many skin problems are readily apparent, social interactions are influenced by it, particularly in a society that places strong emphasis on appearance and glamor. Therefore, the way patient is perceived and accepted by others becomes an important consideration for healthcare professionals, especially nurses, when providing holistic care.

1.3 SKIN PERMEABILITY ^[9]:

In healthy, intact skin, the corneocytes which are keratinized in addition to the non-polar lipid matrix of the stratum corneum. play a significant role in maintaining an effective barrier function. An insufficiency of important fatty acids, particularly linoleic acid, can impair this barrier, resulting in increased permeability and decrease capacity.

Given the wide range of substances which come into touch with human skin, it is important to properly maintain the stratum corneum. Numerous compounds commonly present in cosmetics and personal care products are capable of penetrating into or through the outer layer. Certain materials may also function as penetration enhancers, facilitating the absorption of other substances. A few examples of penetrants are water, aluminum salts, ethanol, glycerol, phenol, essential oils, and urea. Organic solvents like surfactants, dimethyl sulphoxide (DMSO), and propylene glycol and water are some common penetration enhancers.

The skin barrier can be compromised by different factors such as mechanical stress, chemical exposure, UV radiation and microbiological agents such as bacteria, yeast, molds and viruses. Among these, chemical and light-induced damage are particularly significant concerns in cosmetic formulation and product safety.

1.3.1 SKIN TYPES and ITS IDENTIFICATION [7, 8]:

Different criteria are employed to classify different skin types. One widely recognized system is the Fitzpatrick skin phototype classification, which was first introduced in 1975, which categorizes skin based on its colour and response to UV exposure. This system is commonly used to determine appropriate sun protection measures and to identify the skin cancer risk.

However, from a clinical point of view, skin can be classified mainly based on the characteristics of its physical and biological balance like level of hydration, sensitivity, and production of sebum. Every type of skin exhibits distinct characteristics and therefore requires specific care and treatment approaches. Although skin type is largely determined by genetic factors, it can be influenced by over time by environmental conditions, hormonal changes and lifestyle habits.

Based on these parameters, healthy skin is classified into main five types: normal, dry, oily, combination (a mix of oily and dry areas) and sensitive. The key features of all skin types are described as below:

1. **NORMAL SKIN:** The skin type is characterized by an equal level of oil and moisture with moderately sized pores. It is well hydrated, smooth and firm to touch with an equilibrium between sebum production and water content. It is neither excessively dry nor overly oily, it exhibits uniform texture with minimal imperfections by giving a clean, soft and healthy appearance. Typically, this skin type doesn't require any special care.
2. **SENSITIVE SKIN:** It is characterized by an increased tendency to react to stimuli that typically do not affect normal skin. It is generally more fragile and is usually associated with sensations of discomfort like burning, tightness, redness or itching. This skin type may have a compromised barrier function and it allows easier penetration of microorganism and irritant substances with increasing the infection risk and allergic reactions.

Because of its delicate nature, it requires special care to manage dryness, roughness and overall appearance. The terms “sensitive skin” and “irritate skin” are often used interchangeably.

3. **DRY SKIN:** It is temporary in nature and it is generally brought on external factors like low humidity, frequent exposure to hot water, and weather. However, dry skin may linger and become a lifelong or chronic condition for some people. When the skin becomes drier, it can crack, making it more vulnerable to bacterial entry. Although this is not usually severe, inadequate management can lead to secondary skin conditions, such as eczema or increase susceptibility to infections.

General health, underlying factors, and age represents a few variables which may affect the symptoms and signs of the dry skin. It is usually occurred by a sensation of tightness and a rough texture. In many cases, the skin may appear dull or ashy, accompanied by flaking, itching, redness and development of small crakes. In many severe cases, these cracks may deepen and even bleed.

A condition related to this is atopic skin, which is marked by dryness, irritation and scaling and is usually resulting in significant discomfort, particularly itching. Genetic predisposition is the primary cause of this condition, however, different triggering substances which may irritate and aggravate the signs and symptoms like allergens, surrounding environment, nutrient effects, and particular fabric.

4. **OILY SKIN:** It is characterized by an enlarged pore structure, a moist texture and shiny appearance. It is usually occurred by the sebaceous glands which produces excessive amount of sebum, and it can be affected by the genetic and hormonal factors. This skin type is more prevalent among adolescents and individuals under the age of 30 and it is often associated with the production of acne.
5. **COMBINATION SKIN:** The inequitable arrangement of the sebaceous glands and sweat glands leads it to demonstrate the features of both dry and oily skin both, which may depend on the specific area of the skin. The T-zone includes the nose, chin, and forehead and is usually normal but the cheeks can appear dry.

1.4 CARE OF THE SKIN [10, 11, 12, 13];**1.4.1 BASIC SKIN CARE: -**

- The skin is mainly regarded as the body's "sacrificial organ", because it is typically the first to exhibit visible signs of stress. Additionally, skin care represents the largest portion of the beauty industry.
- The situation of neck, face, and hands reflects the general condition of skin including overall health and status of the skin.
- Human skin is an image of overall physical and mental health.

Skin care should not be limited to treatment of skin concerns; rather, it should be prioritized as a daily preventive routine.

1.4.2 The requirements of the customers can be largely classified in to 2 different groups.

Defence From	Repairing a
UVR	Dry skin
IRR	Sunburns
Wind	Acne
Central heating	Wrinkles
Chemicals	Puffy eyes
Cold	Cellulite
Pollution	Skin cancer
Insects	Pigmentation
	Age spots
	Scars

1.4.3 STEPS OF DAILY ROUTINE SKIN CARE: -

This includes main 5 steps:

1. Pre cleanse: Cleanse the skin using warm water by applied with a cotton ball to soften and loosen oil and dirt.
2. Hydrosol (toner): Use a hydrosol to add a water barrier to skin which act as a good foundation.
3. Serum and actives: A serum is applied first, so that it seeps right into the skin.
4. Moisturizer: Helps to work the product into skin and one can feel pampered with a mini-facial massage.
5. Sun screen

ABC of sun safety:

- A-Avoid: Minimize the direct contact with the sunlight or UV rays, especially during the 10 a.m. to 3 p.m.
- B-Block: Wear protective clothing like hats, sunglasses and long-sleeved dresses to protect skin.
- C-Cover: To protect the skin from harmful UV radiation and apply the sunscreen with a broad spectrum with SPF of not less than 15.

1.5 AGEING:

1.5.1 INTRODUCTION:

- Today, life expectancy is significantly increased due to advancements in education, technology, healthcare, food distribution and sanitary conditions improvement.
- Ageing is a continuous lifelong process encompassing both growth and decline and it begins at conception and ends with death.
- Ageing process can be divided in to three parts:
 - A. Growth and development: Occurs in infant years involving physical and mental development.
 - B. Maturation: Take place in teenage and young adults when body reaches its peak functional capacity.
 - C. Senescence: Being after age of 30 and is characterized by gradual physiological decline.

**FIG 1.3 AGEING**

Figure 1.3 shows how facial skin ages structurally and visually, going from youthful, smooth skin to mature, deeply wrinkled skin.

1.5.2 TYPES OF SKIN AGEING ^[14]:

Ageing can be viewed from multiple perspectives, requiring a broader and more comprehensive approach to fully appreciate age-related changes and challenges. Ageing is commonly classified into biological ageing, physiological ageing, social ageing, chronological aging and functional aging.

CHRONOLOGICAL AGEING: Chronological ageing is the number of years an individual has lived. An 85-year-old woman is chronologically older than a 75-years-old man. However, chronological age doesn't match with a person's biological, physiological or social age.

For example, an 85-year-old woman may remain active and engaged in activities such as volunteering, while 75-year-old man may be confined to his home. Sometimes common expressions like "He looks younger than his age" or "She doesn't act her age" reflects this mismatch. In these cases, particular's biological, psychological and social ages may not match their chronological age.

BIOLOGICAL AGEING: Biological ageing refers as gradual decline in cellular functions and the progressive loss of cells over the period of time. With biological ageing, tissues and organs become less efficient and the ability of body to repair and generate slows down, and immune function weakens, and increasing susceptibility to infections. Biological ageing is

also known as physical ageing. Everyone who maintain a healthy lifestyle, including exercise and medical check-ups may appear biologically younger than the others of the same age.

Physiological Ageing: It involves changes in cognitive and emotional functions including memory, learning ability, intelligence, personality and coping mechanisms. Older individuals remain mentally active, acceptable and capable of handling new situations are always considered as psychologically younger.

SOCIAL AGEING: It refers to changes in particular's roles, relationships and societal expectations as the grew older. For example, older adults can take new role such as becoming grandparents or transitions like employment to retirement. These role changes are usually accompanied by social norms that influence how individuals are expected to behave. In some cases, activities like roller skating or bungee jumping may be perceived as inappropriate for older adults due to age-related stereotypes. The social age of an individual, therefore, plays a crucial role in shaping their experience of aging, which can be perceived as either positive or negative depending on personal and social perspectives.

FUNCTIONAL AGEING: Functional ageing mainly refers to how an individual's psychological abilities compare with those of others of similar age. It relates to an ability of individual to perform regular activities and maintain dependence in compare to other group of the same age. It shows how well a person maintains physically and mentally in every day's life. Persons who remain active, independent and capable of managing routine activities are considered functionally active and younger, regardless of their chronological age.

1.5.3 MECHANISM OF AGEING:

Intrinsic process and extrinsic process are two different processes which have been defined as the root cause of the skin ageing.

1.5.3.1 INTRINSIC PROCESS ^[29]:

Intrinsic skin ageing also referred as natural or chronological ageing which is primarily occurs in parts of the body which are protected from the sunlight. Passage of time and genetic factors are reasons of this. At the molecular level, both intrinsic and extrinsic ageing has

same mechanisms including telomere shortening, oxidative stress, mutations in the mitochondrial DNA and hormonal decline.

Reactive Oxygen Species are generated during normal cellular metabolism and are essential for both- chronological ageing and accelerated ageing, in accordance to the free radical theory of ageing. The ROS stimulate signaling pathways like MAPK pathway, that triggers a transcription variable like C jun gene and it leads to ageing and cellular damage.

Intrinsic ageing also involves the gradual degradation of collagen similar to that observed in photoaged skin. In addition, hormonal changes significantly influence this process. The gonads produce the sex hormones, pituitary and adrenal glands begin to decline from the mid-twenties, with a more pronounced decrease in oestrogen and progesterone levels during menopause. A deficiency of oestrogen and androgens contributes to characteristic features of aged skin including dryness, wrinkling, thinning(atrophy), collagen breakdown and reduced elasticity.

1.5.3.2 EXTRINSIC PROCESS ^[30]:

It is mainly caused by environmental and lifestyle factors which includes prolonged exposure to UV radiation, repetitive facial expressions, gravity, positions of sleeping and smoking. Among these, chronical exposure to UV radiation is most important contributor; hence, which is also called as “photo ageing”

This form of ageing is cumulative in nature and largely based on the time spend in the sun exposure and human’s pigmentation of skin. Continuous exposure to sun rays’ leads to structural and physiological modifications in the skin like the stratum corneum becomes more thicker, damage to the epidermis and progressive cellular abnormalities such as dysplasia, atypia and anaplasia. Skin stiffness and elasticity are also decline due to a significant decrease in content of collagen and the collapse of elastic fibers.

1.5.3.3 THE THEORY OF FREE RADICAL ^[15]:

The cumulative oxidative injury of tissues and cells that occurs primarily during normal aerobic metabolism and it is the reason of the free radical theory of ageing. ROS produced during metabolic processes can damage cellular components over time and contribute to the ageing process.

Many lines of evidence support this theory:

1. Variations in lifespan among species which have been connected to the rate of metabolism and levels of antimicrobial defense systems.
2. An expanded lifespan can be linked to increased synthesis of oxidative enzymes in practical models.
3. Free radicals are cause of cellular damage which tends to accumulate with advancing age.
4. Caloric restriction is shown to decrease the production of ROS and is linked to increase longevity.

The buildup of age-related pigments, cell membrane lipid peroxidation, DNA damage, protein cross linking, and diminished mitochondrial functions are some functional and structural changes associated with ageing which may be easily clarified through this theory. Nevertheless, in spite of encouraging data, there are still some number of questions regarding the specific role of oxidative damage in ageing. In ecosystem, free radicals are available in very small amount, and it is challenging to measure them directly in living beings.

Moreover, it has not any evidence that the main cause of ageing is oxidative damage.

Many individuals agree that ageing is a complicated process that is affected by a different environmental, metabolic, and genetic variables. As an outcome, although the free radical theory offers useful information, which proof that is recently present is mostly correlative and not 100% reliable.

1.5.3.4 THEORY OF GENETIC GENE ^[16]:

The genetic theory of ageing proposes that lifespan is largely determined by inherited genetic factors. According to this theory, longevity is influenced from the moment of conception and depends significantly on the genes inherited from parents.

Telomeres, which are repetitive DNA sequences located at the ends of chromosomes, are a important concept in this theory. Genetic material is protected by telomeres during cell division; however, progressively shorten with each division. Cellular ageing and senescence

originate from inability of cells divided properly without damaging vital DNA when telomeres become critically short.

Before exploring the detailed mechanism and supporting or opposing arguments of genetic influences on ageing, it is necessary to know that ageing is a complex process which can be clarified by a different theories. Recently, there is no single theory or categories of theories that can fully account for all aspects of the ageing process.

The molecular pathways and microenvironmental alterations that underlie skin ageing are depicted in the below flowchart and anatomical diagram (Fig. 1.4). It shows how downstream biochemical changes (ROS production, MMP activation, ECM degradation) and their final macroscopic phenotypic results (wrinkling, flaccidity) are connected to upstream ageing factors (intrinsic and extrinsic).

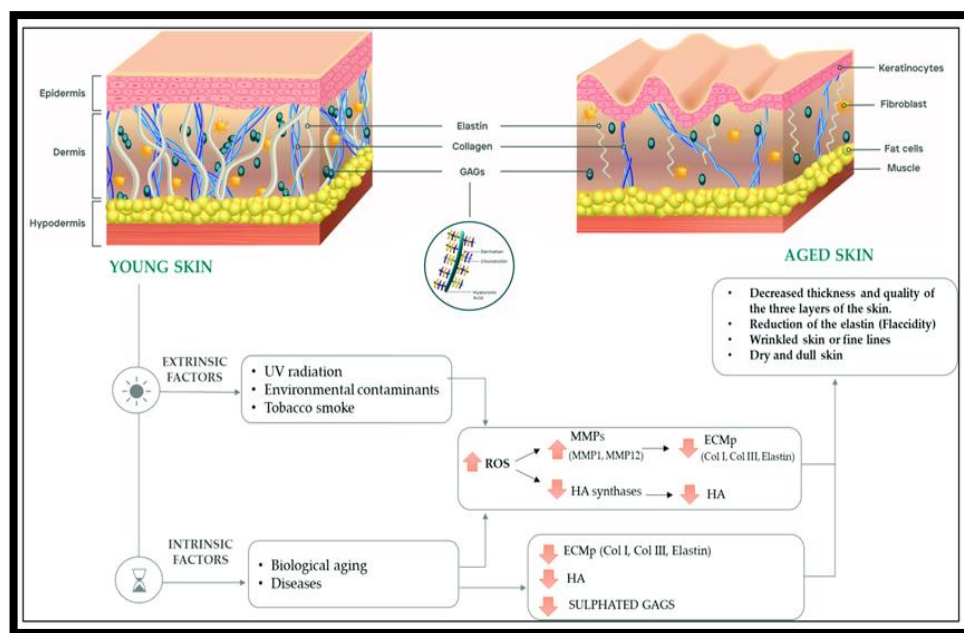


FIG 1.4 COLLAGEN CHANGES DURING AGEING

1.5.3.5 THEORY of MITOCHONDRIAL DNA DAMAGE [17]:

This theory of ageing became available in 1980 by Miguel and Cowalker, who suggested

that death and cellular ageing are mainly caused by damage to mitochondrial DNA. From a cellular stand point, 90% of the vital energy is generated by cells in the form of ATP-a substance that is necessary for maintaining normal cellular functions that derived from mitochondria.

When mitochondrial DNA becomes damaged, the efficiency of energy production decrease, leading to reduced cellular energy availability. This impairment affects negatively to the functions of cells, tissues and organs by contributing their gradual deterioration. Moreover, structural changes such as mitochondrial degeneration, rupture and leakage can exacerbate cellular damage and making them significant contributors to the ageing process.

1.5.4 FACTORS AFFECTING AGEING:

The interrelated characteristics of biological ageing and cellular senescence are depicted in this diagram given below in figure 1.5. It outlines the main cell-intrinsic and cell-extrinsic factors that determine when healthy somatic cells enter a permanent, non-dividing state of senescence.

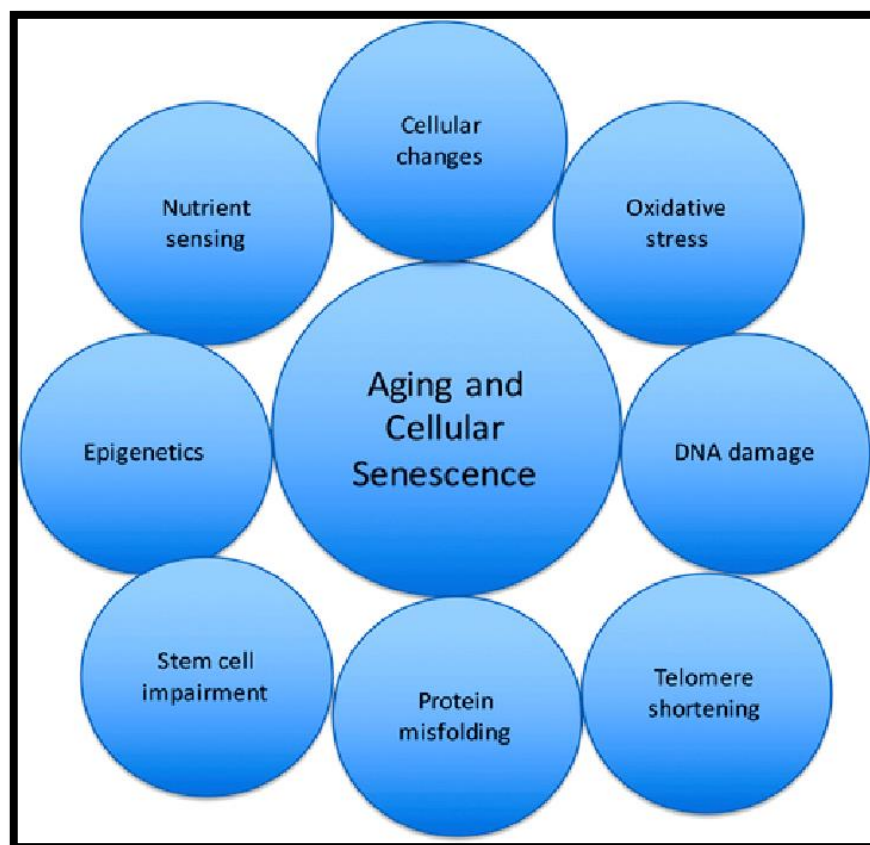


FIG 1.5 FACTORS AFFECTING AGEING

1.5.5 TREATMENTS AVAILABLE IN MARKET and THEIR SIDE EFFECTS ^[18]:

With increasing age, the skin also shows signs of ageing; while this process is inevitable, its effects can be reduced through appropriate care.

- Ways to reduce the signs of ageing skin: As we age, our skin naturally undergoes changes; however, by doing proper care, lifestyle practice, we can minimize visible signs of ageing.
- **Botulinum Toxin Therapy:** Produce pain at the injection site, inflammation and bleeding.
- **Dermabrasion:** Redness, swelling, changes in skin colour, infection, scarring and other skin reactions.
- **Microdermabrasion:** Produces mild tenderness, swelling and redness, minor bruising may also occur.
- **Chemical Peels:** Temporary or permanent change in skin colour, scarring and sores.
- **Fractional Laser Skin Resurfacing:** It can lead to bacterial, viral or fungal infection.
- **Dermal Fillers:** Redness, swelling, itching, rash, pain etc.
- **Non-ablative Skin Rejuvenation:** Acne, scarring, infection etc.
- **Cosmetic surgery:** Causes hematoma, seroma, blood loss, infection, nerve damage, organ damage and and scarring.
- **Retinoid creams:** Produce redness, swelling, crusting or blistering, dryness and irritation to the skin, also skin colour changes.
- **Peptide creams:** Cause skin symptoms such as skin sensitivity, rash, itching and other adverse reactions.

1.6 ACNE:

- Acne-also called as *Acne vulgaris* (AV) is a chronic inflammatory skin disease which is described by the formation of dead skin cells and high production of sebum that blocks the pilosebaceous unit.
- It commonly presents with blackheads, whiteheads, pimples, oily skin and in some cases, scarring.
- Acne is one of the most common skin diseases and it is a multipurpose skin condition that impacts more than 85 percent of teenagers and young adults.

- Research in epidemiological study shows that it can occur at any stage of life, yet it usually occurs during adulthood and it gets frequently worst during teenage life.
- Sebaceous gland hyperplasia aberrant follicular differentiation with higher keratinization, microbial over colonisation of the follicular canal, and higher level of inflammation are a few of the factors that lead to the pathogenesis of acne.
- The adaptive immune system is basically responsible for regulating the inflammatory response.
- An acne-affected follicle undergoing comedogenesis and microbial infection is contrasted with a healthy pilosebaceous unit in this schematic diagram (Fig. 1.6).

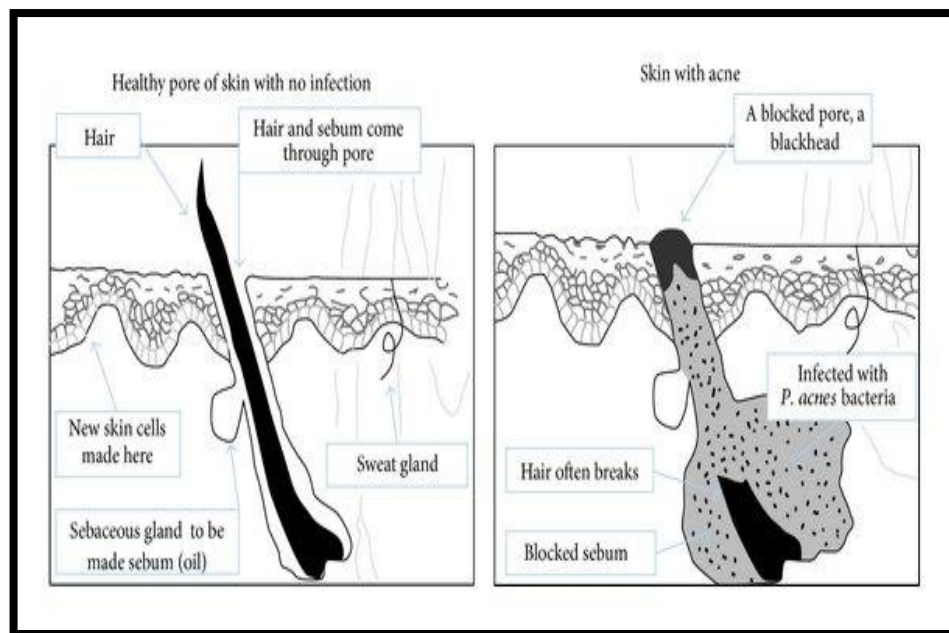


FIG 1.6 DIFFERENCE BETWEEN NORMAL SKIN and SKIN WITH ACNE

1.6.1 TYPES OF ACNE ^[19]:

NON-INFLAMMATORY ACNE: Appearance of blackheads and whiteheads without swelling.

Blackheads (Open Comedowns): It occurs when sebum and dead skin cells come to gather to block a pore.

Whiteheads (Closed Comedowns): It develops when dead skin cells and sebum clog pores. Because the pore remains closed rather than open, they are considered as opposite of

blackheads. In clinical terms, they appear as tiny elevated lumps on the surface of the skin.

Since the closed pores limits the content exposure to the external environment, Whiteheads are often more difficult to treat.

INFLAMMATORY ACNE:

Inflammatory acne refers to pimples that appear red, swollen and often painful. While sebum and acne are an outcome of skin cells, involvement of bacteria is also highly significant in clogging pores and triggering inflammation. The presence of bacteria can lead to infection beneath the skin surface, resulting in deep, painful lesions that are often difficult to treat.

Types of Inflammatory Acne:

- **Papules:** When the walls surrounding the pore deteriorate as an effect of severe inflammation, papules form. Hard and clogged pores are painful and sensitive to touch. Generally, the neighbouring skin appears reddish brown.
- **Pustules:** Pustules form similarly to papules but contain pus. These lesions are raised, red bumps with a visible white or yellow centre and are commonly referred to as “pimples”
- **Nodules:** Nodules occur when clogged and inflamed pores extend deeper into the skin and enlarge. These lesions are firm, painful and located beneath the skin surface. Due to their depth, nodules usually require prescription medications and cannot be effectively treated with home remedies.
- **Cysts:** When pores become extremely clogged with a combination of dead skin cells, bacteria, and sebum, cysts called severe lesions occur. They are large, soft and painful lumps located deep within the skin. Cystic acne is often associated with severe infection and carries a high risk of permanent scarring.

1.6.2 CAUSES ^[20]:

Acne is occurred by clogged pores, which are tiny openings on the surface of the skin.

Each of the pores opens into a follicle, which contains an oil gland and a hair follicle. The oil in oil glands maintain skin supple and helps in getting rid of dead skin cells.

Blockage of these glands can occur due to the accumulation of sebum and dead skin cells, forming a plug known as a comedo. When the surface of the plug remains closed, it appears as a whitehead, whereas an open and oxidized plug forms a blackhead. Acne might occur if bacteria get stuck in the plug and the immune system of the body responds by producing inflammation.

In more serious cases, acne spots may develop deeper into the skin, developing painful and hard cysts. In this condition, it is said as Nodulocystic acne.

Even though, it can impact people of all ages, including newborns. Acne is most commonly seen in young people. It also tends to have a genetic susceptibility and can occur in families. A three-dimensional cross-section of human skin is shown in this anatomical diagram (Fig. 1.7), which highlights the pilosebaceous unit's structure. It illustrates the physical connection between the superficial aperture known as the cutaneous pore, the attached sebaceous gland, and the hair follicle.

The following aspects could cause acne:

- Changes in hormones that increase sebum production, such as those occurring during puberty, menstrual cycles, pregnancy, or due to stress
- Using oil based cosmetic products
- Some drugs such as testosterone, phenytoin, Oestrogen, and steroids; some drug-containing intrauterine devices (IUDs) may also exacerbate acne
- Excessive sweating and high humidity
- Frequent touching, rubbing, or pressure on the skin.

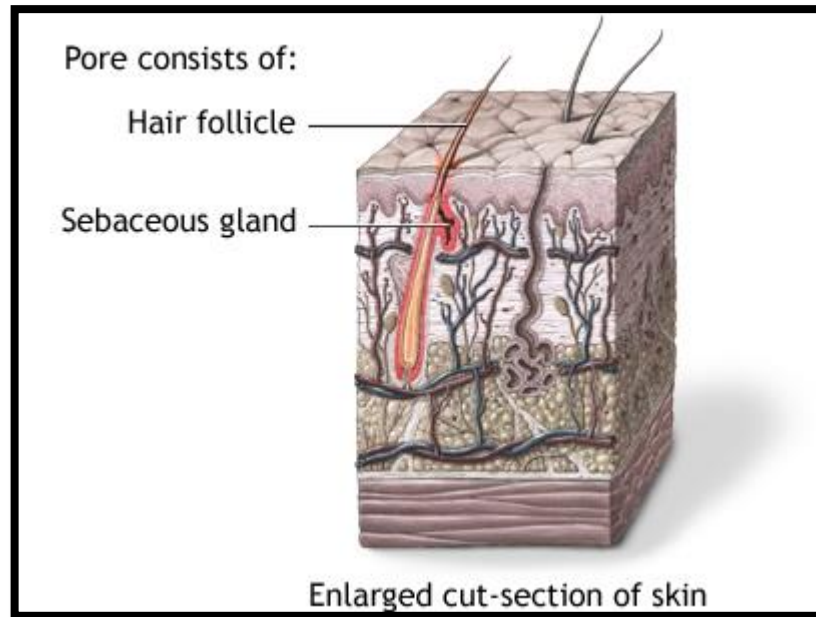


FIG 1.7 ENLARGED CUT-SECTION OF SKIN

1.6.3 SYMPTOMS ^[20]:

Although, acne may also occur on the legs, trunk, Buttocks, and arms, acne generally occurs on face and shoulders. The following skin modifications are associated:

- Bumps on the skin which crust cysts
- Tiny red bumps which are called as papules
- Tiny red lumps called as pustules contain white or yellow pus
- A reddened appearance on the skin
- Skin damage, Black heads and White heads

1.6.4 ETIOLOGY ^[21, 22, 31]:

Acne develops as a result of multiple interrelated factors. These include the glands that produce sebum, the sebaceous ducts having become hyper cornified, *Propionibacterium acnes* occupying the pilosebaceous unit and the ensuing inflammation.

Seborrhea and modifications in the follicular infundibulum are specifically related to the severity of acne. Comedown can be caused by hyper keratinization, hyper cornification, and reduced keratinocyte desquamation in the infundibulum in mild acne. More critical

circumstances result in the follicular infundibulum rupturing, which discharges sebum into the dermis and initiates an inflammatory response.

Acne pathogenesis begins with follicular blockage due to hyper keratinization and keratin plug formation, resulting in the formation of micro comedown. Increased androgen levels favor sebaceous gland growth and stimulate sebum production. These micro comedone may ultimately develop as a result of dead skin cells and sebum forming up inside the sebaceous glands.

1.6.5 EPIDEMIOLOGY ^[23, 24, 31]:

Acne is a highly prevalent dermatological condition, affecting more than 80% of adolescent worldwide. A significant proportion of those affected are teenagers. Although acne is often considered a normal part of maturation process, it can have substantial behavioral, emotional, and psychological impacts. Studies indicate that approximately 80-90% of adolescents, particularly in Western populations, experiencing some degree of physical and psychological effects associated with acne.

Around 60% of affected adolescents present with mild acne and typically manage their condition using non-prescription treatments without seeking medical advice. The vast majority of patients are the remaining 40 percent, who suffer from moderate to severe acne and required clinical attention.

In comparison to Caucasian people, American, Asian, and African people are considered to possess lower incidence of acne. About 20% of people who suffers from severe acne, which can produce scars that can persist for a lifetime.

In the figure 1.8, with the red bars indicating a higher persistence in one group (typically females due to post-adolescent hormonal acne), this precise distribution usually illustrates how acne prevalence or severity peaks in adolescence and steadily declines through adulthood.

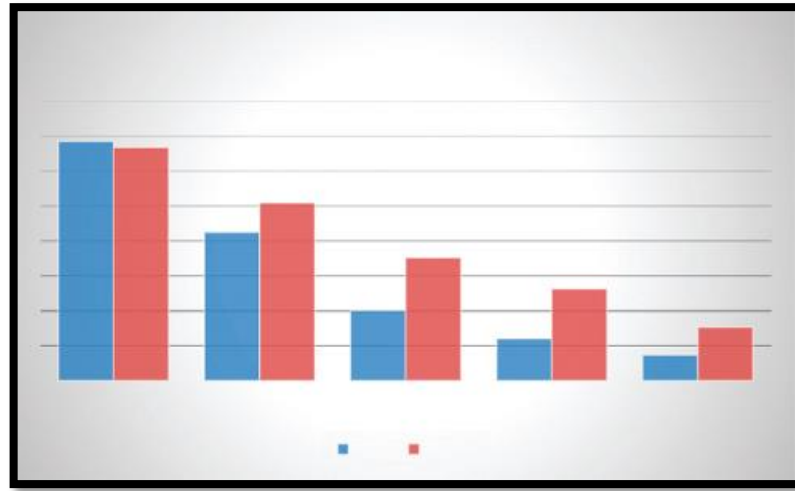


FIG 1.8 ACNE EPIDEMIOLOGY

In adolescents, overweight and obesity have been reported to show an inverse, dose-dependent association with acne prevalence. However, this relationship appears to be gender-specific, b'coz being overweight is directly co-related with pimples in girls among the age of 18-29, but there is no significant association in boys.

Certain acne subgroups, such as pomade acne, and post-inflammatory hyperpigmentation are uncommon in individuals with dark skin tone. Acne vulnerability is also strongly affected by genes, with estimated heritability between first-generation family members reaching approximately 80%.

Additionally, lifestyle factors contribute to acne exacerbation, with approximately 47.3% of patients attributing flare-ups to specific food items and around 40% linking worsening symptoms to the use of cosmetic products.

1.6.6 PATHOPHYSIOLOGY ^[25, 26, 27, 31]:

Acne vulgaris is a chronic, complex inflammatory disease of the pilosebaceous unit involving complex interactions between hormonal, microbial, immunological and environmental factors.

1. **Role of Hormones and IGF-1 Signaling:** Acne is considered an insulin-like factor (IGF-1)-mediated disease, influenced by dietary habits and lifestyle factors such as smoking, which enhance insulin/IFG-1 signaling pathways. The primary hormones

involved in acne development include androgens, insulin, and insulin-like growth Factor-1. These hormones activate oil gland enlargement and increase sebum production.

Additional medicators such as corticotropin-releasing hormone (CRH), α -melanocyte-stimulating hormone (α -MSH), and substance P also contribute to sebaceous gland activity and inflammation.

2. **Sebum production and sebaceous gland activity:** Sebaceous glands hypertrophy and too much sebum secretion are prompted by high androgen levels during the stage of puberty. Changes in the sebaceous lipid profile, influenced by hormonal fluctuation, stress, cosmetics, and diet, further contribute to acne development.

Sebum-rich environments promote the growth of microorganism and create favorable conditions for lesion formation.

3. **Follicular hyper keratinization and micro comedown formation:** A key event in acne pathogenesis is abnormal follicular keratinization. Hyper keratinization and reduced desquamation of keratinocytes in the follicular infundibulum led to blockage of the follicle and formation of keratin plugs (Micro comedown).

This Micro comedown may develop into:

- Open comedown (blackheads)
- Closed comedown (whiteheads)

In severe cases, rupture of the follicular wall releases sebum and keratin into the dermis, triggering inflammation.

4. **Microbial Colonization:** Microbial colonization plays a crucial role in acne progression. Key organisms involved include:

- *Propionibacterium acnes*
- *Staphylococcus epidermis*
- *Malassezia furfur*

Among these, *P. acnes* are the most significant contributor. It multiplies within the sebum rich follicle and produces lipase, hyaluronidase, protease and chemotactic substances along with other pro inflammatory molecules.

5. **Inflammatory Response:** The innate as well as adaptive immune response are provoked by *Propionibacterium acnes* development:

- Immune cell activation
- Release inflammatory mediators
- Complement system activation

Inflammatory lesions like pustules, papules, nodules, and cysts can create as an effect of rupturing of distended follicles and secreting pro-inflammatory substances into the dermis.

6. **Skin barrier dysfunction and epidermal changes:** Androgens influence the skin barrier function, and disruption of this barrier can stimulate epidermal DNA synthesis. This results in epidermal hyperplasia and contributes to follicular hyperkeratosis, further aggravating acne severity.

