

DEVELOPMENT OF HERBAL FORMULATIONS FOR SKIN CARE

Ph.D. Synopsis

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Title: Development of herbal formulations for skin care.

Abstract:

Nano-cosmetics are formulations that incorporate nanomaterials, which possess at least one dimension between 1 and 100 nm. These nanomaterials can enhance the performance, stability, and delivery of active ingredients. Herbal nano-cosmetic formulations combine the therapeutic benefits of herbal ingredients with the improved drug delivery potential of nanotechnology. The aim of the present research work was to formulate and evaluate a herbal cream containing solid lipid nanoparticles (SLNs). 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*, *Averrhoa carambola*, *Nigella sativa*, and *Sphaeranthus indicus*.

SLNs loaded with herbal extracts (kalonji, Gorakhmundi, Kamrakh, lotus) were synthesized using the microemulsion method, and their physicochemical properties were evaluated. Particle size was measured using a particle size analyser, while morphology was assessed by scanning electron microscopy (SEM). Compatibility between the pure extracts and SLN-loaded extracts was evaluated using FTIR. SEM images confirmed the spherical morphology of the SLNs. The mean particle size of the formulations ranged from 48–68 nm, 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 (O/W) herbal cream. The oil phase consisted of stearic acid (4%), glyceryl monostearate (2%), and Cetyl alcohol (2%), melted at 70 °C. The aqueous phase—containing paraffin oil (8%), Tween 80 (2%), triethanolamine (0.25%), and water (77.75%)—was also heated to 70 °C. The aqueous phase was added to the oil phase with continuous stirring, followed by cooling to room temperature. Perfume and methyl paraben (preservative) were added at the end, and 4% of the SLN–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.

Brief description on the state of the art of the research topic:

Nowadays, common skincare problems often arise from a combination of environmental factors, lifestyle habits, and underlying health conditions. The most prevalent issues include acne, dryness or eczema, hyperpigmentation, and signs of premature ageing. Acne is one of the most widespread concerns, affecting both teenagers and adults due to clogged pores, excess sebum production, bacterial growth, and hormonal fluctuations. Adult acne, which frequently appears around the jawline and chin, is often triggered by stress or the use of comedogenic (pore-clogging) skincare and makeup products. Signs of ageing—such as fine lines, wrinkles, and sagging skin—can be accelerated by sun exposure, smoking, and an unhealthy diet. A major contributing factor is the gradual decline in natural collagen production, which typically begins in the mid-twenties.

In recent years, herbal cosmeceuticals have gained significant popularity because they do not contain synthetic chemical ingredients that may cause adverse effects. These products utilise various plant parts and extracts and are increasingly preferred over chemical-based formulations. Many individuals, especially women, opt for herbal skincare products because they are perceived as safer, provide essential nutrients to the skin, promote overall skin health, and generally have fewer side effects. Herbal cosmetics have become a modern trend in the beauty and personal care industry.

Compared to conventional formulations, nano-cosmetics provide a more efficient and targeted approach to skincare. Nanoparticles enable precise delivery of active ingredients to specific skin layers, improving efficacy and enhancing treatment outcomes for various skin concerns.

In the present work, we developed a herbal skincare formulation containing solid lipid nanoparticles (SLNs). The nanoparticles were prepared using the microemulsion method, and a simple procedure was used to formulate an oil-in-water (O/W) herbal cream. In vitro anti-acne activity was evaluated against *Staphylococcus epidermidis* and *Propionibacterium acnes*, while anti-ageing potential was assessed using *C. elegans*. The main objective of this research was to develop a safe and effective herbal skincare product with enhanced therapeutic performance.

Definition of the Problem:

Acne is a common skin condition that occurs when hair follicles become clogged with excess oil and dead skin cells, leading to the development of blackheads, whiteheads, pimples, or deeper cystic lesions. It most frequently appears on the face, neck, chest, and back. Although acne is most common during adolescence due to hormonal fluctuations, it can affect individuals of all ages.

The primary goals of acne treatment are to prevent scarring, reduce the duration and severity of the condition, and minimize its recurrence. Effective management also aims to lessen the psychological impact of acne, which affects at least half of those suffering from the condition. Therefore, strong clinical evidence demonstrating the efficacy of therapeutic agents plays a crucial role in guiding appropriate acne treatment and improving patient outcomes.

Ageing is a natural process characterized by gradual physical, emotional, and mental changes that occur as we grow older. The term can also refer to the process of something reaching maturity or becoming suitable for use, such as *the ageing of cheese*, or even the intentional process of making an object appear older for aesthetic purposes.

As our age grow, our skin undergoes noticeable changes as well. The skin gradually loses elasticity, moisture, and firmness due to reduced collagen and elastin production. These changes lead to common signs of ageing such as fine lines, wrinkles, dryness, uneven tone, and sagging.

Objectives and Scope of study:

The present study is an effort to develop and evaluate herbal skincare products by combining well known medicinal plants having anti-aging and anti-acne activity, which besides being safe and efficacious, would be able to overcome the drawbacks of existing synthetic products and treatment options available.

The present research focused on developing combinations of plant-derived extracts rich in phytoconstituents through both in vitro study.

The results underscore the potent antimicrobial activity of the extract mixtures, likely attributable to their rich phytochemical content, including flavonoids, tannins, saponins, sesquiterpenoids, alkaloids, and terpenoids. Their multitargeted action also lowers the

likelihood of resistance development.

The extracts demonstrated strong activity against *S. epidermidis* and *P. acnes*, both of which are major opportunistic pathogens associated with biofilm formation and antibiotic resistance. The extract's performance, comparable to or exceeding certain standard antibiotics, suggests its potential as a natural antimicrobial agent for topical formulations, wound dressings, or antimicrobial coatings.

Treatment with the extracts significantly prolonged lifespan, enhanced mobility, and improved both oxidative and heat stress tolerance. Additionally, a measurable reduction in lipofuscin levels was observed compared to the control group. Reduced pharyngeal pumping and shorter body length further supported the anti-ageing effects, indicating physiological responses aligned with longevity pathways.

Overall, this study confirms that the developed herbal SLN-based cream possesses significant anti-acne, antimicrobial, and anti-ageing activity and holds strong potential for future therapeutic and cosmetic applications.

Original contribution by the thesis:

The Contributions of our studies include:

1. The particle size and polydispersity index (PDI) of the solid lipid nanoparticle (SLN) formulations were determined using dynamic light scattering (DLS) with a Zetasizer Nano-ZS (Malvern Instruments, UK). The zeta potential of the formulations was measured using a Horiba Scientific SZ-100 system. Morphological characteristics of the SLNs were examined through scanning electron microscopy (SEM) using a JEOL JSM-7600F field emission SEM. Fourier-transform infrared spectroscopy (FTIR) was performed using a Shimadzu FTIR 7800A instrument to identify functional groups and evaluate possible chemical interactions within the formulation.
2. We have performed stability study parameters for solid lipid nanoparticles. The changes that transpired over the 9 months test period have been characterized. Zeta-potential measurements of SLNs fluctuated over the 9 months period with the changes per formulation but it is in acceptable range. At the end of the 9-month testing period, all the average particle sizes were measured. PDI values of all SLNs were also checked.
3. We have performed chromatographic study for all of our plant extracts. 2g of accurately weighed formulation was refluxed with methanol for 1hr and then filtered. This filtrate is

used for TLC spotting. The chromatographic profile of plant extracts and their bioactive constituents was developed and compared with the formulations.

4. We formulated and evaluated a skin care product intended for the management of acne and aging. The cream was prepared using a simple emulsification method. Physicochemical parameters of the formulation were assessed to determine its quality and performance. Stability studies were conducted at room temperature and at accelerated conditions (40 °C / 75% RH) for a period of 90 days.

5. Anti-acne activity of the plant extracts was evaluated against *Staphylococcus epidermidis* and *Propionibacterium acnes* using the agar well diffusion method and the tube dilution method.

For the agar well diffusion assay, test concentrations of 100%, 75%, 50%, 25%, and 12.5% (w/v) were prepared by dissolving the extract in sterile distilled water. The zones of inhibition were measured for each concentration to assess antibacterial activity.

For the tube dilution method, twofold serial dilutions were prepared from 100% to 0.78% (w/v), resulting in concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and 0.78%. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined based on visible growth inhibition and absence of bacterial growth, respectively.

6. Anti-aging activity was evaluated using *Caenorhabditis elegans* (*C. elegans*), a widely recognized model organism in aging and stress-biology research. The nematodes were treated with the plant extracts, and several parameters related to aging were assessed. Oxidative stress and heat stress tolerance assays were performed to determine the protective effects of the extracts under stress-induced conditions. In addition, lifespan and mobility analyses were conducted to evaluate their impact on longevity and locomotor activity. Body length measurements and lipofuscin accumulation levels were also determined as indicators of physiological aging in *C. elegans*.

