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Design and Development of Polyherbal Gel for Antifungal Activity

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ABSTRACT

The study reveals that a polyherbal antifungal gel combining Cassia tora leaf extract, Azadirachta indica leaf extract, Ficus benghalensis extract, and Hinokitiol is effective in tropical climates. The formulation is prepared using polyherb-loaded nanostructured lipid carriers (NLC) and is characterized by particle size, polydispersibility index, zeta potential, and physicochemical parameters.

INTRODUCTION

Drug delivery systems (DDS) based on lipids are a well-researched, proven, and economically feasible method of producing medications in various dose forms[1]. To function, multiple components must be included in lipid formulations such as Nano lipids carriers (NLCs). The two primary criteria that may be improved using formulations such as NLCs are the bioavailability and solubility of the insoluble medicines[2,3]. Numerous pharmaceutical companies have created well-established industrial processes for producing large-scale batches of nanostructured lipid carriers; however, many variables still affect these processes, including the choice of lipids, surfactants, and other crucial excipients and preparation techniques. These variations affect parameters like phase transition, solubility, and drug bioavailability, among others, as well as particle shape and size[4,5]. The unique qualities that lipid nanoparticles exhibit are necessary and crucial to their therapeutic activity. The remarkable characteristics of nanoparticles (NP), such as their surface-to-mass ratio and capacity to bind and transport substances, make them more advantageous for usage as medical products[6].

Fungal infections of the skin are among the most prevalent dermatological conditions today. Stable dosage, semisolid dose, and liquid dose formulations are only a few of the available treatment alternatives[7]. Gels have been used for a very long time as a topical treatment in cosmetics. The use of gels in the principal class of semisolid arrangements has expanded in both scientific and cosmetic applications[8,9].

Among the various health concerns, fungal infections pose a significant challenge, affecting millions of individuals worldwide. Antifungal herbal formulations have emerged as a promising solution, offering a holistic approach to combat these infections while minimizing the side effects associated with synthetic drugs[10].

Topical treatments are mixtures that are smeared, rubbed, sprayed, or instilled directly onto an outside body surface. To treat skin disorders locally or to create systemic pharmacological effects, the topical route of administration has been used. Transparent gels are one of the main categories of semisolid preparations, and their application in pharmaceutical and cosmetic preparations has grown[11].

Topical treatments are concoctions applied directly to the skin, such as by smearing, rubbing, spraying, or injecting. Topical application has been used to treat skin problems locally or to provide systemic pharmacological effects. One of the primary types of semisolid preparations is transparent gels, which are increasingly being used in pharmaceutical and cosmetic preparations[12]. Regardless of the drug's water solubility, gels often provide a faster release of the active component than creams and ointments. They are very biocompatible, easy to give, and unlikely to cause irritation or adverse responses. They also don't need to be removed.

Polyherbal formulations are defined as herbal preparations that comprise two or more herbal medications. Due to the polyherbal formulation's great efficacy against several infectious disorders, it is well recognized. In Ayurveda, drug formulation is founded on two principles: using many drugs and using one drug, which is referred to as polyherbal formulation. Known as polyherbalism or polypharmacy, this is a traditional medical herb technique that makes use of mixing many medicinal plants to obtain additional therapeutic activity[13,14].

MATERIALS AND METHODS

Plant Material

Cassia tora leaves, *Azadirachta indica* leaves, and aerial roots of *Ficus benghalensis* were collected from the local area of the Sawarde and authenticated by Sharadchandraji Pawar College of Agriculture, Kharawate and Hinokitiol purchased from Dhamtech Pharma and Consultants. All other chemicals used in the research work are provided by the institution and were purchased from Loba Chemie Pvt. Ltd., Mumbai.

Plant Extract

Cassia tora leaves and aerial roots of *Ficus benghalensis* were conducted by the Soxhlet extraction technique using methanol as solvent and for *Azadirachta indica* ethanol was used as solvent and was dried by distillation process.

Preformulation Evaluation

Phytochemical Screening

The phytochemical screening of the methanol extracts of the *Cassia Tora* leaves, aerial roots of Ficus benghalensis, and ethanol extract of *Azadirachta Indica* leaves. The preliminary test (test for primary metabolites) tested organic steroid alkaloid and saponin, flavonoid, carbohydrates, tannin, phenol, protein, and glycosides plant extracts.

Determination of λ- max

The absorption maximum of *Cassia tora* leaves extract, Azadirachta indica leaves extract, aerial roots of ficus benghalensis, Hinokitiol, and a combination of Herbs were carried out in phosphate buffer pH 7.4.

Compatibility Study

The compatibility study of the cassia tora leaves extracts, Azadirachta indica leaves extract, aerial roots of ficus benghalensis, Hinokitiol, and polymers was conductedby FTIR and DSC.

FTIR

The compatibility studies were carried out at room temperature using FTIR spectroscopy to determine the drug-drug interaction, and drug excipients/ polymer interactions used in formulation. The spectra of the extract, physical mixture, and formulation were compared.

DSC

Differential scanning calorimetry or DSC is a thermoanalytical technique in which difference in account of heat required to increase the temperature of sample and reference are measured as a function of temperature. Both the sample and reference were mentioned at nearly sample temperature throughout the experiment.