7. **Role of lipids and sphingolipids:** Lipid metabolism has a major part in acne pathobiology. Sphingolipids, including ceramides, sphingomyelin, and glycosphingolipids, must be maintained to preserve the structure and performance of the skin.

Ceramides make up about half of the lipids in the stratum corneum and are crucial in preventing trans epidermal water loss. Alterations in lipid profiles have been observed in acne patients, including:

- Increased levels of C16 sphingomyelin and Ceramide 1-phosphate (C1P)
- Decreased levels of C24 ceramide

These changes may serve as potential biomarkers for disease progression.

8. **Role of foam cells:** Foam cells, which are lipid-laden macrophages and neutrophils formed through the uptake of oxidized lipids, have been identified in acne lesions. Their presence indicates an ongoing inflammatory process and links acne pathogenesis with lipid oxidation mechanism.
9. **Sebum composition and bacterial growth:** Sebum is abundant in area with a high density of *Propionibacterium acnes*. Components such as oleic acid promote bacterial growth. Increased sebum production, combined with follicular hyperkeratosis, enhance microbial proliferation and inflammation.

1.6.7 TREATMENTS and THEIR SIDE EFFECTS ^[28, 31, 32]:

1.6.7.1 TROPICAL TREATMENT:

Benzoyl peroxide:

One common topical medication commonly available for the treatment of acne is benzoyl peroxide. It predominantly functions as an antiseptic, decreasing the number of bacteria on the skin surface. Likewise, it also has anti-inflammatory characteristics and aids in the decreasing the number of comedown formation (Black heads and White heads).

Benzoyl peroxide is commonly obtainable in the form of creams or gels and is typically applied once or twice daily. It should be used after cleansing and applied to all acne-affected areas of the face rather than as a spot treatment.

Care should be taken to use it in small amounts, as excessive application may lead to skin irritation. The medication can also increase sensitivity to sunlight; therefore, patients are advised to limit sun exposure, avoid artificial ultraviolet (UV) sources are sunbeds, and use appropriate sun protection.

An important precaution is its bleaching effect, which may discolour hair, clothing, or fabrics upon contact.

Common side effects:

- Dryness and tightness of the skin
- A burning, itchiness and uncomfortable feeling
- Skin peeling and red spots

Most of the time, the adverse effects are minor and tend to resolve after discontinuation or adjustment of treatment.

Duration of Treatment:

A typical course of treatment lasts approximately 6 weeks, during which most patients experience significant improvement or clearance of acne. In some cases, continued use at a reduced frequency may be recommended to prevent recurrence.

Tropical Retinoids:

Tropical retinoids are frequently used for the treatment of acne. They work by exfoliating the skin, removing dead skin cells from the surface and preventing them from accumulating within hair follicles.

Common topical retinoids include tretinoin and adapalene. These are typically applied once in a day, ideally right before bed, and available in the form of gel or cream.

Topical retinoids can be applied to all acne-prone areas of the face for better results, about 20 minutes after washing the face. Only a small amount should be used, as excessive application may lead to irritation.

It is important to avoid excessive exposure to sunlight and UV radiation while using these medications, as they can increase skin sensitivity.

Redness, stinging, dehydration, and slight skin irritation are the most common side effects. Usually temporary, these effects become more effective with continued use.

A minimum treatment of a period of 6 weeks is mandatory to observe noticeable improvement. In some cases, continued use at a reduced frequency may be advised for maintenance.

Tropical Antibiotics:

Tropical antibiotics are used in the treatment of acne to reduce or eliminate bacteria present in your skin, which particularly leads to clogged hair follicles.

They are available in the form of lotions or gels and are usually applied once or twice daily, depending on the severity of the condition and medical advice.

In general, a course of treatment from six to eight weeks is recommended. To minimize the possibility of developing antibiotic resistance, therapy is often discontinued after this time. Long-time use might lower the possibilities that bacteria will become susceptible to antibiotics. The prolonged use can make resistant to bacteria which may aggravate acne and eventually results in more infections.

Clindamycin and erythromycin are 2 usual topical antibiotics used for the treatment of acne.

Although they are very rare and usually considered mild, adverse effects may consists of:

- A slight irritation of the skin
- A burning of skin and red feeling
- Skin redness or lack of moisture

Azelaic acid:

When the side effects of different acne treatments, like benzoyl peroxide or tropical retinoids, are become too painful or irritating, azelaic acid is often used as an alternative.

It allows to keep pores clear by removing dead skin cells and preventing the growth of bacteria causing acne.

Normally applied twice a day, azelaic acid is supplied in the form of a cream or gel. It may be considered to apply once a day to patients with normal to sensitive skin.

Unlike some other acne treatments, azelaic acid **does not increase sensitivity to sunlight**, so normal sun exposure is generally safe.

Improvement in acne is usually seen after about **1 month of consistent use**.

Usually, negative reactions may consist of following:

- Burning or stinging sensation
- Itching
- Dryness of the skin
- Redness

Oral Antibiotics (Antibiotic Tablets):

For the management of moderate to severe acne, oral antibiotics are usually advised typically in combination with topical treatments to achieve maximum efficiency.

The most frequently used group is the tetracyclines, which includes drugs such as doxycycline and lymecycline.

However, for individuals who are **pregnant or breastfeeding**, erythromycin is usually recommended as a safer alternative.

Improvement in acne is typically seen after **6 weeks** of therapy.

The period of the time of the oral antibiotic course can range from four to six months, based on how the patient responds.

Important considerations:

- Tetracyclines may make increase **sensitivity to sunlight and UV radiation**, so sun protection is advised.
- They may also **reduce the effectiveness of oral contraceptive pills** during the initial weeks of treatment.
- As a result of this, it is suggested to use an alternative method of birth control, such as condoms, over this period.

1.6.7.2 HORMONAL TREATMENT:

Hormonal therapies can be beneficial for women with acne, particularly when breakouts are linked to hormonal fluctuations, such as those occurring during menstrual cycles, or conditions like polycystic ovary syndrome.

One commonly used option is the combined oral contraceptive pill, which helps regulate hormone levels and reduce excess androgen activity-within the most common causes of acne.

This type of therapy can significantly improve acne in many women. However, it may take **several months to up to one year** to observe the full benefits.

Co- cyprindiol:

A hormonal medication known as co- cyprindiol is utilized to treat mild to severe acne, particularly when oral antibiotics are not working. It works by reducing androgen activity, thereby decreasing sebum (oil) production.

Patients are usually required to take this medication for **2 to 6 months** before noticeable improvement in acne occurs.

Risks and Safety considerations:

- The continuous use is relatively associated with a greater probability of breast cancer.
 - Among 10.000 women who have never used co-cyprindiol, about 16 may develop breast cancer by age 35.
 - This risk increases slightly to 17-18 cases in women who are in their twenties and thirties and who have regularly used it for minimum five years.
- There is also a minor chance of blood clots, and that is expected to happen in approximately one in 2,500 women per year,

Contraindications:

- Not considered safe during pregnancy or breastfeeding.
- A pregnancy test may be required before a starting treatment.

Possible side effects:

- Headaches
- Breast tenderness

- Mood changes
- Diminished libido level (a lack of interest in sex)
- Increasing or decreasing weight

Isotretinoin:

For moderate to severe acne, isotretinoin is a powerful oral treatment, particularly when other treatments are failed to work.

It is taken in capsule form and works through multiple mechanisms:

- Normalizes sebum production and significantly reduces oil secretion
- Prevents clogging of hair follicles
- Decrease the number of acne-causing microbes found in the skin
- Lessens swelling and redness that surround lesions

Despite its effectiveness, isotretinoin related to a different possible adverse reaction, which is why its use is restricted to severe cases.

Important considerations:

- It is **only prescribed under the supervision of a special doctor** (such as a dermatologist)
- Regular monitoring is required during treatment due to possible adverse effects.

1.6.7.3 NON-PHARMACEUTICAL TREATMENT:

Several acne treatments do not involve medications and may be considered as adjunct or alternative options, although their effectiveness can vary.

Photodynamic therapy (PDT) is one specific method that focuses on bacteria and inflammation on the skin to help to reduce acne symptoms.

Some additional non-pharmacological treatments include the followings:

- Comedown extractor: a small, pen shaped instrument for eliminating the presence of whiteheads and blackheads on the skin
- Chemical peels: This process involves applying a chemical solution to the face causing the outermost layer of the skin to peel off and become replaced by fresh healthy skin.

Although these treatments may provide some benefits, their results are often **inconsistent**, and they are **not routinely recommended** as primary treatments for acne.

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CHAPTER – 2. INTRODUCTION TO PLANTS

2.1 *Nelumbo nucifera* (LOTUS):

2.1.1 TAXONOMICAL CLASSIFICATION ^[1, 2]:

The botanical name of the Lotus is *Nelumbo nucifera* places it in the kingdom Plantae and subkingdom Tracheobionta. It is classified as a flowering dicotyledon under the Magnoliophyta division and Magnoliopsida class. Historically divided into the subclass Magnoliidae and order Proteales, it is currently the primary species of Nelumbonaceae family under the genus Nelumbo.



FIG 2.1 PLANT AND FLOWER OF *Nelumbo nucifera*

This image (Fig. 2.1) provides an excellent ecological field overview of a *N. nucifera* colony displaying asynchronous development. Unlike an isolated studio photograph, these graphic captures multiple lifecycle phases simultaneously within a single aquatic canopy: emerging conical flower buds, fully expanded blossoms, mature green receptacles, and dried, senescent seed pods.

This above image shows a fully developed sacred lotus (*N. nucifera*) flower with pink petals, a yellow receptacle in the centre that holds embryonic seed carpels, surrounding stamens, and green foliar surfaces in the background.

2.1.2 VERNACULAR NAMES ^[3]:

Sanskrit: Pankaja

Hindi: Kamal

Gujarati: Suriya Kamal

English: Lotus

Punjabi: Kanwal, Pampo

Bengali: Padma

Parts used: Flower

2.1.3 CHEMICAL CONSTITUENTS (FLOWER) ^[4, 5, 6]:

N. nucifera Plant stamens consist of numerous flavonoids, including kaempferol, nelumboside A, and nelumboside B. The stamens additionally have isorhamnetin, glycosides, and non-flavonoid compounds like arbutin, adenine and myo-inositol.

2.1.4 GEOGRAPHICAL DISTRIBUTION ^[7]:

This species is widely distributed into different phytogeographical regions of India exhibiting wide phenotypic variation. Cultivars of *N. nucifera* are found in other countries like Sri Lanka, Thailand, Japan, Korea, Indonesia, China, Brazil, USA and Australia.

The American Lotus (*N. lutea*) or the yellow Lotus is a native of North America and West Indies. It has a wide distribution throughout North America (New York, Southern Ontario, Florida and Texas), Central America, Mexico, parts of Columbia and West Indies. Also, this species is found to grow abundantly along the delta of Mississippi river.

2.1.5 DESCRIPTION ^[8]:

- *N. nucifera* is a perennial aquatic herb with rhizomes.
- The stems are slender, elongated, branched, and creeping with nodal roots.
- The leaves are peltate, orbicular, floating and concave to cup shaped. They are ultra hydrophobic which results in self-clearing.
- Petioles are long, rough, with small prickles.

- Flowers are ranged from white to rosy, sweet-scented, and solitary. They measure 10-25 cm in diameter.
- Fruits are ovoid with nut-like achenes.
- Seeds are black, hard, ovoid in shape.
- Varieties are: *N. nucifera* var. alba (white lotus) and *N. nucifera* var. rubra (red lotus).
- It has an excellent seed longevity.

2.1.6 USES ^[3]:

Medicinal Uses:

It contains a different medicinal property. The roots help in elimination of metabolic waste and lower the body heat. Roots and rhizomes are typically used in the treatment of smallpox, throat diseases, skin pigmentation issues and diarrhoea. Cooked roots are believed to be beneficial in the digestive system and reproductive organs. Paste of rhizome can be used topically for the treatment of ringworm and other skin diseases. The stem has been suggested to promote normal foetal growth. Leaf paste is used directly in the management of fever and inflammatory skin conditions. Moreover, the leaves and flowers have proven successful in treating a many types of bleeding disorders. Flowers area also used in promotion of conception.

Ceremonial Uses:

N. nucifera flowers have religious and cultural importance. They are generally used as offerings in temples. The fibres of the lotus plants are used for specially weaving for Buddha images. Flowers symbolize purity and fortune in Buddhism. Additionally, *N. nucifera* flowers have an important role in Durga Puja in West Bengal.

2.2 *Nigella sativa* (KALONJI):

2.2.1 TAXONOMIC CLASSIFICATION ^[9, 10]:

Nigella sativa is both historically as well as botanically classified as a species of plantae kingdom and especially the Tracheobionta subkingdom because of its vascular nature. It is a flowering dicotyledon classified under Magnoliophyta and Magnoliopsida. It belongs to the family Ranunculaceae and order Ranunculales. This annual herb is well

known around the world for its medicinal and pharmaceutical properties. It is classified particularly in the genus *Nigella*.

The macroscopic morphology of *N. sativa*, also known as black seed, black cumin, or kalonji, is shown in the figure 2.2, split-panel image, with its distinctive angular black seeds on the right and its pale blue/white flower on the left. The fruit morphology and seed dehiscing mechanism of *N. sativa* (black seed) are also depicted in this image. The mature, dark angular seeds that have been released from the locules are displayed alongside the characteristic green, multi-chambered follicle fruit capsules.

2.2.2 VERNACULAR NAMES ^[10, 11, 12, 13]:

Sanskrit: Krushna Jira

Hindi: Kalonji

Gujarati: Kali Jiri

English: Black cumin

Punjabi: kalonji

Bengali: Kali Jira

Parts used: Seeds



FIG 2.2 *Nigella sativa* PLANT and SEEDS

2.2.3 CHEMICAL CONSTITUENTS ^[13, 14]:

Major Compound: Thymoquinone

Physicochemical Parameters:

- Total content of ash: 3.8 to 5.30%.
- The range of acid-insoluble ash is 0. to 0.50%.
- The volatile oil contains ranges from 0.5% to 1.30%.
- Fatty oil is available in 35.0 - 41.60 percent
- Alcoholic acidity ranges from 3.40 to 6.30 %

Volatile Oil Composition:

- Yellowish-brown oil (steam distillation)
- Contains:
 - ♦ Carvone
 - ♦ D-Limonene
 - ♦ P-Cymene
 - ♦ Nigellone

Other Phytochemicals

- Nigellin which has bitter principle
- Proteins, Tannins and Resins
- Glucose-Reducing sugars
- Saponins
- Organic acids soluble in alcohol

Presented Amino Acids are Cystine, Lysine, Aspartic acid, Alanine, Glutamic acid, Valine, Leucine, Tryptophan

Poisonous Component

Mellenthin- Saponin → **Melanthigenin-** through hydrolysis

Storage Note:

- Alcohol-soluble acids increase rapidly in powered seeds during storage.

2.2.4 GEOGRAPHICAL DISTRIBUTION ^[15, 16]:

It is commonly found across the world. It is usually found in India and Pakistan. The various species also grown in Syria, Israel, Parts of Southern Europe, Lebanon, and Bangladesh. Kalonji is normally grown as a winter crop, such as wheat. In India, it is widely cultivated in Himachal Pradesh, The Gangetic Plains, Punjab, Assam, Bihar, and Maharashtra.

2.2.5 DESCRIPTION ^[17, 18]:**Morphology of *N. sativa***

- Small, prostrate annual herb
- Height: 44-60 cm

Leaf

- Arrangement: Opposite
- Separated into different sections that are linear up to 3 cm.
- Lower leaves: Small, petiolate
- Upper leaves: Longer, elongated

Flower

- Solitary and light blue in colour
- Borne on long pendules
- Terminal position

Fruits

- Capsule

Seeds

- Trigonal (triangular)
- Initially white → turn black upon exposure to air

- Known as black seeds
- Pungent odour
- Rich in oil content

Reproduction

- Primarily self-pollination

2.2.6 USES ^[19, 20]:

This versatile natural remedy is widely used as an aromatic spice, carminative, and condiment, and it possesses significant traditional medicinal properties, acting as a diuretic, diaphoretic, stomachic, digestive, and hepatoprotective liver tonic. Gastrointestinally, it helps treat diarrhoea, indigestion, dyspepsia, sour belching, bad breath, and excessive salivation, and it can even be combined with buttermilk to relieve hiccups. Its other internal applications include addressing loss of appetite, vomiting, dropsy (edema), puerperal disorders, obesity, dyspnoea, and intermittent fevers. It also acts as an emmenagogue and galactagogue, though it can function as an abortifacient in high doses. For respiratory and neurological relief, inhaling it alleviates colds and catarrh, while direct applications help soothe headaches and migraines. Externally, it is used in oil decoctions or as a paste to treat swellings and various skin conditions, including eczema, alopecia, leukoderma, freckles, and pimples. Additionally, it exhibits anthelmintic and antibacterial activities, making it useful in treating sores, leprosy, and mercury poisoning.

2.3 *Averrhoa carambola* (KAMRAKH):

2.3.1 TAXONOMICAL CLASSIFICATION ^[21, 22, 23, 24]:

The botanical name of starfruit is *Averrhoa carambola* L., which belongs to the kingdom Plantae. As a vascular-seed bearing plant, it is belonging to the subkingdom Tracheobionta and subdivision Spermatophyta. It has been categorized as a flowering dicotyledon under the Magnoliophyta division and Magnoliopsida class. Taxonomically, it relates to the subclass Rosidae and the order is Geraniales. It is an important member of the Oxalidaceae family, especially the genus *Averrhoa*.



FIG 2.3 *Averrhoa carambola* PLANT and FRUIT

The macroscopic morphology and cross-sections of starfruit (*A. carambola*) are shown in the above image (Fig. 2.3), which highlights the fruit's characteristic star-shaped transverse slices and five-angled longitudinal ridges. The starfruit, *A. carambola*, is shown growing in its natural arboreal canopy in this field photo. It depicts dense layers of dark green, compound leaves encircling clusters of mature, golden-yellow fruits hanging pendant-style from woody branches.

2.3.2 VERNACULAR NAMES ^[24, 25]:

Sanskrit: Karma Ranga

Hindi: Kamrakh, Karmal

Gujarati: Kamrakh

English: Starfruit, Chinese gooseberry

Punjabi: Kamrakh

Bengali: Kamranga

Part used: Fruit

2.3.3 CHEMICAL CONSTITUENTS ^[26, 27, 28, 29]:

Major Phytochemicals:

- Saponins

- Alkaloids
- Flavonoids
- Tannins

Phenolic Compounds include Proanthocyanidins, Gallo tannins, Vit. C, Epicatechin

Sterols consist of Campesterol, Isofucosterol, β sitosterol, Lupeol

Fatty acids present are Oleic Acid, Palmitic acid, Linolenic acid and Linoleic acid

Flavonoids available are Rutin, Quercetin 3 O β D glycoside

Nutritional Components:

- Reducing as well as non-reducing sugars are present
- Minerals are Iron, calcium, phosphorus
- Nutritional fibre
- Pectin and cellulose, hemicellulose
- Volatile flavour compounds
- Carotenoids

2.3.4 GEOGRAPHICAL DISTRIBUTION [30, 31]:

A. carambola commonly known as starfruit, is considered native to Ceylin and Moluccas. It is widely grown in Southeast Asia, especially in Malaysia and nearby countries.

The plant is extensively grown in southern parts of China, especially in provinces such as Fujian, Guangdong, and Guangxi, as well as in India and Taiwan.

Kamrakh is also well known in the Philippines and Queensland. It is harvested on many South Pacific islands, including Tahiti, Guam, New Caledonia and Hawaii.

In India, the plant is found throughout the country, with significant cultivation in Gujarat and Maharashtra. Overall, *A. carambola* is widely distributed across tropical regions and is considered to be native to Malaysia.

2.3.5 DESCRIPTION [32, 33, 34, 35]:**Plant Habit**

- Small, evergreen tree/shrub
- Height: 5-7 m (up to 10 m)
- Spread: 20-25 ft
- Short trunk (= 15 cm diameter)
- Bushy, rounded crown

Leaves

- Length: 15-25 cm
- Arrangement: Alternate, spiral
- Type: Imparipinnate (5-11 leaflets)
- Leaflets:
 - Size: 2-9 cm x 1-4.5 cm
 - Upper surface: smooth
 - Lower surface: whitish
- Sensitive to:
 - Light (fold at night)
 - Mechanical shock

Flowers

- Colour: Purple to bright purple
- Position: Axillary clusters
- Size: 6 mm wide
- Structure:
 - 5 petals (curved)
 - 5 sepals

Fruits

- Unripe: Green, Ripe: Yellow/Orange
- Shape: Oblong, 5-6 angled
- Size: 5 to 15 cm in length, up to 9 cm in width
- Texture: Crisp, juicy

- Cross-section: **star shaped**
- Taste: **Sour to sweet**
- Odour: **Oxalic acid-like**

Seeds

- Number: **0-12 per fruit**
- Shape: **Flat, thin (=5 mm)**
- Colour: **Brown**
- Enclosed in **gelatinous aril**
- **Short viability**
- Propagation: **By seeds**

2.3.6 USES [36, 37, 38, 39]:

General Health Benefits: *A. carambola* is valued for its nutritional and therapeutic properties. The fruit is low in calories (approximately 30 Kcal per fruit) and rich in dietary fibre, making it beneficial for weight management. The flowers of the sweet variety are traditionally used in the treatment of coughing.

Starfruit is a great source of B9 vit. (folic acid) that plays a significant role in reducing the chance of cardiovascular disorders such as heart disease and stroke. It also contains B-complex vitamins that contribute to hair development and help maintain general well-being. Additionally, the fruit has been considered to have blood-purifying properties, contributing to improved skin health and a radiant complexion.

This versatile natural remedy is popular as a home remedy for hangovers and sunburns, while also effectively treating coughs, fevers, ulcers, and sore throats. Its leaves are specifically used to improve digestion and treat stomach ulcers. In India, the ripe fruit is traditionally used to control haemorrhage and relieve bleeding haemorrhoids, while the dried fruit or its juice is commonly administered to reduce fever.

2.4 *Sphaeranthus indicus* (GORAKHMUNDI):

2.4.1 TAXONOMICAL CLASSIFICATION [40]:

Sphaeranthus indicus is a plant species belonging to the genus *Sphaeranthus* within the sunflower family, Asteraceae. It is classified under the kingdom Plantae and subkingdom Viridaeplantae. Its broader evolutionary lineage places it within the division Phanerogamae and super division Spermatophyta. At more specific taxonomic levels, it belongs to the class Magnoliopsida, subclass Asteridae, super order Asteranae, and order Asterales.

The macroscopic morphology of *Sphaeranthus indicus*, also referred to as East Indian globe thistle or Gorakhmundi, a significant medicinal herb in traditional Ayurvedic medicine, is depicted in the below image (Fig. 2.4). Its characteristic winged stems, serrated, obovate leaves, and spherical, purple-to-magenta composite flower heads are highlighted in the picture. The harvested, sun-dried flower heads (capitula) of *S. indicus*, also known as East Indian globe thistle, Gorakhmundi, or Mundi, are shown in the image.



FIG 2.4 *Sphaeranthus indicus* PLANT

2.4.2 VERNACULAR NAMES ^[41, 42]:

Sanskrit: Mundi, Sravani, Bhumi Kadamba

Hindi: Mundi, Gorakhmundi

Gujarati: Gorakhmundi

English: East Indian globe-thistle

Punjabi: Gorakhmunda

Bengali: Surmuriya, Mudmudiya

Parts used: Whole Plant

2.4.3 CHEMICAL CONSTITUENTS ^[43]:

Flavonoids

- 5-hydroxy-7-methoxy-6-C-glycosylflavone(**C-glycoside**)

Alkaloids and oils

- **Sphaeranthine (alkaloid)**
- Volatile oil (steam distillation)

Sesquiterpenes and Derivatives

- 7 α -hydroxyeudesm-4-en-6, 12-olide
- 2-hydroxycostic acid
- β eudesmol
- Ilicic acid

Essential Oil Components (GC/GC-MS)

- 2,5-dimethoxy-*p*-cymene, 18.25%
- α – agarofuran, 11.82%
- 10-*epi*- γ -eudesmol, 7.91%
- Selin-11-en-4 α -ol, 12.70%

Other Oil Constituents

Cadinene, ocimene, citral, *p* – methoxycinnamaldehyde, Geraniol, eugenol, Geranyl acetate

Newly Isolated Compounds

- Eudesmanolides:
 - 11 α , 13-dihydro-3 α ,7 α -dihydroxyfrullanolide
 - 2 α -hydroxycostic acid
 - Cryptomeridiol, 4-epicryptomeridiol

-
- Hydroxyeudesmanolides

Biological Activity: Anti-bacterial properties against both *Staphylococcus aureus* and *Staphylococcus albus*.

Also E. coli., Fungus species, Helminthosporium genus.

2.4.4 GEOGRAPHICAL DISTRIBUTION ^[44]:

S. indicus is widely distributed throughout India, occurring at altitudes of up to approximately 1500 m above mean sea level. It is commonly found in regions such as Chhindwara district, particularly in areas like Rater.

In tropical regions, like Garhwal Himalaya, this plant is also usual in wet and humid weather. It commonly grows easily in moist areas such as, riverbanks, marshes and other environments that maintain water.

2.4.5 DESCRIPTION ^[45]:

Plant Habit

- Strongly scented, annual herb
- Much-branched with tapering roots
- Toothed wings are arranged on a cylindrical base

Leaf

- Decurrent and sensile
- Size: 2 to 7 cm to 1 to 1.5 cm
- Shape: Obovate-oblong in shape
- Surface: Glandular hairy
- Margin: Spinous-serrate or dentate
- Colour: Greenish-brown

Flowers

- Terminal, solitary, globose heads
- Colour: Purple

- Bracts: Short, slender, acuminate

Floral Structure

- Outer florets:
 - Female, fertile
- Central florets:
 - Bisexual, fertile/sterile
- Involucre: Narrow
- Receptacle: Small, naked

Corolla

- Female flowers:
 - Purple, tubular, 2 to 3 teeth
- Hermaphrodite flowers:
 - Purplish-white in colour, cylindrical or funnel shaped
 - 4 to 5 teeth

Reproductive parts

- Anthers: Sagittate base
- Style: Filiform and sometimes a connate

Fruit

- No pappus
- An oblong achene

Odour

- Slightly aromatic
- Fades on storage

2.4.6 USES ^[43]:

This medicinal plant possesses a wide array of therapeutic benefits, functioning as a tonic, deobstruent, alternative, and aphrodisiac that is highly beneficial for treating nervous debility and eye disorders. Internally, it is used to manage varied conditions such as leprosy, diabetes,

fever, pectoralgia, gastroenteritis, hernia, helminthiasis, haemorrhoids, and dyspepsia. Its respiratory applications are diverse; the seeds are utilized to treat asthma, bronchitis, cough, fever, and pertussis, while a decoction of the plant relieves insomnia and chest disorders, and is reported to have anti-tubercular activity.

The plant's individual components also hold distinct values. The leaves can be consumed as a pot-herb, and their powder serves as a nervine tonic and skin disease treatment. The roots function as a diuretic, febrifuge, expectorant, and stomachic, with the root oil specifically applied to treat scrofula, and the root bark powder used locally as a paste or taken to treat bleeding piles. Externally, a paste of the plant made with oil is applied to soothe painful swellings and pruritus (itching). Beyond human medicine, it has practical agricultural and ecological uses, acting as a natural fish poison and a pest control agent to kill crabs.

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CHAPTER – 3.**INTRODUCTION TO SOLID LIPID NANOPARTICLES:****3.1 SOLID LIPID NANOPARTICLES (SLNs) AS NEXT-GENERATION COLLOIDAL CARRIERS: ENHANCING STABILITY and CONTROLLED DRUG RELEASE:**

For the delivery of lipid-based compounds in specific cases, lipids are being widely used as an alternative for polymer-based nanoparticles. solid lipid nanoparticles (SLNs), another name used for lipid nanoparticles, since they were first introduced in 1991, they proved to be a viable substitute for conventional colloidal-based transport system such as, Liposomes, emulsions, and polymer-based micro and nano-particles.

Solid bio-degradable lipids compose the matrix of solid lipid nanoparticles that are water-based colloidal dispersion. These systems are typically made of sphere shaped solid-lipid particles which are dispersed in water or aqueous solution of surfactant and vary in size between 10-1000 nm. Physiological properties of SLNs like particle size, zeta potential, scanning electron microscopy have a significant effect on both their in vivo and in vitro activity.

The growing interest in lipid-based delivery systems is attributed to several advantages, including:

- Enhanced oral bioavailability with reduced plasma variability
- Improved characterization and safety profile of lipid excipients
- Ease of technology transfer and scalability for industrial production

As a substitute of polymeric nanoparticles, solid lipid nanoparticles are seemed to be among the most promising potential- new colloidal carrier system. Structurally, they resemble oil-in-water emulsions used in parenteral nutrition; however, in SLNs, the lipid liquid phase is replaced with as solid lipid, resulting in improved stability and controlled drug release properties as given in the figure below (Fig. 3.1 and 3.2).

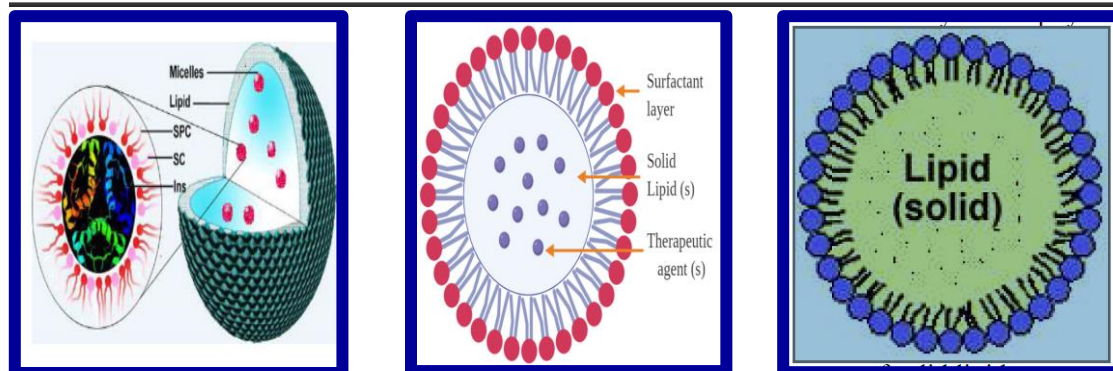


FIG 3.1: REPLACEMENT OF LIQUID LIPID BY SOLID LIPID

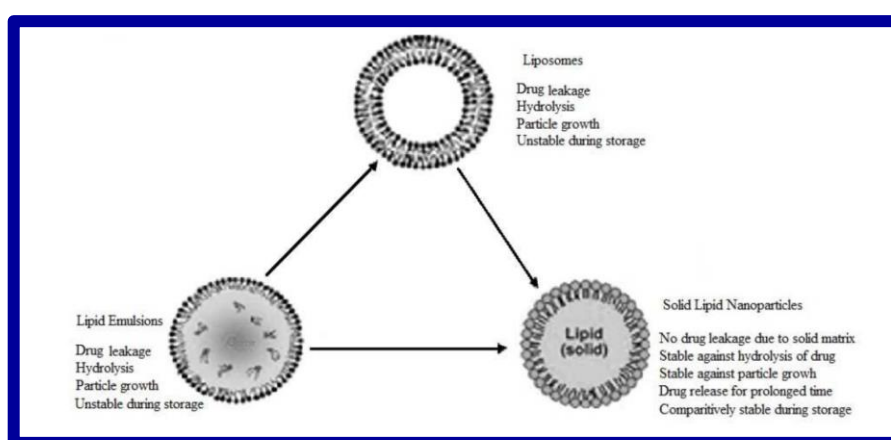


FIG 3.2: A REPRESENTATION of SLNs OVER LIPOSOME and EMULSION

3.2 SOLID LIPID NANOPARTICLES AS COSMECEUTICALS [3, 5, 7, 11]:

Solid lipid nanoparticles (SLNs) have been successfully incorporated into sunscreen formulations and are widely used as carriers for UV blockers and anti-aging activities. An *in vivo* study demonstrated a significant increase (approximately 31%) in skin hydration after 4 weeks when 4% SLNs were incorporated into a conventional cream base.

SLNs have been proven to be innovative topical delivery systems, offering controlled drug release along with pronounced occlusive properties. A significant development in nano cosmetics was made in 2005 when the 1st cosmetics made of solid lipid nanoparticles were introduced in the market place.

Advantages of SLNs for dermal applications in cosmetic products include:

- Occlusive properties that enhance skin hydration
- Modified and regulated release of active ingredients
- Improved penetration into deeper skin layers
- Enhanced UV protection
- Reduced systemic absorption of active compounds
- Prolonged and long-lasting effects
- Improved sensory and finish quality of formulations

3.3 ADVANTAGES OF SLNs ^[2, 3]:

- High loading capacity for functional compounds
- Lyophilization possible
- Manage and target the drug release at specific sites.
- High-level of biological compatibility
- An increase in pharmacological stability
- Higher and enhanced drug content
- Easily able to sterilize and scale up
- Chemical defence of incorporated substances which are labile in nature
- Very much easy to produce than biologically polymeric nanoparticles
- No specific solvent is necessary

3.4 NEGATIVE ASPECTS OF SLNs ^[2, 3]:

- Inadequate capacity of drug loading
- Drug may expel during polymorphic transitions in storage (sometimes resulting in burst release).
- Particle growth
- Unforeseen polymeric transition motion
- Comparatively the dispersions' high-water content- 70 to 99.99%
- Unpredictable gelation tendency

3.5 EXCIPIENTS USED IN SLNs:

Table 3.1 and 3.2 outline the specific structural and functional roles each chemical category plays within a Solid Lipid Nanoparticles (SLNs) matrix.

TABLE 3.1 VARIETIES OF CHEMICALS USED IN THE SLNs PREPARATION

Lipids		Surfactants and Co-surfactants	
Triacylglycerols	Trimyristin Tripalmitin Tristearin Tricaprin Trilaurin	Phospholipids	Egg lecithin Phosphatidylcholine Soy lecithin
Acylglycerols	Glycerol behenate Glycerol monostearate Glycerol palmitostearate	Ethylene oxide/propylene oxide co polymers	Poloxamer 407 Poloxamer 182 Poloxamer 188 Poloxamine 908
Waxes	Cetyl palmitate	Alkylaryl polyether alcohol polymers	Tyloxapol
Fatty acids	Palmitic acid Stearic acid Behenic acid Decanoic acid	Sorbitan polyoxyethylene fatty acid esters:	Polysorbate 80 Polysorbate 20 Polysorbate 60
Cyclic complexes	Cyclodextrin	Bile salts	Sodium glycocholate Sodium Tauro deoxycholate Sodium cholate Taurocholic acid sodium salt Sodium taurocholate

Hard fat types	Witepsol H 35 Witepsol W 35	Alcohols	Butanol (11) Ethanol

TABLE 3.2 OTHER MATERIALS USED IN THE PREPARATION OF SLNs

Category	Examples	Functions
Cryoprotective Agents	Glucose, Trehalose, Maltose, Glycine, Lactose, Gelatine, Mannitol, Polyvinyl pyrrolidone, Mannose and so on	The maintain the structure stable during lyophilization and prevents the aggregation of nanoparticles
Surface Charge Alterants	Dimyristoyl Phosphatidyl Glycerol-DMPG, Stearyl amine, Diacetyl phosphate, Dipalmitoyl phosphatidyl choline-DPPC	Modifying zeta potential for particular cell uptake and enhanced physical stability
Stealth Components	polyethylene glycol- PEG, Poloxamer	Improve blood circulation time by postponing phagocytic clearance and opsonisation

3.6 DIFFERENT METHODS FOR PREPARATION OF SLNs [2, 3, 7]:

The method of preparation has an enormous effect on key variables such as particle size, drug loading capacity, drug release profile and formulation stability.