Methodology of Research and Results:

1. All plants extracts were collected from Bhagvati Herbals & Healthcare private limited, Vapi.

Solid Lipid Nanoparticles were prepared using microemulsion technique. Stearic acid was placed into a conical flask, which was kept in a water-filled porcelain dish and heated on a magnetic stirrer at 70 °C. The drug extract, Tween 80, and soy lecithin were then added

and stirred at 1000 rpm until completely mixed. The resulting microemulsion was diluted dropwise with ice-cold water and stirred at 3000–3100 rpm for 2–3 hours in a cold-water bath. The prepared suspension was sonicated for half an hour. Sonicated suspension was centrifuged at 14500 RPM at 8°C for 5 minutes to separate probable nanoparticles and the nanoparticles were collected using methanol and dried until a completely dry powder was obtained.

The SLNs were lyophilized to prolong the shelf-life of the Herbal extract-loaded SLNs. Lyophilization of particles was performed using 5% (w/v) mannitol as cryoprotectant to minimize the risk of particle aggregation. SLN's emulsion was frozen at -40°C for 24 hours, and then lyophilization was done. Particle size, zeta potential, and polydispersity index were evaluated after freeze-drying again to ensure no significant size enlargement was happening.

The mean particle size and polydispersity index of the SLN formulations were measured using dynamic light scattering (DLS) (Zeta Sizer Nano-ZS; Malvern Instruments) method. Zeta potential was measured using a Horiba Scientific SZ-100. The morphological examination of SLNs was performed by SEM (JEOL JSM 7600F FE-SEM). FTIR is used for identifying functional groups, studying chemical reactions. FTIR was done by FTIR 7800A- A model from Shimadzu.

PS ranged from 48-68 nm & PDI ranged from 0.17-0.37. The volume distribution showed that approximately 82%, 95%, 98%, and 100% of particles were within a narrow size range for lotus, Kamrakh, kalonji, and Gorakhmundi extracts, respectively. All formulations exhibited PDI values < 0.5, indicating a uniform and stable particle size distribution.

The measured potential is the most appropriate potential, which is known as the zeta potential. Zeta potential of the prepared SLNs ranged from -5.0 mV to -19.1 mV. All formulations showed a negative zeta potential which is expected due to the presence of fatty acid residues from stearic acid and the non-ionic nature of Tween 80. All SLNs were found to be negatively charged due to fatty acid residues of stearic acid. SEM images revealed that all SLNs were near-spherical, smooth-surfaced, and uniformly distributed.

The particle sizes were consistently below 100 nm, validating the DLS results. The

SEM results revealed spherical NPs morphology for SLNs, which confirmed the size results with no evident sign of aggregation. The IR spectra of pure drug extract show principal peaks that are mostly near the principal peaks of the prepared SLNs. For Gorakhamundi, SLNs show major peaks at 2833.94, 2109.77, 1641.28, 1491.30, 1245.63, 1081.37, 1027.09, & 941.68 cm^{-1} , which are mostly near the principal peaks of pure drug extract. For Kalonji, SLNs show major peaks at 2818.23, 1648.42, 1498.44, 1349.90, 1252.77, 1084.23, 1027.09 & 942.82 cm^{-1} , which are mostly near the principal peaks of pure drug extract. For Kamrakh, SLNs show major peaks at 2109.77, 1654.13, 1349.90, 1257.06, 1084.23, 1031.38 & 947.11 cm^{-1} , which are mostly near the principal peaks of pure drug extract. For Lotus, SLNs show major peaks at 2923.93, 1655.56, 1475.69, 1349.90, 1296.62, 1252.77, 1099.94, 1024.24, 948.53 & 710.00 cm^{-1} , which are mostly near the principal peaks of pure drug extract. The major characteristic peaks of the extracts were retained in the SLN formulation without significant shifts, confirming the absence of major chemical interactions between extracts and excipients (stearic acid, soy lecithin, Tween 80). Therefore, the components were found to be compatible.

SLNs	Particle Size (nm)	PDI
Gorakhamundi	56.90	0.172
Kalonji	48.77	0.251
Kamrakh	50.18	0.285
Lotus	68.75	0.374

Table PARTICLE SIZE & PDI VALUES

SLNs	Zeta Potential value(mv)
Gorakhamundi	-5.0
Kalonji	-17.8
Lotus	-19.1
Kamrakh	-9.1