Antifungal Activity

The antifungal activity of the pure medicine was assessed using the cup-plate technique. Distilled water was used to create a nutrient agar medium, which was then autoclave-sterilized for 15 minutes at 121°C. The medium should then be aseptically transferred to a sterile Petri plate and allowed to solidify for a few minutes. Once the culture has been set, add 100 µl of the test microbe, Candida albicans, to each plate and disseminate the culture with a spreader. Next, wells were created on plates using a borer. 100 µl of the test sample (pure medicine) should be added aseptically to each well. The plates were analyzed to ascertain the zone of inhibition surrounding the well after being incubated for 24 hours at 37 °C.

Preparation of Polyherb Loaded NLC

Polyherb-loaded NLC was prepared using the Hot high-pressure homogenization method. The liquid and solid lipids are blended, and heated above the solid lipid's melting point, and then the medicine is added to create a drug-disperse lipid melt. By mixing enough tween 80 with deionized water, the aqueous phase is produced independently. The temperature of this phase and the lipid melt are the same. These two phases are combined and heated to high-shear homogenization for a limited period to create pre-emulsion. Instantaneously, the pre-emulsion is passed through HPH three to five times at different pressures. The intended average droplet size of the nano-emulsion often dictates the number of cycles. After that, the emulsion is stirred and chilled to room temperature. The solid lipid recrystallizes, causing the droplets to solidify.

Table 1: Formulation Table of NLC

Sr. No.	Ingredients	F1	F2	F3	F4
1	Combination of Herbs (0.5gm Each Herb)	2 gm	2 gm	2 gm	2 gm
2	Stearic Acid	0.8 gm	0.8 gm	0.8 gm	0.8 gm
3	Oleic Acid	0.2 gm	0.2 gm	0.2 gm	0.2 gm
4	Tween 80	0.5 gm	1.0 gm	1.5 gm	2.0 gm
5	PEG 400	0.25 gm	0.25 gm	0.25 gm	0.25 gm
6	Water (Q.S.)	50 ml	50 ml	50 ml	50 ml

Preparation of Gel

50 milliliters of pure water mixed with weighed amounts of sodium carboxyl methyl cellulose in a beaker while constantly swirling. It was necessary to dissolve a certain amount of propyl and methylparaben in 5 milliliters of distilled water by boiling it in a water bath. It was provided to the abovementioned mixture after cooling. Gradually, the propylene glycol was added to create a uniform mass. After a specific medication extract mixture was supplied, water was added to make the volume reach 100 ml. To create a gel with the necessary consistency, triethanolamine was finally added to the mixture dropwise.

Formulation Table

Table 2: Composition of Polyherbal Gel

INGREDIENTS	F1	F2	F3	F4	F5	F6

Combination of Herbs	2 gm	2 gm	2 gm	-	-	-
Polyherb Loaded NLC	-	-	-	1gm	1gm	1gm
Sodium CMC	1.5gm	2.0gm	2.5gm	1.5gm	2.0gm	2.5gm
Methyl Paraben	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm	0.3gm
Propyl Paraben	0.1gm	0.1gm	0.1gm	0.1gm	0.1gm	0.1gm
Propylene Glycol	5ml	5ml	5ml	5ml	5ml	5ml
Triethanolamine	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.
Distilled water	Q.S. to 100ml	Q.S. to 100ml	Q.S. to 100ml	Q.S. to 100ml	Q.S. to 100ml	Q.S. to 100ml

Evaluation of Polyherb NLC

Particle Size

The performance of the nanostructured lipid carrier is influenced by the particle size as it controls the pace and volume of medication release as well as absorption. Before measurement, 10 times as much pure water was added to 1 mL of each Polyherb-loaded NLC mixture. Dynamic light scattering (DLS)was used to measure the particle size and polydispersibility index of the resulting nanostructured lipid carrier. A photon correlation spectrometer was used to analyze the variations in light scattering causedby the Brownian motion of the particles, and a Nano sizer SZ-100 (Horiba nano size analyser, Japan) was used to measure particle sizes between 10-3000 nm. At a scattering angle of 900, light scattering was seen at 25ºC.

Zeta Potential

A crucial method for figuring out the charge on the particles of nanostructured lipid carriers is zeta potential. Using a zeta size analyzer and the dynamic light scattering method, the zeta potential of the NLC formulation containing a combination of herbs was ascertained. In the UK, Malvern Instrument Ltd. Clear, disposable zeta cells were used to hold the samples, and the outcomes were noted. Before each experiment, cuvettes were cleaned using the sample that was going to be analyzed and then rinsed withmethanol.

Scanning Electron Microscopy (SEM)

Morphological evaluation of the selected Herb-loaded NLC formulation was carried out in Scanning Electron Microscope (SEM).

***IN-Vitro* Study**

Using USP Type II equipment and 900 cc of pH 6.8 phosphate buffer as a medium at 37±0.5ºC, a drugrelease study of pure drug and Polyherb-loaded NLC was carried out. The paddle's rotational speed was set at 50 revolutions per minute. An aliquot of five milliliters of the sample was taken after the Polyherb-loaded NLC, was loaded in DialysisMembrane 12 KDa Himedia and placed in the dissolving equipment. The sample was taken at predefined intervals of one hour, two hours, three hours, and up to twelve hours. To keep the volume and sink conditions constant, the removed sample was substituted with equal quantities of dissolving media. Following filtering, the sample was examined using UV spectroscopy to determine the percentage of drug release.