Different methods or preparation are being developed to manufacture SLNs, with high pressure homonization and microemulsion Dilution having been the most commonly used methods. SLNs are usually formulated with lipids, emulsifiers, and water or organic solvents, depending on the preparation method.

Various methods for preparing SLNs are explained below.

1. High Pressure Homogenization:

High Pressure Homogenization (HPH) is a reliable and efficient technique for manufacturing solid lipid nanoparticles. This technique involves pushing a liquid through a narrow gap (between the micrometer range) at pressure up to 100-2000 psi. This method accelerates the fluid over a relatively short distance to extremely high velocities- > 1000 km/hr which leads to the formation of nanoparticles.

Usually, 5 to 10% concentrations of lipids are used in this method, however, formulations with lipid content as high as 40 percent have also been explored.

There are mainly 2 types of HPH: Hot homogenization and Cold homogenization.

2.Ultra Sonic method and High-Speed Homogenization:

In order to get smaller size of particles in solid lipid nanoparticles, high speed homogenization and ultrasonication have been widely used. This technique boosts a particle size reduction through the combination of acoustic energy and mechanical shear.

The use of this method can reduce shear stress more effectively than other methods with high energy; although it has some limitations. These involve the possibility of metal contamination from the probe and physical instability of the formulation like growth of particles while on storage. To promote nanoparticle formation, bath sonicator and probe sonicator are used.

It consists of 2 types:

A. Probe ultrasonication

B. Bath ultrasonication

3.Evaporating the solvent method:

In this process, the lipid material is dissolved in a water based insoluble organic solvent such as cyclohexane. This organic phase is then emulsified into an aqueous phase by using appropriate surfactants. The emulsion resulting is homogenized under high pressure in order

to form a fine o/w water emulsion. After that the organic solvent is eliminated by evaporation at low pressure of approx. 40-60 mbar.

During solvent evaporation, lipid participates in the watery medium which results in a dispersion of nanoparticles. This process is capable of manufacturing nanoparticles with an average particle size of close to 25 nm.

4.The solvent-emulsification diffusion method:

This technique can produce nanoparticles with particle size of 30 to 100 nanometre. It has an advantage of not needing high temp procedure that can make it suitable for thermolabile drugs. Thus, the absence of heat during the procedure is an important benefit of this technique. It requires dissolving of the lipid in an organic phase via low heating, e.g., heating in a water bath at 50 °C in order to assure complete solubility. The organic phase is added in to an acidic water phase containing suitable stabilizers. The adjustment in pH induces coacervation, which results in the development of solid lipid nanoparticles.

5. Supercritical Fluid (SCF) Technology- RESS method:

It has a significant benefit of being solvent free which makes it an ecologically friendly and safe choice for the preparation of nanoparticles. This platform itself has several variants for the production of nanoparticle and powders.

Solid lipid nanoparticles can be produced by using the rapid Expansion of supercritical fluid method. It requires dissolving of the lipid in a supercritical fluid and then quickly expanding it by means of nozzle, triggering in the formation of nanoparticles because of a sudden reduction in solubility.

Carbon dioxide (CO₂, 99.99% purity) is the one most frequently employed solvent in this method since it is nontoxic and non-flammable and it can be readily expelled under mild conditions.

6. Microemulsion Dilution:

This method involves dilution of microemulsion for manufacturing solid lipid nanoparticles. For preparing an optically clear microemulsion, stir at 65-70 °C. The structure of this system is ideally made up of a low melting point fatty acids [such as stearic acid], emulsifiers [such as polysorbate 20, Soy phosphatidylcholine, polysorbate 60, sodium tauro deoxycholate], co-emulsifiers [such as sodium monoethylphosphate] and water.

The hot microemulsion is dispersed dropwise into ice cold water with vigorously stirring. The unexpected dilution forces the lipid phase to precipitate and SLNs to develop.

An average volume ration of hot microemulsion to chilled water is 1:25-1:50. The chemical composition of the microemulsion system on the productivity of formation of nanoparticles along with final particle characteristics.

7. Double Microemulsion (w/o/w):

Warm water-in-oil-in-water[w/o/w] double microemulsions are prepared in 2 steps for production of SLNs.

In the 1st step, w/o microemulsion is formed by the addition of an aqueous drug solutions into a mix of melted lipid, surfactant, co-surfactant at a temp slightly above the melting point of lipid which results in a clear system. In the 2nd step, the primary w/o microemulsion is dispersed in an aqueous phase composed of surfactant, co-surfactant, resulting in a clear w/o/w double microemulsion.

SLNs are subsequently produced by dispersing the warm double microemulsion into an ice cold aqueous medium and purifying an ultra-sonification system to remove unwanted stabilisers and surfactant.

A number of emulsins are inherently prone to instability caused by the factors such as coalescence of internal water droplets in the oil phase, the coalescence of oil droplets and the rupture of the interfacial film that covers the internal droplets. But for SLNs production, requirement of stability is only for a short period of time specifically, among the formation of the clear double microemulsion and its rapid quenching in a cold aqueous medium that can be successfully achieved

8.Precipitation method:

Solid lipid nanoparticles can also be prepared by the precipitation method that employs the use of organic solvents. Glycerides are dissolved in a water insoluble organic solvent, e.g., chloroform. The resultant organic phase is further emulsified into an aqueous phase containing appropriate surfactants.

Next to emulsification, the evaporation of organic solvent occurs which causes the precipitation of lipid in the aqueous solution and formation of nanoparticles,

9.Solvent Evaporation-Film Formation method:

This method involves dissolving of the lipid and drug in to an appropriate organic solvent in order to produce a homogenous solution. After that, this solution is being evaporated under a lower pressure and constant rotation, producing a thin film of lipid.

This lipid film is further processed with a liquid phase which contains emulsifiers. To make dispersion easier, the system is ultrasonicated along with a probe sonicator. As an outcome, small and uniform size nanoparticles are formed.

10.Solvent Injection method (Solvent Diffusion method):

This approach is a novel for the preparation of solid lipid nanoparticles and it offers multiple advantages across traditional methods, including the application of therapeutically compatible organic solvents, convenience of handling, and a fast production process which does not call for expensive equipment.

The method is based on the precipitation of a lipid form its dissolution state in an organic solvent. In this manner, solid lipid is dissolved in water based miscible solvent such as acetone, ethanol, isopropanol and/or a combination of these.

The resulting lipid solvent is immediately injected using a needle into a vigorously stirred aqueous phase which might contain a suitable surfactant. Following an injection, the solvent is dispersed into the aqueous phase, thereby precipitating the lipid with formation of nanoparticles.

The use of an emulsifier in the water phase encourages the creation of fine droplets at the injection site and helps in stabilization of SLNs by decreasing the surface tension between the water and solvent till the diffusion of solvent is complete. Lastly, the dispersion will be filtered to eliminate unused or unprecipitated lipid, forming a stable formulation with SLN.

11. Membrane contracting method:

The membrane contractor method is a unique approach for making solid lipid nanoparticles which is particularly appropriate for mass production.

In this, the lipid phase gets heated higher than its melting point and compressed through the membrane pores, which results in the generation of a fine droplets of lipid. Meanwhile, the aqueous phase travels throughout the membrane module with continuously sweeping away droplets which are formed at the pore outlets.

When the dispersed material is brought to room temperature, lipid droplets become solid that resulting in the creation of SLNs. The resultant nanoparticles have characteristics like particle size and PDI, are greatly affected by multiple process variables including aqueous phase cross flow velocity, aqueous and lipid phase temperatures, membrane pore size and pressure of lipid phase.

3.7 CHARACTERIZATION AND ASSESSMENT OF SLNs ^[3, 7]:

1. Particle size measurement and shape:

Laser diffraction [LD] and photon correlation spectroscopy [PCS] are 2 of the highly effective and frequently utilized techniques for measuring the particle size in solid lipid nanoparticles. PCS also referred to as dynamic light scattering [DLS], which measures alterations in the intensity of light scattered due to brownian motion of particles.

It can determine the particle size from a few nanometers to about 3 micrometers.

Although PCS is highly reliable for nanoparticle characterization, it has limitation in accurately detecting larger microparticles.

Laser diffraction (LD), on the other hand, is useful for measuring a broader size distribution, including larger particles, complementing the limitations of PCS.

In comparison with PCS and LD, EM techniques (SEM and TEM) offer a direct representation of morphology, size and shape of particles which allows a detailed structural analysis.

When stored properly, optimised SLN dispersions are usually reported having a physical stability of a year or more.

2.Measurement of Zeta Potential and pH:

Zeta Potential measurement:

When determining the surface charge of particles in colloidal systems such as solid lipid particles, zeta potential is a key parameter. It is necessary for estimating the physical stability of dispersion because of the electrostatic repulsion between equally charged particles works to prevent aggregation.

The measurement of zeta potential is typically carried out instruments such as a zeta potential analyser or zetameter. The malvern zetasizer is one of the most widely used instruments for this purpose.

The magnitude of electrostatic repulsion or attraction between particles in an aqueous SLNs suspension is represented by the zeta potential. Generally, it has values below -25 mV or above +25 mV indicated sufficient electrostatic stabilization of the formulation, ensuring good storage stability.

pH:

The hydrogen ion (H^+) activity in hydrophilic liquids is capable of being measured scientifically by using a pH probe. It offers important information about the acidity of a substance or alkalinity, a key consideration in the description of colloidal systems like SLNs.

Electrochemical potential measurement is the base of a pH probe's operation. which is quantitatively described by the Nernst equation. This relationship allows the determination of pH from the measured potential difference.

3.Shape and Surface Morphology:

A number of sophisticated microscopic methods can be used to study the surface morphology of SLNs. These are atomic force microscopy [AFM], optical microscopy, scanning electron microscopy [SEM] and transmission electron microscopy [TEM].

These techniques provide detailed information regarding particle shape, surface characteristics, size distribution, and structural organization, thereby performing an important part in the comprehensive characterization of SLNs.

4. Analysis of Degree Crystallinity:

X-ray diffractometry [XRD] which evaluate the geometric scattering of radiation off internal crystal planes was employed to track the crystallinity of the formulation. This technique enabled it to distinguish between amorphous and crystalline lipid phases, thus allowing it simpler to quantify the particles' structural order.

Another important technique used for crystallinity analysis is differential scanning calorimetry (DSC). Although its application differs slightly from that in bulk materials, DSC is useful for determining the nature and polymorphic state of lipids within nanoparticles. It provides information on glass transition and melting temperatures, along with their associated enthalpy changes, which helps in understanding the crystallinity and thermal of SLNs.

5.Determination of Incorporated Drug:

Lots of process and formulation affects the drug loading capacity, size distribution and particle size of nanoparticles. It includes the kind of the type of lipids [triglycerides, fatty acids, waxes], emulsifier [anionic, cationic or non-ionic] and the technique of preparation used.

The parameters affecting drug loading capacity are as follows:

- **Thermodynamic Factors:** It includes the miscibility of the drug in the lipid melt and the saturation solubility.

- **Structural Parameters:** It is basically defined by the heterogeneity of the solid lipid matrix or homogeneity of the chemical.
- **Kinetic Factors:** Basically, the rate of polymorphic transitions during the cooling and storage phases that influences the degree of crystal perfection.

6. Measurement of modification of lipid:

Polymorphism and Stability of Lipid Matrix in SLNs

Thermodynamically stable state and lipid density at packing improve, but capacity of drug incorporation decreases, in the sequence given below:

Supercooled molten state < α modification < β' modification < β modification

This indicates that more ordered and stable lipid forms (especially β -modification) possess higher packing density but offers less space for drug incorporation.

Because of the presence of emulsifiers and Particles of small size, polymorphic transitions in SLNs are often significantly retarded compared to bulk lipids. However, such transformations can still occur over time and influence drug release and stability.

X-ray scattering and dynamic scanning calorimetry [DSC] are regularly used techniques for evaluating the polymorphic state on the lipids in SLNs. DSC depends on the idea that different lipid alterations have various melting points and enthalpies allowing for the identification of polymorphic forms.

This provides structural details about the lipid matrix by establishing the lattice's short and long spacings. It is highly recommended to monitor fluctuations in SLN dispersion over time, as reactions such as removal of solvent may cause polymorphic transitions, impacting the overall stability of the formulations and effectiveness.

7. *In Vitro* Drug Release:

There are different techniques employed for evaluation of the *in vitro* drug release profile of solid lipid nanoparticles (SLNs). Commonly used techniques include:

- Dialysis tubing method

- Inverse method of dialysis
- The diffusion of cell of Franz

These methods help in understanding the drug release kinetics, mechanism of release, and overall performance of SLNs formulations under controlled laboratory conditions.

8. Density:

A gas pycnometer is an analytical tool that employs Boyle's law and Archimede's principle to determine the precise density of solid materials.

In this technique, an inert gas-most commonly helium-is used instead of a liquid displacement medium. The gas penetrates into the pores and void spaces of the material, allowing for accurate measurement of the true volume and, consequently, the true density of the sample.

This approach has a number of positive aspects like excellent accuracy and quick analysis, making it a reliable technique for density determination of solid lipid nanoparticles (SLNs) and other particulate systems.

9.Viscosity:

The viscosity of a fluid under a single flow condition can be measured by a viscometer. For fluids with variable flow behavior, a rheometer is preferred as it measures viscosity over a range of shear rates.

In viscosity measurement, either the fluid or an object moves relative to the other, usually under laminar flow conditions (Low Reynolds Number).

U-tube, falling sphere, Krebs, and quartz viscometers are some common viscometer types.

10. Molecular Weight:

Analytes have been divided according to their size of molecules by using gel permeation chromatography (GPC), which is also referred to as size exclusion chromatography.

11.Surface Hydrophobicity:

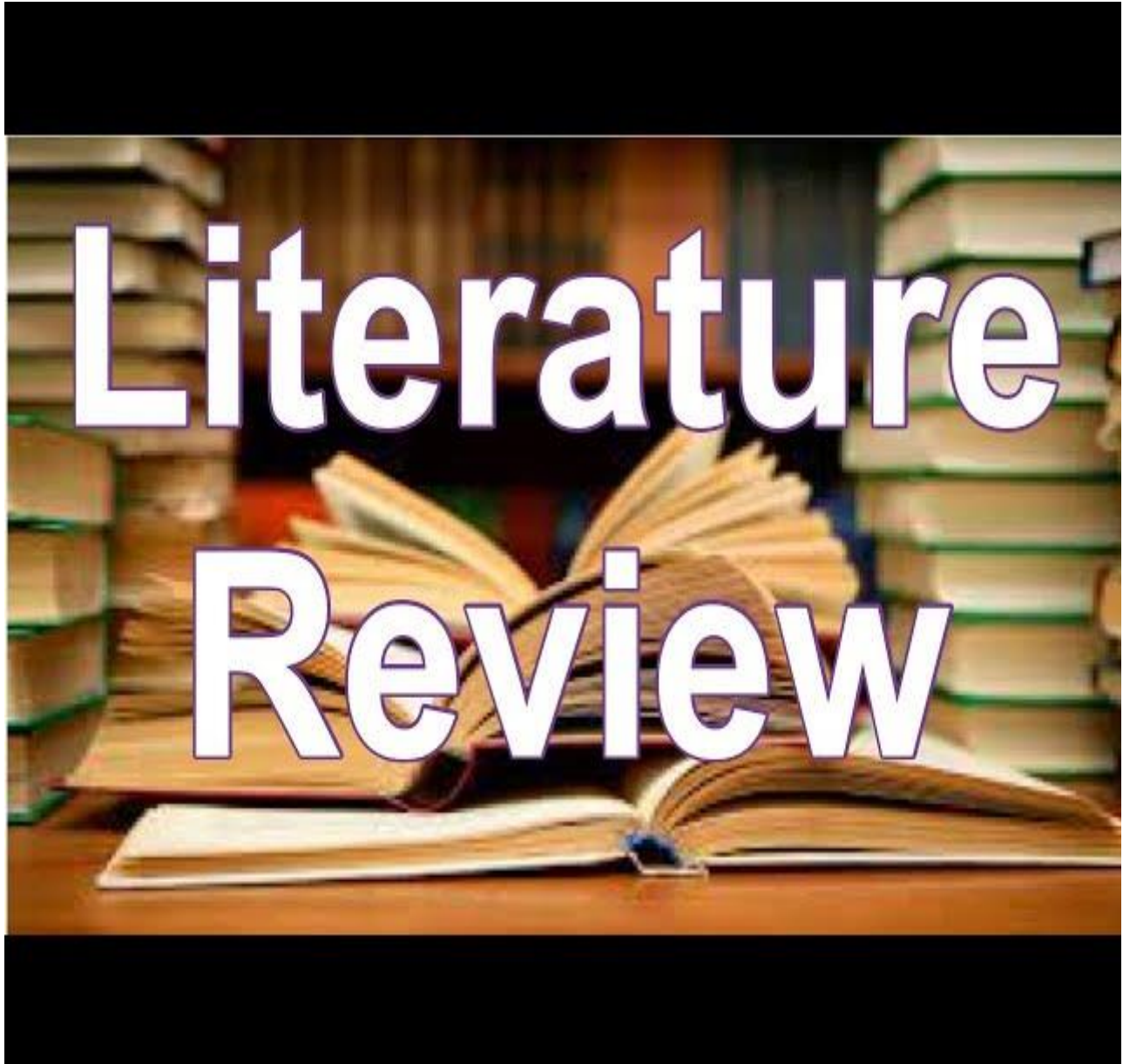
The characteristics of the surface of solid lipid nanoparticles, especially their hydrophobicity and hydrophilicity are assessed by using 2 phase partitioning and contact angle measurement.

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CHAPTER 4. LITERATURE REVIEW



CHAPTER – 4.
REVIEW OF LITERATURE

TABLE 4.1 LIST of MEDICINAL PLANTS FUNCTIONING AS AN ANTI-AGEING
[1, 2, 3].

SN	Botanical Name	Part used	Chemical Constituents	Activity
1	<i>Emblica officinalis</i>	Fruit	Ascorbic acid and also contains fat, phyllembelin and tannins	The fruit's gallic acid, tannins, ellagic acid along with other compounds possess the ability to scavenge free radicals
2	<i>Curcuma longa</i>	Rhizome	Curcuminoside, Curcumin in the quantity of 50 to 60 percent, volatile oil, resin, camphor, camphene etc are also present	Curcumin works as a singlet oxygen quencher and superoxide scavenger
3	<i>Acanthopanax koreanum</i>	Leaves, Root, Stem	Acanthopanic acid, rutin and saponins	Free radical scavenging activity
4	<i>Angelica archangelica</i>	Root	Furanocoumarins	Inhibit elastase and Collagenase
5	<i>Caesalpinia pulcherrima</i>	Flower	Gallic acid, Catechin, Rutin and Ellagic Acid	ABTS cation radical scavenging activity, DPPH radical scavenging
6	<i>Calendula officinalis</i>	Flower	Triterpenes, Sterols,	Inhibiting activity/secretion of

			Flavonoids, Carotenes	MMP-2 and MMP-9 Decrease trans epidermal water loss
7	<i>Citrus sinensis</i>	Fruit	Phenolic compounds and Ascorbic acid	NF-B and AP-1 translocation and procaspase-3 cleavage
8	<i>Coriandrum sativum</i>	Seeds	Petroselinic acid triglycerides	Skin barrier functioning and Prevent moisture loss
9	<i>Crataeva nuruvula</i>	Stem bark	Lupeol	Antioxidant action and keratinocyte proliferation
10	<i>Diospyros discolor</i>	Leaves	Phenolic compounds	Inhibit matrix- metalloproteinase in human skin fibroblast
11	<i>Terminalia chebula</i>	Fruits	Hydrolysable tannins	Hyaluronidase, elastase inhibition and collagen synthesis, anti-oxidative and MMP-2 inhibition
12	<i>Glycyrrhiza glabra</i>	Stem	Flavonoids	Reduce the fine lines and wrinkles
13	<i>Glycine max</i>	Seeds	Isoflavones	Prevent skin cell apoptosis, erythema
14	<i>Punica granatum</i>	Fruits	Flavonoids, Anthocyanins, Catechin	Anti-oxidant activity
15	<i>Curcuma xanthorrhiza</i>	Rhizome	Xanthorrhizol	Increases the Suppression of type-1 pro-collagen suppression and UVB

				included MMP-1
16	<i>Camellia japonica</i>	Oil	High concentration of vitamin E and Rutin	Anti-oxidant, Skin emollient and moisturizing properties
17	<i>Oscimum basilicum</i>	Leaves	Phenolics and Flavonoids	Anti-ageing properties
18	<i>Rosmarinus officinalis</i>	Leaves	Rosemarinic acid, Caffeic acid, Carnosic acid.	Hold off the process of ageing
19	<i>Coffea arabica</i>	Seeds	Chlorogenic acid, Caffeine, Melanoids	Anti-inflammatory and prevent ageing
20	<i>Ilex paraguariensis</i>	Extract	Phenolics	Anti-oxidant
21	<i>Opuntia ficus-indica</i>	Extract	Three flavonoids, quercetin, (+)-dihydroquercetin, and quercetin 3-methyl ether	Skin hydrating properties Antioxidative neuroprotectants that exhibiting protective actions

TABLE 4.2 LIST of MEDICINAL PLANTS FUNCTIONING AS AN ANTI-ACNE ^[4-10].

SN	Botanical Name	Part used	Chemical Constituent	Activity
1	<i>Camellia sinensis</i>	Extract	polyunsaturated fatty acid, Polyphenol	5 α -reductase inhibitory activity, Anti-inflammatory activity

2	<i>Commiphora mukul</i>	Gugu lipid is a standardized oleoresin extract	Oleoresin	Anti-bacterial activity
3	<i>Hippophae rhamnoides L.</i>	Extract of fruit	Organic acids, Vitamins C and E, polyunsaturated fatty acid, macronutrients	Inhibitory activity of Type 1 α reductase
4	<i>Lens culinaris</i>	Complex extracts and powder	Polyphenol	Anti-bacterial, Anti-inflammatory, Anti-androgen and Anti-oxidant properties
5	<i>Aloe barbadensis</i>	Extracts and Powder complex	Polysaccharide	Anti-oxidant, anti-inflammation, anti-androgen, and anti-bacterial activities
6	<i>Vitex negundo</i>	All parts	Flavonoids	Anti-bacterial, anti-oxidant, anti-inflammatory, and anti-androgen properties
7	<i>Butyrospermum paradoxum</i>	Oil	Alkaloid, Anthraquinones, flavones, saponins, steroids & tannins	Anti-bacterial activity
8	<i>Andrographis paniculata</i>	Leaves	Diterpenoids, flavonoids and polyphenols	Anti-androgen, anti-bacterial, anti-inflammatory, and anti-oxidant activities

9	<i>Melaleuca alternifolia</i>	Oil	Terpinen-4-ol, γ -Terpinene, γ -Terpinene	Anti-inflammation and anti-bacterial activity
10	<i>Curcuma longa</i>	Extracts	Essential oil, curcumin, curcuminoids,	Anti-bacterial and anti-inflammatory activity
11	<i>Azadirachta indica</i>	Extracts	Tri-terpenoids and tetra-nor triterpene nimbolin A and B, nimbin, tannin and volatile oil in the bark and leaf	Anti-inflammation and anti-bacterial activity
12	<i>Withania somnifera</i>	Extracts	Withanolides, glycowithanolides	Anti-inflammation and anti-bacterial activity
13	<i>Aloe vera</i>	Extracts	Minerals, sugars, vitamins, saponins, enzymes, amino acids and salicylic acid	Anti-inflammation and anti-microbial activity
14	<i>Psoralea corylifolia</i>	Seeds	Bakuchiol	Antifilarial activity
15	<i>Hemidesmus indicus</i>	Extracts	Lupeol, its octacosanoate, α amyirin, β amyirin, their acetate, hexatriacontane and sitosterol	Anti-inflammation and anti-bacterial Activity

16	<i>Punica granatum</i>	Fruit extracts	punicalin, strictinin A, and granatin B	Anti-oxidant
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4.1 LITERATURE REVIEW of HERBAL FORMULATIONS:

Tiwari *et al.* (2024)^[1] offered a modern framework in recent advancements in Phyto pharmaceuticals in their comprehensive research on the production and evaluation of plant based anti-ageing emulsions. Their results highlight the application of modern pharmaceutical approaches to enhance the stability and delivery of Phyto-oxidants. The study shows detailed criteria of physicochemical evaluation.

Sharma *et al.* (2024)^[2] investigated Phyto therapeutic alternatives to artificial comedolytics by creation of a special herbal cream for *Acne vulgaris*. The main findings of the study are demonstrating the synergistic anti-microbial of the formulation against *Propionibacterium acnes* and its potent sebum regulating action. The researchers have successfully validated a bio-compatible delivery matrix that is capable of entering the sebaceous glands by physiochemically rigorous profiling, particularly optimizing trans-follicular diffusion and rheological stability.

Rangila *et al.* (2024)^[3] provided a detailed critical review of recent anti-acne formulation methods with a focus on the transition from standard basis to enriched bioactive delivery systems. The highlights of the study involve an assessment of the synergistic potential of different phytochemicals and their potential to treat acnes.

Choudhary *et al.* (2024)^[4] centred on the methodical formation of an anti-acne topical product, focusing on the physicochemical integrity of the formulation. The research points out the effective integration of bioactive compounds with strong inhibitory impacts of pathogens causing acne while preserving high skin compatibility.

Jadhav *et al.* (2024)^[5] successfully develop an herbal topical system by using a triple action pharmaceutical approach to manage the complex nature of *Acne vulgaris*. The study illustrates the simultaneous anti-inflammatory, anti-comedogenic and anti-bacterial activities of the formulation that offers a holistic replacement to chemical-based treatments.

Aleena *et al.* (2025)^[6] studied *camellia sinensis* and *Eclipta alba* for their beneficial properties in a unique herbal formulation for acne. The analysis further emphasizes the dual

action approach of the system that uses the polyphenolic anti-oxidants presented in green tea to lower sebum oxidation as well as identifying the potent anti-microbial properties of *Eclipta alba*.

Sharma *et al.* (2025)^[7] contributed to the rapid development of natural therapeutics by generating a specific herbal matrix for the clinical management of *Acne vulgaris*. The research project shows the fruitful physical and chemical characterization of the formulation, via focus on improving viscosity for optimum follicular permeability.

Mohite *et al.* (2025)^[8] has studied the dermatological potential of liquorice by preparing the targeted herbal cream for treating acne. The study shows the use of glycyrrhizin and flavonoids the formulation for giving a strong dual tasking effect: preventing the growth of bacteria causing acne while also decreasing post inflammatory erythema and hyper pigmentation.

4.2 LITERATURE REVIEW of PLANTS:

4.2.1 *N. nucifera*:

Pharmacognostical review:

Rao *et al.* (2005)^[9] validated the hepatoprotective potential of *N. nucifera* flowers. Floral extracts returned endogenous antioxidant levels and regulated liver biomarkers in responding to cell injury that is induced by oxidative stress.

Phytochemical review:

Tungmunnithum *et al.* (2018)^[10] provided a complete phytochemical identification of *N. nucifera* and determining specific flavonoid that are responsible for the therapeutic effect. It also validated the broad-spectrum pharmacological activities of the plant which include anti-inflammatory and anti-oxidant properties, that established a scientific ground work for the transition from traditional medicine to standard beauty applications.

Chen *et al.* (2019)^[11] established a connection between current pharmaceutical applications and ancient ethnobotany of *N. nucifera*; identified the diverse phytochemical profile containing polyphenols and alkaloids; and demonstrated its function in improving systemic

wellness and dermal health through anti-ageing mechanisms and biologically tested antioxidants.

Dubey *et al.* (2019)^[12] performed an accurate phytochemical screening of *N. nucifera* flower extracts to determine the essential secondary metabolites; substantial concentrations of flavonoid and phenolic content were found, which correlated these particular phytoconstituents to the potent free radical scavenging ability of the plant and compatibility for standard herbal formulations.

Sranuji *et al.* (2021)^[13] explored the molecule-based interaction between human immune cells and *N. nucifera* flower extracts. Certain phytochemicals were obtained to possess a powerful immunomodulatory effect by controlling cytokine production in macrophages, suggesting that the plant has the ability to treat inflammation related skin diseases and promote localised dermal immunity.

Pharmacological review:

Dubey *et al.* (2017)^[14] provided a comprehensive review of pharmacological profile of *N. nucifera*, highlighting its broad therapeutic activity; elaborated on its clinically significant properties, such as anti-oxidant, anti-microbial and anti-inflammatory properties; determined its potential as a highly valuable botanical product for the invention of modern drug delivery system.

Islam *et al.* (2018)^[15] assessed the metabolic impact of *N. nucifera* flower extracts in *in vivo* models; significantly higher hypolipidemic and hypoglycaemic effects were detected by lowering lipid profiles and blood glucose, suggesting the potential of the plant for regulating metabolic disorders and also its use in holistic, health-friendly formulations.

Bhat *et al.* (2023)^[16] provided an up-to-date synthesis of phytochemical architecture and therapeutic versatility of *N. nucifera*; mapped the beneficial interaction between its flavonoids and alkaloids that are bioactive, confirming its value in treatment of multi factorial conditions through potent anti-inflammatory, anti-oxidant and cardioprotective mechanisms.

4.2.2 *N. sativa*:

Pharmacognostical review:

Sharma *et al.* (2011) ^[17] established accurate pharmaceutical standards for *N. sativa* seeds to assure authenticity of raw material; systemic phytochemical and physicochemical assessments were done to measure bioactive content and essential oil content, thereby providing the basic QC parameters needed for the standardisation of *N. sativa* in pharmaceutical products.

Rashid *et al.* (2018) ^[18] used advanced pharmacological and microscopic techniques to produce a final standardisation protocol for *N. sativa*, via focus on determining diagnostic anatomical characteristics and cellular markers in order to avoid manipulation and assure the botanical quality and purity and uniformity essential for therapeutic based herbal drugs.

Thakur *et al.* (2022) ^[19] conducted a deep evaluation of kalonji seeds by focusing on their cultural and ethnopharmacological role on reproductive wellness; discovered the association between certain phytochemical substances and contraceptive activity, offering clinical safety data and pharmacological proof regarding the plant's effects on uterus physiology.

Phytochemical review:

Qayyum *et al.* (2020) ^[20] systemically evaluated the effectiveness of different extraction techniques for extracting biologically active substance from kalonji; associated to specific phytochemical products to modern and traditional applications, emphasising the importance of plant as a potent anti-oxidant and anti-inflammatory molecule in both therapeutic and clinical settings.

Dalli *et al.* (2021) ^[21] synthesised a current advance [2019 to 2021] in phytochemistry of *N. sativa*, alongside a focus on thymoquinone. The seeds have broad spectrum pharmacological activities- which include immunomodulatory, anti-viral and anti-neoplastic activities were confirmed and new patterns in its therapeutic application were identified.

Manoharan *et al.* (2021) ^[22] published an integrated review of *N. sativa*'s phytochemical structure, mapping out the therapeutic potential of its various biologically active 2° metabolites; focused on the multiple pharmacological profile of the plant- especially its anti-inflammatory, anti-oxidant and anti-microbial effects as the foundation for its application in nutraceutical and novel drug delivery systems.

Pharmacological review:

Javed *et al.* (2012)^[23] performed a comparison of phytochemical and nutritional profiles of kalonji and *Trachyspermum ammi* was performed and their synergistic pharmaceutical properties were verified, with a spotlight on the high level of phenolic compounds and essential oils, leading to drive their dual anti-microbial and anti-oxidant efficacy in modern and therapeutic purposes.

Dajani *et al.* (2016)^[24] synthesised the preclinical evidence supporting *N. sativa* as a potent complimentary therapeutic effect. Its historical importance was explored alongside the latest pharmacological validation, by focus on its anti-hypertensive, anti-diabetic and gastroprotective properties and it was recommended for its inclusion into a clinical management based on an efficacy profile and safety.

Aklaque *et al.* (2018)^[25] assessed a therapeutic role of kalonji within the context of the Unani system of medicine, conventional humoral concepts were integrated with modern pharmacological research findings to demonstrate its potency in the treatment of digestive, chronic respiratory and inflammatory ailments, which reinforced its status as a corner stone of holistic Greco Arabis medicine.

Islam *et al.* (2019)^[26] delivered a broad scholarly update on therapeutic landscape of *N. sativa*; properly classified its bioactive components and their associated pharmacological effects. Detected significant gaps in existing literature to advice upcoming formulations and clinical studies.

4.2.3 *A. carambola*:

Pharmacognostical review:

Vargas-Madriz *et al.* (2022)^[27] synthesised a phenolic architecture of star fruit, determining high amounts of polyphenols and organic acids; discovered a direct connection between these particular secondary metabolites and excellent anti-oxidant power of fruit; recommended for its greater application as an essential component in anti-ageing formulations.

Pongener *et al.* (2022)^[28] evaluated the physicochemical characteristics and biodiversity of *A. carambola* varieties indigenous to the regions of the Nagaland; observed notable

differences in fruit's acidity, weight and Vit C amount among various accessions, giving a vital information for choosing high-quality germplasm for regional breeding and processing in industries into valuable botanical products.

Phytochemical review:

Luan *et al.* (2021) ^[29] carried out a holistic review of *A. carambola*, filling the gap between medicinal applications and recent pharmaceutical validation; analysed the synthesis of important bioactive compounds like organic acids and flavonoids, proving their efficacy in the management of inflammation, oxidative stress associated disorders and diabetes.

Hul Jannah *et al.* (2023) ^[30] revealing an abundance of tannins, alkaloids, saponins; biochemical analysis demonstrated its high enzymatic activity and nutritional value, further confirming its potential as an effective base for nutritional and therapeutic supplements.

Pharmacological review:

Gowrishankar *et al.* (2018) ^[31] gave a thorough summary of *A. carambola*'s therapeutic and botanical properties; elaborate its traditional use among different cultures for treating health conditions like cough and jaundice; summarised the most recent evidence of science concerning its pharmacological role as a highly potent anti-oxidant anti-pyretic and wound healing activity.

Saha *et al.* (2018) ^[32] executed a full pharmacological evaluation of *A. carambola* plant, illustrating its systemic actions in many biological systems; offered scientifically proved validation of hepatoprotective, anti-hyperlipidaemic and immunomodulatory activities, all while focusing on the therapeutic margins and safety profile of its multiple solvent-based extracts.

4.2.4 *S. indicus*:

Pharmacognostical review:

Vikani *et al.* (2008) ^[33] presented a detailed explanation of pharmacognostical description of Gorakhmundi in order to develop clinical identification indicators recorded significant

micro-morphological and macro-morphological aspects, such as cellular arrangements and leaf architectural features, that act as a definitive quality assurance standard to make sure the authenticity of the herb and plant-based integrity in conventional dosage forms.