Table ZETA POTENTIAL VALUES FOR SLNs

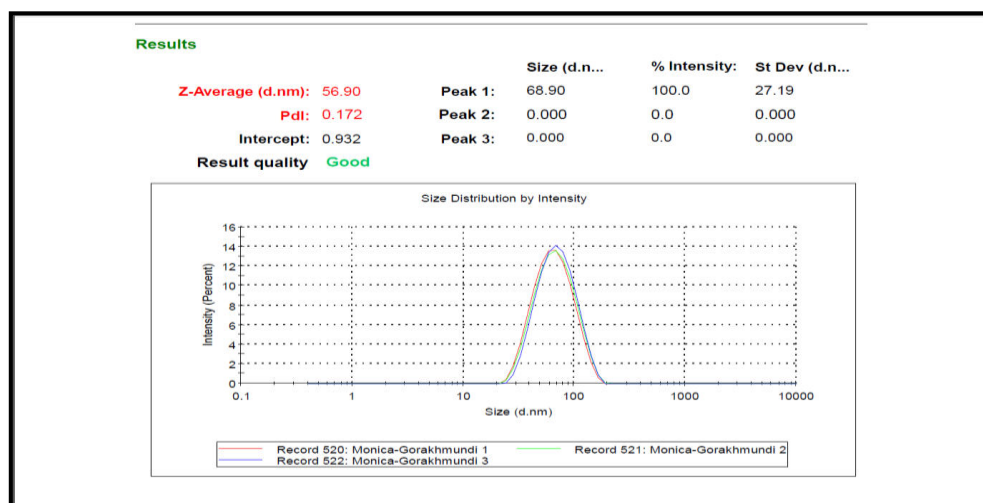


Fig - PARTICLE SIZE & PDI REPORT OF GORAKHMUNDI

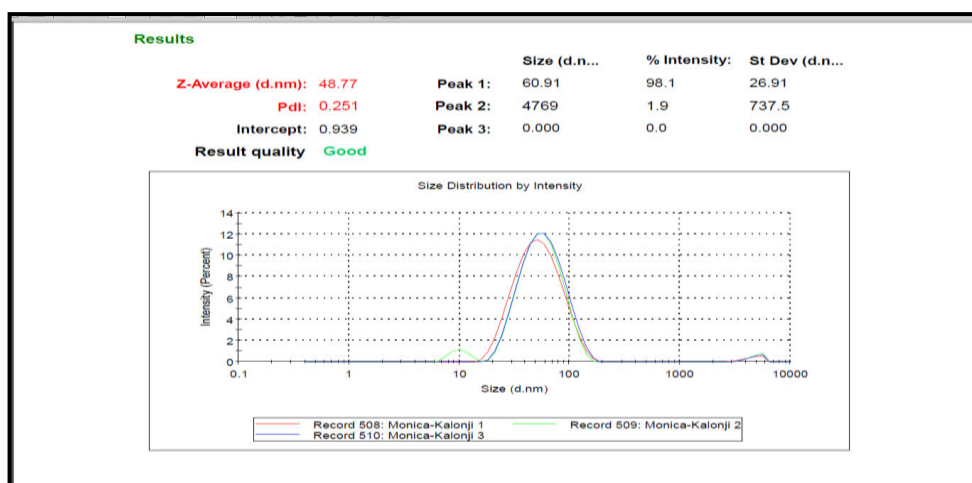


Fig - PARTICLE SIZE & PDI REPORT OF KALONJI

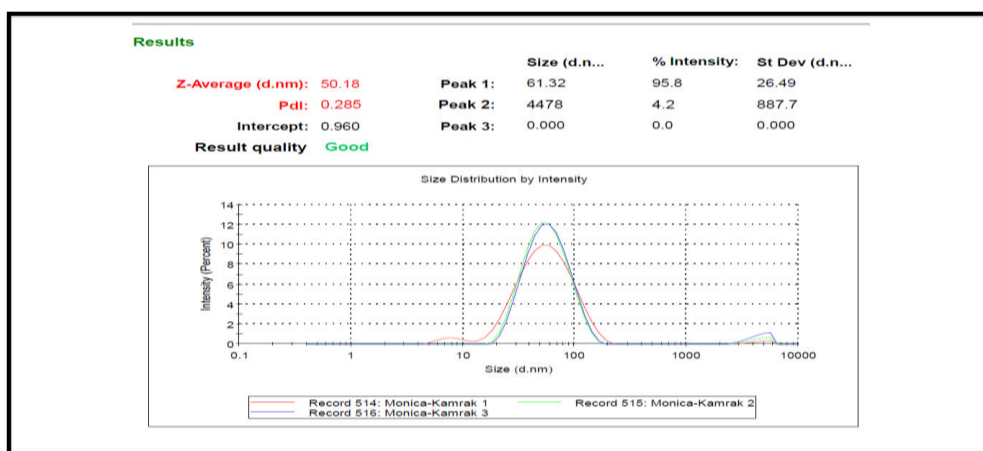


Fig - PARTICLE SIZE & PDI REPORT OF KAMRAKH

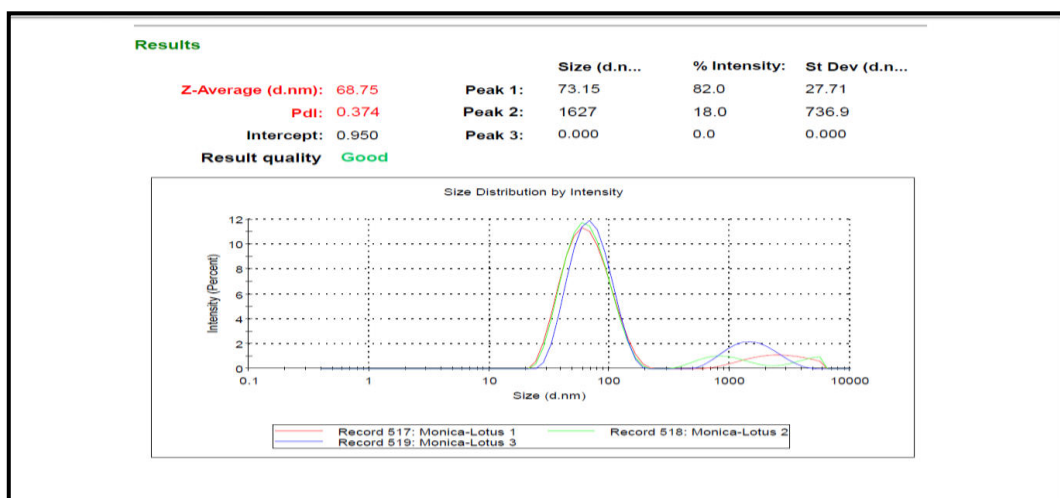


Fig - PARTICLE SIZE & PDI REPORT OF LOTUS

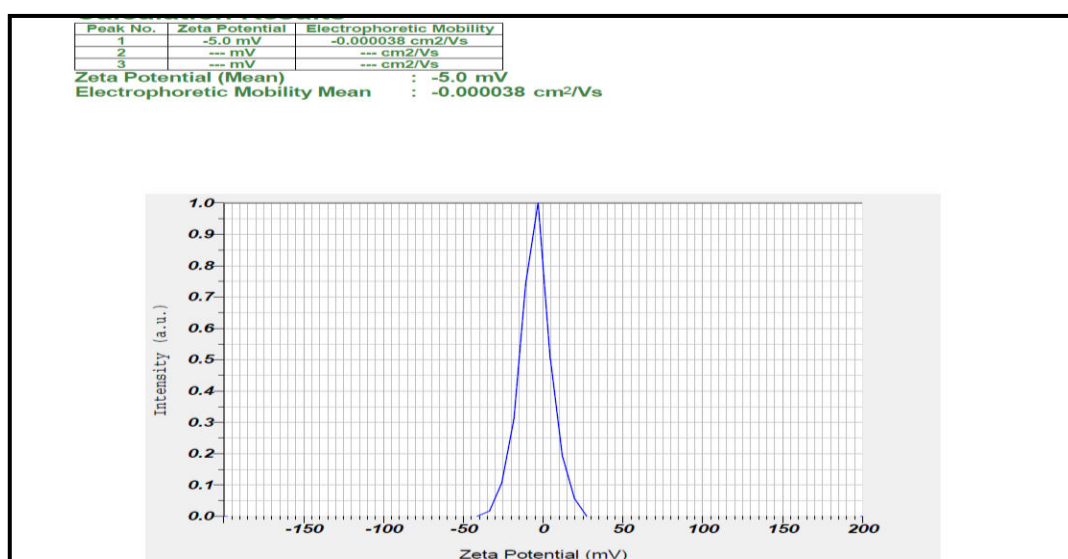


Fig - ZETA POTENTIAL REPOT OF GORAKHMUNDI

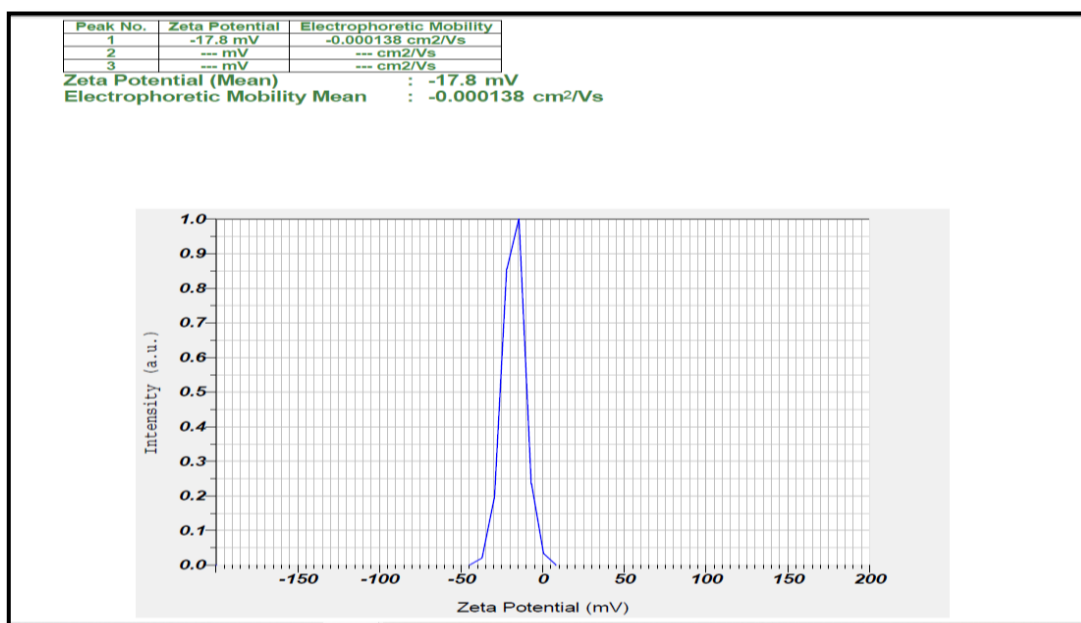


Fig - ZETA POTENTIAL REPORT FOR KALONJI

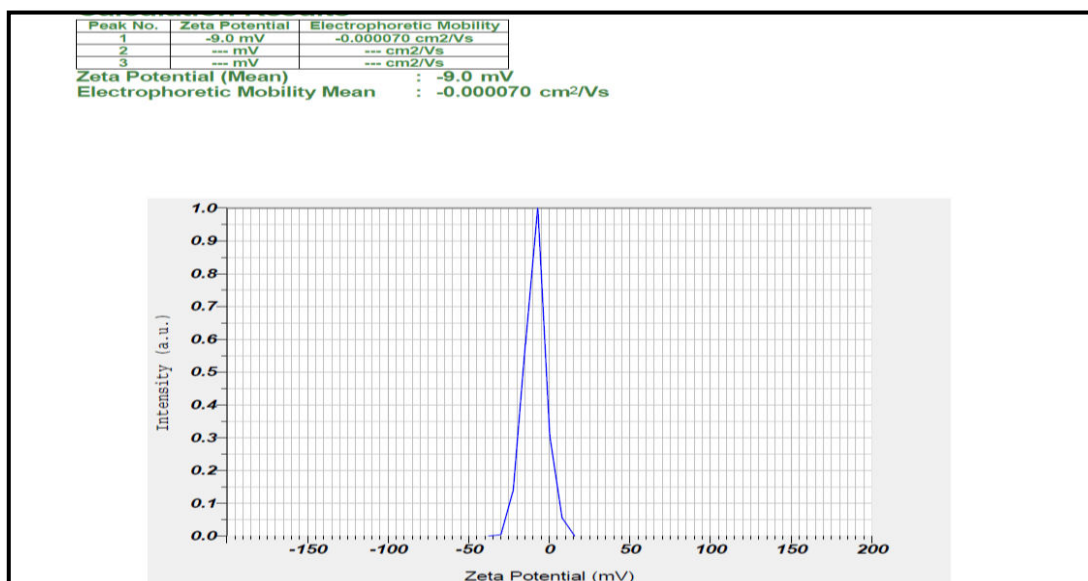


Fig - ZETA POTENTIAL REPORT FOR LOTUS

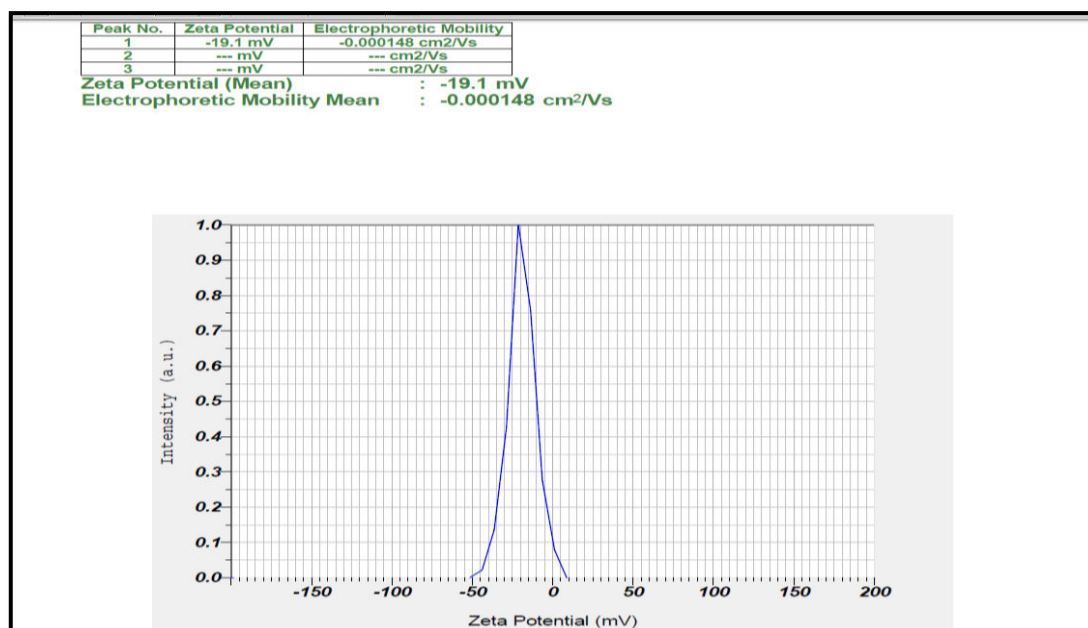


Fig - ZETA POTENTIAL REPORT FOR KAMRAKH

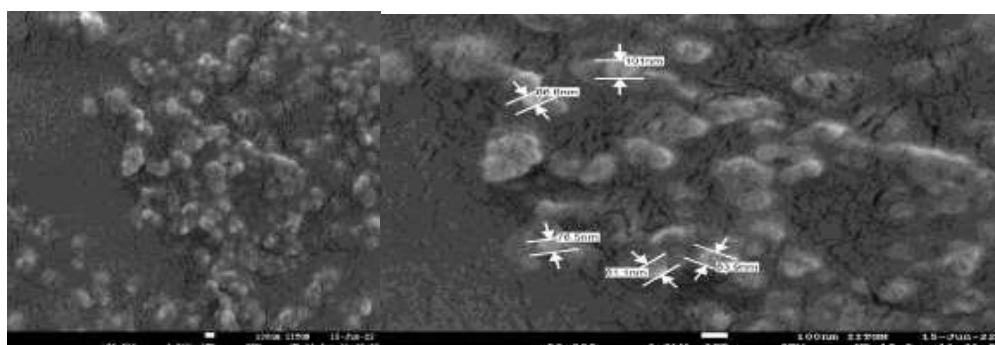


Fig - SEM IMAGES OF KALONJI



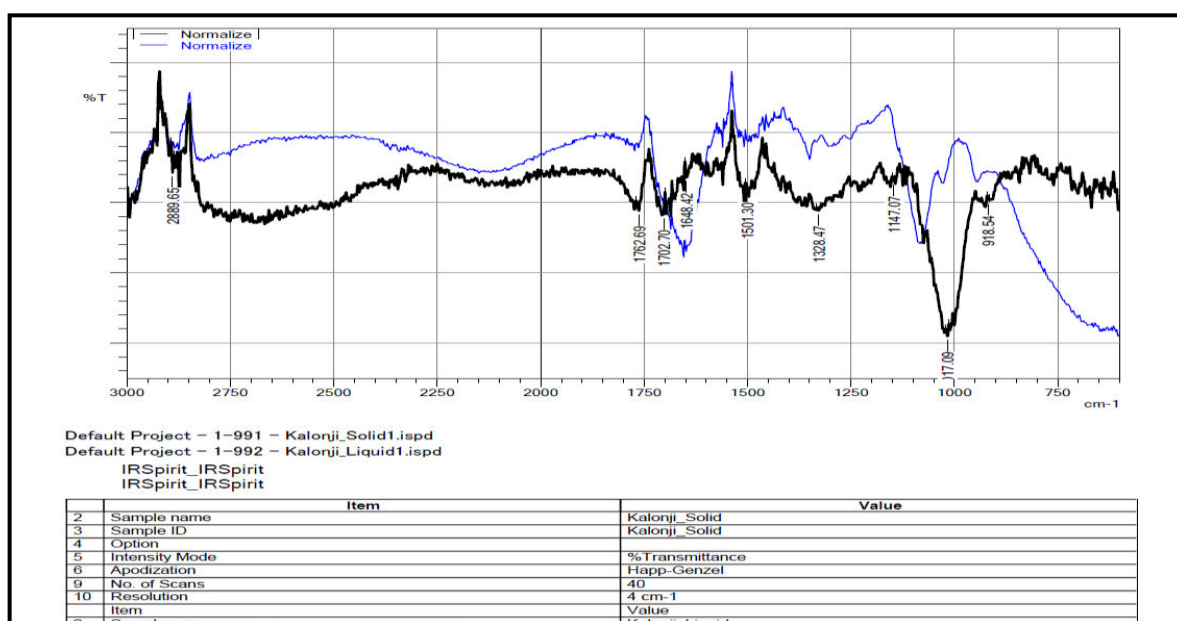


Fig - FTIR REPORT OF KALONJI

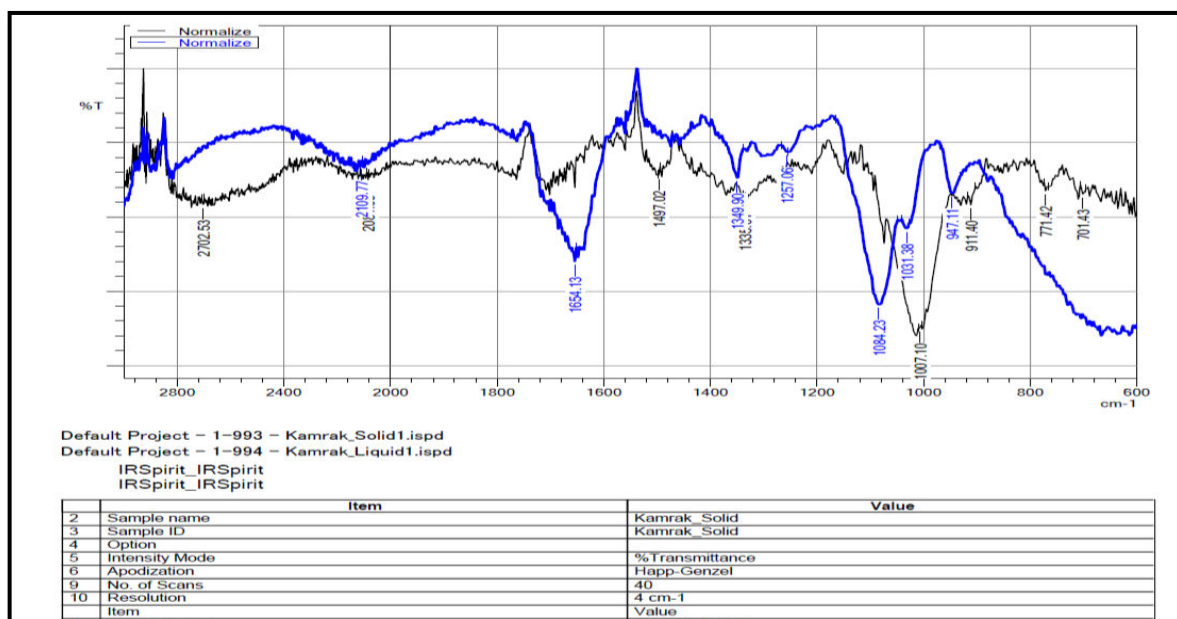