Evaluation of Gel Formulation

Physical Evaluation

Physical characteristics including colour and uniformity were examined manually.

Washability

After applying the product by hand, it was examined under flowing water.

pH

A digital pH meter was used to measure the formulation's 1% aqueous solution at a steady temperature.

Spreadability

Spreadability was measured using in-house designed and manufactured equipment. The apparatus consists of a wooden block with two glass slides, one of which is attached to a weight pan that is moved on a pulley and positioned horizontally about the fixed slide. The other glass slide is movable. The spreadability of the formulation was measured using the gel's "slip and drag" characteristics. A surplus of gel (about 2g) was being examined on this ground slide. The gel was then placed in between two slides. A one-kilogram weight was placed on top of each of the two slides for five minutes to press out air and produce a uniform layer of gel between them. I scraped out the extra gel from the edges. 50 g of pull-off was then applied to the upper plate. With the help of a thread that is attached to the hook, note how long it took the top slide to travel 7.5 cm (measured in seconds). A shorter period suggested better spreadability. The formula for calculating

spreadability was S = M*L/T. where T is the amount of time (measured in seconds) required to completely separate the slides from one another, L is the length traveled by the glass slide, M is the weight in the pan (connected to the top slide), and S is for spreadability.

Extrudability

One popular empirical test is to measure the force required to remove the substance from the tube. When the applied shear in the rhea gram zone is calculated using a method that results in a shear rate greater than the yield value, plug flow is seen. The evaluation method used in the current study to assess formulations for extrudability is based on the number in percentage of formulations and formulations extruded from lacquered aluminum collapsible tubes on the application of weight in grams required to extrude at least 0.5 cm of formulations in 10 seconds. More extrusion leads to enhanced extrudability. Extrudability of each formulation is measured three times, with average results displayed.

Homogeneity

After the gels were put inside the field, all advancing gels were examined for visual homogeneity. Their appearance had been put to the test.

In vitro diffusion study

The arbitrary movement of molecules through an area brought on by a concentration gradient from high to low concentration is known as diffusion. In vitro, diffusion is often defined as the passive diffusion of a permeant from a vehicle in the donor chamber over an artificial or biological membrane into a receptor fluid in the receptor chamber, excluding delivery techniques such as iontophoresis and microneedles. The term "permeant" refers to the kind of molecular species that permeates a tissue or membrane. During the permeation process, which comprises first breaking the membrane and then diffusing through it, the permeant is transferred across it. A substance can enter through the membrane without it diffusing or going through it. The quantity of permeant that enters the circulatory system per unit area and travels through a membrane in that time is known as the "flux" and can be represented in mass, area, or time units. The receptor chamber serves as the mechanism for in vitro permeation. On the other hand, accumulation is the quantity of permeant that flows across a membrane in a predefined period and is expressed in mass or area units.

Result and Discussion

Phytochemical Evaluation

Table 3: Phytochemical Evaluation of Herbs

Sr. No.	Test	Cassia tora leaves extract	Azadirachta indica leaves extract	Aerial roots of Ficus benghalensis extract
1	Alkaloids Test	+	+	+
2	Flavonoids Test	+	+	+
3	Saponins Test	+	+	-
4	Terpenoid Test	-	+	+
5	Glycoside Test	+	-	-
6	Tannins Test	+	+	+
7	Steroids Test	+	+	+
8	Phenols Test	+	+	+
9	Coumarins	-	-	+

Determination of λ- max

The absorption maximum of *Cassia tora* leaves extract was found to be 221 nm, for Azadirachta indica leaves extract 202 nm, for aerial roots of ficus benghalensis extract 214 nm for Hinokitiol 255 nm and for a combination of Herbs 253 nm in phosphate buffer pH 7.4 and 253 nm wavelength was selected and utilized for further studies.

Calibration Curve of *Cassia Tora* leaves extract

Calibration curve of *Cassia tora* leaves extract:

The absorbance data of the standard solutions are shown in Table 3.

The values of Abs. (absorbance) were plotted against respective concentrations (Figure 1). The conc. showed linearity when the curve was plotted indicating it obeyed Beers Lambert law. The regression coefficient value was 0.9622 in phosphate buffer pH 7.4.

Calibration curve of *Azadirachta indica* leaves extract:

The absorbance data of the standard solutions are shown in Table 3.

The values of Abs. (absorbance) were plotted against respective concentrations (Figure 1). The conc. showed linearity when the curve was plotted indicating it obeyed Beers Lambert law. The regression coefficient value was 0.9845 in phosphate buffer pH 7.4.

Calibration curve of aerial roots of *Ficus benghalensis linn* extract:

The absorbance data of the standard solutions are shown in Table 3.

The values of Abs. (absorbance) were plotted against respective concentrations (Figure 1). The conc. showed linearity when the curve was plotted indicating it obeyed Beers Lambert law. The regression coefficient value was 0.9071 in phosphate buffer pH 7.4.