Tiwar *et al.* (2018) ^[34] went through an integrated pharmacognostic verification of *S. indicus*, thereby demonstrating a solid scientific basis for its traditional usage through the identification of vital phyto chemical markers- essential oils and alkaloids and validating their key roles in supplying analgesic, anti-inflammatory and hepatoprotective health benefits.

Singh *et al.* (2019) ^[35] served an in-depth analysis of the pharmacognostical qualities of *S. indicus*; concentrating on established standardised parameters needed for its botanical characterisation; outlined the microscopic and macroscopic features of the plant together with its wide-ranging phytochemical profile including steroids, bicyclic sesquiterpene lactones and glycosides to promote assessment of quality and clinical consistency in the development of herbal drug.

Sharma *et al.* (2020) ^[36] founded detailed standardisation protocol for the aerial portions of *S. indicus*, with an emphasis on physicochemical parameters like extractive values, ash values, and humidity content; offered a comprehensive pharmacological blueprint involving micron scale detailing of vascular bundles and trichomes to use as a reference point to ensure purity of raw materials and quality for pharmaceutical use.

Phytochemical review:

Harathi *et al.* (2017) ^[37] evaluated the in vitro antibacterial efficacy and the phytochemical constituents in *S. indicus*. It highlighted its traditional importance as an important anti-jaundice curative herb; that has been shown its bioactive extracts possess substantial inhibitory properties towards different pathogenically bacterial strains, resulting in pharmacological evidence that favours its use in treating hepatic and infectious diseases.

Sarle *et al.* (2020) ^[38] underwent a preliminary Phytochemical screening of *S. indicus*, methodically determining the existence of both primary and secondary metabolites comprising of proteins, carbohydrates, saponins, tannins etc. that revealed the fundamental chemical profile of the plant belonging to the Asteraceae family; permitting a further quantitative analysis and specific extraction of its bio-active therapeutic substances.

Arumugam *et al.* (2023) ^[39] quantified the phytochemical richness and the antioxidant potential in methanolic extract of leaf of *S. indicus* Linn.; a number of in vitro assays were conducted to demonstrate strong radical scavenging activity, which has been attributed to a high percent of flavonoids and phenolics, thus supporting its growth as a herbal antioxidant resource in cosmetic and pharmaceutical products.

Josh *et al.* (2023) ^[40] worked on a rigorous phytochemical and physicochemical study of the whole *S. indicus* plant; defined important quality standards by estimating extractive yields, loss on drying, ash values; recognised a diversified chemical profile made up of flavonoids, triterpenoids, alkaloids throughout all parts of plant to justify its holistic utilisation in traditional herbal treatments.

Pharmacological review:

Mahajan *et al.* (2015) ^[41] characterised *S. indicus* as a multipotential plant with medicinal activities by means of a detailed examination of its multifaceted pharmacological profile; summarised data indicating its effectiveness as an antimicrobial, neuroprotective, antidiabetic agent; highlighted its classical value in curing a variety of medical conditions that ranged from dermatological disorders to hemicrania.

George *et al.* (2017) ^[42] offered a brief pharmacological information on *S. indicus* presenting recent research on its biological activities; especially noted that its potential in controlling CNS disorders via its anxiolytic and anticonvulsant properties, bringing together data on its outstanding macromorphological and antidiabetic properties for pharmaceutical applications.

Shirode *et al.* (2021) ^[43] assumed a contemporary pharmacological re-evaluation of *S. indicus* plant illustrating its diverse therapeutic effects; more specially, explained its anti-inflammatory functions and capacity in decreasing oxidative stress related damage, whereas improving its safety profile for inclusion in long lasting herbal therapy and incorporated health care models.

Girase *et al.* (2024) ^[44] described an updated study with focusing on its novel contribution in current herbal therapeutics; collected modern data on its method of extraction and chemical stability of its bioactive substances; further demonstrated wide ranging potential as an antimicrobial and anti-inflammatory agent in advance pharmaceutical investigations.

4.3 LITERATURE REVIEW of SOLID LIPID NANOPARTICLES:

Tiyaboonchai *et al.* (2012) ^[45] describe the systemic formulation and characterisation of solid lipid nanoparticles containing of curcuminoids. It showed that this nano delivery system significantly boosts the aqueous solubility and chemical stability of curcuminoids, by offering an effective framework for enhancement of efficacy of lipid based herbal bio actives used in pharmaceutical and topical applications.

Nasrollahi *et al.* (2013) ^[46] executed an *invitro* comparative analysis between a normal tretinoin cream and cream containing SLNs of tretinoin. The outcome concluded that when conventional cream is being compared with SLNs based system, nano-delivery system is significantly decreased skin irritation and regulating the rate of release of the active substances with improving its photosensitivity and penetration to the skin.

Chinembiri *et al.* (2017) ^[47] found the topical delivery of raw extracts of ashwagandha by using SLNs and niosomes. They investigated that both lipophilic carriers greatly improved the capacity of extract to penetrate the skin and retention in compared to conventional formulations, with some SLNs having a unique potential for prolonged release as well as maintenance of the plant's highly sensitive phytochemical elements.

Ki m *et al.* (2017) ^[48] developed a SLNs system that delivers *Houttuynia cordata* extract via oral administration with exceptionally high drug loading capacity; standard in vitro investigations demonstrated that matrix of the SLNs has a controlled, sustained release profile had successfully preventing the volatile and sensitive bio active ingredients from GI degradation.

Vijayanandan *et al.* (2018) ^[49] created and optimized SLNs as a transporter system for a specific herbal extract for improvement of therapeutic efficacy. It has been shown that throughout this study, formulation containing SLNs drastically boosted the antidepressant activity of the extract in comparison to its usual form, possibly due to higher penetration in BBB(Blood-Brain-Barrier) and the secured continuous release of neuroactive phytochemicals.

Diniyanti *et al.* (2018) ^[50] prepared solid lipid nanoparticles as a means for delivery of alpha mangosteen rich mangosteen pericarp extract. High shear homogenisation method was applied to produce a stable nanoparticle. It was observed that nano system thoroughly incorporated the antioxidant rich extract, substantially improving its stability and potential for insertion into a variety of skin care or pharmaceutical goods.

Haghighi *et al.* (2018) ^[51] concentrated on the creation and evaluation of SLNs loaded with Ginkgo biloba extract in an attempt to overcome the low bioavailability of extract. It was demonstrated that formulation of nanoparticles provided an excellent entrapment efficacy and a stable release profile; thereby successfully stimulating the antioxidant and neuroprotective properties of the terpene lactones and flavonoids in compared to pure extract.

Trideva *et al.* (2020) ^[52] mentioned a detailed evaluation of SLNs, outlined several preparation techniques such as HPH, Solvent emulsification evaporation, supercritical fluid method etc., focused on the key evaluation of structural features and morphology of nanoparticles; additionally provided a new perspective on present research developments which focus on the role they play as scalable, biocompatible carriers for boosting the delivery and stability of lipid based therapeutic substances.

Ananth *et al.* (2021) ^[53] designed and evaluated hydrogels consist of SLNs enriched with Fenugreek seed extract for treating alopecia; it was found that the hydrogel system containing SLNs gave more favourable hair growth than normal extracts through improved follicular permeability and long-lasting release of flavonoids and bioactive diosgenin.

Thora *et al.* (2021) ^[54] centred on formulation and characterisation of SLNs of *Hibiscus rosa-sinensis* extract in order to develop a stable nanoparticle-based system, the lipid: surfactant ratio was optimally adjusted. On the basis of the study, encapsulation of SLNs substantially increase the anthocyanins stability and other fragile phytochemicals in the Hibiscus extract, making it an appropriate way of using it in the treatment that facilitate hair growth and perform the role of antioxidants.

Ruo Xin *et al.* (2022) ^[55] developed and characterised gel with *Kappaphycus alvarezii*- red seaweed extract along with solid lipid nanoparticles. It showed that the gel containing SLNs shows great stability of the antioxidant compounds of the seaweed and offering a better hydration of skin and texture characteristics than regular gel bases. The research highlighted ability of SLNs to efficiently transport marine derived bio actives in topical skin care products.

Upadhyay *et al.* (2023) ^[56] examined the concept of SLNs as a highly advanced drug delivery system, describing their ability to combine the advantages of lipid emulsions and polymeric nanoparticles. More specifically, they talked about how SLNs may overcome physiological barriers thereby improving the solubility of poorly water-soluble drugs and

offer a biologically acceptable alternate for artificial polymers that have systemically lower toxic effects.

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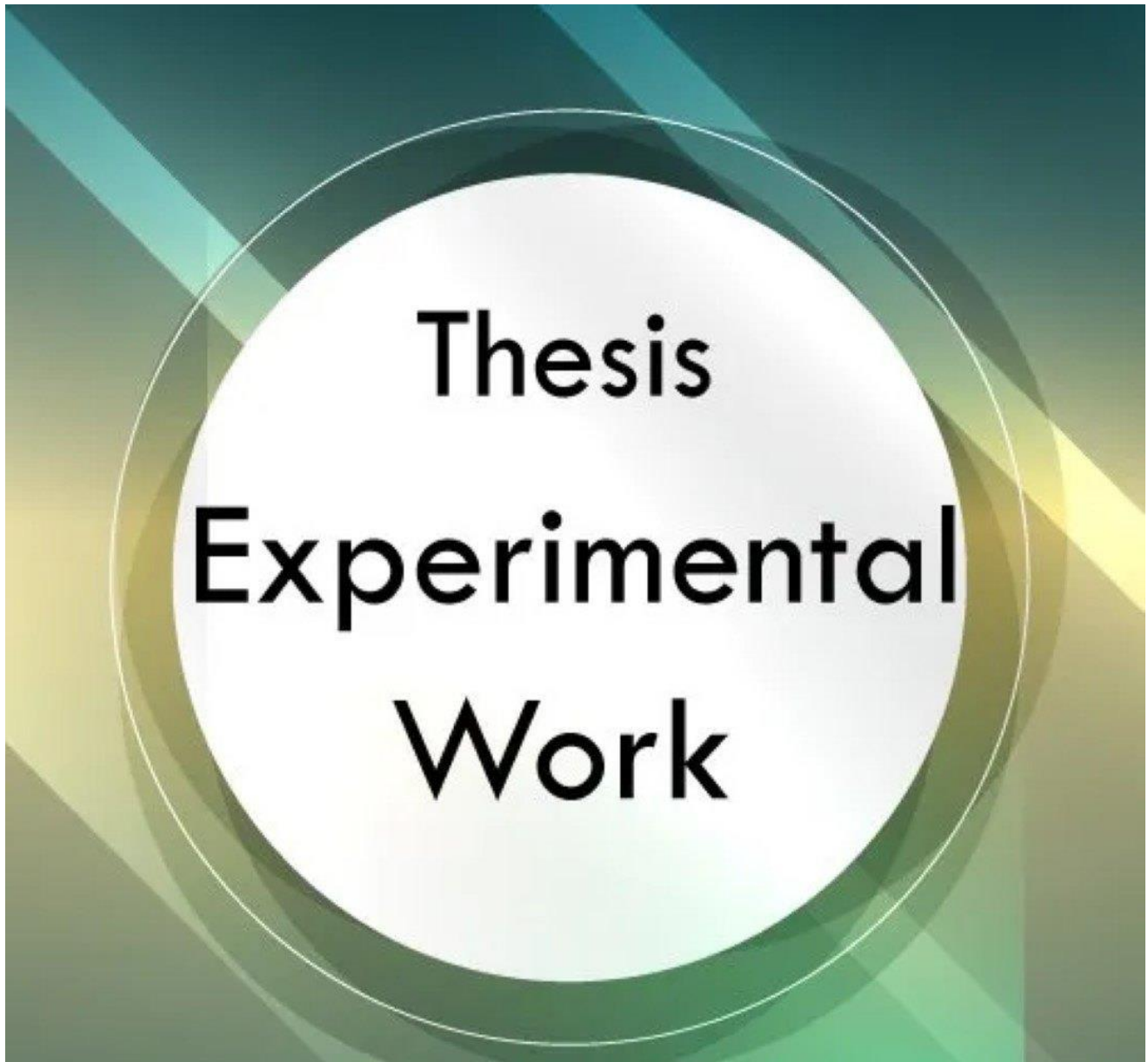
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CHAPTER 4. EXPERIMENTAL WORK



CHAPTER - 5.

EXPERIMENTAL WORK:

5.1 PROCUREMENT and AUTHENTICATION of PLANT MATERIALS:

We procured the botanical raw materials and extracts from **Bhagvati Herbals and Healthcare Pvt. Ltd. (Vapi, Gujarat, India)**. To ensure experimental reproducibility, we cross-examined each batch with its official Certificate of Analysis (CoA) to verify purity, solvent extraction ratios, and phytochemical profiles. Upon receipt, we authenticated the botanical identities, and transferred them to environmentally controlled storage. By maintaining strict temperature regulation and low humidity, we prevented moisture absorption and potential chemical degradation prior to the formulation development phases.

To ensure the purity, quality, and structural reproducibility of these botanical raw materials, the four purchased plant extracts (*N. nucifera*, *S. indicus*, *A. carambola*, and *N. sativa*) underwent rigorous baseline physicochemical evaluation prior to nanoformulations. The organoleptic properties (colour, odour, and taste) along with critical physicochemical parameters—specifically pH value, solubility, and moisture content—were cross-verified against their respective certificates of analysis (CoA). This screening confirmed the complete absence of unwanted external moisture or extraneous impurities in the raw material batches, which was a vital prerequisite to prevent premature destabilization or degradation of the subsequent lipid nanoparticles.

Moreover, this commercial plant extract was formally treated as the active pharmaceutical ingredient (API) baseline for solid lipid nanoparticles (SLNs) synthesis. To guarantee rigorous regulatory compliance and post-formulation shelf-life stability, the supplier's certificate of analysis (CoA) values were independently cross-validated. The raw extracts strictly exhibited a moisture content of <5%, establishing a critical benchmark for preventing unwanted phase separation and ensuring optimal lipophilic blending during the hot micro emulsification matrix phase (utilizing stearic acid, Tween 80, and soy lecithin). Concurrently, manufacturer-provided validation data confirmed that both heavy metal profiles and total microbial loads complied safely with the permissible pharmacopeial boundaries mandated for topical delivery systems. Rather than relying solely on supplier-provided certification, independent laboratory cross-validation was performed

systematically using thin layer chromatography (TLC) against pure analytical reference standards. Prior to initiating nanoparticle nucleation, each botanical agent was assigned a robust, verified chemical identity, baseline purity, and a standardized phenolic fingerprint matrix.

5.1.1 RATIONALE FOR UTILIZING STANDARDIZED COMMERCIAL EXTRACTS OVER IN-HOUSE EXTRACTIONS:

Initial formulation trials were performed using crude botanical extracts prepared in-house via standard laboratory extraction techniques. However, these in-house extracts presented severe technical challenges during the optimization of the solid lipid nanoparticles (SLNs). The laboratory-prepared crude matrices yielded formulations with a mean particle size consistently exceeding 200–300 nm, accompanied by a high polydispersity index (PDI) and frequent colloidal aggregation. This physical instability was mechanically traced to the presence of fluctuating moisture contents, micro-particulate cellular debris, and un-optimized resinous matrix elements within the crude extracts. These impurities structurally disrupted the high-shear hot micro emulsification and sonication phases (5.2), preventing uniform surfactant-lipid orientation at the oil-water interface. Because the primary objective of my PhD research is the engineering of a sub-100 nm topical delivery system to ensure optimal transdermal permeation, achieving an ultra-small, uniform, and stable nano-size was a critical requirement. Consequently, to circumvent these structural barriers, commercially standardized and highly purified extracts were utilized. These industrial-grade matrices feature strict moisture control (<5%) and are depleted of bulk cellular debris, providing the necessary physicochemical uniformity to consistently achieve a stable, sub-100 nm particle size with an optimized PDI.

5.2 PREPARATION TECHNIQUE FOR SOLID-LIPID NANOPARTICLES ^[1-4]:

In this study, the **microemulsion technique** was selected as the formulation method for developing solid lipid nanoparticles (SLNs) due to its distinct thermodynamic and practical advantages over alternative conventional techniques. Unlike high-pressure homogenization (HPH) or ultrasonication, which rely heavily on high-energy mechanical inputs that can cause structural shear stress or instrumental wear, the microemulsion method utilizes the

spontaneous thermodynamic assembly of a lipid, surfactant, and co-surfactant system. The underlying droplet size is fundamentally governed by the formulation composition rather than external mechanical energy, ensuring excellent reproducibility and consistent submicron particle distributions.

Furthermore, this method is fundamentally a solvent-free process when utilizing molten lipids, making it highly desirable from a toxicological, regulatory, and green-chemistry standpoint by passing the necessity of removing hazardous organic residues associated with solvent-diffusion or solvent-evaporation techniques. From an industrial perspective, the microemulsion method offers a practical route for scale-up. The transition from the warm, stable microemulsion to the solidified nanoparticle matrix is achieved through a controlled thermal precipitation step via dilution in cold water, allowing for predictable crystal network formation without complex equipment constraints. Therefore, this technique balances technical simplicity, formulation safety, and nanoscale structural control.

Nanoparticle fabrication was initiated by melting 500 mg of stearic acid in a conical flask at 70 °C using a magnetic stirrer equipped with a water-filled porcelain dish. Next, the drug extract (1 g), Tween 80 (3.4 mL), soy lecithin (1.1 mL), and distilled water (2.1 mL) were introduced into the flask and blended at 1000 RPM until a uniform microemulsion developed. This formulation was subsequently diluted through the dropwise addition of ice-cold water under vigorous, continuous agitation at 3000–3100 RPM for 2–3 hours in a cold-water bath. The resulting suspension was then sonicated for 30 minutes. To isolate the nanoparticles, the sonicated mixture was centrifuged at 14,500 RPM and 8 °C for 5 minutes. Finally, the recovered nanoparticles were rinsed with methanol and thoroughly dried to yield a completely dry powder. The sequential workflow for this nanoparticle preparation is illustrated in Figure 5.1.



FIG 5.1 – STEPS PREPARATION of SLNs

5.2.1 LYOPHILIZATION of SLNs:

To suppress aqueous degradation pathways such as lipid hydrolysis and sedimentation, the Solid Lipid Nanoparticles (SLNs) were formulated into solid dry form via freeze-drying. While this process optimizes shelf life, the thermal and mechanical stresses inherent to freezing and desiccation frequently induce particle coalescence.

Consequently, mannitol (5% w/v) was utilized as a cryoprotective agent. This excipient forms a protective matrix and engages in hydrogen bonding with the surfactant layer, minimizing proximal particle interaction during ice crystal propagation. Solidification was achieved by pre-freezing the samples at $-40\text{ }^{\circ}\text{C}$ for 24 hours. Post-drying, the solid dry form was rehydrated in deionized water to assess the impact of processing on the formulation.

Comprehensive re-evaluation of particle size, polydispersity index (PDI), and zeta potential was conducted to verify that the nanoparticles retained their structural integrity without undergoing agglomeration.

5.2.2. CHARACTERIZATION of SLNs^[5, 6, 7]:

5.2.2.1. ASSESSMENT of PARTICLE SIZE, POLYDISPERSITY INDEX (PDI) and ZETA POTENTIAL:

The mean particle size (hydrodynamic diameter) and polydispersity index (PDI) of the prepared solid lipid nanoparticles (SLNs) were determined by dynamic light scattering (DLS), also known as photon correlation spectroscopy. The measurements were performed using a zeta sizer nano-ZS (Malvern Instruments, UK) at a fixed scattering angle of 173° (backscattering detection) and a controlled temperature of 25 °C.

To prevent multiple scattering effects and ensure optimal signal intensity, the SLNs dispersion was appropriately diluted with ultra-pure water (Milli-Q water) prior to analysis. The PDI was recorded to evaluate the width of the particle size distribution and the homogeneity of the formulation.

Concurrently, the surface charge, expressed as zeta potential, was evaluated to estimate the physical stability of the nano-dispersion. The zeta potential was measured by determining the electrophoretic mobility of the nanoparticles using a horiba scientific SZ-100 analyser. The samples were placed in a specialized capillary cell at 25 °C.

All measurements for particle size, PDI, and zeta potential were performed in triplicate ($n=3$), and the data were expressed as mean $\pm$ standard deviation (SD) to ensure statistical accuracy and reproducibility.

5.2.2.2. MORPHOLOGICAL CHARACTERIZATION of SLNs:

The shape, and structural morphology of the developed solid lipid nanoparticles (SLNs) were evaluated using a high-resolution field emission scanning electron microscopy (FE-SEM) system (JEOL JSM-7600F). For sample preparation, a 100 μ L aliquot of the diluted SLNs dispersion was uniformly applied onto a clean aluminium stub and allowed to undergo vacuum-drying overnight to ensure the complete removal of moisture without disrupting the nanostructure. To prevent sample charging and enhance electrical conductivity during imaging, the dried specimens were sputter-coated with a thin layer of platinum under an inert argon atmosphere. The prepared stubs were subsequently introduced into the microscope chamber and analysed at an accelerating voltage of 15 kV.

Representative micrographs were captured at various magnifications to systematically determine particle sphericity, surface roughness, and overall shape homogeneity.

5.2.2.3 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY**ANALYSIS:**

To identify the characteristic functional groups and investigate potential intermolecular chemical interactions or incompatibilities between the lipid matrix, surfactant, and encapsulated active agent within the formulation, fourier transform infrared (FTIR) spectroscopy was performed. The spectral analysis was conducted using a shimadzu FTIR-7800A spectrophotometer. The infrared profiles of the pure drug and the optimized solid lipid nanoparticle (SLNs) formulation were recorded across a wavenumber spectrum of 4000 to 400 cm^{-1} at a spectral resolution of 4 cm^{-1} . The resulting interferograms were analyzed to monitor shifts, disappearances, or the appearance of new absorption bands, thereby confirming the chemical stability and successful entrapment of the components within the nanoparticle matrix.

5.2.3 LONG-TERM STABILITY and PHYSICOCHEMICAL DYNAMICS of SLNs

18].

The physicochemical stability of the developed solid lipid nanoparticles (SLNs) was systematically evaluated over a 9-month storage period under variable environmental conditions. Zeta potential measurements, which reflect the surface charge and electrostatic repulsive forces of the nanocarriers, exhibited characteristic fluctuations over time that were highly dependent on the specific composition of each formulation. To quantify long-term physical integrity, the cumulative variations in mean hydrodynamic particle size and polydispersity index (PDI) were measured at the conclusion of the 9-month study.

A comparative evaluation between storage regimes revealed that SLNs maintained under refrigerated conditions (4 °C) exhibited superior stability profiles compared to those stored under accelerated stress conditions at 40 °C $\pm$ 2 °C and (75 $\pm$ 2) % Relative Humidity (RH). At elevated temperatures and humidity, the kinetic energy of the system increases, accelerating lipid polymorphic transitions into highly ordered crystalline lattices. This lattice rearrangement fundamentally drives instability through active pharmaceutical ingredient (API) expulsion and irreversible particle aggregation.

The study highlights that optimizing the concentration of tween 80 as the primary surfactant is a critical determinant of formulation longevity. Elevated levels of tween 80 effectively

lower the interfacial tension at the lipid-aqueous interface, forming a robust, sterically hindering mechanical barrier around the lipid core. This optimal surface coverage suppresses droplet coalescence and ostwald ripening, significantly improving physical stability, yielding smaller, more uniformly distributed nanoparticles with a highly controlled PDI.

5.3 THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS:

For comprehensive standardization, each crude botanical extract underwent authentication via thin layer chromatography (TLC) against specific, high-purity chemical reference standards. A dual-marker profiling strategy was executed for the *N. nucifera* extract to verify the simultaneous presence of the flavonoid biomarkers' quercetin and kaempferol. Concurrently, *S. indicus* extract was standardized against pure quercetin, *A. carambola* extract against gallic Acid, and *N. sativa* extract against thymoquinone. Following chromatographic development, the retention factor (*R_f*) values of the resolved bands in the Phyto complex profiles were calculated and directly cross-referenced with the corresponding reference standard spots to definitively confirm biomarker identity.

5.3.1 PHARMACOLOGICAL RATIONALE AND MULTI-TARGET SYNERGY of IDENTIFIED BIOMARKERS:

The specific selection and chromatographic authentication of the target phytoconstituents—quercetin, kaempferol, thymoquinone, and gallic acid—are grounded in rigorous pharmacological evidence supporting their dual anti-acne and anti-ageing therapeutic modalities. The pathogenesis of acne vulgaris is characterized by localized follicular inflammation and opportunistic bacterial proliferation, specifically driven by *Propionibacterium acnes* and *Staphylococcus epidermidis*. Mechanistically, the bioactive fractions of *S. indicus* and *A. carambola* have been extensively documented to exert potent bactericidal and bacteriostatic microbicidal actions. Concurrently, the authenticated biomarkers quercetin and gallic acid downregulate the inflammatory cascade by inhibiting pro-inflammatory cytokines, thereby mitigating clinical erythema and edema at the application site.

Parallely, cutaneous ageing is driven by reactive oxygen species (ROS) accumulation and subsequent extracellular matrix degradation. thymoquinone (from *N. sativa*) and gallic acid

(from *A. carambola*) function as electron-donating antioxidants that scavenge free radicals, while actively suppressing the upregulation of matrix metalloproteinases (MMPs), specifically collagenase and elastase. This enzymatic inhibition preserves structural dermal matrix proteins, preventing wrinkle formation and maintaining skin elasticity.

The integration of these four distinct botanical profiles into a singular nanostructured delivery system creates a multi-target synergistic phyto complex. In this matrix, the concurrent activation of antimicrobial, anti-inflammatory, and anti-senescent biochemical pathways yields a superior, compounded therapeutic outcome that cannot be achieved by the isolated components alone.

5.3.1.1 METHODOLOGICAL PROTOCOL FOR CHROMATOGRAPHIC FINGERPRINTING AND SEMI-QUANTITATION:

Comprehensive chromatographic fingerprinting of the procured botanical extracts was achieved using planar thin layer chromatography (TLC) to chemically define each matrix before nano formulation. The qualitative identification and structural authentication of specific target biomarkers were established by running the crude plant extracts concurrently against their respective pure chemical reference standards on identical pre-coated silica gel plates. The unique chromatographic fingerprint of each extract is defined by the specific spatial distribution, resolution, and sequential migration of resolved bands across the stationary phase.

For initial quantitative profiling, a comparative visual and densitometric evaluation of band intensities was utilized to estimate the relative abundance and baseline density of the target markers within the extract matrixes. While this chromatographic protocol provides a highly reproducible, cost-effective, and legally accepted baseline for chemical standardization, it is recognized as a semi-quantitative screening technique. To achieve absolute analytical precision, high-throughput validation via high-performance thin-layer chromatography (HPTLC) or high-performance liquid chromatography (HPLC) is designated for future optimization phases to map out precise peak-area concentration curves.

5.3.2 EXTRACTION and PREPARATION of TEST SOLUTIONS FROM SLNs FORMULATIONS:

To analyzed the entrapped active constituents from the lipid matrix, a precise sample preparation protocol was established.

An accurately weighed 2.0 g sample of the optimized solid lipid nanoparticles (SLNs) formulation was transferred into a round-bottom flask and subjected to reflux extraction with methanol for 1 hour. The choice of methanol ensured the complete dissolution of the lipid framework and the quantitative extraction of the encapsulated bioactive compounds. Following the extraction period, the mixture was cooled to room temperature and filtered through a 0.45 µm membrane filter to eliminate any insoluble lipid residues. The resulting clear filtrate was collected and utilized as the final test solution for subsequent thin-layer chromatography (TLC) spotting.

Fingerprint analysis of *S. indicus*:

Test solution: Methanol extract of *S. indicus*

Stationary phase: pre-coated silica gel TLC plate G 60F254

Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)

Spotting: 10 x 2 cm plate

Saturation time: 30 minutes

Run time: 20 minutes.

Detection: UV 366 nm.

Identification of quercetin in *S. indicus*:

Test solution: Methanol extract of *S. indicus*

Stationary phase: pre-coated silica gel TLC plate G 60F₂₅₄

Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)

Spotting: 10 x 4 cm plate

Saturation time: 30 minutes

Run time: 20 minutes

Detection: UV 366 nm

Identification of quercetin and kaempferol in *N. nucifera*:

Test solution: Methanol extract of *N. nucifera*

Stationary phase: pre-coated silica gel TLC plate G 60F₂₅₄

Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)

Spotting: 10 x 6 cm plate

Saturation time: 30 minutes

Run time: 20 minutes

Detection: In day light, UV 366 nm

Identification of thymoquinone in *N. sativa*:

Test solution: Methanol extract of *N. sativa*

Stationary phase: pre-coated silica gel TLC plate G 60F₂₅₄

Mobile phase: CHCl₃: Acetone: Glacial acetic acid (15.3:3:1)

Spotting: 10 x 4 cm plate

Saturation time: 40 minutes

Run time: 25-30 minutes

Spraying agent: 5% methanolic KOH

Detection: In day light, UV 366, 254 nm after derivatization

Identification of gallic acid in *A. carambola*:

Test solution: Methanol extract of *A. carambola*

Stationary phase: pre-coated silica gel TLC plate G 60F₂₅₄

Mobile phase: CHCl₃: Ethyl acetate: Formic acid

Spotting: 10 x 4 cm plate

Saturation time: 30 minutes

Run time: 40-45 minutes

Spraying agent: 1% methanolic FeCl₃

Detection: In day light after derivatization

5.4 DESIGN, FORMULATION, and PERFORMANCE EVALUATION of SKIN CARE PRODUCTS:

The primary objective of this phase of the research was to systematically design, develop, and examine the performance of an advanced, topical skin care product incorporating the optimized solid lipid nanoparticles (SLNs). Utilizing nanocarriers within topical delivery

systems aims to overcome the stratum corneum barrier, thereby enhancing the skin penetration, localized retention, and bioavailability of the encapsulated botanical extracts.

The formulation framework was strategically engineered to provide deep dermal delivery while maintaining skin biocompatibility and aesthetic elegance. To establish its therapeutic and commercial viability, the performance of the developed cosmeceutical formulation was thoroughly evaluated through a battery of rigorous characterization parameters.

5.4.1 PREPARATION of THE O/W EMULSION CREAM BASE and SLNs INCORPORATION ^[9, 10]:

To develop a stable and dermatologically acceptable topical delivery vehicle, an oil-in-water (O/W) emulsion cream base was formulated using precisely calculated lipophilic and hydrophilic components. The composition of both phases is detailed below:

- **Lipophilic Phase (Oil Phase):** Consisted of a structural blend of stearic acid (8.0 g), glyceryl monostearate (4.0 g), and Cetyl alcohol (4.0 g). These components were selected to provide the necessary emollient properties, structural consistency, and viscosity to the cream matrix.
- **Hydrophilic Phase (Aqueous Phase):** Comprised light liquid paraffin oil (8.0 mL) as a secondary emollient, tween 80 (2.0 mL) as the primary hydrophilic surfactant to stabilize the emulsion interface, triethanolamine (0.25 mL) as an alkalizing agent to saponify the free fatty acids and adjust the final pH, and purified water (q.s. up to 100 mL) as the continuous medium.
- **Preservative System:** Methylparaben was incorporated into the formulation in an adequate concentration to ensure microbiological stability and prevent microbial proliferation during storage.

5.4.1.1 FORMULATION and DEVELOPMENT of SLNs-LOADED SKIN CARE CREAM:

The fabrication of the topical nanocomposite delivery system was executed via a controlled oil-in-water (O/W) emulsification phase-inversion method, followed by the cold incorporation of the nanocarriers. The step-by-step preparation protocol was carried out as follows:

- **Phase Separation and Thermal Conditioning:** The lipophilic phase—comprising stearic acid (8.0 g), glyceryl monostearate (4.0 g), and cetyl alcohol (4.0 g)—was weighed and heated uniformly to 70 °C until complete melting and homogenization were achieved. Concurrently, the aqueous phase components—consisting of light liquid paraffin oil (8.0 mL), Tween 80 (2.0 mL), triethanolamine (0.25 mL), and purified water (q.s. up to 100 mL)—were combined in a separate beaker and brought to the identical temperature of 70 °C to prevent thermal shock upon mixing.
- **Emulsification Cycle:** Under continuous and constant mechanical agitation, the heated aqueous phase was gradually introduced into the molten oil phase. This controlled addition initiated the phase emulsification process. The resulting warm emulsion was maintained under steady, low-shear stirring while being allowed to cool ambiently to room temperature, yielding a smooth, elegant, and structurally stable cream base.
- **Nanoparticle Incorporation:** Once the emulsion base had cooled completely to room temperature, 4% w/w of the pre-formed SLNs extract mixture was added. The SLNs were meticulously incorporated into cream under mild stirring conditions. This controlled temperature was essential to safeguard the solid lipid matrix of the nanoparticles and prevent premature active ingredient expulsion or crystalline transition.
- **Finishing and Preservation:** In the final step, methylparaben was added as a preservative to ensure microbiological stability, along with a suitable perfume to optimize the organoleptic characteristics of the cosmetic product. The finalized SLNs-loaded cream was homogenized completely and transferred into airtight, light-resistant containers for subsequent stability profiling and skin-permeation testing.

5.5 PHYSICOCHEMICAL EVALUATION of THE FORMULATED CREAM:

To ensure quality control, stability, and dermatological compliance, the developed Solid Lipid Nanoparticles (SLNs)-loaded cream was subjected to a comprehensive battery of physicochemical characterization tests. These evaluations monitor the structural integrity of the emulsion matrix and guarantee its safety and efficacy for topical application.

5.5.1 Physical and Organoleptic Characterization:

The formulated cream was systematically evaluated for its primary macroscopic and organoleptic attributes to ensure batch-to-batch uniformity and aesthetic acceptability. The physical appearance of the formulation was thoroughly assessed based on the following key parameters:

Appearance: The formulation's appearance was assessed by checking its colour, thickness, texture, and pearlescence, as well as testing for any phase separation or segregation.

Washability and Removal Evaluation: The ease of removal of the formulation from the application site is a critical factor governing patient compliance. The removability was evaluated by applying a uniform quantity of the developed formulation (approximately 0.2 g) over a marked area ($2 \times 2 \text{ cm}^2$) on the volar forearm of healthy human volunteers. After allowing the formulation to remain on the skin for a specified period (15 minutes), the application site was washed under running tap water at a controlled flow rate and standard temperature ($25 \pm 2^\circ \text{C}$). The ease of removal was evaluated qualitatively based on the time and manual rinsing effort required to completely clear the formulation from the skin surface, and categorized as highly washable, moderately washable, or poorly washable.

- **After-Feel Evaluation:** The sensory attributes of the formulation post-application were evaluated to assess cosmetic acceptability. A standardized amount of the formulation was applied to the clean skin of the volar forearm of volunteers. The formulation was gently rubbed for 30 seconds to facilitate absorption. The application site was subsequently evaluated for distinct sensory parameters, including smoothness (absence of grittiness or coarse particles), emolliency (moisturising effect without excessive oiliness), and the nature and amount of residue remaining on the skin surface after complete application.
- **Determination of pH:** The pH of the topical formulation was determined to evaluate their compatibility with the physiological pH of human skin. The evaluation was carried out using a digital, calibrated pH meter. Briefly, approximately 0.5 g of the

formulation was accurately weighed using an analytical electronic balance and quantitatively dispersed in 50.0 mL of freshly prepared distilled water. The mixture was stirred continuously using a magnetic stirrer for 15 minutes to ensure uniform dispersion. After stabilization, the electrode was immersed directly into the dispersion, and the pH value was recorded. The instrument was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2 prior to the analysis.