Fig - FTIR REPORT OF KAMRAKH

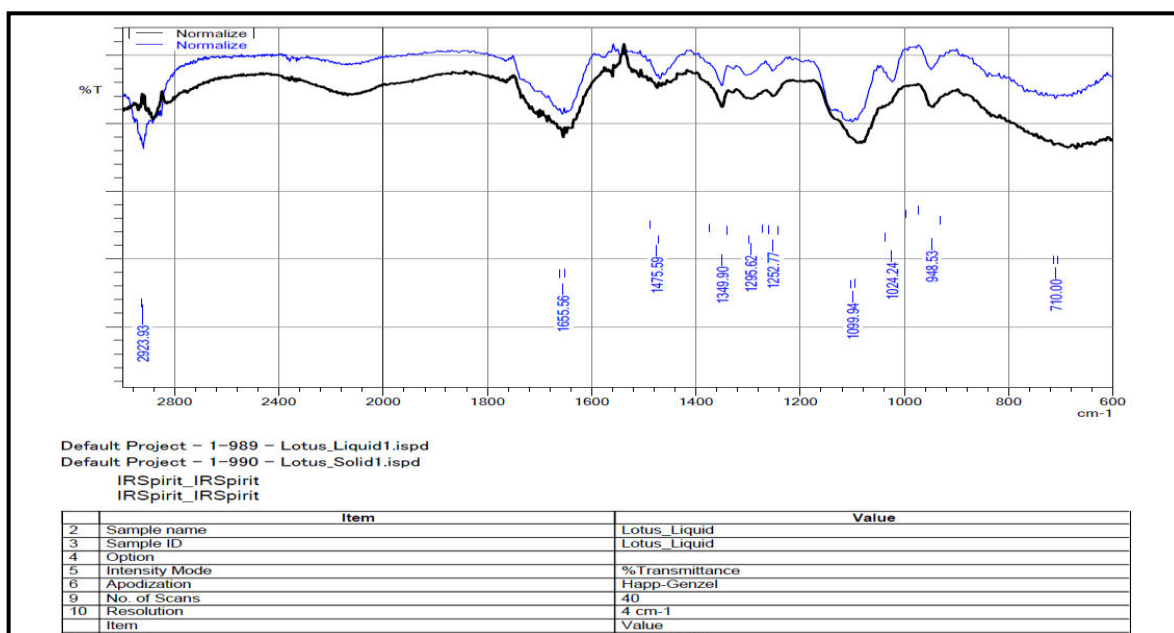


Fig - FTIR REPORT OF LOTUS

2. Stability Parameters of SLNs: The changes that transpired over the 9-month test period have been characterized. Zeta-potential measurements of SLNs fluctuated over the 9 months, with the changes per formulation ranging from -3.72 mV to -7.82 mV, but all values remained within an acceptable range. Particle size remained **below 100 nm**, and PDI values (**0.30–0.49**) indicated that all formulations retained acceptable stability. SLNs stored at 4°C demonstrated better stability as compared to SLNs stored at room temperature.

SN	SLNs	ZP (mv) (3 months)	ZP (mv) (6 months)	ZP (mv) (9 months)
1	Gorakhmundi	-5.17	-4.03	-3.99
2	Kalonji	-7.14	-6.89	-6.71
3	Kamrakh	-7.56	-7.48	-7.29
4	Lotus	-6.82	-6.31	-6.28

Table ZETA POTENTIAL STABILITY PARAMETERS