Calibration curve of Hinokitiol:

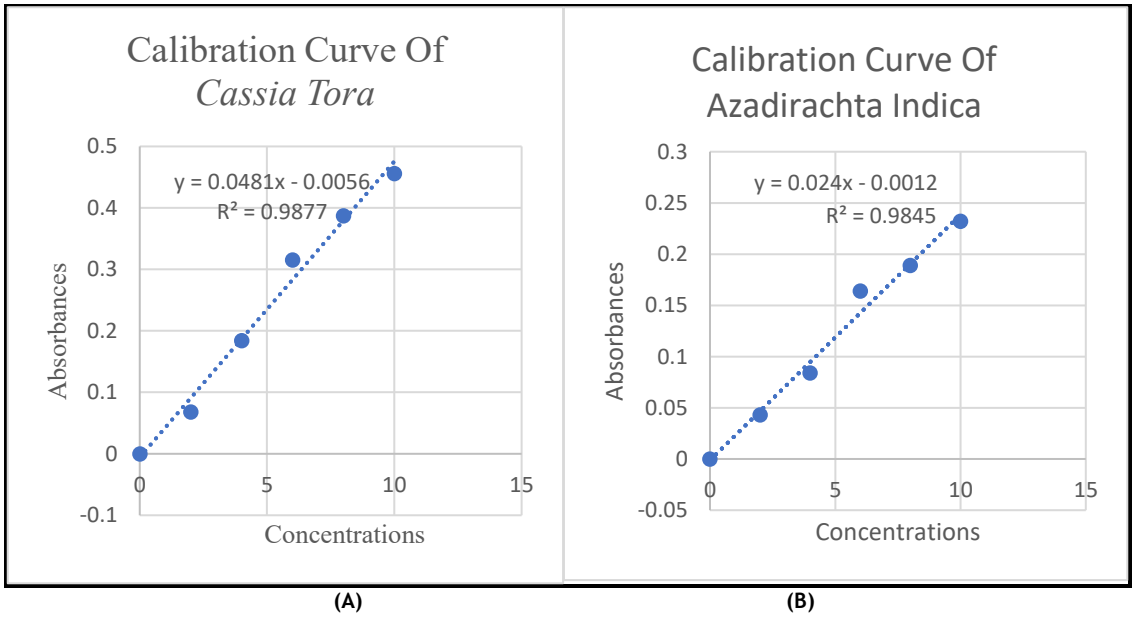
The absorbance data of the standard solutions are shown in Table 3.
The values of Abs. (absorbance) were plotted against respective concentrations (Figure 1). The conc. showed linearity when the curve was plotted indicating it obeyed Beers Lambert law. The regression coefficient value was 0.9882 in phosphate buffer pH 7.4.

Calibration curve of Combination of Herbs:

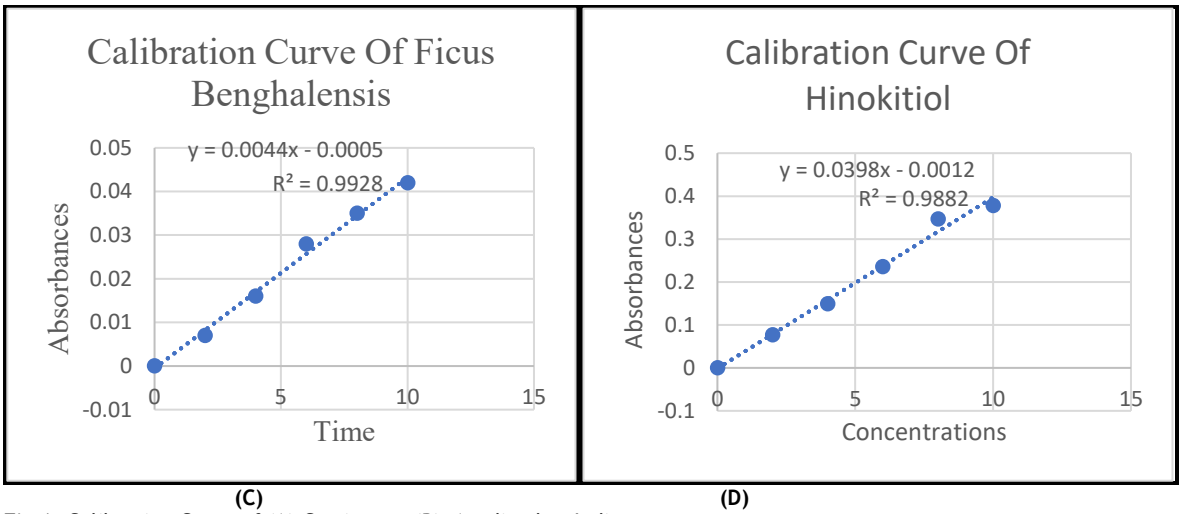
The absorbance data of the standard solutions are shown in Table 3.
The values of Abs. (absorbance) were plotted against respective concentrations (Figure 1). The conc. showed linearity when the curve was plotted indicating it obeyed Beers Lambert law. The regression coefficient value was 0.9859 in phosphate buffer pH 7.4.