- **Smear Evaluation:** Smear testing was conducted to characterize the nature of the film produced upon topical application, which directly influences the drug release profile and barrier properties. A fixed quantity of the cream was applied onto a designated area of the skin with uniform strokes. The type of film generated—whether greasy, non-greasy, occlusive, or transparent—was visually observed and recorded. Additionally, the ease of spread ability and the continuity of the resulting film (absence of clump formation or phase separation during application) were evaluated.
- **Dye test:** To confirm the structural morphology and determine the emulsion type (oil-in-water [O/W] or water-in-oil [W/O]) of the developed cream formulations, a microscopic dye test was performed based on differential solubility principles.
 - ♦ A representative sample of the cream formulation was intimately mixed with uniform quantity of the lipophilic dye, **Scarlet Red (Sudan IV)**. A thin, uniform smear of the dyed cream was subsequently prepared on a clean, grease-free glass microscopic slide and protected with a coverslip. The sample was examined under an optical microscope under appropriate magnification (e.g., 40x or 100x).
 - ♦ The structural configuration was identified based on the localization of the colorant:
 - ♦ **Oil-in-Water (O/W) Emulsion:** The formulation displayed distinctly red-stained dispersed oil droplets distributed evenly throughout a clear, colourless continuous aqueous background.
 - ♦ **Water-in-Oil (W/O) Emulsion:** The formulation exhibited colourless dispersed aqueous droplets visible against a uniformly red-stained continuous oily background.

- **Homogeneity:** The structural uniformity and homogeneity of the developed formulations were rigorously evaluated to ensure consistent drug distribution, elegant aesthetic appearance, and the absence of phase separation.
- **Tube Extrudability:** Cream extrudability was assessed by measuring the amount of product discharged from a collapsible tube under a specific force; higher extrusion percentages reflect easier dispensing.

To test this, the prepared sample was filled into a clean, lacquered aluminium collapsible tube with a one-ounce capacity and a 5 mm nozzle. The tube was securely held between two glass slides before a constant 1 kg load was applied to press out the cream. The extruded material was then collected, weighed, and used to calculate the total percentage of extrusion.

- **Primary Skin Irritation Test:** The application site was cleaned with surgical spirit before applying the formulation. The cream was then applied to the designated area of the skin. The site was observed for signs of erythema and edema at intervals of 24, 48, and 72 hours after application.

The purpose of this test was to evaluate the potential of the formulation to cause skin irritation. The lack of erythema and edema suggested that the formulation was non-irritating and safe for topical use.

- **Determination of Acid Value:** About 2 h of the formulation was accurately weighed and dissolved in 50 mL of a 1:1 mixture of ethanol (95% v/v) and ether, previously neutralized with 0.1 N KOH. The mixture was refluxed with gentle heating and constant agitation until fully dissolve. Then, 1 mL of phenolphthalein indicator was added, followed by titration with 0.1 N KOH until a stable light pink colour appeared for 30 seconds. The acid value was determined using:

$$\text{Acid value} = (5.61 \times N) / W$$

Where N represents the volume of KOH used and W is the sample weight(g).

- **Determination of Saponification Value:** Approximately 2 g of the formulation was accurately weighed and refluxed with 25 mL of 0.5 N alcoholic KOH for 30 mins. The mixture was then cooled to room temperature, followed by the addition of 1 mL phenolphthalein indicator. It was titrated against 0.5 N HCl until the endpoint was obtained. A blank determination was conducted simultaneously under the same conditions without the sample. The saponification value was calculated as:

$$\text{Saponification value} = 28.5 \times (b-a) / w$$

Where, a represents the volume of HCl used for the sample, b for the blank and w is for the sample weight (g).

5.5.2 TEXTURE ANALYSIS of CREAM:

Texture analysis was performed by QTS Brookfield texture analyser. Texture analyser is an instrument that measures the response of a sample to compression. Quantifying extrudability is important to determine the ease of removal and application of creams. In forward extrusion the sample is forced through an orifice in sample container. To push or force something out of something it requires extrudability. In the test force is applying to a product so it flows direct to an outlet and in the form of one or more holes that are in present in test cell. The sample or formulation is compressed until the structure of the sample is suspended and it release through these outlets. The peak or maximum force was considered as a measure of the firmness or hardness of the formulation. The area under the force-time curve was taken as an indicator of the consistency of the formulation. When product gives the high value that means firmer the sample. while sample contains more consistency or thickness it gives high value. Maximum peak in the positive area shows the maximum sample firmness. In the graph, the peak force represented sample hardness, while the area under the curve represented consistency.

Instrumentation:

Equipment Name: QTS Brookfield Texture
Analyzer Equipment Make: Brookfield CNS
Farnell Equipment Id: Texture PRO V (2.1)

Testing criteria/parameters

Test type : Compression
Trigger point : 5 g
Target value : 20mm
No. of cycle : 1
Hold time : 0 sec
Recovery time : 0 sec

TPB support span: 0 mm

Figure 5.2 shows a brookfield QTS texture analyzer, a precision laboratory instrument used to determine the texture profile analysis (TPA) of a sample. A sample placed beneath the compression probe on the testing stage is clearly highlighted in this image of a QTS texture analyser taken from a different angle.



FIG 5.2 TEXTURE ANALYSIS

5.5.3 ESTIMATION of PHYTOCONSTITUENTS:

5.5.3.1 PREPARATION of TEST SOLUTION:

2gm of formulations were extracted with 25 mL methanol for 1 hour using reflux and filtered.

5.5.3.2 ESTIMATION of TOTAL PHENOLIC CONTENT ^[11, 12]: Total phenolic content was determined using the Folin-Ciocalteu method.

Method: A volume of 1 mL of methanolic extract was mixed with 0.1 mL of Folin-Ciocalteu reagent and 0.9 mL of distilled water in a 10mL volumetric flask and kept in the dark for 5 minutes. Afterwards, 1 mL of 7% (w/v) sodium carbonate and 0.4 mL distilled water added. The mixture was incubated in the dark for 30 minutes for colour development. Absorbance was recorded at 765 nm using methanol as a blank. The total phenolic content was quantified using a gallic acid standard curve and expressed as mg GAE per 100 g of dry weight. All experiments were performed in triplicate.

5.5.3.3 METHODOLOGICAL JUSTIFICATION FOR FORMULATION-CENTRIC PHYTOCHEMICAL ESTIMATION:

The quantitative evaluation of total phenolic content (TPC) was intentionally restricted to the final nano-emulsion cream formulation rather than conducted individually for each raw botanical extract. This analytical strategy is scientifically and methodologically justified by four key parameters. First, in alignment with the core tenets of ayurveda and polyherbal synergy, the combination of multiple botanical matrixes (*N. nucifera*, *N. sativa*, *A. carambola*, and *S. indicus*) induces complex chemical interactions that alter the overall phytochemical dynamics. Quantifying the individual raw extracts in isolation fails to capture these synergistic or antagonistic interactions, whereas measuring the final formulation reflects the actual therapeutic dose available to the end consumer. Second, since the qualitative presence and identity of the specific phenolic biomarkers (quercetin, kaempferol, gallic acid, and thymoquinone) had already been verified against pure reference standards via TLC, repetitive individual quantitative assays would represent an inefficient allocation of laboratory resources.

Finally, according to international regulatory frameworks for standardized commercial herbal products, the critical quality control marker and batch uniformity are defined by the biomarker concentration within the finished product rather than the raw materials. Therefore, focusing exclusively on the final formulation establishes a clinically relevant, regulatory-compliant baseline for the delivery system.

5.5.3.4 ANALYTICAL RATIONALE FOR COLLECTIVE PHENOLIC STANDARDIZATION OVER ISOLATED MARKER QUANTIFICATION:

The quantitative evaluation of the delivery system was systematically targeted toward total phenolic content (TPC) via spectrophotometric estimation, rather than isolating individual chromatographic entrapment efficiency (EE%) calculations for each separate botanical marker. This specific analytical design was selected to mitigate severe technical risks associated with multi-step destructive extraction protocols required to isolate individual markers from the dense, multi-component O/W cream matrix. Attempting to quantitatively isolate individual, chemically diverse markers (such as quercetin, kaempferol, gallic Acid, and thymoquinone simultaneously) introduces a high probability of extraction-induced analyte degradation, incomplete phase partitioning, or volatile recovery loss, which would heavily skew the precision of the data. To ensure analytical accuracy, TPC was utilized as a highly reliable collective surrogate biomarker matrix. This method circumvents the limitations of destructive physical extraction by quantifying the overall bioactive capacity directly from the solubilized formulation. This approach effectively encompasses the total phenolic contributions from all four integrated botanical sources, establishing a comprehensive, non-destructive, and scientifically rigorous parameter for the chemical standardization of the final nano-delivery vehicle.

5.5 ACCELERATED and LONG-TERM STABILITY KINETICS of THE DEVELOPED FORMULATION:

To assess the structural integrity, physicochemical robustness, and shelf-life potential of the optimized topical formulation, stability testing was conducted in strict accordance with the International Council for Harmonisation (ICH) Q1A(R2) guidelines.

The formulation was packaged in hermetically sealed, light-resistant containers to mimic commercial storage conditions and prevent moisture loss. Samples were then distributed across two distinct environmental chambers maintained under the following strictly monitored storage regimes for a total duration of 90 days:

Ambient Storage Condition (Real-Time): Maintained at controlled room temperature (25 $\pm$ 2 °C) and ambient relative humidity.

Accelerated Storage Condition: Maintained inside an environmental stability chamber set to an elevated temperature of (40 °C ± 2 °C) and a regulated relative humidity of 75 ± 2) % RH.

5.6 ANTI-ACNE ACTIVITY:

5.6.1 TEST MICROORGANISM:

Microorganism: *Staphylococcus Epidermidis*, *Propionibacterium acnes* (ATCC 6919)

Inoculum preparation: Microorganisms were grown on agar medium to obtain fresh cultures.

Test Antimicrobial Agent

Reference item: Vancomycin for *S. aureus*, Cefoxitin for *P. acne*

Test item Name: Mixture of *S. indicus* and *A. carambola* extract

Test item Code: 250EDU05, 250EDU04

Batch No.: BH/SI/DE/323/24

Date of Mfg.: January 2025

Exp. Date: December 2027

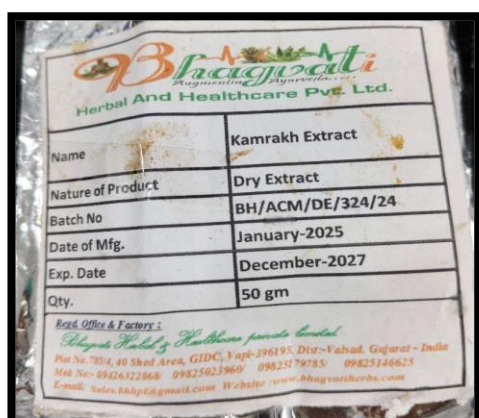


FIG 5.3 *A. carambola* and *S. indicus* EXTRACTS

5.6.2 CONCENTRATIONS TESTED: 100%, 75%, 50%, 25%, and 12.5%

(w/v).

SN	Test item concentration tested
1	100%
2	75%
3	50%
4	25%
5	12.5%
6	Positive Control
7	Negative Control

The test item was mixed in sterile distilled water to achieve the desired concentrations.

Tube Dilution: Twofold serial dilutions from **100% to 0.78% (w/v) (100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, 0.78%)**.

5.6.3 METHOD ^[17, 18, 19,20,21].

The antimicrobial activity was assessed using two complementary, CLSI compliant methods: the agar well diffusion assay for qualitative screening of antibacterial activity and the tube dilution assay for qualitative determination of MIC and MBC, ensuring a comprehensive evaluation of the extract's efficacy.

5.6.3.1 AGAR WELL DIFFUSION METHOD:**5.6.3.1.1 PREPARATION OF AGAR PLATES:**

Muller-Hinton agar was prepared as per the manufacturer's guidelines and sterilized in an autoclave at 121 °C and 15 psi for 15-20 mins. The sterilized medium was cooled to 45-50 °C in a laminar airflow environment. It was then poured into sterile 90 mm petri plates to obtain a consistent depth of 4 ± 0.5 mm, which was checked using a depth gauge. The plates were left to solidify at room temperature (25 ± 2 °C) for approximately 30 min. under aseptic conditions.

5.6.3.1.2 INOCULATION OF AGAR PLATES:

For each bacterial strain, a standardized inoculum (10^6 CFU/mL) was prepared and evenly

distributed on the agar surface using a sterile cotton swab. After dipping into the inoculum, the swab was pressed against the tube wall to eliminate excess liquid. The agar surface was streaked in a crisscross pattern in three directions, approximately 60° apart, to achieve a confluent lawn.

The inoculated plates were left to dry at 25 ± 2 °C for 5-10 min. to promote bacterial attachment and remove residual moisture.

Wells measuring 6mm in diameter and 4 mm in depth were made aseptically using a sterile cork borer, which was sterilized with 70% ethanol and flaming between uses. The agar plugs were removed with a sterile needle and disposed of appropriately.

5.6.3.1.3 SERIAL DILUTION and APPLICATION of TEST ITEM (AGAR WELL DIFFUSION):

Serial Dilution Preparation:

A stock solution (100%W/V) was prepared by dissolving 10 g of lyophilized extract in 10 mL sterile distilled water (pH 7.0 ± 0.2). Further dilutions (75%, 50%, 25% and 12.5%) were obtained by mixing appropriate volumes of the stock solution with sterile distilled water (e.g. 7.5 mL stock with 2.5 mL water for 75%). The solutions were vortexed for uniform mixing and subsequently filtered through a 0.22 µm syringe to ensure sterility.

Application to Wells: Using a calibrated micropipette, 100 µL of each test concentration (100%, 75%, 50%, 25%, 12.5%), Reference item (30 µg/mL), and sterile distilled water was dispensed into designated wells. Each plate included all test conditions and controls, arranged in a hexagonal pattern to minimize diffusion overlap. Wells were filled carefully to avoid spillage, ensuring consistent diffusion into the agar matrix.

5.6.3.1.4 INCUBATION of PLATES:

The plates were incubated at 37 °C for 24 hrs. following the addition of test samples into the wells, allowing microbial growth and interaction with the test substances.

The incubation temperature of 37 °C was selected as it represents the optimal growth

condition for most bacterial species.

5.6.3.2 TUBE DILUTION METHOD ^[13, 14, 15,]

To quantify MIC and MBC of extract and reference against bacteria.

5.6.3.2.1 PROCEDURE:

Twofold serial dilutions of extract were prepared in sterile each 5 mL glass test tube was filled with 1 mL of Mueller-Hinton broth (pH 7.3 ± 0.2), starting from 100% (w/v) to 0.78% (w/v) (100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, 0.78%).

Reference item was diluted from 30 $\mu\text{g/mL}$ to 0.47 $\mu\text{g/mL}$ in parallel.

An inoculum of bacteria standardized to 5×10^5 CFU/mL (derived from 0.5 McFarland standard) was introduced at 100 μL per tube, producing a final volume of 1.1 mL with an approximate bacterial density of 4.5×10^5 CFU/mL.

Control tubes included: (1) growth control (MHB + inoculum, no test substance), (2) sterility control (MHB alone), and (3) vancomycin positive control.

The samples were incubated at 37 ± 1 °C for 24 ± 2 hrs while being gently agitated at 100 RPM to ensure even mixing and proper oxygen availability.

5.6.3.2.2 MIC DETERMINATION:

Post-incubation, tubes were inspected for turbidity using a spectrophotometer (OD600). The minimum inhibitory concentration (MIC) corresponded to the lowest concentration showing no visible growth, with OD600 values less than 0.05 in comparison to the growth control.

5.6.3.2.3 MBC DETERMINATION: Samples (100 μL) taken from each clear tube were spread onto Muller-Hinton agar plates using a sterile L-shaped spreader and incubated at 37 ± 1 °C for 24 ± 2 hrs. post-incubation, the minimum bactericidal concentration (MBC) was determined as the lowest concentration with no visible colony growth, corresponding to $\geq 99.9\%$ bacterial killing. Colony counts were verified using a colony counter.

5.6.3.2.4 QUALITY CONTROL: All procedures were conducted in triplicate, with

duplicates per experiment. Sterility was confirmed using uninoculated MHB and agar plates. Reference item MIC/MBC values were cross-checked against CLSI reference ranges for bacteria (MIC: 1–4 µg/mL).

5.6.3.3. OBSERVATION and MEASUREMENT of ZONE of INHIBITION:

5.6.3.3.1 AGAR WELL DIFFUSION ASSAY: After incubation, plates were examined under diffused lighting for zones of inhibition, defined as clear, circular areas around wells indicating no bacterial growth.

A digital calliper was used to measure the inhibition zones, with reading taken to the nearest 0.1 mm. Two perpendicular measurements were averaged to account for asymmetry, and results were recorded from triplicate experiments.

Photographs were taken using a digital camera for documentation, with a ruler included for scale.

5.6.3.3.2 TUBE DILUTION ASSAY:

Turbidity was assessed visually and quantified at OD600 using a spectrophotometer. Tubes with OD600 < 0.05 were considered growth negative.

For MBC determination, agar plates inoculated from clear tubes were examined for colony growth under a colony counter. Absence of colonies (0 CFU) indicated bactericidal activity. Data were recorded from triplicate experiments, with duplicates per condition, ensuring statistical robustness.

5.7 ANTI-AGEING ACTIVITY [22, 23, 24, 25, 26];

5.7.1 AVAILABILITY, MAINTENANCE, and CULTURING CONDITIONS of *C. elegans*:

5.7.1.1 RATIONALE FOR THE MODEL ORGANISM:

To evaluate the *in vivo* biocompatibility, physiological impacts, and anti-aging potential of the developed plant extracts, *Caenorhabditis elegans* (*C. elegans*) was employed as a premier nematodal model organism. *C. elegans* provides a highly tractable platform for biogerontological screenings owing to its well-defined traits, including a short and predictable lifespan, rapid reproductive cycle, ease of axenic or monoxenic laboratory cultivation, and an anatomically simple body plan. Crucially, its completely sequenced genome shares high genetic homology with human metabolic and longevity pathways, rendering it a predictive surrogate for identifying cytoprotective interventions.

5.7.1.2 SOURCE and AVAILABILITY:

The wild-type *C. elegans* Bristol N2 strain and pathogenicity-attenuated *Escherichia coli* OP50 were sourced from the National Centre for Biological Sciences (NCBS). Cryopreserved stocks were thawed and established in accordance with standard international nematode research protocols to ensure genetic integrity before initiating the experimental cohorts.

5.7.1.3 MAINTENANCE and ENVIRONMENTAL CONDITIONS:

The *C. elegans* cultures were maintained under strictly controlled environmental parameters:

- **Temperature:** Cultured inside a precision biological incubator at a constant 20 °C to ensure stable growth kinetics.
- **Illumination:** Kept under continuous dark or low-light conditions to minimize light-induced stress, eliminate UV-driven compound degradation, and prevent the unwanted proliferation of photographic contaminants such as molds and algae.
- **Exclusion Criteria:** During the experimental observation window, physical monitoring was routinely performed. Individual nematodes exhibiting abnormal physiological phenotypes—such as internal hatching (bagging), vulval rupturing, desiccation due to migration to the dry walls of the plates, or individuals that penetrated/burrowed beneath the agar medium were strictly omitted from subsequent statistical analysis.

5.7.1.4 CULTURING MEDIUM and AGE SYNCHRONIZATION:

- Nematodes were propagated on nematode growth medium (NGM) agar plates freshly seeded with a live lawn of *E. coli* OP50, which served as the primary monoxenic nutrient source.
- To eliminate developmental variations across treatment groups, age-synchronous populations of hermaphroditic nematodes were established. Gravid adults were subjected to a standard alkaline hypochlorite bleaching lysis protocol using a freshly prepared solution of 5M Sodium Hydroxide (NaOH) and Sodium Hypochlorite (NaClO). This process selectively dissolved the maternal carcasses while leaving the egg shells intact. The isolated eggs were allowed to hatch overnight in sterile M9 buffer in the absence of food to arrest them at the L1 larval stage. The synchronized L1 larvae were then transferred onto seeded NGM plates and grown concurrently until they reached the identical developmental milestone (L4 larval stage or young adulthood) to commence extract exposure.

5.7.2 ASSESSMENT OF LIFESPAN and MOBILITY of *C. elegans*:**5.7.2.1 EXPERIMENTAL SETUP AND TREATMENT COHORTS:**

To evaluate the anti-ageing efficacy and impact on physical longevity of the developed plant extracts, age-synchronized L4-stage *C. elegans* hermaphrodites were randomly allocated into experimental groups. For each assay, a fixed population of 50–70 nematodes were transferred onto standard nematode growth medium (NGM) agar plates pre-seeded with a monoxenic lawn of *E. coli* OP50. The treatment groups were exposed to varying concentrations of the plant extracts:

- **Experimental Concentrations: 50, 100, 500, and 1000 µg/mL**, incorporated directly or top-spread uniformly across the nutrient lawn.
- **Negative Control Group:** Cultured on NGM plates treated with sterile, deionized water to establish baseline survival metrics.
- **Positive Control Group:** Cultured on NGM plates treated with 50 µg/mL of spermidine (Spd), a well-documented longevity-extending autophagy inducer.

To eliminate data confounding caused by overlapping generations, surviving nematodes were systematically relocated to freshly prepared NGM plates every 48 hours during their

active reproductive window, ensuring optimal environmental and nutritional conditions. All experimental conditions were maintained in triplicate ($n=3$) to guarantee reproducibility and statistical rigor.

5.7.2.2 LIFESPAN MONITORING and VIABILITY CRITERIA:

Nematode survival and mortality kinetics were monitored and logged daily at a fixed time interval. Longitudinal viability was assessed via tactile mechanical stimulation using a sterilized, fine platinum-wire worm picker.

- **Viable Classification:** Nematodes exhibiting intentional locomotive, sinusoidal, or head-withdrawal actions in response to light physical prodding.
- **Mortality Classification:** Individuals failing to elicit any discernible neuromuscular or Behavioral response after consecutive, gentle tactile stimuli applied to both the anterior and posterior regions were scored as dead and promptly removed from the plates.

5.7.2.3 SEMI-QUANTITATIVE MOBILITY and HEALTH SPAN SCORING:

To evaluate structural health span and neuromuscular degradation over time, the locomotor capacity of the nematodes was assessed at predefined temporal milestones. The quality of worm movement was graded semi-quantitatively using a standardized four-tier mobility classification index:

- **Grade A (Spontaneous Mobility):** Nematodes exhibiting coordinated, continuous, and unprovoked sinusoidal swimming or crawling locomotion across the agar surface.
- **Grade B (Stimulated Locomotion):** Nematodes appearing stationary or sluggish at rest but demonstrating normal, coordinated locomotion upon receiving a gentle mechanical touch with the worm picker.
- **Grade C (Impaired Mobility):** Nematodes displaying severe neuromuscular decline, characterized by exclusive head movement or localized twitching without any forward or backward displacement of the body axial trunk following mechanical stimulation.
- **Grade D (Deceased):** Nematodes showing absolute immobility and complete absence of neuromuscular response to tactile prodding (classified as dead).

The cumulative daily survival records and periodic mobility distributions were subsequently compiled for survival curve analysis and health span profiling.

5.7.3 QUANTIFICATION of *IN VIVO* LIPOFUSCIN ACCUMULATION and NEMATODE BODY LENGTH:

To evaluate the impact of the extract on aging biomarkers and physical development, autofluorescent lipofuscin accumulation and total body length were quantified following a 10-day treatment period. Synchronized *C. elegans* populations from each experimental group—comprising control (0 µg/mL) and treatment groups exposed to 50, 100, and 500 µg/mL of the extract—were harvested for analysis.

From each treatment cohort, ten individual nematodes were randomly selected and transferred onto a microscope slide prepared with a 1% (w/v) agarose pad. To ensure complete immobilization and prevent movement artifacts during image acquisition, the nematodes were anesthetized by applying a 7 mM levamisole hydrochloride solution directly to the pad.

Visualisation of endogenous lipofuscin, which serves as a classical marker of cellular senescence, was performed using a fluorescence microscope equipped with a standard green fluorescent protein (GFP) excitation/emission filter set. Micrographs of the immobilized worms were captured under identical exposure and gain settings across all groups to maintain quantitative consistency.

Subsequent image processing and morphometric analyses were conducted using ImageJ software. The total body length of each nematode was traced and calculated from head to tail along the midline. Concurrently, the relative lipofuscin accumulation was determined by measuring the mean fluorescence intensity integrated over the entire anatomical area of the worm, subtracting background fluorescence. To ensure the reliability and reproducibility of the experimental findings, the entire procedure was executed in triplicate across three independent biological replicates.

5.7.4 EVALUATION OF OXIDATIVE and HEAT STRESS TOLERANCE ASSAYS IN *C. elegans*:

To determine the capacity of the plant extract to enhance physiological resilience against environmental stressors, oxidative and heat stress tolerance assays were performed using synchronized populations of *C. elegans*.

5.7.4.1 PRE-TREATMENT PHASE:

Synchronized L4-stage hermaphrodite nematodes were transferred onto Nematode Growth Medium (NGM) plates seeded with *E. coli* OP50 and supplemented with varying concentrations of the plant extract (0, 50, 100, and 500 µg/mL). The cohorts were maintained under standard environmental conditions at 20°C for a duration of 6 days to allow for sufficient physiological absorption and systemic distribution of the bioactive compounds. Each experimental group comprised a minimum of 50 nematodes per replicate.

5.7.4.2 PARAQUAT-INDUCED OXIDATIVE STRESS ASSAY:

To evaluate intracellular antioxidant defences, extract-pretreated and untreated control worms were transferred to freshly prepared NGM plates containing 70 nM paraquat dichloride (methyl viologen), which induces lethal oxidative stress via intracellular superoxide radical generation. The plates maintained the respective extract concentrations matching the initial 6-day pre-treatment profiles. Nematode survival was monitored and documented at strict 12-hour intervals starting from the onset of exposure. Worms were scored as dead if they exhibited a total lack of movement and failed to respond to gentle physical prodding with a platinum wire pick. Survival tracking was maintained continuously until absolute mortality was achieved across all experimental cohorts.

5.7.4.3 THERMAL STRESS TOLERANCE ASSAY:

For the assessment of thermotolerance, pre-treated nematodes were subjected to acute thermal shock by shifting the incubation temperature from their physiological baseline. The assay plates were sealed to prevent desiccation and transferred to a bacteriological incubator maintained at 35 °C for an acute duration of 4 hours. Following the thermal challenge, the nematodes were returned to the baseline temperature of 20 °C for a 12-hour recovery period.

Survival status was subsequently evaluated using mechanical stimulation, recording the ratio of surviving to deceased individuals within each treatment group.

5.7.4.4 STATISTICAL VERIFICATION:

To validate the reliability, consistency, and statistical significance of the stress-response kinetics, all survival assays were executed in triplicate across three independent biological replicates.

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CHAPTER 5. RESULTS AND DISCUSSION



**RESULTS and
DISCUSSION**

CHAPTER - 6.

RESULT & DISCUSSION:

6.1 CHARACTERIZATION of DEVELOPED SLNs:

6.1.1 PARTICLE SIZE DYNAMICS and POLYDISPERSITY ANALYSIS

[1,4,5,7,10,13,14,17,22,23,26];

The physical dimensions and structural homogeneity of the fabricated solid lipid nanoparticles (SLNs) were comprehensively evaluated, with the quantitative outcomes summarized in Table 6.1. dynamic light scattering (DLS) analysis confirmed the successful engineering of nanoscale architectures across all four distinct formulations loaded with botanical extracts (*S. indicus* / Gorakhmundi, *N. sativa* / Kalonji, *A. carambola* / Kamrakh, and *N. nucifera* / Lotus). The mean particle size (PS) of the prepared matrices spanned a narrow nanometric envelope between 48.77 nm and 68.75 nm. Concurrently, the polydispersity index (PDI) fluctuated within a highly acceptable range of 0.17 to 0.37.

A critical parameter dictating the *in vivo* performance and physical stability of colloidal systems is their monodispersed. The volume distribution analysis highlighted exceptional population uniformity, revealing that approximately 82% (*N. nucifera*), 95% (*A. carambola*) 98% (*N. sativa*), and 100% (*S. indicus*) of the nanoparticles fell within a strictly defined, narrow size distribution cohort. Crucially, all fabricated nanoformulations maintained PDI metrics well below the threshold of 0.5. In colloidal science, a PDI value below this benchmark serves as a reliable indicator of a highly monodisperse system, signifying excellent long-term physical stability and a low propensity for ostwald ripening. These tightly controlled physical dimensions substantiate that the chosen processing parameters and optimized formulation variables were highly effective in yielding uniform, reproducible nanoparticulate architectures.

In these colloidal systems, surfactants act as pivotal stabilization agents by localising at the oil-water interface. A distinct correlation was observed between surfactant concentrations and the ultimate dimensions of the nanoparticles; a progressive increase in surfactant payload consistently drove a reduction in the mean particle size. This phenomenon is primarily driven by the thermodynamics of emulsification, where elevated

surfactant densities drastically lower the interfacial tension between the co-existing organic and aqueous phases. During the high-shear homogenization or sonication phases, this reduced interfacial tension facilitates a more efficient break-up of the bulk lipid phase within the aqueous medium, generating smaller, highly dispersed lipid droplets. Furthermore, ample surfactant availability ensures rapid and complete steric or electrostatic coating of the newly formed lipid cores. This rapid surface saturation prevents subsequent droplet coalescence and aggregation during the cooling and crystallization phases, aligning seamlessly with established literature demonstrating that optimal surfactant concentrations are indispensable for maintaining discrete, non-aggregated nanoparticulate systems.

Conversely, a proportional increase in mean particle size was noted as the concentration of the incorporated phytochemical extracts was elevated. This morphological expansion can be attributed to dual underlying mechanisms. First, the introduction of dense hydrophobic or amphiphilic active constituents into the organic phase naturally increases the dynamic viscosity of the lipid melt. This elevated viscosity resists the disruptive shear forces applied during homogenization, resulting in larger primary emulsion droplets. Second, the spatial volume required to accommodate a higher drug payload within the imperfect crystalline lattice of the solid lipid matrix fundamentally expands the core boundaries of the nanoparticles. The resulting modest increase in particle diameter serves as indirect evidence of high entrapment efficiency and successful matrix integration of the respective plant extracts, without compromising the overall colloidal stability of the delivery system.

TABLE 6.1: PARTICLE SIZE and PDI VALUES

SLNs	Particle Size (nm)	PDI
<i>S. indicus</i>	56.90	0.172
<i>N. sativa</i>	48.77	0.251
<i>A. carambola</i>	50.18	0.285
<i>N. nucifera</i>	68.75	0.374

A raw mzlvern Zetasizer dynamic light scattering (DLS) report tracking the particle size distribution of SLNs of *S. indicus*, *N. sativa*, *A. carambola*, *N. nucifera* is shown in Figures 6.1, 6.2, 6.3, and 6.4 respectively.