SN	SLNs	PS (nm) (3 months)	PS (nm) (6 months)	PS (nm) (9 months)
1	Gorakhmundi	84.07	83.59	83.76
2	Kalonji	77.64	71.40	71.20
3	Kamrakh	72.20	71.83	71.40

4	Lotus	92.19	94.53	92.19
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Table PARTICLE SIZE STABILITY PARAMETERS

SN	SLNs	PDI (3 months)	PDI (6 months)	PDI (9 months)
1	Gorakhmundi	0.425	0.422	0.411
2	Kalonji	0.302	0.321	0.313
3	Kamrakh	0.360	0.360	0.357
4	Lotus	0.493	0.474	0.486

Table PDI STABILITY PARAMETERS

3. TLC study of extracts: Each extract and its marker compound showed distinct single spot, confirming the presence of phytoconstituents and suitability for fingerprinting.

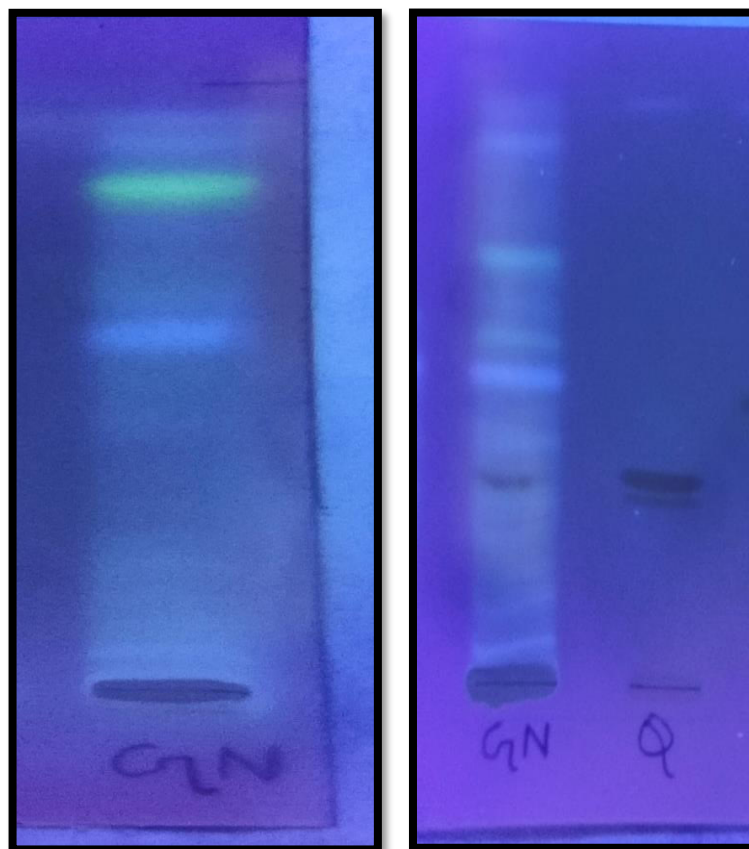


Fig- Fingerprint analysis TLC & Identification of Quercetin for *Sphaeranthus indicus* UNDER 366 nm

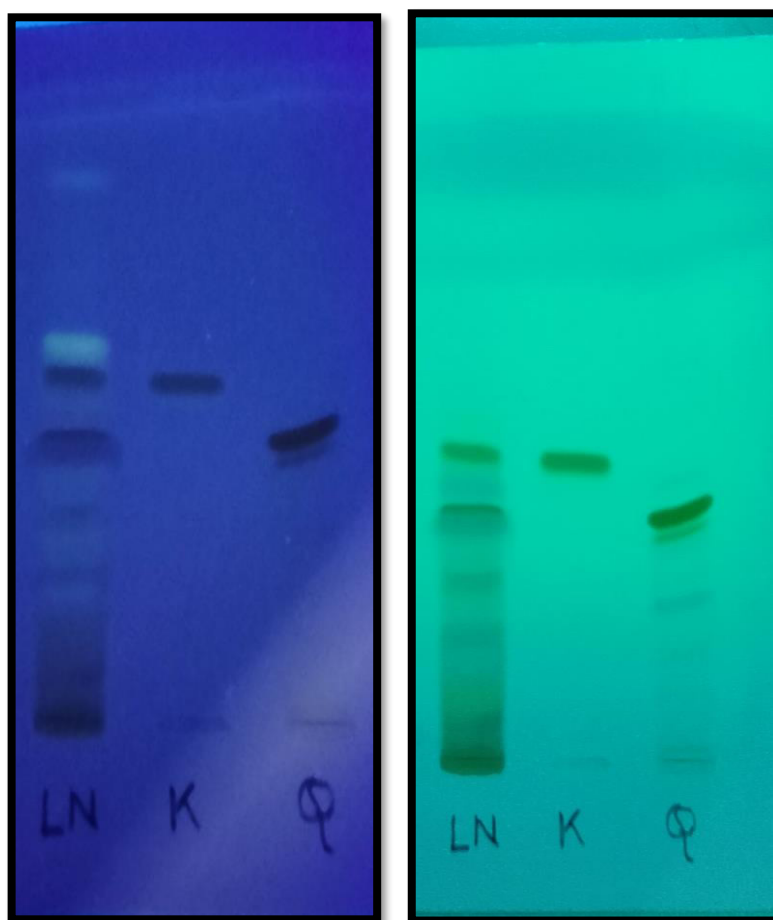


Fig - TLC study of Quercetin & Kaempferol in *Nelumbo nucifera* under 366 nm & under day light