Table 4: Calibration curve of Herbs

Sr. No.	Concentration (mcg/ml)	Absorbances (Phosphate buffer pH 7.4)			
		Cassia Tora	*Azadirachta Indica*	*Ficus benghalensis*	Hinokitiol
01.	0	0	0	0	0
02.	2	0.068	0.043	0.007	0.077
03.	4	0.184	0.084	0.016	0.149
04.	6	0.315	0.164	0.028	0.236
05.	8	0.387	0.189	0.035	0.347
06.	10	0.456	0.232	0.042	0.378



(A) **(B)**



(C) **(D)**

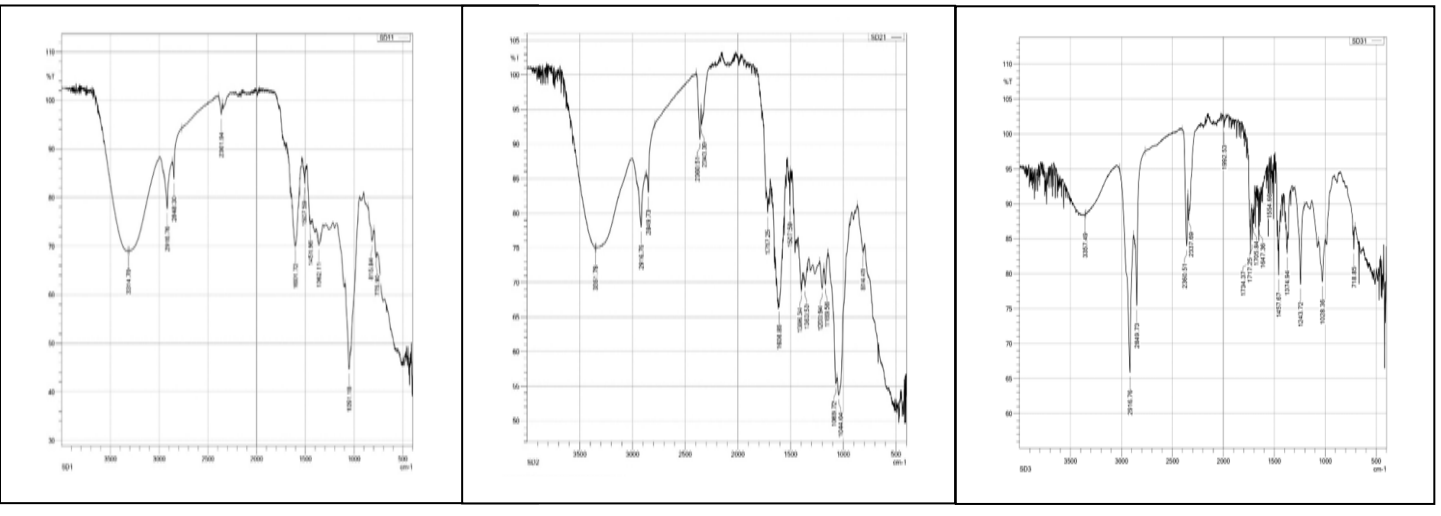
Fig 1: Calibration Curve of (A) Cassia tora (B) Azadirachta Indica (C) Ficus benghalensis (D) Hinokitiol

Compatibility Study

The compatibility study of Herbs and excipients was carried out using FTIR and DSC methods. The results are shown below.

FTIR

- The *Cassia Tora* leaves extract showed significant bands at 3314.70 cm^{-1} for OH- stretch. The C-H stretching was observed at 2916.76 cm^{-1}. The C=O stretching was observed at 1601.72 cm^{-1}. The C=C and C-O stretching was observed at 1507.59 cm^{-1} and 1051.18 cm^{-1} respectively.
- The *Azadirachta Indica* leaves extract showed significant bands at 3359.78 cm^{-1} for OH- stretch. The C-H stretching was observed at 2916.76 cm^{-1}. C=N stretching was observed at 1608.86 cm^{-1}. The C=C and C-O stretching was observed at 1507.59 cm^{-1} and 1169.56 cm^{-1} respectively.
- The Aerial roots of *Ficus benghalensis Linn* extract showed significant bands at 3357.49 cm^{-1} for O-H stretching. The C-H stretching was observed at 2916.76 cm^{-1}. The C=O stretching was observed at 1734.37 cm^{-1}. C=N stretching was observed at 1647.36 cm^{-1}. The C=C and C-O stretching was observed at 1554.66 cm^{-1} and 1028.36 cm^{-1} respectively.
- The drug Hinokitiol showed significant bands at 3199.17 cm^{-1} for OH- stretch. The C-H stretching was observed at 2921.04 cm^{-1}. The C=C and C-O stretching was observed at 1540.39 cm^{-1} and 1219.48 cm^{-1} respectively. The C-H bending was observed at 818.69 cm^{-1}.
- The combination of Herbs showed significant bands at 3356.06 cm^{-1} for OH- stretch. The C-H stretching was observed at 2916.76 cm^{-1}. The C=O stretching was observed at 1717.25 cm^{-1}. C=N stretching was observed at 1653.07 cm^{-1}. The C=C and C-O stretching was observed at 1507.59 cm^{-1} and 1243.72 cm^{-1} respectively. The C-H bending was observed at 822.97 cm^{-1}.
- The Physical Mixture demonstrated the distinct peak representing OH- stretching at 3257.65 cm^{-1}. The C=O stretching was observed at 1708.70 cm^{-1}. C=N stretching was observed at 1607.43 cm^{-1}. The C=C and C-O stretching was observed at 1514.72 cm^{-1} and 1113.93 cm^{-1} respectively.
- The formulation of Gel containing *Cassia tora* leaves extract, *Azadirachta indica* leaves extract, aerial roots of *Ficus benghalensis linn* extract, Hinokitiol, Sodium CMC, Methyl paraben, Propyl paraben, and Triethanolamine shows significant peaks at 3338.94 cm^{-1} for OH- stretching. The C=N and C-O stretching was observed at 1637.38 cm^{-1} and 1166.71 cm^{-1} respectively. The C-H bending was observed at 837.23 cm^{-1}.