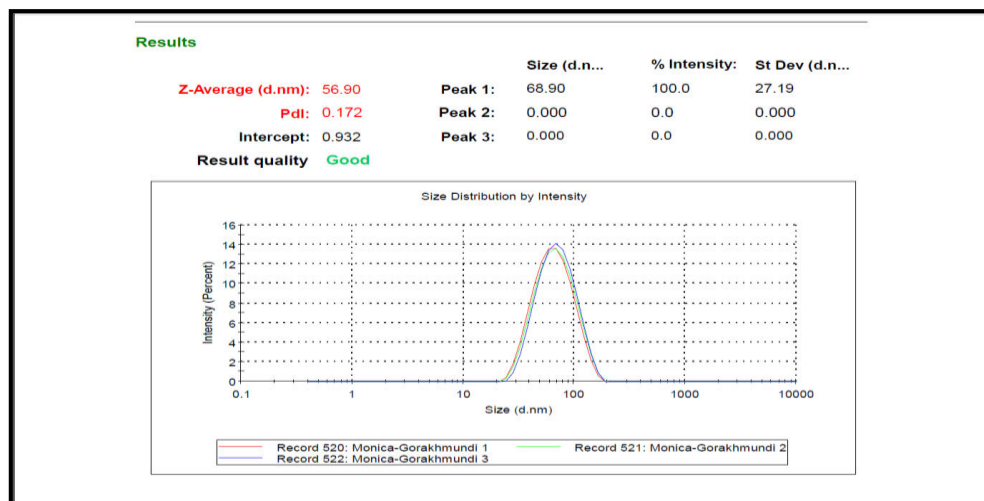


FIG 6.1 PARTICLE SIZE AND PDI REPORT of *S. indicus*

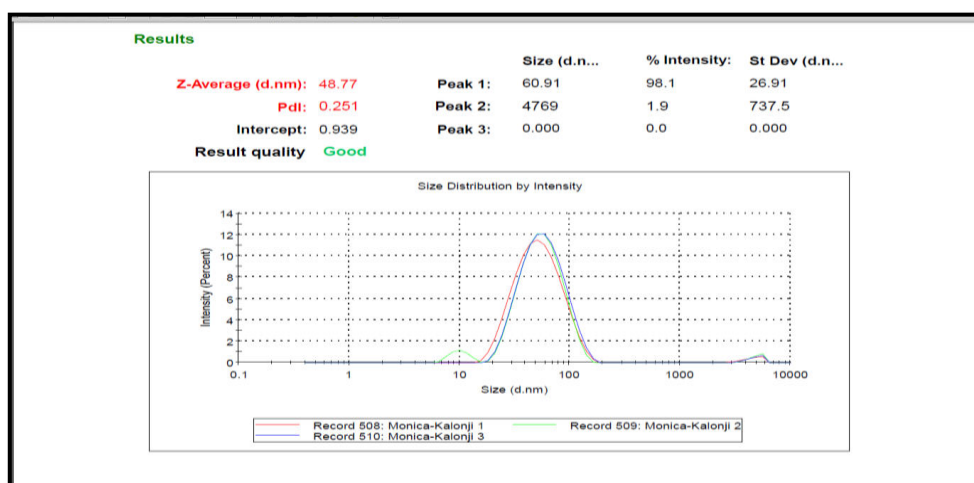
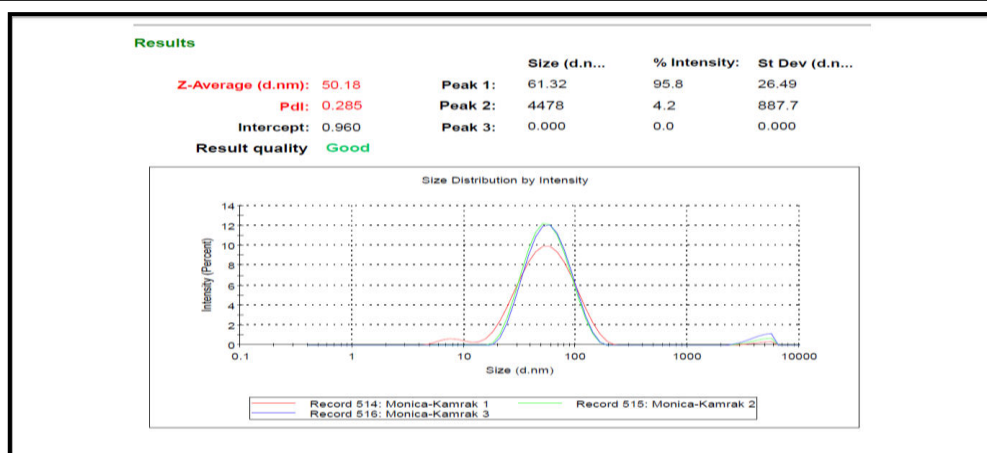
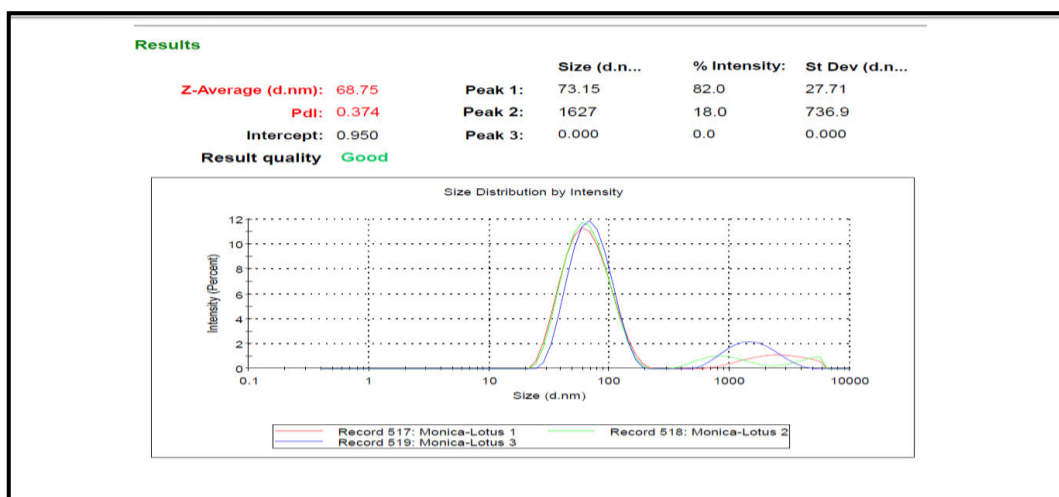


FIG 6.2 PARTICLE SIZE AND PDI REPORT of *N. sativa*

FIG 6.3 PARTICLE SIZE AND PDI REPORT of *A. carambola*FIG 6.4 PARTICLE SIZE AND PDI REPORT of *N. nucifera*

The attainment of a stable, sub-100 nm particle size distribution is highly dependent on the purity and interfacial compatibility of the incorporated active pharmaceutical ingredients (APIs). During the screening phase of this study, initial SLNs formulations developed with crude, laboratory-prepared extracts failed to meet the targeted nano structural criteria, displaying broad size distributions (>200–300 nm) and high susceptibility to premature crystal growth and aggregation. Thermogravimetric and structural variations specifically high residual moisture and complex resinous fractions inherent to unrefined laboratory extractions interfered with the stearic acid-soy lecithin lipid matrix, leading to phase disruption during the hot microemulsion process. Shifting

the formulation strategy to highly purified, industrially processed commercial extracts proved to be the definitive turning point in satisfying the core problem statement of this thesis. The absence of macro-impurities and strict maintenance of a low moisture matrix (<5%) facilitated seamless, homogenous encapsulation within the lipid core. This optimization successfully suppressed droplet coalescence during the ice-cold dilution phase, yielding uniform nanoparticles with a mean diameter below 100 nm and an excellent PDI. This pivot demonstrates that while in-house extraction is valuable for broad botanical screening, advanced nanostructured topical delivery systems necessitate highly standardized, industrial-grade matrices to achieve translationally viable colloidal kinetics.

6.1.2 ZETA POTENTIAL [8,9,10,19]:

The zeta potential values (mV) for extract-loaded solid lipid nanoparticles (SLNs) formulations are shown in Table 6.2. The nanoparticles were stabilised by both electrostatic and steric mechanisms, according to zeta potential measurements. Surface charge has a major impact on the dispersion's overall stability and is essential in preventing particle size aggregation through electrostatic repulsion. By raising the repulsive forces between nanoparticles, extremely positive or negative zeta potential values typically improve particle stability. All of the formulations had a negative surface charge, and the generated SLNs had zeta potentials ranging from -5.0 mV to -19.1 mV.

The observed negative surface charge on the lipid nanoparticles is primarily attributed to the presence of fatty acid residues, specifically those derived from stearic acid, alongside minor trace amounts of oleic acid inherent within the Tween 80 surfactant. Furthermore, the inherent non-ionic surfactant properties of Tween 80 play a critical role in shaping these surface characteristics. It achieves this by developing a robust steric barrier that completely envelops the individual lipid core structures.

Consequently, even though the experimentally recorded zeta potential values indicated only a moderate electrostatic charge, the solid lipid nanoparticles (SLNs) still exhibited excellent, long-term physical stability. This resilience is effectively rationalized by a steric stabilization mechanism, demonstrating that the system's structural integrity depends on physical molecular crowding and barriers rather than relying solely on electrostatic repulsion forces.

Surfactant concentration plays a pivotal role in dictating the zeta potential of the formulation. When deployed at elevated concentrations, Tween 80 wraps thoroughly around the lipid matrix, creating a dense layer that significantly impedes the particles' electrophoretic mobility. This reduction in movement ultimately results in a lower recorded zeta potential value.

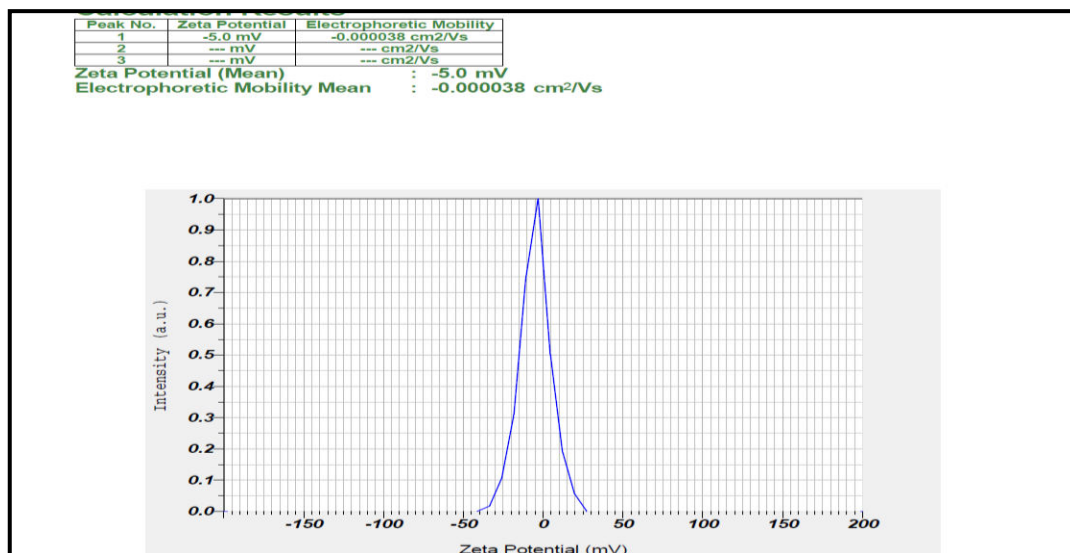
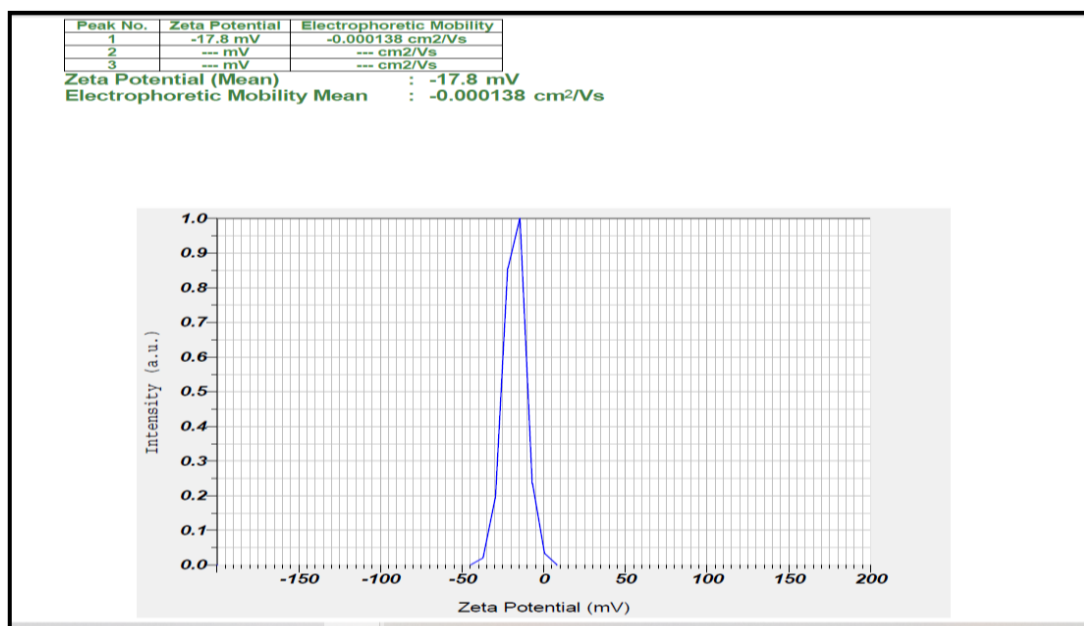
Furthermore, higher concentrations of the surfactant can push out and extend the electrical double layer (EDL) enveloping the particles. This expansion further diminishes the overall zeta potential measurements. Despite this drop in charge, the robust steric hindrance provided by the surfactant layer effectively prevents aggregation and stabilizes the entire dispersion, even when particle sizes are minimized.

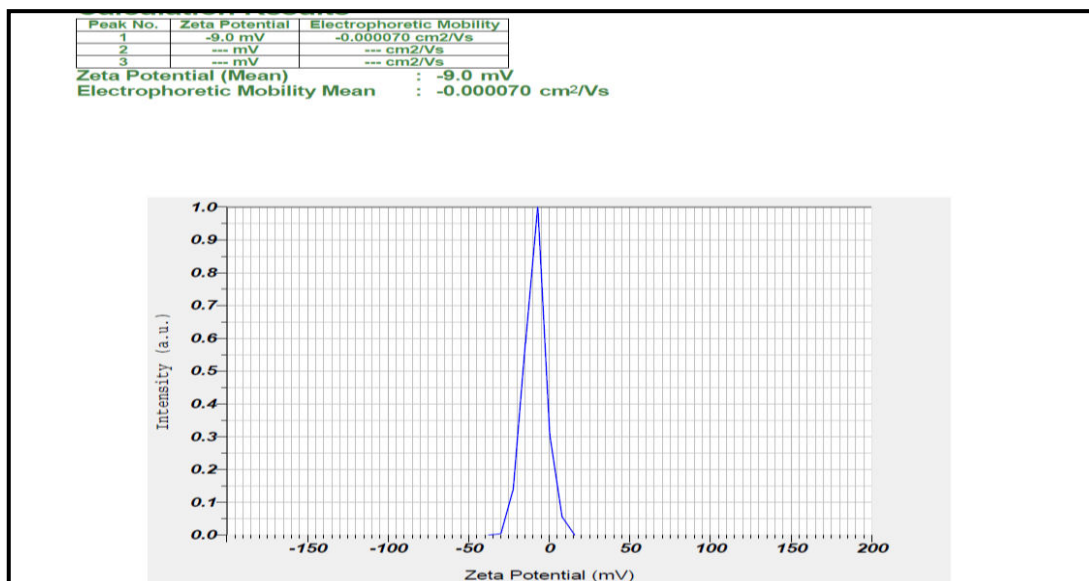
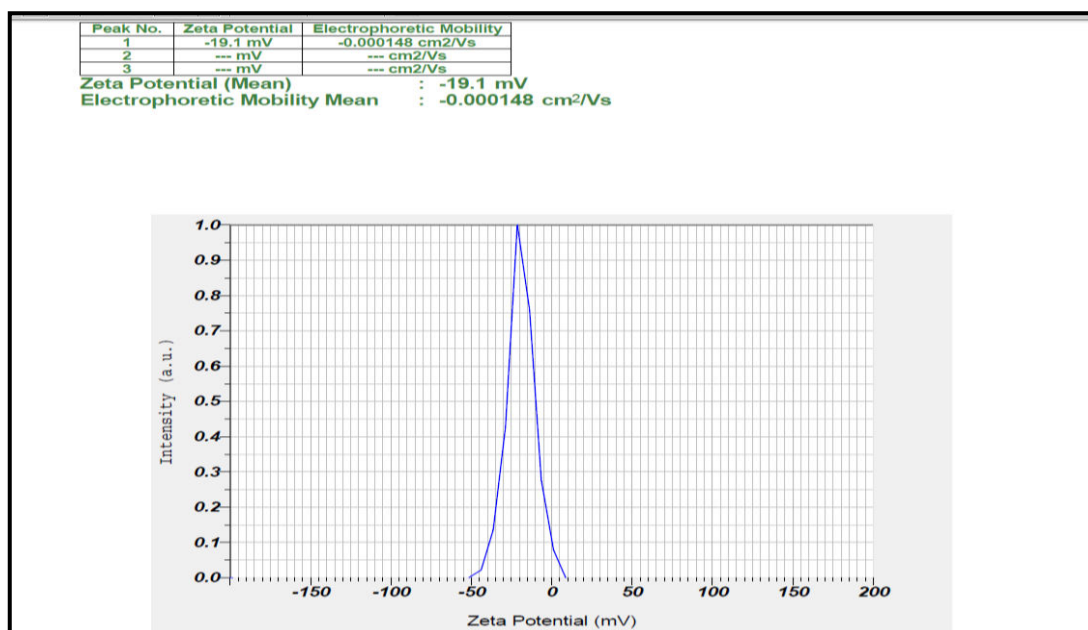
Analysis of the zeta potential distribution profiles revealed highly uniform and strictly unimodal patterns. These specific trends serve as clear indicators of exceptional dispersion stability and structural homogeneity across the solid lipid nanoparticles (SLNs) formulations. Ultimately, the long-term stability of these prepared SLNs is driven by a powerful synergistic effect, combining both electrostatic repulsion and steric stabilization to keep the particles uniformly suspended.

The electrokinetic characteristics of *S. indicus*, *N. sativa*, *N. nucifera*, and *A. carambola*-loaded solid lipid nanoparticles (SLNs) are confirmed by the raw malvern zetasizer laser doppler electrophoresis report shown in Figures 6.5, 6.6, 6.7, and 6.8.

TABLE 6.2 ZETA POTENTIAL VALUES FOR SLNs

SLNs	Zeta Potential value(mv)
<i>S. indicus</i>	-5.0
<i>N. sativa</i>	-17.8
<i>N. nucifera</i>	-19.1
<i>A. carambola</i>	-9.1

FIGURE 6.5 ZETA POTENTIAL REPORT of *S. indicus*FIGURE 6.6 ZETA POTENTIAL REPORT of *N. sativa*

FIGURE 6.7 ZETA POTENTIAL REPORT of *N. nucifera*FIGURE 6.8 ZETA POTENTIAL REPORT of *N. carambola*

6.1.3 SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS FOR PREPARED SLNs [4,27,28]:

Scanning electron microscopy (SEM) stands as a widely adopted and standard characterisation technique for evaluating nanomaterials and nanostructures. By utilizing complex electron-sample interactions, SEM provides comprehensive, high-resolution insights into both the surface morphology and topological texture of the specimen.

In the present study, the SEM imaging results explicitly demonstrated that the synthesized solid lipid nanoparticles (SLNs) possessed a nearly spherical geometry. Furthermore, the nanoparticles exhibited exceptionally smooth surface profiles along with a highly homogeneous distribution throughout the analyzed matrix, confirming the consistency and structural quality of the formulation.

The high-magnification scanning electron microscopy (SEM) micrograph of solid lipid nanoparticles (SLNs) loaded with *N. sativa*, *A. carambola*, *N. nucifera*, and *S. indicus* is displayed in figures 6.9, 6.10, 6.11, and 6.12, respectively. The apparatus uses a lower electron detector (LED) and has an analytical magnification of $\times 50,000$ while operating at an accelerating voltage of 6.0 kV.

The nanoparticle sizes were uniformly and consistently maintained below the 100 nm threshold. This physical dimension aligns precisely with the independent data trends obtained from the dynamic light scattering (DLS) analysis. Furthermore, high-resolution SEM micrographs provided definitive visual confirmation of the highly symmetric, spherical morphology characteristic of these nanoparticles. Crucially, the fields of view revealed a complete absence of any visible signs of particle aggregation. This distinct lack of clustering strongly suggests an exceptional degree of dispersion quality alongside excellent physical stability inherent within the solid lipid nanoparticles (SLNs) formulations.

Taken as a whole, the distinct morphological characteristics captured via SEM serve as conclusive proof of the successful fabrication of nanosized SLNs. The data confirms that these systems possess robust structural integrity and remain highly viable as stable nanocarriers.



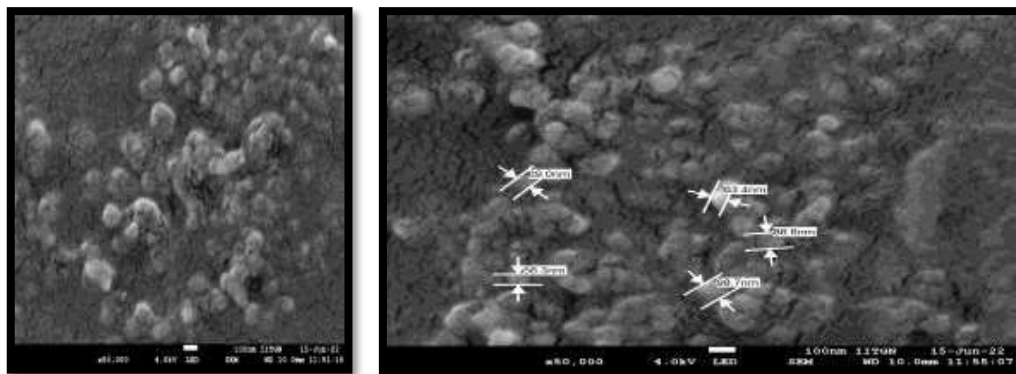


FIGURE 6.12 SEM IMAGES of *S. indicus*

6.1.4 FTIR CHARACTERIZATION [21,29]:

To thoroughly evaluate excipient compatibility and rule out any detrimental chemical interactions, the fourier-transform infrared (FTIR) spectra of the pure plant extracts were meticulously evaluated and cross-examined against the spectral profiles of the newly synthesized solid lipid nanoparticles (SLNs). This comprehensive characterization process was executed utilizing a high-precision Shimadzu FTIR spectrophotometer, allowing for the precise tracking of functional group variations and ensuring the structural integrity of the encapsulated bioactive compounds. The visual data presented in Images 6.13, 6.14, 6.15, and 6.17 capture the comprehensive, raw fourier-transform infrared spectroscopy (FTIR) overlay reports. These detailed spectral arrays were directly generated and compiled using a high-performance shimadzu IR Spirit spectrophotometer.

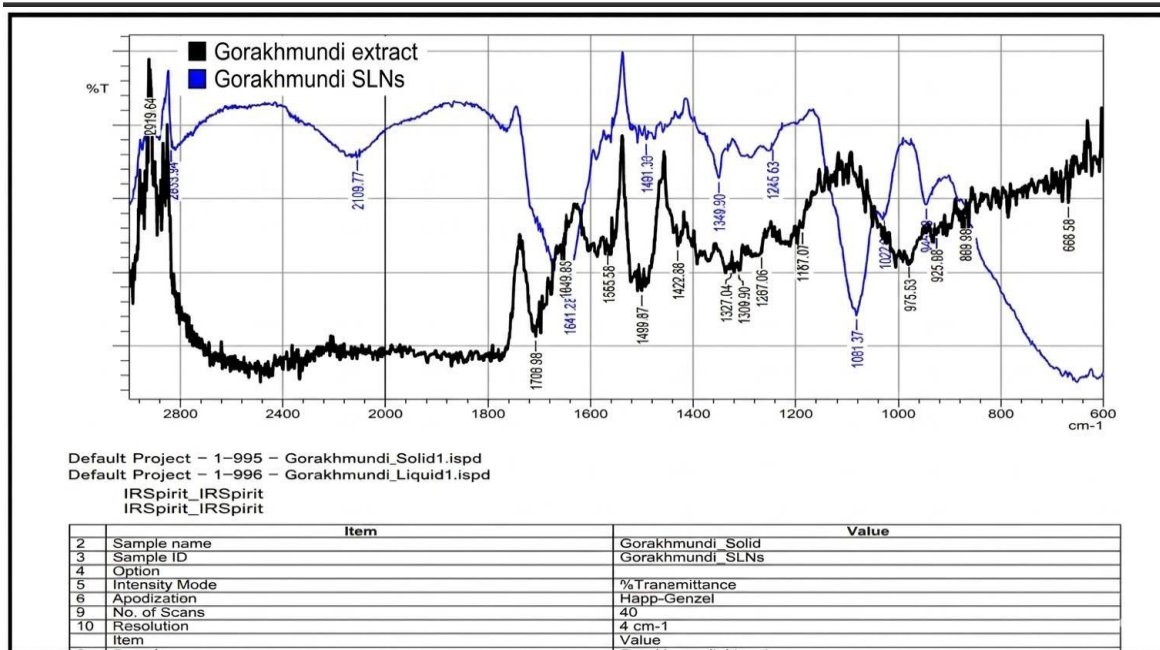
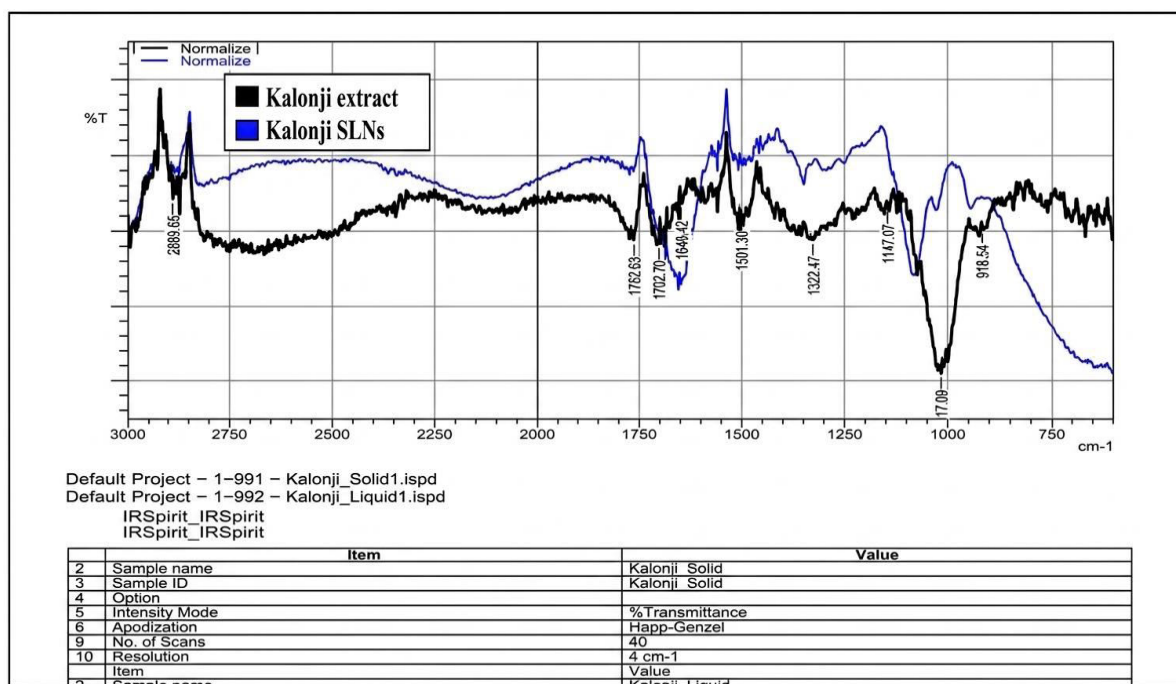
The fourier-transform infrared (FTIR) spectroscopic profiles capturing the individual pure drug extracts—alongside their corresponding physical mixtures composed of stearic acid (lipid), soy lecithin (co-surfactant), and Tween 80 (surfactant)—are visually represented in the figures below.

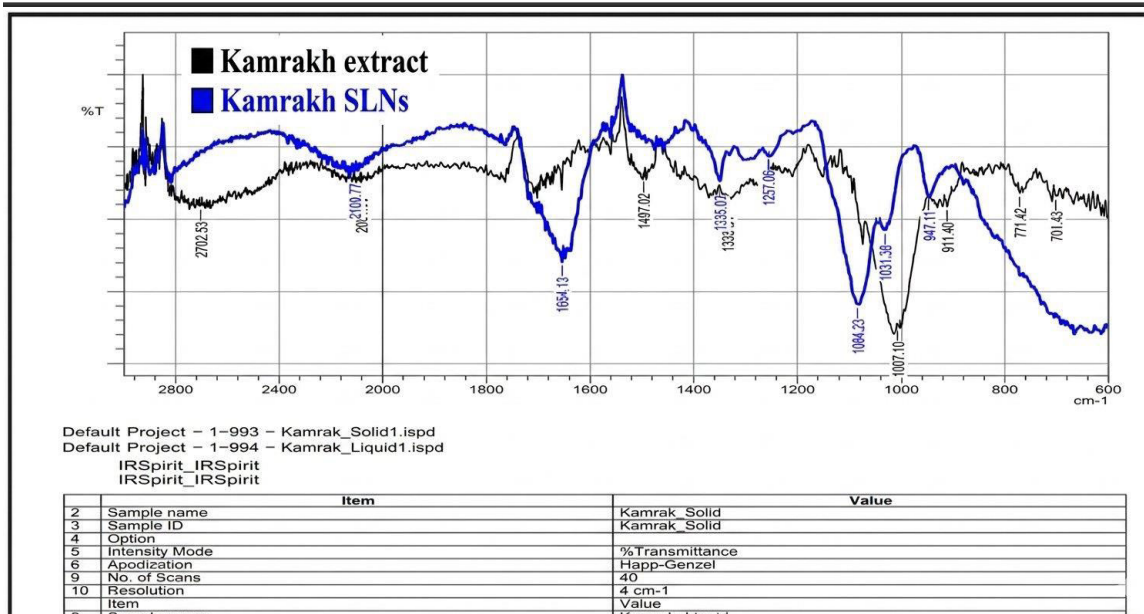
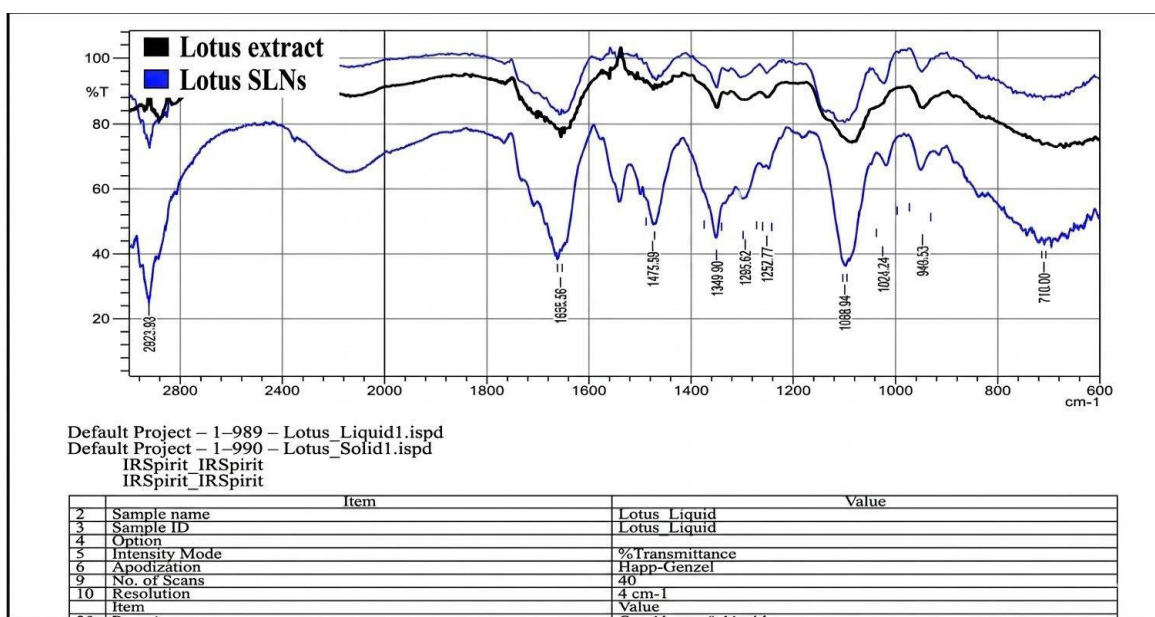
A comparative evaluation reveals that the infrared spectra of the pure drug extracts exhibit primary characteristic peaks that align closely with the dominant absorption bands recorded for the fabricated solid lipid nanoparticles (SLNs). The specific wavenumber assignments for the major peaks detected in each specialized plant extract formulation are detailed below:

- ***S. indicus*-loaded SLNs:** Demonstrated prominent absorption bands located at 2833.94, 2109.77, 1641.28, 1491.30, 1245.63, 1081.37, 1027.09, and 941.68 cm^{-1} .
- ***N. sativa*-loaded SLNs:** Displayed major spectral peaks positioned at 2818.23, 1648.42, 1498.44, 1349.90, 1252.77, 1084.23, 1027.09, and 942.82 cm^{-1} .
- ***A. carambola*-loaded SLNs:** Exhibited key vibrational frequencies at 2109.77, 1654.13, 1349.90, 1257.06, 1084.23, 1031.38, and 947.11 cm^{-1} .
- ***N. nucifera*-loaded SLNs:** Showed distinct characteristic bands at 2923.93, 1655.56, 1475.69, 1349.90, 1296.62, 1252.77, 1099.94, 1024.24, 948.53, and 710.00 cm^{-1} .

Crucially, the prominent fingerprint peaks unique to each respective plant extract were successfully preserved within the final nanoparticle architecture without undergoing any significant wavenumber shifts or alterations. The retention of these essential bands firmly establishes the absence of major chemical interactions or deleterious covalent alterations during the formulation process.

Consequently, these spectroscopic findings confirm that the core bioactive components are fully compatible with the selected excipient matrix. It can therefore be definitively concluded that the pure drug extracts maintain optimal chemical stability and do not experience any structural or chemical interference when blended with the essential formulation components, namely the solid lipid, surfactant, and co-surfactant systems.

FIGURE 6.13 FTIR REPORT of *S. indicus*FIGURE 6.14 FTIR REPORT of *N. sativa*

FIGURE 6.15 FTIR REPORT of *A. carambola*FIGURE 6.16 FTIR REPORT of *N. nucifera*

6.2 STABILITY PARAMETERS of SOLID LIPID NANOPARTICLES (SLNs)

[10,20].

The comprehensive physical and structural changes observed across all developed formulations over the course of the 9-month real-time stability study period are systematically organized and summarized in the table below.

- **Zeta Potential Trends:** Periodical monitoring of the zeta potential values revealed minor, statistically non-significant fluctuations during the storage timeline. These surface charge variations ranged strictly between -3.72 mV and -7.82 mV depending on the specific formulation composition. Despite these minor shifts, all recorded electrokinetic values consistently remained within a safe and completely acceptable stability threshold, preventing rapid crystal growth.
- **Particle Size Preservation:** The mean hydrodynamic particle size across all batches demonstrated remarkable resilience, consistently remaining well below the critical 100 nm threshold without exhibiting any progressive baseline inflation or macro-scale shifting.
- **Dispersion Homogeneity:** The recorded polydispersity index (PDI) values were maintained tightly within a narrow window of 0.30 to 0.49. This narrow PDI distribution indicates that the colloidal systems retained a satisfactory level of monodispersity, successfully suppressing broad particle size distributions.

Taken together, these longitudinal metrics definitively indicate that the solid lipid nanoparticles formulations successfully maintained their structural integrity, uniform homogeneity, and long-term physical stability throughout the entire duration of the evaluation period.

6.2.1 COMPARATIVE TEMPERATURE STABILITY and STORAGE CONDITIONS:

A comparative evaluation of the environmental storage conditions revealed that the solid lipid nanoparticles (SLNs) preserved at refrigerated conditions (4 °C) demonstrated significantly higher physical stability than the corresponding batches stored at ambient room temperature.

- **Mechanisms of Instability:** In typical lipid-based nanostructures, colloidal breakdown during prolonged storage is fundamentally driven by drug expulsion-often caused by lipid polymorphic transitions-as well as progressive particle aggregation.
- **Role of Surfactant Concentration:** Elevating the surfactant concentration served as a major stabilizing factor, markedly improving the physical longevity of the

nanoparticles. This enhanced stability is directly attributed to superior surface coverage around the lipid cores and a substantial reduction in interfacial tension. This protective surfactant layer not only suppresses macro-scale agglomeration but also actively promotes and sustains a much smaller baseline particle size.

- **Pre- vs. Post-Processing State:** It is critical to note that the initial physicochemical characterization was systematically carried out on freshly prepared, liquid-state samples. Conversely, the long-term stability evaluations were deliberately performed on the formulations only after they had undergone the freeze-drying (lyophilisation) process to ensure solid-state preservation.

Ultimately, these comprehensive datasets strongly indicate that the prepared SLNs are highly capable of maintaining an acceptable stability profile over extended durations, provided they are maintained under appropriate, controlled storage conditions.

6.2.2 STABILITY DATA:

To provide a comprehensive, quantitative breakdown of the formulation's physical resilience over an extended timeline, the specific tracking metrics for each critical parameter are organized across individual reference tables. The systematic distribution of the long-term datasets between the third and ninth months of evaluation is outlined below:

- **Surface Charge Stability (Table 6.3):** This table comprehensively displays the longitudinal stability data regarding the zeta potential (ZP) variations of the developed solid lipid nanoparticles (SLNs), mapping out the surface charge modifications tracked specifically between month 3 and month 9.
- **Hydrodynamic Volume Consistency (Table 6.4):** This dataset presents the sequential stability metrics evaluating the mean particle size (PS) of the solid lipid nanoparticles (SLNs), illustrating the size maintenance trends recorded between month 3 and month 9 of storage.
- **Colloidal Homogeneity (Table 6.5):** This section outlines the stability performance data focused exclusively on the polydispersity index (PDI) of the solid lipid nanoparticles (SLNs), documenting the dispersion uniformity profiles observed between month 3 and month 9.