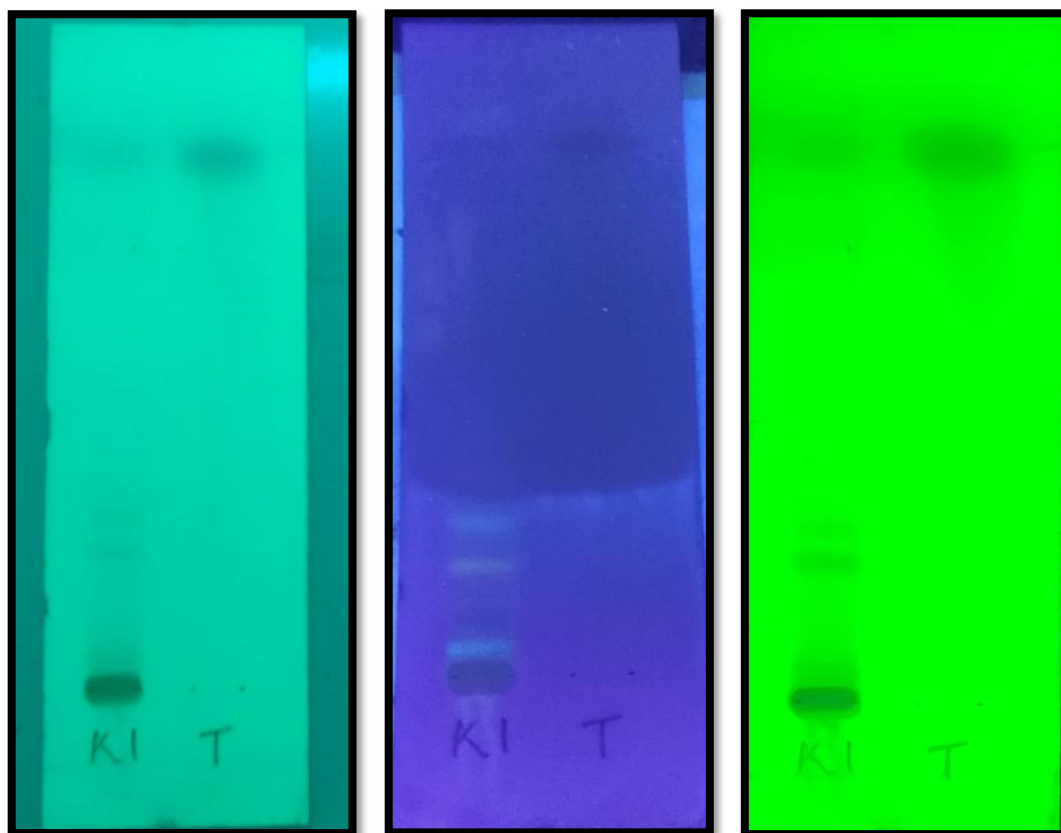


Fig - TLC study of Thymoquinone in *Nigella sativa* under day light, 366 nm & 254 nm



Fig - TLC study of Gallic acid in *Averrhoa carambola* under day light

4. Development of skin care product: The oil phase (stearic acid, glyceryl monostearate, and Cetyl alcohol) was melted at 70 °C. The aqueous phase (paraffin oil, Tween 80, triethanolamine, and water) was separately heated to 70 °C. The aqueous phase was added to the oil phase with continuous stirring. The mixture was cooled to room temperature with continuous stirring. After cooling, 4% SLN extract mixture was incorporated. Perfume and methyl paraben were added at the end before transferring the cream to suitable containers.

5. Physio chemical evaluation of the formulation: Physicochemical parameters like pH, Spreadability, acid value, etc. were carried out. Optimum physicochemical parameters are obtained as a result.

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

Table PHYSICOCHEMICAL EVALUATION OF CREAM

Days	Phenolic Content
0	0.013%
30	0.015%
60	0.013%
90	0.011%

Table TOTAL PHENOLIC CONTENT OF FORMULATION

The cream exhibited a peak load of 467 g, indicating good firmness. A higher peak load corresponds to a firmer formulation.

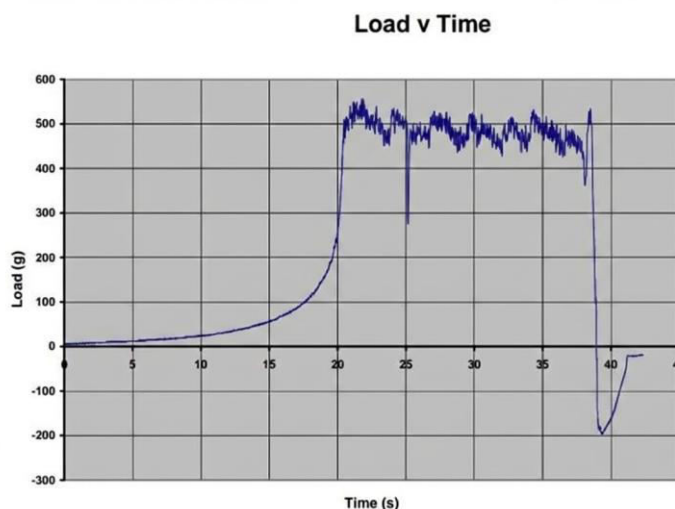


Fig Cream strength curve

6. Stability study of formulation: Stability studies conducted at room temperature and 40 °C / 75% RH for 90 days showed no significant changes in colour, pH, after-feel, or homogeneity. This confirms that the cream formulation is physically stable.

Day	Temperature	Formulations	Parameter			
			Color	pH	After Feel	Homogeneity
0	RT	Cream	NCC	6.02	E	G
	40°C ±2° C 75 RH±2RH	Cream	NCC	6.03	E	G
30	RT	Cream	NCC	6.04	E	G
	40°C ±2° C 75 RH±2RH	Cream	NCC	6.03	E	G
60	RT	Cream	NCC	6.02	E	G
	40°C ±2° C 75 RH±2RH	Cream	NCC	6.04	E	G
90	RT	Cream	NCC	6.03	E	G
	40°C ±2° C 75 RH±2RH	Cream	NCC	6.02	E	G

Table STABILITY STUDIES OF FORMULATION

NCC: Not change in colour,

E: Emollient,

G: Good

7. Anti-acne activity: 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 quantitative determination of MIC and MBC, ensuring a comprehensive evaluation of the extract's efficacy.

By using Agar well diffusion method, Extract mixtures demonstrated strong antimicrobial activity against *Staphylococcus epidermidis* and *Propionibacterium acnes* with inhibition zones increasing in a concentration-dependent manner.

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 (Gorakhmundi, Kamrakh)	100% (w/v)	17.0 ± 0.4
	75% (w/v)	16.0 ± 0.3
	50% (w/v)	14.0 ± 0.4
	25% (w/v)	13.0 ± 0.5
	12.5% (w/v)	7.0 ± 0.3

Table Zones of Inhibition for *Staphylococcus epidermidis* (Agar Well Diffusion)

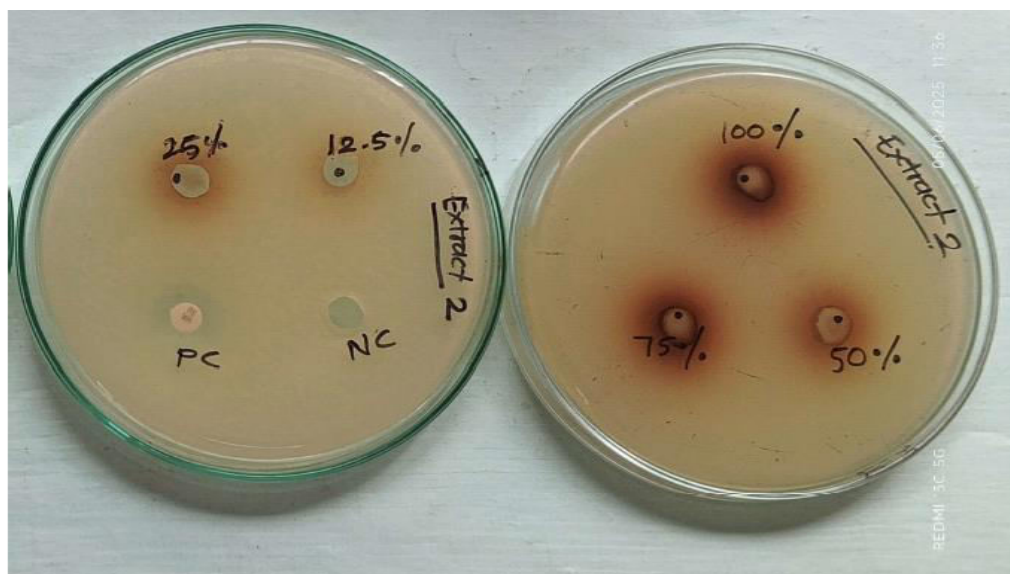


FIG *Staphylococcus Epidermidis*: PC: Positive control, Negative control: NC;
Plate I: 100% Concentration; 75% Concentration; 50% Concentration;
Plate II: 25% Concentration; 12.5% Concentration, PC and NC