(A) (B) (C)

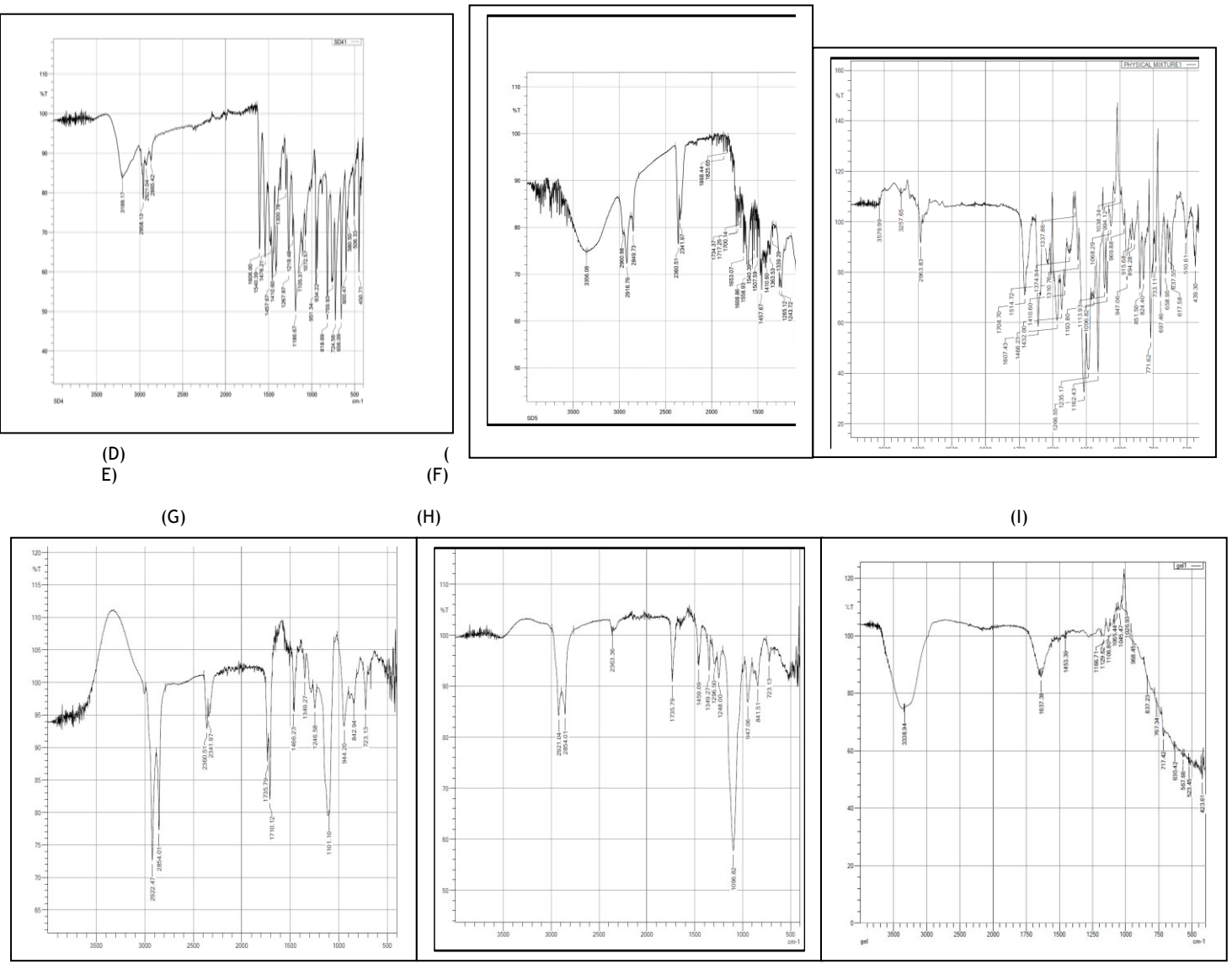


Fig 2: FTIR of (A) Cassia tora (B) Azadirachta Indica (C) Ficus benghalensis (D) Hinokitiol (E) Combination of Herbs (F) Physical Mixture of NLC (G) Formulation of NLC (H) Physical Mixture of Gel (I) Formulation of Gel

DSC

The thermal behaviors of Herbs a Combination of Herbs Physical Mixture Gel Formulation are shown in the figure.

The DSC thermogram of *Cassia Tora* leaves extract showed a characteristic endothermic peak at 144.3ºC.

The DSC thermogram of *Azadirachta Indica* leaves extract showed a characteristic endothermic peak at 151.5ºC.

The DSC thermogram of Aerial roots of *Ficus benghalensis* extract showed a characteristic endothermic peak at 141.2ºC.