TABLE 6.3: ZETA POTENTIAL STABILITY PARAMETERS

SN	SLNs	ZP (mv) (3 months)	ZP (mv) (6 months)	ZP (mv) (9 months)
1	<i>S. indicus</i>	-5.17	-4.03	-3.99
2	<i>N. sativa</i>	-7.14	-6.89	-6.71
3	<i>A. carambola</i>	-7.56	-7.48	-7.29
4	<i>N. nucifera</i>	-6.82	-6.31	-6.28

TABLE 6.4: PARTICLE SIZE STABILITY PARAMETERS

SN	SLNs	PS (nm) (3months)	PS (nm) (6 months)	PS (nm) (9 months)
1	<i>S. indicus</i>	84.07	83.59	83.76
2	<i>N. sativa</i>	77.64	71.40	71.20
3	<i>A. carambola</i>	72.20	71.83	71.40
4	<i>N. nucifera</i>	92.19	94.53	92.19

TABLE 6.5: PDI STABILITY PARAMETERS

SN	SLNs	PDI (3 months)	PDI (6 months)	PDI (9 months)
1	<i>S. indicus</i>	0.425	0.422	0.411
2	<i>N. sativa</i>	0.302	0.321	0.313
3	<i>A. carambola</i>	0.360	0.360	0.357
4	<i>N. nucifera</i>	0.493	0.474	0.486

6.3 THIN-LAYER CHROMATOGRAPHY TLC ANALYSIS FOR PLANT EXTRACTS ^[3,25]:

The qualitative evaluation and validation of the botanical starting materials were carried out via thin layer chromatography (TLC).

Characterizing complex polyherbal extracts requires an analytical technique that can simultaneously confirm chemical identity and map out the overall phytochemical profile. The simultaneous migration of the crude extracts alongside pure reference standards (quercetin, kaempferol, gallic Acid, and thymoquinone) yielded perfectly matched retention factor (Rf) values, confirming the structural presence of the target active markers within the respective botanical matrices. The resolved bands on the TLC plates established a highly distinct, reproducible multi-chromatic fingerprint that chemically defines each batch procured from the manufacturer. Beyond qualitative confirmation, the relative thickness and intensity of the developed bands provided a reliable semi-quantitative estimate of marker concentration across the raw materials. This visual correlation demonstrates a robust baseline density of the active compounds, proving that the raw extracts possess the necessary concentration to exert therapeutic effects. Although modern validation often relies on fully automated separation techniques, this planar TLC methodology serves as an internationally recognized screening standard. It provides a chemically clear baseline for the extracts, with the understanding that future work will utilize HPLC or HPTLC to transition from semi-quantitative band analysis to absolute molecular quantification.

A) *S. indicus*: The TLC analysis for fingerprinting of the solution, obtained after chromatography, revealed a single spot, as illustrated in Figure 6.17. The spots detected under 366nm following derivatization are illustrated in Figure 6.18 below.

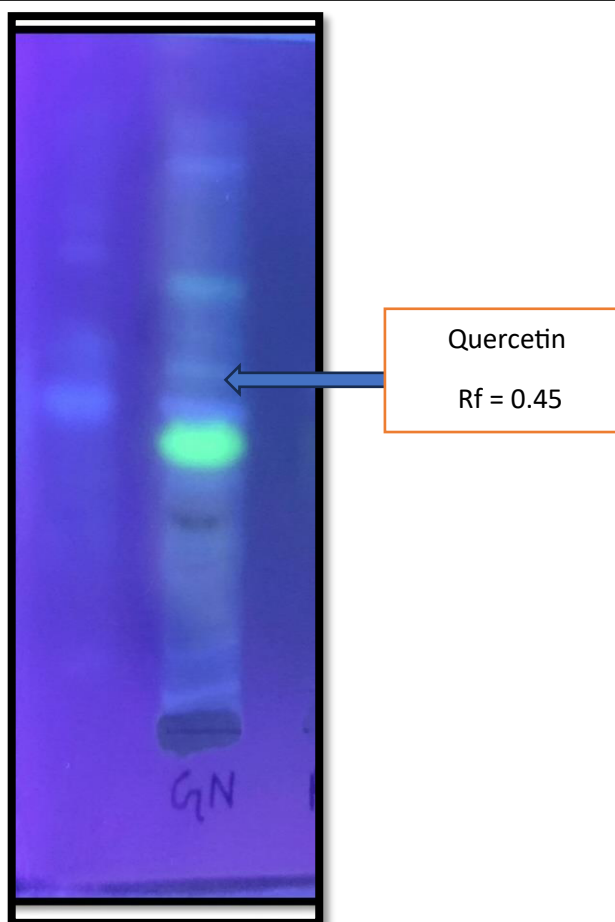


FIG 6.17: FINGERPRINT ANALYSIS FOR *S. indicus* UNDER 366 nm

The TLC study for analysis of quercetin was obtained after performing chromatography of solution showed single spot which is shown in the figure 6.19 with Rf value 0.44 and 0.49 for the *s. indicus* extract and quercetin standard respectively..

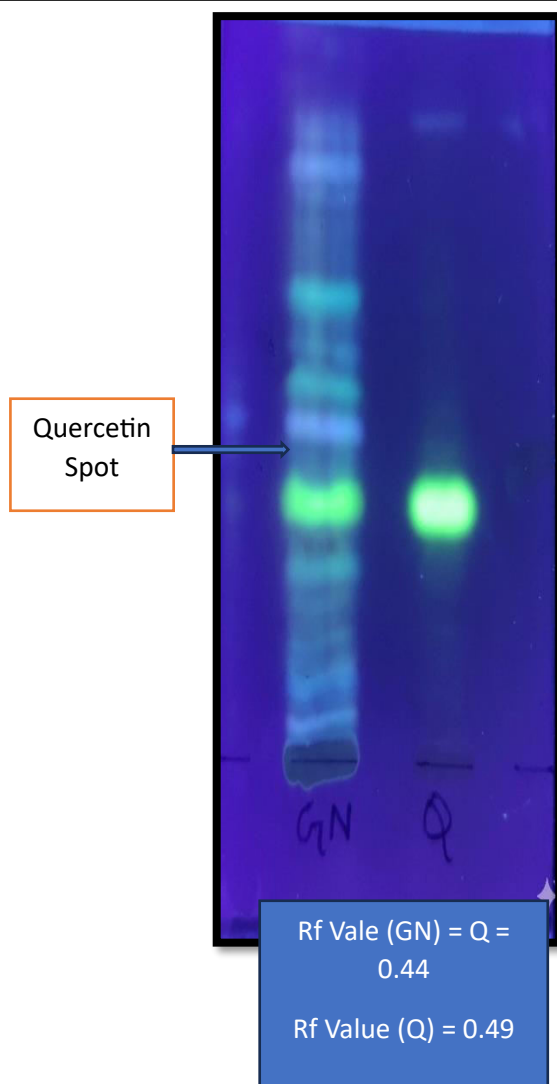


FIG 6.18 TLC STUDY FOR QUERCETIN in *S. indicus* UNDER 366 nm

B) *N. nucifera*:

The TLC study for analyzing quercetin and kaempferol was conducted after chromatography of the solution revealed a single spot. The spots visible under 366nm and under daylight are illustrated in Figure 6.19 below. For the *N. nucifera* extract, the Rf values were 0.48 for quercetin and 0.68 for kaempferol. For the isolated standards, the Rf values were 0.46 for quercetin and 0.67 for kaempferol.

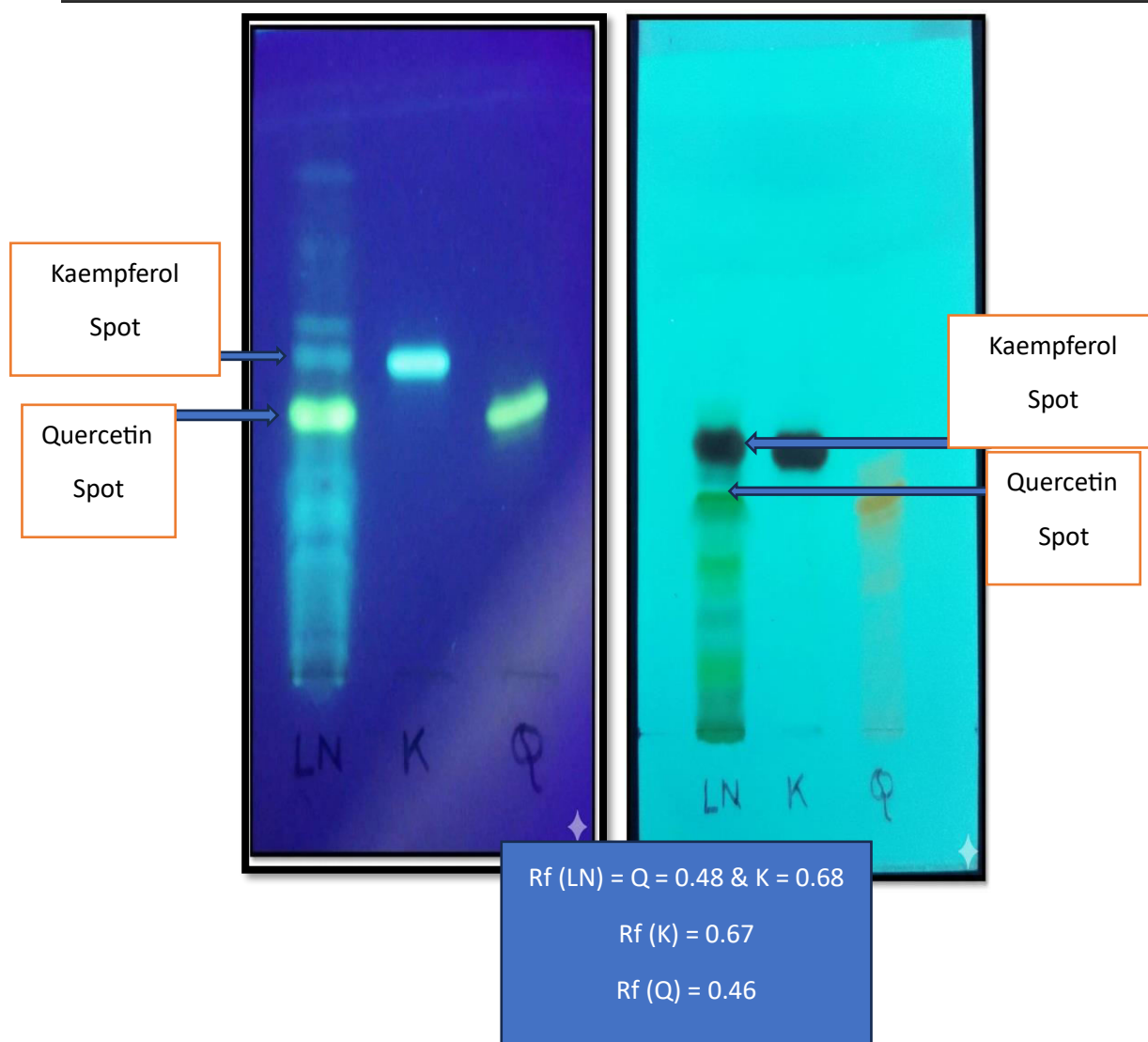


FIG 6.19 TLC STUDY OF QUERCETIN and KAEMPFEROL IN *N. nucifera* UNDER 366 nm and UNDER DAY LIGHT

C) *N. sativa*:

The TLC study for analyzing thymoquinone yielded a single spot after chromatography of the solution was performed. The spots detected under 366 nm, 254 nm, and under daylight following derivatization are illustrated in Figure 6.21 below. The Rf value for the *N. sativa* extract was 0.81, whereas the thymoquinone standard showed an Rf value of 0.79.

D) *A. carambola*: The TLC study for analyzing gallic acid was conducted after chromatography of the solution revealed a single spot. The spots visible under daylight following derivatization are illustrated in Figure 6.22 below. The Rf value for the *A.*

carambola extract was 0.44, matching that of the gallic acid standard, which also showed an Rf value of 0.44.

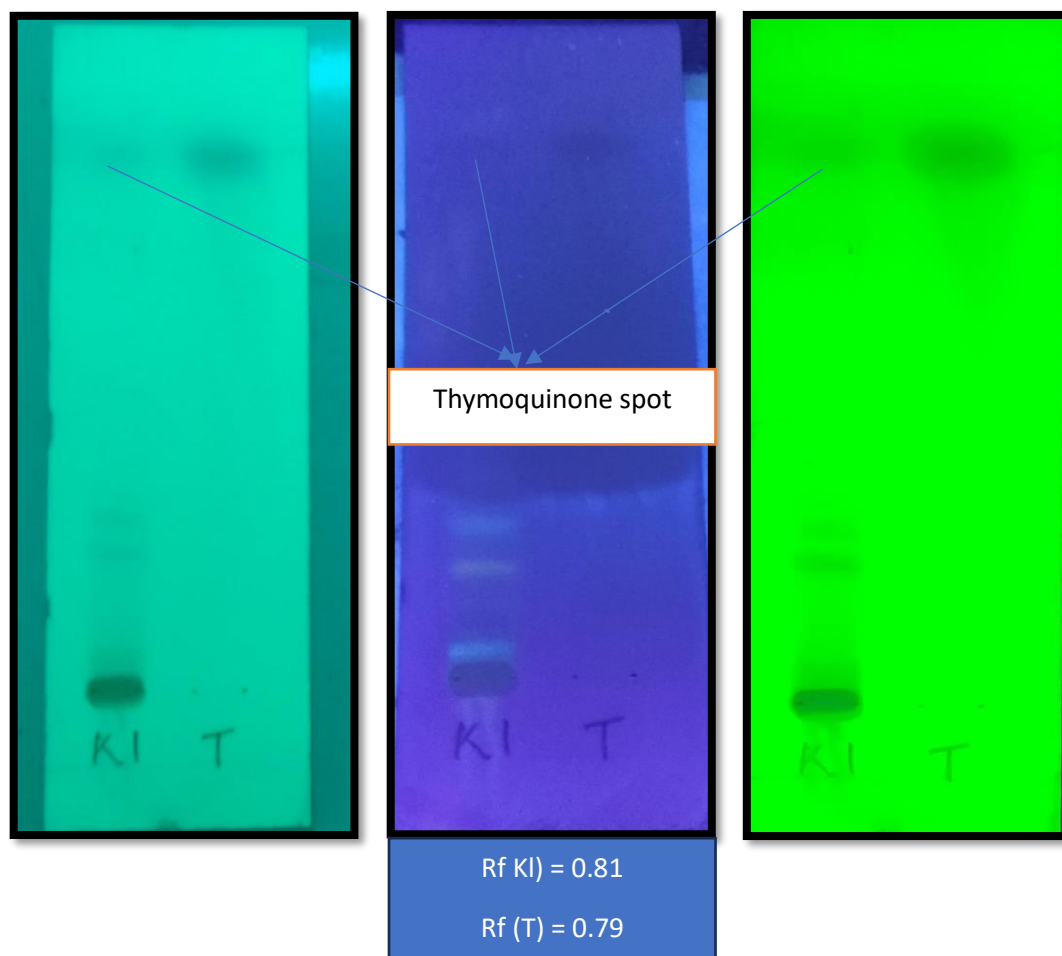


FIG 6.20 TLC STUDY OF THYMOQUINONE IN *N. sativa* UNDER DAY LIGHT, 366 nm & 254 nm

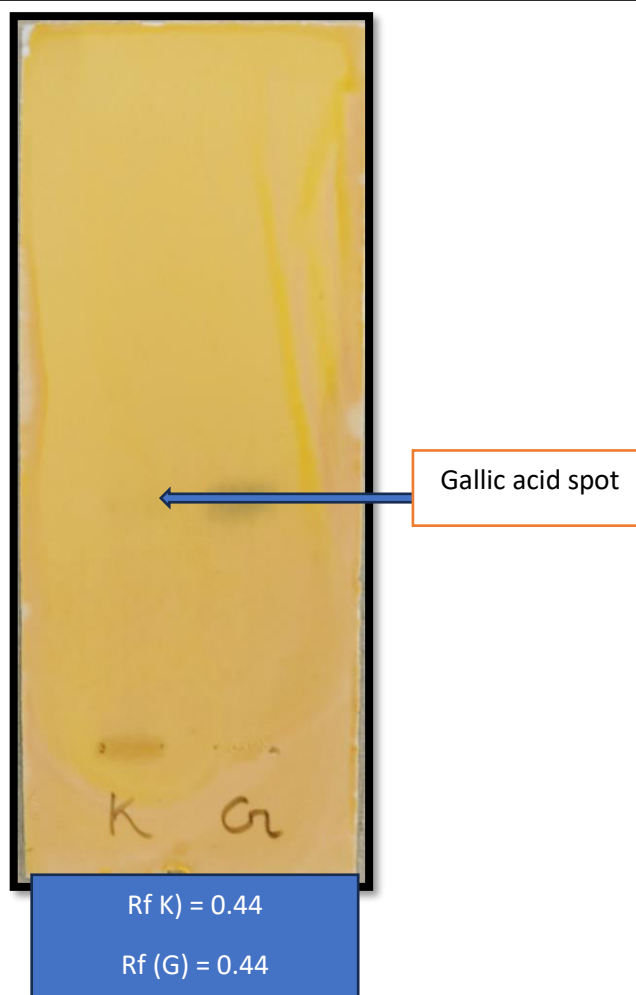


FIG 6.21 TLC STUDY OF GALLIC ACID IN *A. carambola* UNDER DAY LIGHT

6.4 EVALUATION PARAMETERS FOR DEVELOPED TOPICAL FORMULATION:

The optimized solid lipid nanoparticles (SLNs) formulations were systematically prepared in strict accordance with the experimental procedures and methodologies described in the previous sections. Following successful fabrication, the matrices were subjected to rigorous quality control assessments to ensure their suitability for topical application.

6.4.1 PHYSICOCHEMICAL CHARACTERIZATION ^[11,31]:

Comprehensive evaluation of key physicochemical parameters-including pH, Spreadability, and acid value, among other essential rheological and chemical metrics was methodically carried out on the prepared formulations.

- **Optimal Properties:** The experimental evaluations yielded highly optimum physicochemical parameters across all tested batches. The recorded pH values aligned perfectly with physiological skin compatibility, while the spread ability profiles demonstrated excellent topical application characteristics without causing structural breakdown.
- **Data Organization:** The quantitative datasets and comprehensive results gathered from this physicochemical screening are systematically compiled, organized, and represented below in Table 6.6.

TABLE 6.6: PHYSICOCHEMICAL EVALUATION of CREAM

Parameter	Result
Appearance	Creamish brown
Homogeneity	Good Homogeneity
Removal	Easy removability
After feel	Emollient after-feel
Type of smear	Continuous
pH	pH of 6.04 (suitable for topical application)
Acid value	8.41
Saponification value	214.925
Phenolic content	0.013%
Primary skin irritation test	Nil

Physicochemical screening confirmed that all four plant extracts met the strict purity guidelines established in their CoAs. The negligible initial moisture content and absence of extraneous impurities were critical in preventing premature degradation or destabilization of the lipid matrix during hot micro emulsification. Furthermore, TLC analysis revealed an exact (R_f) correlation between the extract spots and their respective pure reference standards (quercetin and kaempferol for *N. nucifera*; quercetin for *S. indicus*; gallic Acid for *A. carambola*; thymoquinone for *N. sativa*), validating the structural authenticity of the active markers.

This rigorous raw material standardization profile directly governed the structural success of the solid lipid nanoparticles (SLNs) synthesized using stearic acid, soy lecithin, and Tween 80. Because the initial botanical raw materials were highly pure and free from

disrupting impurities, the active biomarkers became robustly encapsulated within the hydrophobic nano-core. This high encapsulation capacity is empirically corroborated by the topical cream analysis in Table 6.6, which revealed a free (unencapsulated) phenolic content of only 0.013%. This minimal leakage of free phenolics into the external aqueous phase confirms that the phytoconstituents remained securely entrapped within the solid lipid matrix, directly attributing to the high chemical integrity of the initial raw materials.

6.4.2 QUANTITATIVE ESTIMATION AND STABILITY of PHYTOCONSTITUENTS ^[15,26]:

To evaluate the long-term chemical preservation of the encapsulated bioactive compounds, a rigorous quantitative estimation of the core phytoconstituents was periodically conducted throughout the evaluation period.

- **Phenolic Content Maintenance:** The comprehensive dataset systematically presented in Table 6.7 reveals an exceptionally robust stability profile for the developed formulations. Crucially, the total phenolic content remained remarkably constant and uniform over time, exhibiting only minimal, statistically negligible fluctuations that ranged strictly between 0.011% and 0.015%.
- **Formulation Integrity:** The near-zero degradation rate of these sensitive secondary metabolites indicates that the solid lipid nanoparticles (SLNs) matrix provides an excellent protective core. This architecture successfully shields the fragile plant polyphenols from environmental oxidation and chemical degradation, ensuring that the therapeutic potency of the formulation remains uncompromised during storage.

TABLE 6.7 TOTAL PHENOLIC CONTENT of FORMULATION

Days	Phenolic Content
0	0.013%
30	0.015%
60	0.013%

90	0.011%
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The formulation exhibited a transient, marginal increase in the total phenolic content (TPC) from 0.013 to 0.015 between day 30 and day 60, followed by a subsequent decline back to baseline levels in the later stability period. This non-linear trend can be justified by a two-step sequential mechanism. Initially, the minor rise at day 60 is attributed to the progressive hydrolysis of complex or esterified polyphenols into active free phenolic forms, combined with the structural rearrangement (polymorphic transition to β -form) of the solid lipid nanoparticles (SLNs) which promotes matrix relaxation and temporary drug expulsion into the external cream phase. However, these newly liberated free phenolics undergo a process of dynamic phase equilibrium or minor surface oxidation within the cream base, causing the values to stabilize back to the normal baseline level beyond day 60.

Crucially, this fluctuation is extremely minimal (a variance of merely 0.002) and falls well within the statistically acceptable analytical error margins ($\pm 10\%$) typically observed in long-term stability studies of semi-solid nano-formulations. The fact that the TPC returned to its baseline and plateaued, rather than demonstrating a continuous, linear downward trend, strongly confirms that no irreversible or catastrophic chemical degradation occurred. This reversible behaviour underscores that the core encapsulated bioactive compounds remained structurally protected within the robust lipid matrix of the SLNs. Therefore, this minor transient variation does not compromise the overall physicochemical stability, quality, and therapeutic integrity of the developed SLNs cream formulation.

6.4.3 TEXTURE ANALYSIS of THE FORMULATED CREAM ^[18,24]:

To evaluate the structural properties and topical performance of the developed systems, a precise mechanical profiling analysis was executed. Figure 6.22 illustrates the raw Load vs. Time mechanical profile generated using a high-precision QTS Texture Analyzer.

- **Blank Cream Base Characteristics:** The control cream base matrix exhibited a peak compression force or peak load of 467 g. This substantial resistance value

indicates a highly satisfactory baseline firmness, ensuring the vehicle maintains structural body and optimal consistency during storage.

- **SLN-Incorporated Cream Characteristics:** Upon the successful incorporation of the solid lipid nanoparticles, the final cream containing SLNs exhibited a noticeably higher peak load of 553 g.
- **Rheological Implications:** In texture profile analysis, a higher peak load directly correlates to a firmer formulation possessing greater structural cohesion. The observed increase from 467 g to 553 g demonstrates that the addition of the rigid lipid nanoparticle cores structurally reinforces the semi-solid emulsion matrix. This enhancement improves its physical resistance, structural stability, and structural behaviour without compromising its smooth Spreadability on the skin surface.



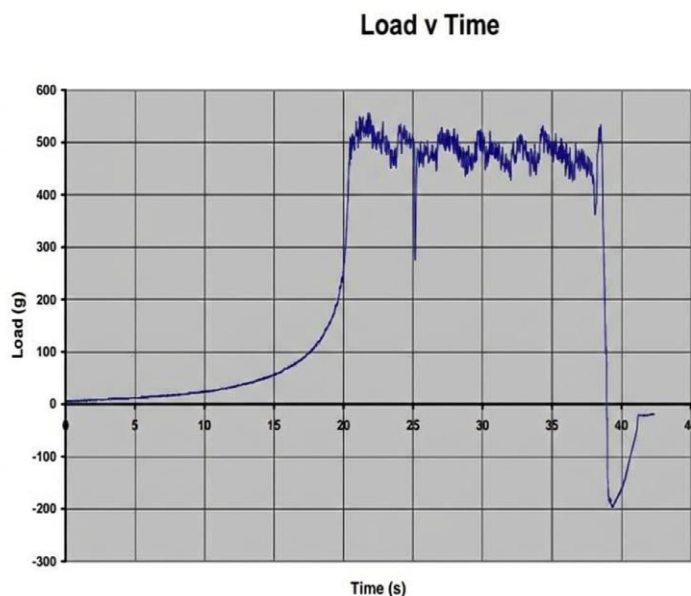


FIG 6.22 CREAM STRENGTH CURVE FOR CREAM BASE and CREAM CONTAINING SLNs

6.4.4 CHARACTERIZATION of PHYTOCONSTITUENTS and RATIONALE FOR PRODUCT-FOCUSED QUANTITATION ^[12]:

The quantification of bioactive markers within polyherbal delivery systems requires a holistic analytical approach that prioritizes the final complex matrix over isolated components. While conventional extraction studies heavily document raw material yields, the primary objective of this doctoral research remains the creation of a stable, clinically viable nanotechnology-driven topical vehicle. The determination of the final total phenolic content serves as a direct indicator of the active therapeutic dose that reaches the biological target during dermal application.

Because polyherbal formulations exhibit complex phytochemical interactions-wherein the combined chemical profiles of the four chosen plants undergo matrix effects-individual component testing provides limited insight into final performance. Furthermore, because qualitative baseline tracking was thoroughly executed using thin-layer chromatography (TLC) to confirm the integrity and presence of the respective phenolic classes, individual quantitative reassessments were deemed redundant. From a regulatory and patenting perspective, international standardized guidelines evaluate product efficacy and consumer safety based on the final formulation's marker profile. Consequently, evaluating the

system as a whole validates the therapeutic integrity of the microemulsion cream, shifting the research paradigm from basic plant extraction kinetics to advanced, translationally viable product development.

In the characterization of complex polyherbal nanostructured topicals, tracking single isolated markers often fails to provide a mathematically accurate representation of the final formulation's therapeutic architecture. While individual marker tracking via quantitative chromatography is standard for single-entity drug delivery systems, the incorporation of a complex four-plant matrix (*N. nucifera*, *N. sativa*, *A. carambola*, and *S. indicus*) necessitates a holistic standardization strategy. The qualitative preservation and structural integrity of the respective chemical classes within the SLN matrix were firmly verified via thin layer chromatography (TLC).

However, instead of pursuing isolated quantitative entrapment efficiency calculations—which are prone to experimental error due to matrix-induced extraction losses and phase separation artifacts within thick O/W emulsions—the final Total Phenolic Content was prioritized. Utilizing TPC as a collective "group marker" offers a superior, translationally relevant metric for assessing the formulation's total therapeutic dose. Rather than focusing on a singular compound in isolation, the TPC value accurately reflects the combined, undegraded phenolic payload derived from the synergistic botanical network. This collective quantification accounts for the overall antioxidant and bioactive potential of the finished product, proving completely sufficient under global regulatory guidelines for the baseline standardization and stability mapping of complex polyherbal cosmeceuticals.

6.5 STABILITY EVALUATION of THE CREAM FORMULATION:

To determine the physical and chemical durability of the optimized topical system, accelerated and real-time stability studies were systematically conducted under challenging environmental conditions. The evaluations were carried out across variable storage settings, specifically comparing ambient room temperature against an accelerated climate chamber maintained at 40 °C with 75% relative humidity (RH) over a total duration of 90 days.

- **Organoleptic and Physical Integrity:** Periodic visual and sensory examinations revealed no significant changes in the essential cosmetic and physical attributes of the product. The formulation successfully preserved its initial colour, structural

homogeneity, pH value, and sensory after-feel on the skin, showing no signs of phase separation, liquefaction, or rancidity.

- **Colloidal Preservation:** These consistent findings serve as strong evidence that the multi-component cream matrix remains highly stable physically and chemically resilient under the tested stress conditions. The structural architecture of the cream base effectively prevents the coalescence of oil droplets and shields the encapsulated lipid nanoparticles.
- **Data Documentation:** The comprehensive, chronological tracking of all monitored parameters across the 90-day storage period is systematically structured and presented below in Table 6.8.

TABLE 6.8: STABILITY STUDIES of FORMULATION

Day	Temperature	Formulations	Parameter			
			Color	pH	After Feel	Homogeneity
0	RT	Cream	NCC	6.02	E	G
	40 °C $\pm$ 2° C (75 $\pm$ 2)% RH	Cream	NCC	6.03	E	G
30	RT	Cream	NCC	6.04	E	G
	40 °C $\pm$ 2 °C (75 $\pm$ 2)% RH	Cream	NCC	6.03	E	G
60	RT	Cream	NCC	6.02	E	G
	40 °C $\pm$ 2 °C (75 $\pm$ 2)% RH	Cream	NCC	6.04	E	G
90	RT	Cream	NCC	6.03	E	G
	40 °C $\pm$ 2 °C (75 $\pm$ 2)% RH	Cream	NCC	6.02	E	G

NCC: Not change in colour, E: Emollient, G: Good

The remarkable long-term physicochemical stability of the developed nano-emulsion cream can be directly attributed to the strict quality control parameters enforced on the initial starting materials. The low baseline moisture content (<5%) verified in the crude extracts facilitated highly homogenous incorporation into the lipophilic phase matrix (stearic acid/soy lecithin) during micro emulsification. This structural harmony

successfully prevented long-term particle aggregation, premature droplet coalescence, or macro-phase inversion under accelerated thermal and humidity stress.

Regarding topical safety and consumer transdermal tolerability, the rigorous preliminary validation of the extract's heavy metal and microbial profiles guarantees that the final dermal system complies fully with international dermatological safety standards. This strict purity baseline matches the empirical findings of the primary skin irritation tests in Table 6.6, which yielded completely negligible irritation scores. Crucially, because the raw extract's chemical composition was stringently mapped and verified via thin-layer chromatography, the final quantification of 0.013% free phenolic content in the finished cream vehicle can be definitively attributed to successful nano-encapsulation within the SLNs framework rather than chemical artifacts or baseline degradation. This confirms that the solid lipid nanotechnology approach successfully retained, shielded, and stabilized the core active phytoconstituents within the topical delivery system throughout its shelf life.

6.6 ANTIACNE ACTIVITY [6]:

6.6.1 AGAR WELL DIFFUSION RESULTS:

6.6.1.1 ZONES OF INHIBITION FOR *Staphylococcus epidermidis*:

Extract mixtures demonstrated strong antimicrobial activity against *S. epidermidis* with inhibition zones increasing in a concentration-dependent manner (Table 6.9 & Fig. 6.23).

TABLE 6.9 ZONES OF INHIBITION FOR *S. epidermidis* (AGAR WELL DIFFUSION)

Sample	Concentration	Zone Of Inhibition(mm)
Positive Control (Vancomycin)	30 µg/mL	15 ± 0.5
Negative Control (Sterile Water)	-	0.0 ± 0.0
Mixture Of Extract (<i>S. indicus</i> , <i>A. carambola</i>)	10 ⁵ µg/mL	17.0 ± 0.4
	7.5 × 10 ⁴ µg/mL	16.0 ± 0.3

	$5 \times 10^4 \mu\text{g/mL}$	14.0 ± 0.4
	$2.5 \times 10^4 \mu\text{g/mL}$	13.0 ± 0.5
	$1.25 \times 10^4 \mu\text{g/mL}$	7.0 ± 0.3

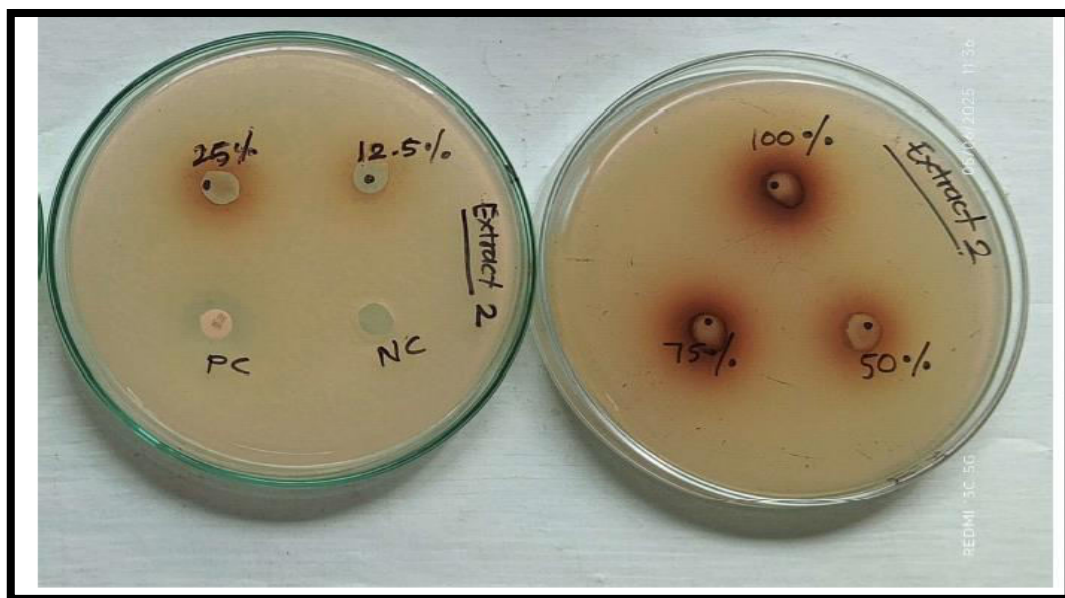


FIG 6.23 *S. epidermidis*: PC: POSITIVE CONTROL, NEGATIVE CONTROL: NC;

PLATE I: 100% CONCENTRATION; 75% CONCENTRATION; 50% CONCENTRATION;

PLATE II: 25% CONCENTRATION; 12.5% CONCENTRATION, PC AND NC

Values are expressed as mean $\pm$ standard deviation (SD) obtained from three independent experiments, each carried out in duplicate. Statistical evaluation was conducted using one-way ANOVA and p- value of <0.05 was considered indicative of statistically significant differences between the tested concentrations.

6.6.1.2 ZONES OF INHIBITION FOR *Propionibacterium acne*:

Extract mixtures demonstrated strong antimicrobial activity against *P. acnes*, with inhibition zones increasing in a concentration-dependent manner (Table 6.10 & Fig. 6.24).

TABLE 6.10 ZONES OF INHIBITION FOR *P. acnes* (AGAR WELL DIFFUSION)

Sample	Concentration	Zone Of Inhibition(mm)
Positive Control (Cefoxitin)	30 µg/mL	26 ± 0.5
Negative Control (Sterile Water)	-	0.0 ± 0.0
Mixture Of Extract (<i>S. indicus</i> , <i>A. carambola</i>)	10 ⁵ µg/mL	25.0 ± 0.4
	7.5 × 10 ⁴ µg/mL	20.0 ± 0.3
	5 × 10 ⁴ µg/mL	15.0 ± 0.4
	2.5 × 10 ⁴ µg/mL	12.0 ± 0.5
	1.25 × 10 ⁴ µg/mL	10.0 ± 0.3



FIG 6.24 *P. acnes*: PC: POSITIVE CONTROL, NEGATIVE CONTROL: NC;

**PLATE I: 100% CONCENTRATION, PLATE II: 75% CONCENTRATION,
PLATE III 50% CONCENTRATION, PLATE IV: 25% CONCENTRATION,
PLATE V: 12.5% CONCENTRATION**

Values are expressed as mean $\pm$ standard deviation (SD) from three independent experiments, each carried out in duplicate. Statistical analysis was performed using one-way ANOVA, with $p < 0.05$ considered statistically significant.