Sample	Concentration	Zone Of Inhibition(mm)
Positive Control	30 µg/mL	26 ± 0.5

(Cefoxitin)		
Negative Control (Sterile Water)	-	0.0 ± 0.0
Mixture Of Extract (Gorakhmundi, Kamrakh)	100% (w/v)	25.0 ± 0.4
	75% (w/v)	20.0 ± 0.3
	50% (w/v)	15.0 ± 0.4
	25% (w/v)	12.0 ± 0.5
	12.5% (w/v)	10.0 ± 0.3

Table Zones of Inhibition for *Propionibacterium acne* (Agar Well Diffusion)

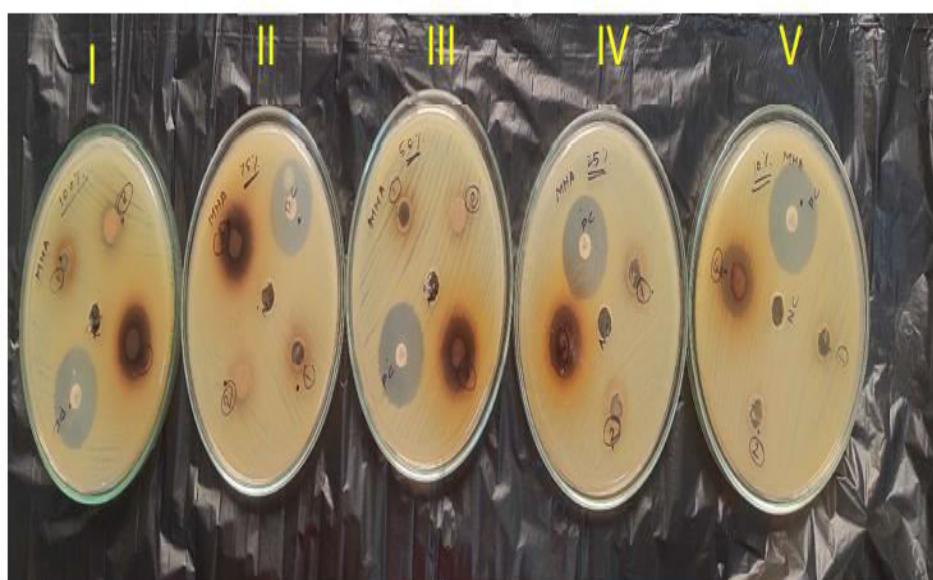


FIG *Propionibacterium acne*: PC: Positive control, Negative control: NC;

Plate I: 100% Concentration, Plate II: 75% Concentration, Plate III 50%

Concentration, Plate IV: 25% Concentration, Plate V: 12.5% Concentration

Values represent the mean ± standard deviation (SD) of three independent experiments, each in duplicate. Statistical analysis (one-way ANOVA, $p < 0.05$) confirmed significant differences between concentrations.

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

Sample	MIC	MBC
Vancomycin	2 µg/mL	4 µg/mL
Mixture of	6.25% (w/v)	12.5% (w/v)

extracts (Gorakhamundi, Kamrakh)		
Negative Control (Sterile Water)	No Inhibition	No Bactericidal effect

**Table MIC and MBC of Mixture Of Extracts and Vancomycin Against
Staphylococcus epidermidis (Tube Dilution)**

Sample	MIC	MBC
Cefoxitin	2 µg/mL	4 µg/mL
Mixture of extracts (Gorakhamundi, Kamrakh)	12.5% (w/v)	25% (w/v)
Negative Control (Sterile Water)	No Inhibition	No Bactericidal effect

**Table MIC and MBC of Mixture Of Extracts and Vancomycin Against
Propionibacterium acne (Tube Dilution)**

MIC and MBC values are based on triplicate experiments with duplicates. MIC was determined as the lowest concentration with OD600 < 0.05. MBC was the lowest concentration yielding no colony growth. Vancomycin values align with CLSI standards (MIC: 1–4 µg/mL).

8. Anti-aging activity: The nematode *Caenorhabditis elegans* (*C. elegans*) is a prominent model organism used to determine anti-aging activity. The *Caenorhabditis elegans* strains (N2) and *E. coli* OP50 used in this study were obtained from NCBS. *Caenorhabditis elegans* was raised on plates of Nematode Growth Medium (NGM) or Supplemented with *E. coli* OP50 and maintained at 20°C. It was kept in a dark or low-light environment for minimizing light reduces unwanted growth (e.g., Mold, algae). Nematodes that exploded, moved to the wall of the plate, and drilled into the medium were eliminated from further data analysis.

Life span and mobility analysis: The specific quantity number of synchronous L4-stage nematodes were transferred onto NGM plates containing OP50 and the extract concentrations (50 µg/mL, 100 µg/ mL, 500 µg/mL, or 1,000 µg/mL), water, or 50

$\mu\text{g/mL}$ spermidine (Spd). A gentle prod with a picker was used to check the survival of the nematodes. And dead individuals were counted daily. The mobility of nematodes was measured at different cohort levels. Plant extracts prolonged *Caenorhabditis elegans* lifespan and promoted mobility.

On the 30th day, in the control group, the proportion of motion Grade A nematodes decreased from 90.05% to 36.77%, while that in the other groups remained greater than 60%.

In the group of extracts at 100 $\mu\text{g/mL}$, the proportion of motion Grade A individuals was 89.04%. This result indicates that treatment of plant extracts prolonged the lifespan of *C. elegans*, moreover, these individuals exhibited better autonomous locomotion at their middle-late life stages.