The DSC thermogram of *Cassia Tora* leaves extract showed a characteristic endothermic peak at 54.5ºC.

The DSC thermogram of a combination of herbs showed a characteristic endothermic peak at 128.6ºC.

The DSC thermogram of a physical mixture containing a Combination of Herbs, tween 80, stearic acid, oleic acid, PEG 400 showed the characteristic peak at 131.2ºC. The DSC analysis of herbs and physical mixture revealed a negligible change in the melting point of herbs when mixed with other excipients, indicating no interaction between the herbs and excipients.

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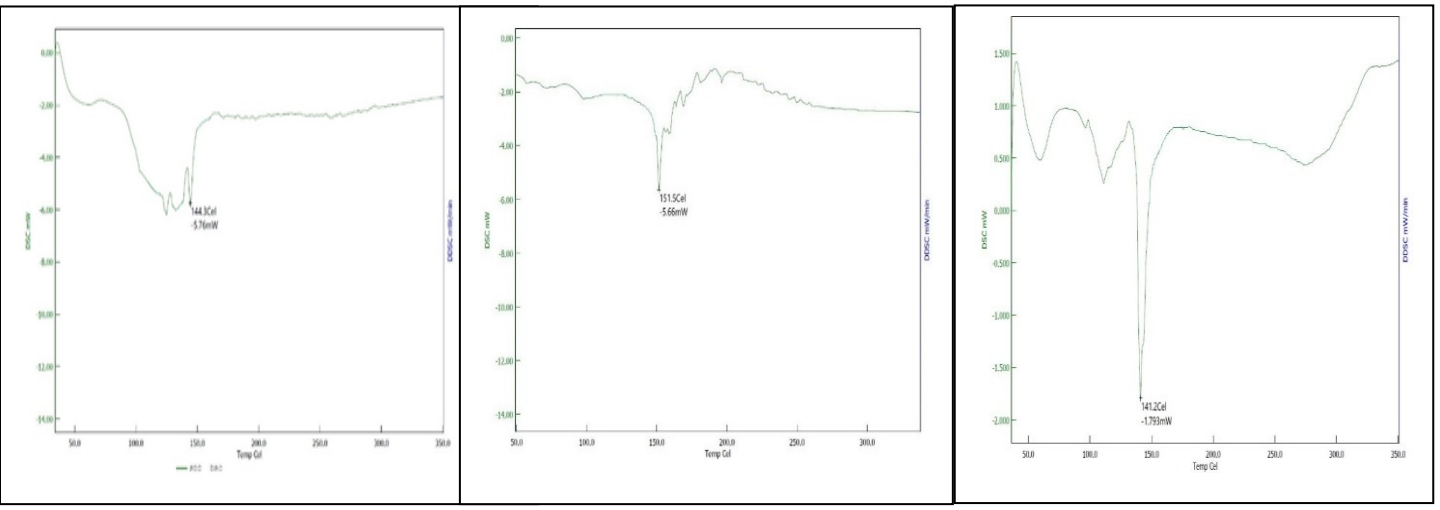
The DSC thermogram of the prepared NLC formulation showed a characteristic endothermic peak at 133.2ºC which revealed that there was no change in the melting point of herbs when mixed with other excipients, indicating some modification or interaction between the drug and excipients in the final formulation.

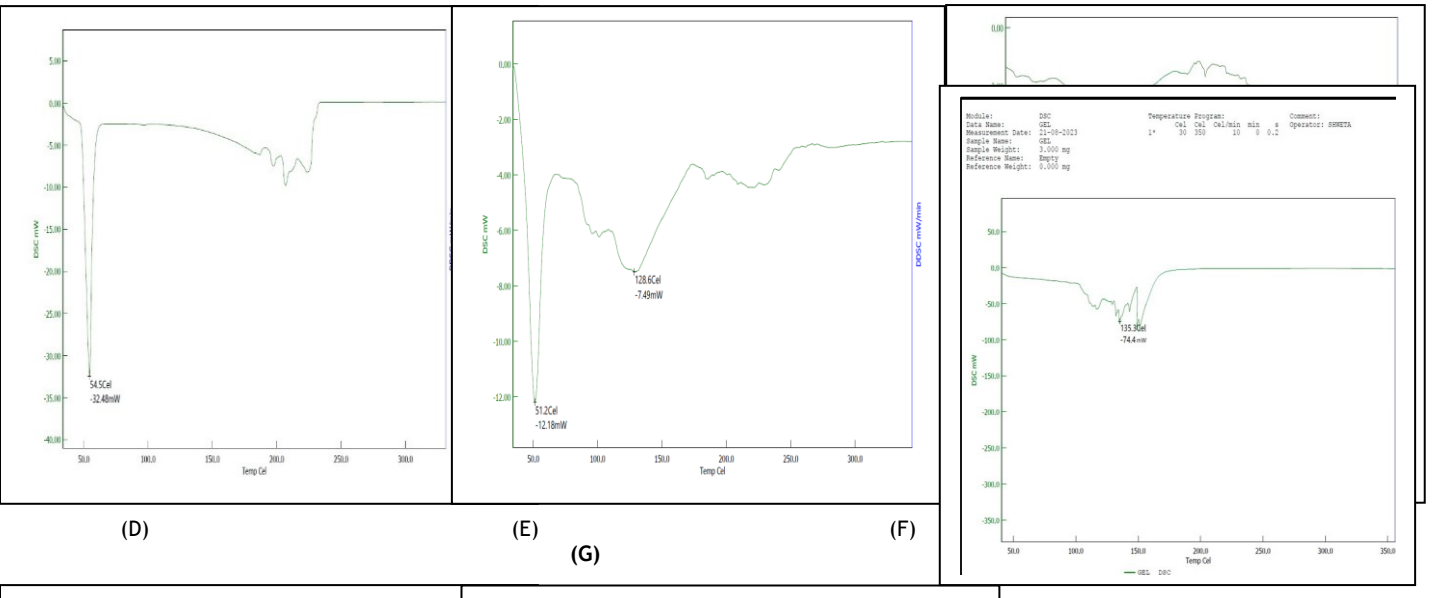
The DSC thermogram of a physical mixture containing a Combination of Herbs,