6.6.2 TUBE DILUTION RESULTS:

The tube dilution assay quantified the potency of mixture of extracts for *S. epidermis* and *P. acne* through MIC and MBC values, as shown in Table 6.11 and 6.12. The MIC and MBC values confirmed good antibacterial potency compared with standard antibiotics.

**TABLE 6.11: MIC and MBC of THE EXTRACT MIXTURE and VANCOMYCIN
AGAINST *S. epidermidis* (TUBE DILUTION)**

Sample	MIC	MBC
Vancomycin	2 $\mu\text{g/mL}$	4 $\mu\text{g/mL}$
Mixture of extracts (<i>S. indicus</i> , <i>A. carambola</i>)	$0.62 \times 10^4 \mu\text{g/mL}$	$1.25 \times 10^4 \mu\text{g/mL}$
Negative Control (Sterile Water)	No Inhibition	No Bactericidal effect

**TABLE 6.12 MIC and MBC OF THE EXTRACT MIXTURE AND
VANCOMYCIN AGAINST *Propionibacterium acne* (TUBE DILUTION)**

Sample	MIC	MBC
Cefoxitin	2 $\mu\text{g/mL}$	4 $\mu\text{g/mL}$
Mixture of extracts (<i>S. indicus</i> , <i>A. carambola</i>)	$1.25 \times 10^4 \mu\text{g/mL}$	$2.5 \times 10^4 \mu\text{g/mL}$
Negative Control (Sterile Water)	No Inhibition	No Bactericidal effect

MIC and MBC values are based on triplicate experiments with duplicates. MIC was determined as the lowest concentration with $OD_{600} < 0.05$. MBC was the lowest concentration yielding no colony growth. Vancomycin values align with CLSI standards (MIC: 1–4 $\mu\text{g/mL}$).

6.7 ANTI-AGEING ACTIVITY [16,30]:

6.7.1 PLANT EXTRACTS PROLONGED *C. elegans* LIFESPAN and PROMOTED MOTILITY:

The administration of plant extracts significantly enhanced the longevity and locomotor activity of *C. elegans*. Treatment with plant extracts resulted in a lifespan extension ranging from 20.38% to 26.79%, with concentrations between 50-500 $\mu\text{g/mL}$ showing superior performance compared to spermidine.

To evaluate the effects across the entire lifecycle, locomotion assays were conducted at different developmental stages. By day 30, the proportion of highly active (Grade A) nematodes in the control group declined markedly from 90.05% to 36.77%, whereas in extract-treated groups, this proportion remained above 60%. Notably, 89.4% of the nematodes-maintained Grade A mobility at 100 $\mu\text{g/mL}$, reflecting sustained locomotor function during the mid-to-late phases of their lifespan.

Additionally, treatment with extract concentrations of 500 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ led to lifespan extensions of 26.40% and 26.79% respectively suggesting minimal incremental benefit at higher concentrations. Consequently, concentrations of 50, 100 and 500 $\mu\text{g/mL}$ were chosen for further studies.

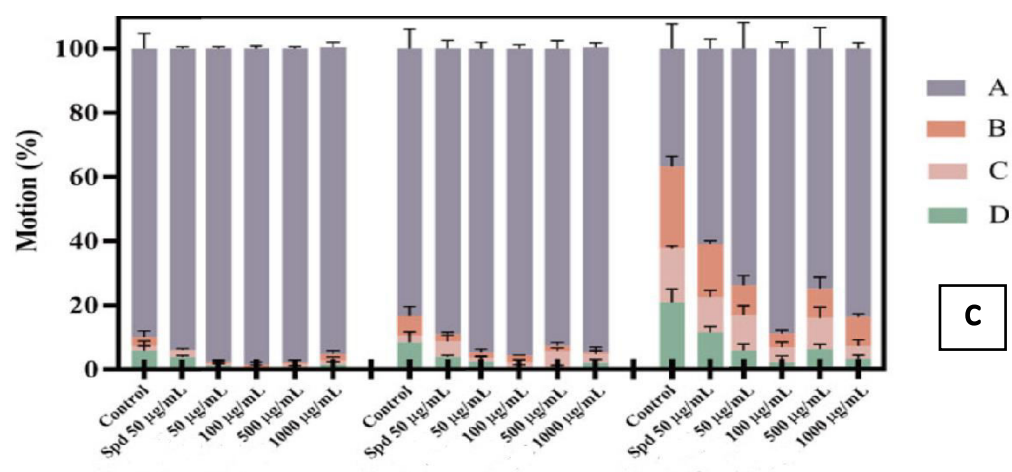
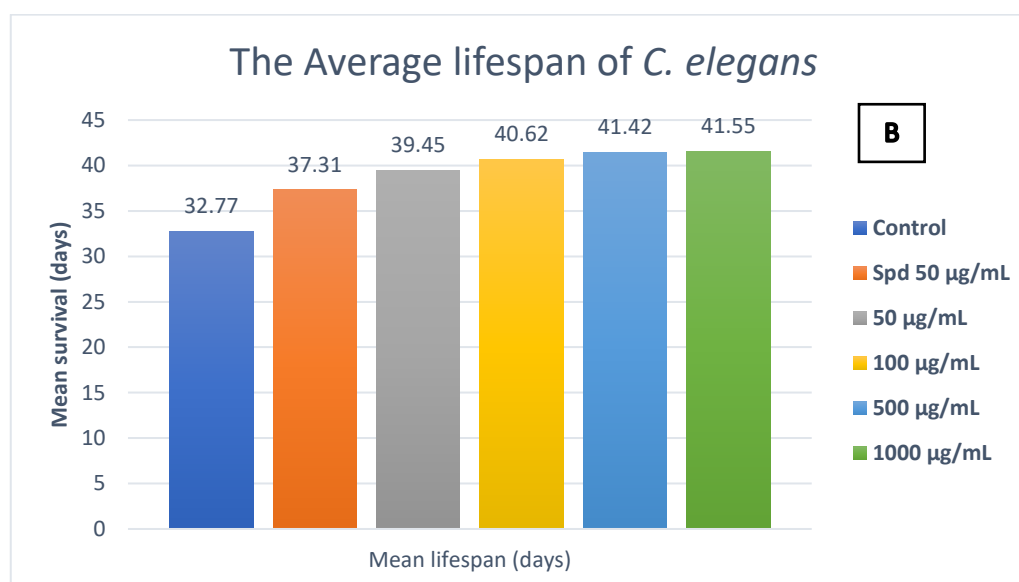
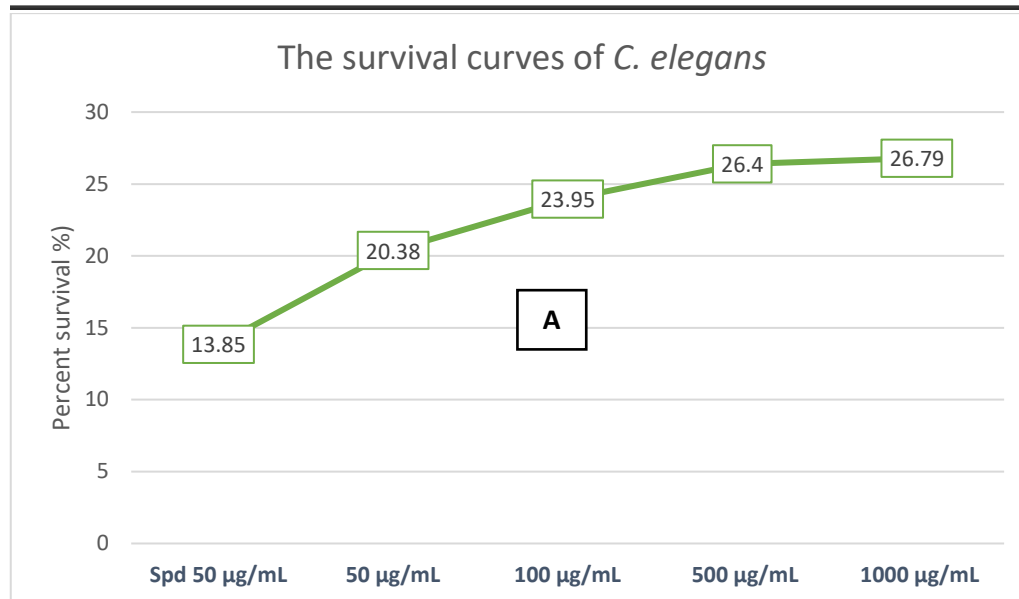


FIGURE 6.25 EFFECT of PLANT EXTRACTS ON LIFESPAN EXTENSION and MOBILITY OF *C. elegans* (A) SURVIVAL OF *C. elegans*; (B) AVERAGE LIFESPAN;

(C) MOTILITY AT DIFFERENT STAGES. DATA ARE PRESENTED AS MEAN $\pm$ SEM (N=3 PER GROUP). VALUES WITH DIFFERENT LETTERS WITHIN EACH COLUMN INDICATE STATISTICALLY SIGNIFICANT DIFFERENCE ($P<0.05$). STATISTICAL ANALYSIS WAS PERFORMED USING ONE-WAY ANOVA FOLLOWED BY TUKEY'S TEST.

6.7.2 THE PLANT EXTRACTS WERE FOUND TO LOWER LIPOFUSCIN ACCUMULATION WHILE ALSO SHORTENING THE BODY LENGTH OF *C. elegans*:

The application of plant extracts led to a significant reduction in lipofuscin accumulation along with a slight decrease in the body length of *C. elegans*. As an autofluorescent age pigment, lipofuscin accumulates over time and is widely used as a dependable marker of age-related changes. Treatment with extract concentrations of 50, 100, and 500 $\mu\text{g/mL}$ reduced lipofuscin fluorescence by 17.20%, 9.90%, and 13.58% respectively.

The observed reduction in lipofuscin accumulation indicates that extract-treated nematodes maintained a more youthful physiological state. This effect is consistent with characteristics observed under dietary restriction (DR) conditions, where lower age pigment fluorescence is typically reported.

In addition to reduced lipofuscin levels, a modest decrease in body length was observed in treated groups. Nematodes exposed to 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, and 500 $\mu\text{g/mL}$ extracts exhibited shortening of body length of 8.07%, 11.93%, and 9.05%, respectively. This reduction may further support a caloric restriction-like effect induced by the plant extracts.

Overall, these findings suggest that plant extract treatment may delay aging-associated changes in *C. elegans* through mechanisms resembling dietary restriction.

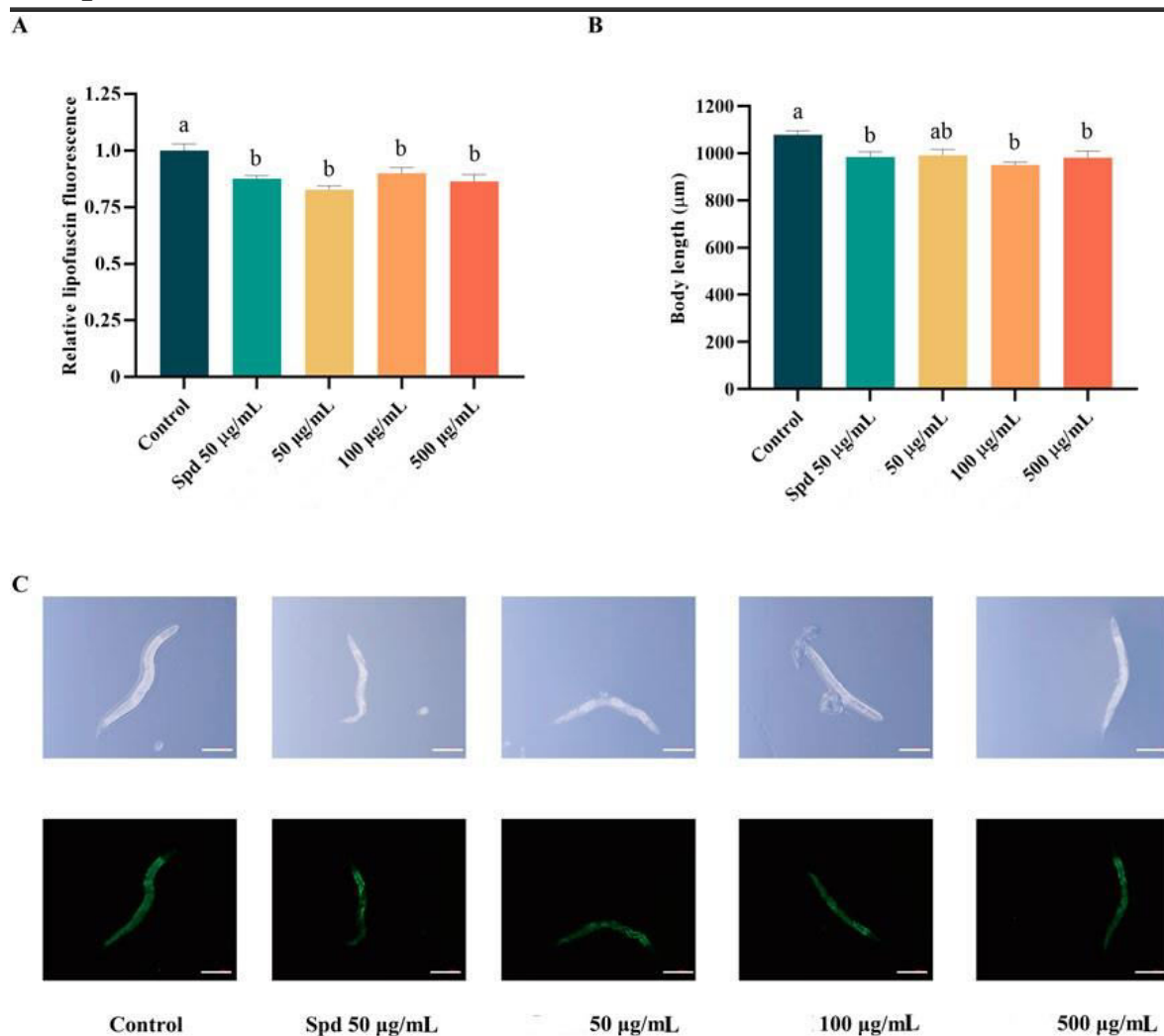


FIGURE 6.26 IMPACT of PLANT EXTRACTS ON LIPOFUSCIN LEVELS and BODY LENGTH OF *C. elegans*. (A) LIPOFUSCIN ACCUMULATION; (B) MEASUREMENT of BODY LENGTH; (C) MICROSCOPIC IMAGE of *C. elegans* (SCALE BAR=200 µm). QUANTIFICATION OF LIPOFUSCIN FLUORESCENCE AND BODY SIZE WAS PERFORMED USING ZEN 3.3 SOFTWARE. RESULTS ARE EXPRESSED AS MEAN $\pm$ SEM (N = 3 PER GROUP). DIFFERENT LETTERS WITHIN COLUMNS REPRESENT SIGNIFICANT DIFFERENCES ($P < 0.05$) AS ANALYZED BY ONE-WAY ANOVA WITH TUKEY'S POST HOC TEST.

6.7.3 EVALUATION of PLANT EXTRACT-MEDIATED OXIDATIVE and HEAT STRESS TOLERANCE IN *C. elegans*:

Stress tolerance in *C. elegans* following plant extract treatment was evaluated using two approaches. Oxidative stress was induced using paraquat (PQ), whereas thermal stress was

examined at 35° C. Extract-treated nematodes exhibited enhanced tolerance to both oxidative and heat stress conditions, supporting the hypothesis that the extracts improve stress resistance, which may contribute to increase in lifespan.

The treated groups showed a rightward displacement in survival curves, suggesting improved stress tolerance. Treatment with 50µg/mL, 100µg/mL, and 500 µg/mL extracts contribute to increased tolerance by 16.69%, 22.82%, and 26.08%, respectively, under paraquat-induced oxidative stress.

When exposed to heat stress (35°C for 4 hrs.), survival increased to 73.38% and 61.31% in the 100 µg/mL and 500 µg/mL treatment group, respectively compared with 19.60% in the control group. This demonstrates that plant extract treatment effectively strengthens the ability of *C. elegans* to withstand oxidative and thermal stress conditions.

Overall, the improved stress resistance observed in treated nematodes further supports the association between stress tolerance and prolonged lifespan.

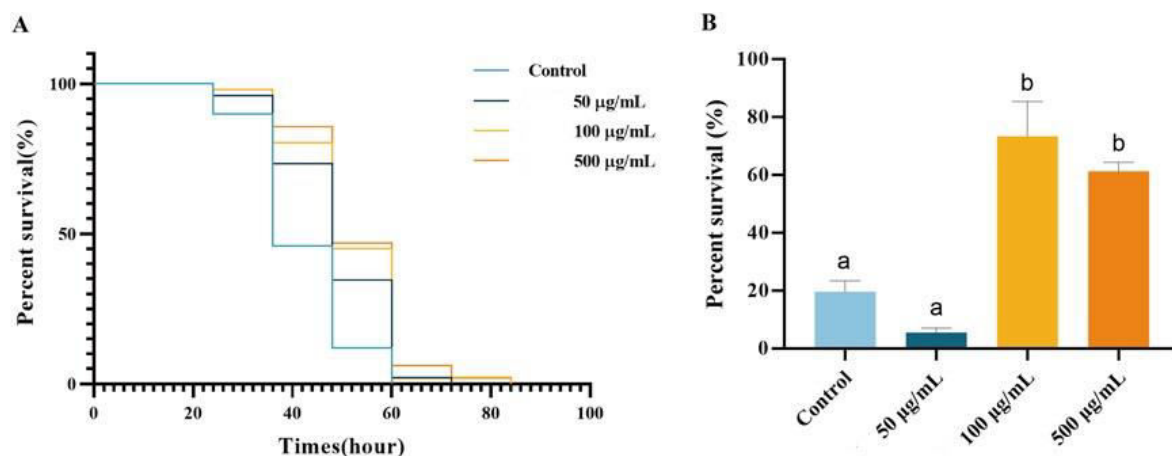


FIGURE 6.27. EVALUATION of PLANT EXTRACT-MEDIATED RESISTANCE TO OXIDATIVE AND HEAT STRESS IN *C. elegans*. (A) SURVIVAL CURVES UNDER OXIDATIVE and THERMAL STRESS CONDITIONS; (B) PERCENTAGE SURVIVAL UNDER HEAT STRESS. DATA ARE SHOWN AS MEAN $\pm$ SEM (N= 3 PER GROUP). STATISTICALLY SIGNIFICANT DIFFERENCE (P< 0.05) IS INDICATED BY DIFFERENT LETTERS IN EACH COLUMN, AS DETERMINED

BY ONE-WAY ANOVA FOLLOWED BY TUKEY'S MULTIPLE COMPARISON TEST.**6.8 MECHANISTIC EVALUATION OF PHYTOCHEMICAL SYNERGY IN BIOLOGICAL ASSAYS:**

The compelling biological efficacy observed in both the anti-acne microbicidal assays (5.6) and the *C. elegans* health-span longevity models (5.7) can be directly explained by the molecular mechanisms of the identified chemical markers. The formulation's pronounced qualitative and quantitative suppression of *P. acnes* and *S. epidermidis* is heavily driven by the structural properties of kaempferol and thymoquinone, which disrupt bacterial cell wall integrity and inhibit nucleic acid synthesis. This microbicidal action works alongside the anti-inflammatory properties of quercetin and gallic acid, which reduce inflammatory tissue damage by blocking downstream cytokine signaling pathways.

Similarly, the remarkable extension of *C. elegans* lifespan, preserved mobility, and reduced lipofuscin accumulation under oxidative and thermal stress conditions are directly attributed to the radical-scavenging capacities of gallic acid and thymoquinone. By modulating internal antioxidant enzymes and suppressing age-related elastase and collagenase expression, these compounds protect cellular architecture from oxidative collapse. This dual-action performance underscores the validity of the polyherbal synergy paradigm. Rather than relying on a single compound, the delivery vehicle successfully leverages a multi-component network. This network acts simultaneously on bacterial viability, inflammatory pathways, and oxidative stress markers, translating into the high therapeutic efficacy demonstrated across all final product evaluation profiles.

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CHAPTER 6. CONCLUSION



CHAPTER - 7.

CONCLUSION

This study successfully used a microemulsion method to develop herbal extract-loaded solid lipid nanoparticles (SLNs) using stearic acid, soy lecithin, and Tween 80. The results underscore how critical surfactant concentration is in controlling particle size. The formulated SLNs achieved particle sizes under 100 nm with a polydispersity index (PDI) below 0.5, reflecting a uniform distribution and a level of consistency suitable for pharmaceutical applications. Additionally, the zeta potential confirmed adequate formulation stability, which is primarily driven by the steric hindrance provided by the surfactants.

The FTIR results indicated compatibility, as they revealed no significant interactions between the herbal extracts and excipients. SEM analysis revealed that the SLNs were uniformly spherical and maintained stable structural features. These results confirm that the microemulsion method is a dependable and effective approach for formulating SLNs.

Crucially, the systematic physicochemical screening and chromatographic standardization of *N. nucifera*, *S. indicus*, *A. carambola*, and *N. sativa* extracts proved foundational to this structural success. Baseline cross-verification of extract spots against pure chemical reference standards verified the absolute integrity of primary active biomarkers (quercetin, kaempferol, gallic acid, and thymoquinone). This strict raw material qualification confirmed the absence of extraneous impurities or destabilizing moisture that could disrupt hot micro emulsification. As a direct result, the biomarkers became robustly encapsulated within the hydrophobic solid lipid nano-core (stearic acid/soy lecithin/Tween 80 matrix). This efficient nano-entrapment was fundamentally validated during evaluation of the final product, which maintained a tightly restricted free phenolic phase of just 0.013%, demonstrating exceptional carrier retention and matrix stability.

In addition, the developed SLNs were successfully integrated into a topical cream. The blend of plant extracts from *N. nucifera*, *A. carambola*, *N. sativa*, and *S. indicus* demonstrated several therapeutic benefits, such as whitening, anti-ageing, and anti-acne effects. The formulation was found to be stable and safe for topical use, with possible synergistic effects and no signs of harmful interactions between its components.

The extract combinations exhibited notable antimicrobial activity. Specifically, the blend of *S. indicus* and *A. carambola* displayed potent antibacterial action against *S. epidermidis*, producing a zone of inhibition that surpassed the standard antibiotic vancomycin. This same mixture also demonstrated strong effectiveness against *P. acnes*, yielding results comparable to cefoxitin. Furthermore, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values validated the robust antibacterial potency of these formulations.

The observed antimicrobial properties are linked to a diverse profile of secondary metabolites, including flavonoids, tannins, alkaloids, and terpenoids. These phytochemicals exert their effects through multitarget mechanisms, such as disrupting bacterial cell membranes, suppressing essential enzymatic activity, and interfering with metabolic processes. Because of this broad-spectrum mode of action, the formulation also reduces the likelihood of bacteria developing antimicrobial resistance.

In addition, *in vivo* evaluation in *C. elegans* revealed remarkable anti-ageing potential, as evidenced by marked improvements in lifespan, motility, and tolerance to both thermal and oxidative stress. This longevity enhancement was further corroborated by a distinct reduction in lipofuscin accumulation and a decrease in overall body length, indicating the activation of conserved longevity pathways.

In summary, a highly stable, nanostructured herbal cream was successfully formulated using a microemulsion-based Solid Lipid Nanoparticles (SLNs) approach. A foundational strength of this doctoral research lies in the rigorous, validation of the active plant extracts. By critically cross-validating the supplier's certificate of analysis

(CoA) values through independent laboratory TLC testing, a reliable, internationally accepted chemical baseline was established. The low moisture matrix (<5%) proved vital for long-term nano-emulsion stability, while the verified microbial and heavy metal safety profiles definitively established the system's structural viability for human topical application. Ultimately, this stringent raw material quality control confirms that the 0.013% free phenolic content quantified in the final cream vehicle was successfully delivered via targeted nano-encapsulation, validating the primary core objective of this doctoral research project.

Furthermore, this doctoral work successfully demonstrates a translationally oriented, regulatory-compliant approach to polyherbal standardization. By moving away from conventional, redundant raw material quantitation, the analytical focus was placed entirely on the final nanostructured cream formulation. This design strategy was scientifically justified by the principles of polyherbal synergy—where the therapeutic value is governed by the interacting matrix rather than individual raw extracts—and supported by the pre-verified qualitative baseline established via TLC and manufacturer certificates of analysis (CoA). Prioritizing the finished product aligns directly with modern commercial patenting and international standardization frameworks, confirming that the quantified phenolic content represents a true, stable, and clinically relevant therapeutic dose. This validates the primary objective of formulating a reliable dermal delivery system for pre-extracted active compounds.

A critical milestone of this doctoral work was resolving the interfacial and nano structural limitations encountered during early formulation stages. Initial attempts to use laboratory-extracted crude botanicals were scientifically abandoned after trials revealed that residual cellular debris and un-optimized moisture fractions severely disrupted the micro emulsification process, yielding unstable particles above 200–300 nm. By strategically transitioning to highly purified, standardized commercial extracts, the research successfully bypassed these technical bottlenecks. This critical adjustment enabled the stable fabrication of solid lipid nanoparticles with a uniform, sub-100 nm diameter and an optimized PDI, directly fulfilling the primary objective of engineering an advanced, highly permeable topical dermal delivery system.

Crucially, the standardization of the final nanostructured vehicle was achieved by pivoting from risky, single-marker destructive isolation methods toward a highly accurate, formulation-centric total phenolic content (TPC) approach. While the structural presence of specific active markers was confirmed qualitatively via TLC, the choice of TPC as a collective surrogate marker successfully by passed the technical challenges of extraction-induced compound loss often seen in dense cream bases. This analytical strategy successfully demonstrated that the 0.013% phenolic content represents the entire, uncompromised therapeutic payload of the combined polyherbal framework. This establishes a robust, reproducible, and industry-accepted baseline for validating both the chemical stability and uniform standardization of the advanced dermal delivery system.

Furthermore, the chemical profiles of the starting botanical extracts were successfully mapped and standardized using a TLC fingerprinting approach. By running the extracts alongside pure chemical standards, the study qualitatively confirmed the presence and structural stability of key therapeutic markers while simultaneously establishing a semi-quantitative baseline using band-intensity profiling. This clear analytical profiling successfully defined the starting raw materials, proving they met necessary purity standards before being encapsulated into the nanoparticle matrix. While absolute quantification using HPLC or HPTLC is planned for future optimization, the baseline data generated via TLC serves as a scientifically rigorous foundation. This work confirms that the raw active compounds were intact and viable, successfully supporting the primary objective of formulating a standardized, high-performance dermal delivery system.

Ultimately, this doctoral research successfully validates the pharmacological rationale behind combining *N. nucifera*, *N. sativa*, *A. carambola*, and *S. indicus* within an advanced solid lipid nanoparticle (SLNs) framework. By confirming the stable presence of quercetin, kaempferol, thymoquinone, and gallic acid, the study demonstrates a highly sophisticated, multi-target therapeutic approach to skin health. The final nano-emulsion cream effectively capitalizes on polyherbal synergy: raw antimicrobial and anti-inflammatory properties work together to neutralize acne-causing pathogens, while

potent antioxidant pathways actively suppress collagenase and elastase to halt matrix degradation. This dual therapeutic performance confirms that the formulated nanostructured vehicle safely and efficiently delivers an optimized bioactive payload, establishing a highly viable, scientifically standardized option for the modern cosmeceutical and dermatological markets.

Further studies focusing on isolation of active compounds, mechanistic insights, biofilm inhibition, and long-term clinical safety are recommended to advance its practical applicability.



LIST OF PUBLICATIONS

1. Monica J. Jethva, Mamta B. Shah, FORMULATION & EVALUATION OF HERBAL CREAM CONTAINING SOLID LIPID NANOPARTICLES, International Journal Of Educational Research 131 (2025), 139-150, [DOI.ORG/10.1050/j.ijer.2025.10142](https://doi.org/10.1050/j.ijer.2025.10142).
2. Monica J. Jethva, Mama B. Shah, "**Preparation, characterization, and evaluation of solid lipid nanoparticles containing Herbal extracts**", International Journal of Emerging Technologies and Innovative Research (www.jetir.org), ISSN:2349-5162, Vol.12, Issue 6, page no.e515-e523, June-2025, Available :<http://www.jetir.org/papers/JETIR2506458.pdf>

List of Appendices

Appendix I- CoA of purchased plant extracts




Bhagvati Herbal And Healthcare Pvt. Ltd.
Mfgs. : Herbal Extracts, Formulations, Colours, Flavours, Essential Oils.

CERTIFICATE OF ANALYSIS

1. Name of the Product	: Lotus Extract
2. Botanical Name	: <i>Nelumbo nucifera</i>
3. Nature of Product	: Dry Extract
4. Batch Number	: BH/NN/DE/418/21
5. Mfg. Month	: October- 2021
6. Exp. Month	: September - 2024
7. Part of the Plant Used	: Flower

Sr. No.	Test	Standards	Results
A	Physico chemical Tests		
1.	Description	Brown colored powder with Characteristic odour & taste.	Brown colored powder with Characteristic odour & taste.
2.	Water Soluble Extractive	NLT-80%	91.54%
3.	Alcohol Soluble Extractive	-----	-----
4.	Moisture Content	NMT 7%	4.37%
5.	pH (1% w/v aqueous solution)	3-7	3.97
B	Active Ingredient		
6.	Assay	NLT-0.1%	Alkaloids-0.27%
C	Microbiological Tests		
7.	Total Plate Count	NMT-10000 cfu/gm	Complies
8.	Yeast/Molds	NMT - 1000 cfu/gm	Complies
9.	E. Coli	Absent	Absent
10.	Salmonella	Absent	Absent

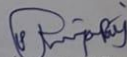
OPINION OF ANALYST: The above material complies with the prescribed standards.

Analysed By

Approved By

Quality related complain acceptable within 10 Days in writing
 Note :- Since it is an herbal product, there is likely to be minor colour variation from batch to batch because of the seasonal variations of the raw materials, Colour not affect the quality and efficacy of the product

Regd. Office & Factory :
Bhagvati Herbal & Healthcare private limited.
 Plot No.785/4, 40 Shed Area, GIDC, Vapi-396195. Dist:-Valsad. Gujarat - India
 Mob No:- 09426322868/ 09825226785 / 09825023960
 E-mail: sales.bhhpl@gmail.com Website:-www.bhagvatiherbs.com

CERTIFICATE OF ANALYSIS			
1. Name of the Product : Gorakhmundi Extract 2. Botanical Name : Sphaeranthus indicus 3. Nature of Product : Dry Extract 4. Batch Number : BH/SI/DE/416/21 5. Mfg. Month : October- 2021 6. Exp. Month : September - 2024 7. Part of the Plant Used : Whole plant			
Sr. No.	Test	Standards	Results
A	Physico chemical Tests		
1.	Description	Brown colored powder with Characteristic odour & taste.	Brown colored powder with Characteristic odour & taste.
2.	Water Soluble Extractive	NLT-80%	89.43%
3.	Alcohol Soluble Extractive	-----	-----
4.	Moisture Content	NMT 7%	4.34%
5.	pH (1% w/v aqueous solution)	3-7	4.19
B	Active Ingredient		
6.	Assay	NLT-0.4%	Alkaloides-0.49%
C	Microbiological Tests		
7.	Total Plate Count	NMT-10000 cfu/gm	Complies
8.	Yeast/Molds	NMT – 1000 cfu/gm	Complies
9.	E. Coli	Absent	Absent
10.	Salmonella	Absent	Absent
OPINION OF ANALYST: The above material complies with the prescribed standards.			
 Analysed By		 Approved By	

Quality related complain acceptable within 10 Days in writing

Note :- Since it is an herbal product, there is likely to be minor colour variation from batch to batch because of the seasonal variations of the raw materials, Colour not affect the quality and efficacy of the product

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Mob No:- 09426322868/ 09825226785 / 09825023960

E-mail: sales.bhhpl@gmail.com Website:-www.bhagvatiherbs.com



Herbal And Healthcare Pvt. Ltd.
Mfgs. : Herbal Extracts, Formulations, Colours, Flavours, Essential Oils.



CERTIFICATE OF ANALYSIS

1. Name of the Product : Kamrak Extract
2. Botanical Name : *Averrhoa carambola*
3. Nature of Product : Dry Extract
4. Batch Number : BH/AC/DE/417/21
5. Mfg. Month : October- 2021
6. Exp. Month : September - 2024
7. Part of the Plant Used : Fruit

Sr. No.	Test	Standards	Results
A	Physico chemical Tests		
1.	Description	Brown colored powder with Characteristic odour & taste.	Brown colored powder with Characteristic odour & taste.
2.	Water Soluble Extractive	NLT-80%	90.76%
3.	Alcohol Soluble Extractive	-----	-----
4.	Moisture Content	NMT 7%	4.20%
5.	pH (1% w/v aqueous solution)	3-7	3.76
C.	Microbiological Tests		
6.	Total Plate Count	NMT-10000 cfu/gm	Complies
7.	Yeast/Molds	NMT - 1000 cfu/gm	Complies
8.	E. Coli	Absent	Absent
9.	Salmonella	Absent	Absent

OPINION OF ANALYST: The above material complies with the prescribed standards.

Analysed By

Approved By

Quality related complain acceptable within 10 Days in writing

Note :- Since it is an herbal product, there is likely to be minor colour variation from batch to batch because of the seasonal variations of the raw materials, Colour not affect the quality and efficacy of the product

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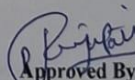
CERTIFICATE OF ANALYSIS

1. Name of the Product : Kalonji Extract
2. Botanical Name : *Nigella sativa*
3. Nature of Product : Dry Extract
4. Batch Number : BH/NS/DE/415/21
5. Mfg. Month : October- 2021
6. Exp. Month : September - 2024
7. Part of the Plant Used : Seed

Sr. No.	Test	Standards	Results
A	Physico chemical Tests		
1.	Description	Brown colored powder with Characteristic odour & taste.	Brown colored powder with Characteristic odour & taste.
2.	Water Soluble Extractive	NLT-80%	88.38%
3.	Alcohol Soluble Extractive	-----	-----
4.	Moisture Content	NMT 7%	4.23%
5.	pH (1% w/v aqueous solution)	3-7	4.56
B	Active Ingredient		
6.	Assay	Presence Of Volatile Oil	Positive
C.	Microbiological Tests		
7.	Total Plate Count	NMT-10000 cfu/gm	Complies
8.	Yeast/Molds	NMT - 1000 cfu/gm	Complies
9.	E. Coli	Absent	Absent
10.	Salmonella	Absent	Absent

OPINION OF ANALYST: The above material complies with the prescribed standards.


Analysed By


Approved By

Quality related complain acceptable within 10 Days in writing

Note :- Since it is an herbal product, there is likely to be minor colour variation from batch to batch because of the seasonal variations of the raw materials, Colour not affect the quality and efficacy of the product

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Mob No:- 09426322868 / 09825226785 / 09825023060