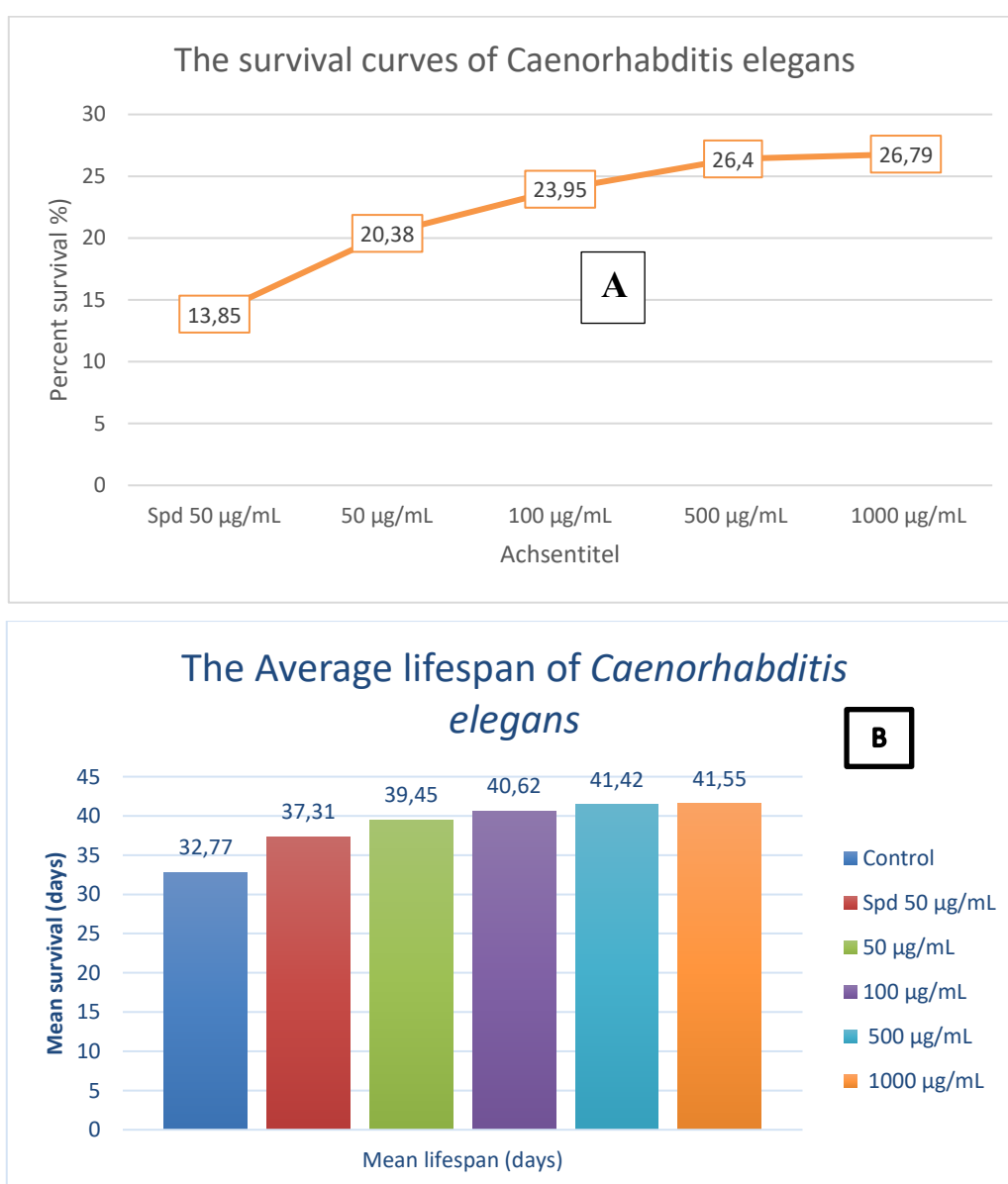


Figure Effects of plant extracts on prolonging the lifespan and promoting mobility

of *Caenorhabditis elegans*. (A) The survival curves of *Caenorhabditis elegans*; (B) The Average lifespan of *Caenorhabditis elegans*.

Determination of body length and lipofuscin level: After 10 days, 10 nematodes treated with 0 µg/mL, 50 µg/mL, 100 µg/mL, or 500 µg/mL of extracts were placed onto a 1% agarose coated microslide. Then, they were anesthetized with levamisole hydrochloride (7 mM). The lipofuscin fluorescence intensity was detected with fluorescence microscopy at GFP channel. The lipofuscin fluorescence intensity and body length of *C. elegans* were assessed with the ImageJ software. Each treatment was implemented three times.

Plant extracts reduced the accumulation of lipofuscin and shortened the body length of *Caenorhabditis elegans*. Auto-fluorescent lipofuscin accumulates with the age of *Caenorhabditis elegans*. Compared with the control group, the relative lipofuscin fluorescence of *Caenorhabditis elegans* was diminished under the 50, 100, and 500 µg/mL doses, yielding 17.20%, 9.90%, and 13.58%, respectively. Extracts **reduced lipofuscin accumulation** (9.9–17.2%) and modestly reduced body length, indicating a possible caloric-restriction-like effect. The body length of the nematodes was also recorded, which was shortened partly by treatment with extracts in 50, 100, and 500 µg/mL doses (by 8.07%, 11.93%, and 9.05%, respectively).

Oxidative and heat stress tolerance assay: *Caenorhabditis elegans* individuals at L4-stage were moved to the NGM plates containing 0 µg/mL, 50 µg/mL, 100 µg/mL, or 500 µg/mL of extracts, and kept at 20°C for 6 days. Extract-treated nematodes showed **greater tolerance** to paraquat-induced oxidative stress and heat stress at 35 °C, supporting the hypothesis that extracts enhance stress resistance, contributing to increased lifespan. The life curves moved rightward with extracts treatment. The 50, 100, and 500 µg/mL extracts resulted in 16.69%, 22.82%, and 26.08% of increments in tolerance, respectively. After heat stress treatment at 35°C for 4 h, the survival rates of the 100 and 500 µg/mL groups were 73.38% and 61.31%, while it was only 19.60% in the control group.

Achievements with Respect to Objectives:

1. Stable solid lipid nanoparticles (SLNs) were successfully developed using the microemulsion method. The particle size (PS) of the formulations ranged from 48 to 68 nm, with polydispersity index (PDI) values between 0.17 and 0.37. Volume distribution

analysis indicated that approximately 82%, 95%, 98%, and 100% of particles fell within a narrow size range for lotus, Kamrakh, kalonji, and Gorakhmundi extracts, respectively. All formulations exhibited PDI values below 0.5, demonstrating a uniform and stable particle size distribution.

The zeta potential of the SLNs ranged from -5.0 mV to -19.1 mV. Morphological analysis showed that the nanoparticles were near-spherical, smooth-surfaced, and uniformly dispersed. FTIR spectra confirmed that the pure plant extracts exhibited no major interactions with the formulation components such as the lipid, surfactant, and co-surfactant, indicating their compatibility within the SLN system.

2. Qualitative phytochemical analysis was performed using Thin Layer Chromatography (TLC), which confirmed the presence of key phytoconstituents such as quercetin, kaempferol, thymoquinone, and gallic acid in the plant extracts.

3. Solid lipid nanoparticles were successfully incorporated into the cream formulation, and a skin care product targeting acne and aging was successfully developed and evaluated.

4. Anti-acne activity was successfully evaluated against *Staphylococcus epidermidis* and *Propionibacterium acnes* using both the agar well diffusion method and the tube dilution method. The plant extract mixtures demonstrated strong antimicrobial activity against both microorganisms, indicating their potential effectiveness in acne management.

5. Anti-aging activity was successfully evaluated in *Caenorhabditis elegans*. Treatment with the plant extracts significantly prolonged the lifespan of the nematodes, enhanced their mobility, and improved both oxidative and heat stress tolerance. In addition, the extracts reduced lipofuscin accumulation and resulted in a shorter body length, indicating a potential anti-aging and caloric-restriction-mimicking effect.

Conclusion:

In this research work, herbal extract-loaded SLNs were successfully prepared using the microemulsion technique with stearic acid, soy lecithin, and Tween 80. All SLNs exhibited particle sizes below 100 nm and PDI values lower than 0.5, which are considered acceptable in the pharmaceutical field. The zeta potential values indicated

that the prepared SLNs possessed adequate stabilization, supported by steric effects arising from the surfactant. FTIR studies showed no major interaction between the pure extracts and the formulation components, confirming compatibility. SEM analysis demonstrated that the SLNs were spherical with uniform morphology and good stability. Thus, SLNs can be effectively prepared using the microemulsion technique.

The results further confirmed that the solid lipid nanoparticles of all herbal extracts were successfully incorporated into a cream suitable for topical application. The combined extracts of *Nelumbo nucifera*, *Averrhoa carambola*, *Nigella sativa*, and *Sphaeranthus indicus* exhibited multiple beneficial effects, including skin-whitening, anti-ageing, and anti-acne activity. The findings from the experimental work and evaluation parameters indicate that the SLN-based herbal cream is effective, safe, and easy to use.

The antimicrobial efficacy of the mixture of *Sphaeranthus indicus* and *Averrhoa carambola* extracts (Code: 250EDU04 & 250EDU05) was rigorously assessed against *Staphylococcus epidermidis* and *Propionibacterium acnes* using agar well diffusion and tube dilution assays following CLSI guidelines (M07-A11, 2018). At 100% (w/v) concentration, the mixture produced a zone of inhibition of 17.0 ± 0.4 mm, surpassing the positive control vancomycin ($30 \mu\text{g/mL}$, 15.0 ± 0.5 mm) and 25.0 ± 0.4 mm at 100% (w/v), comparable to cefoxitin (26.0 ± 0.5 mm at $30 \mu\text{g/mL}$) respectively. The MIC and MBC were found to be 6.25% (w/v) and 12.5% (w/v) for *Staphylococcus epidermidis* and $12.5 \mu\text{g/mL}$ (1.25% w/v) and $25 \mu\text{g/mL}$ (2.5% w/v) for *Propionibacterium acnes* confirming strong antibacterial activity.

The extracts demonstrated strong activity against *S. epidermidis* and *P. acnes*, both of which are major opportunistic pathogens associated with biofilm formation and antibiotic resistance. The extract's performance, comparable to or exceeding certain standard antibiotics, suggests its potential as a natural antimicrobial agent for topical formulations, wound dressings, or antimicrobial coatings.

The anti-ageing potential of the extracts was evaluated using *Caenorhabditis elegans*. Treatment with the extracts significantly prolonged lifespan, enhanced mobility, and improved both oxidative and heat stress tolerance. Additionally, a measurable reduction in lipofuscin levels was observed compared to the control group. Shorter body length further supported the anti-ageing effects, indicating physiological responses aligned with longevity

pathways.

Overall, this study confirms that the developed herbal SLN-based cream possesses significant anti-acne, antimicrobial, and anti-ageing activity and holds strong potential for future therapeutic and cosmetic applications.

Publications:

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