Sodium CMC, Methyl Paraben, Propyl Paraben, and Triethanolamine showed the characteristic peak at 132.9ºC. The DSC analysis of herbs and physical mixture revealed a negligible change in the melting point of herbs when mixed with other excipients, indicating no interaction between the herbs and excipients.

The DSC thermogram of the prepared Gel formulation showed a characteristic endothermic peak at 135.3ºC which revealed that there was no change in the melting point of herbs when mixed with other excipients, indicating some modification or interaction between the drug and excipients in the final formulation

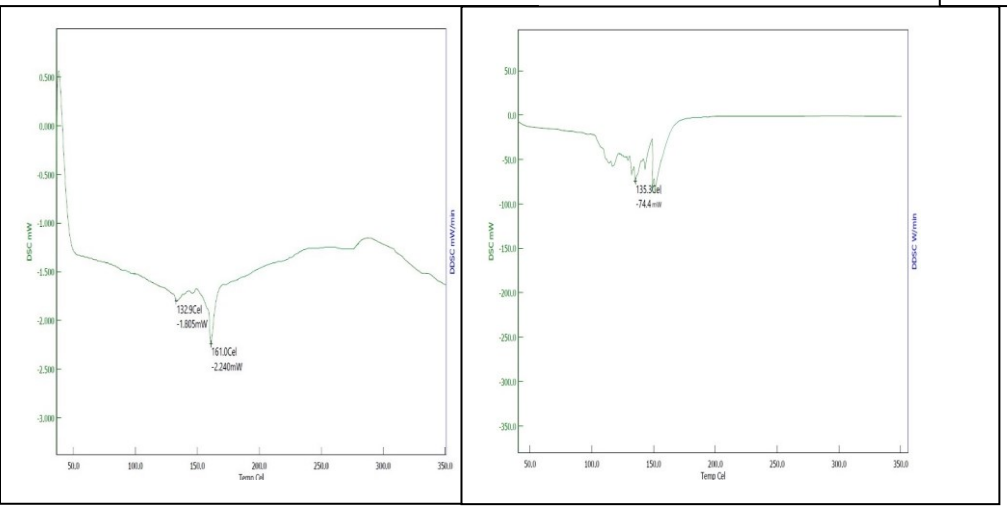


(A) (B) (C)



(D) (E) (F)

(G)



(H) **(I)**

Fig 3: DSC of (A)*Cassia tora* (B) *Azadirachta Indica* (C) *Ficus benghalensis* (D) Hinokitiol (E) Combination of Herbs (F) Physical Mixture of NLC (G) Formulation of NLC (H) Physical Mixture of Gel (I) Formulation of Gel

Antifungal Activity of Herbs

Anti-fungal activity of herbs was done using the cup plate assay method. The antifungal activity showed results for ethanol extract of *Cassia tora leaves*, *Azadirachta Indica leaves*, *aerial roots of ficus benghalensis*, *and* hinokitiol against the fungal strain of Candida albicans at the different concentrations of 50 mg/ml, 100 mg/ml.

Table 5: Antifungal Activity of Herbs against Candida Albicans

Sr. No.	Sample	Zone of Inhibition (mm)

		Cassia Tora	*Azadirachta Indica*	*Ficus Bnghalensis*	Hinokitiol	Combination of Herbs
1.	50 mg	4 mm	6 mm	5 mm	8 mm	12 mm
2.	100 mg	7 mm	8 mm	6 mm	10 mm	19 mm

Table 6: Antifungal Activity of Herbs against Aspergillus Niger

Sr. No.	Sample	Zone of Inhibition (mm)				
		Cassia Tora	*Azadirachta Indica*	*Ficus Bnghalensis*	Hinokitiol	Combination of Herbs
1.	50 mg	3 mm	7 mm	4 mm	6 mm	10 mm
2.	100 mg	5 mm	6 mm	7 mm	8 mm	17 mm

Evaluation of Polyherb NLC

Particle Size and Polydispersibility Index

The Particle size of the Polyherb Loaded Nanostructured Lipid Carrier was found in range of 115.5nm to 193.4 nm and polydispersibility index in range of 0.365 to 0.624.

The formulation F4 shows lowest Particle size which is 115.5 nm with polydispersibility index of 0.365. Since the value of polydispersibility index is less than 1 indicates uniform distribution of droplets throughout the formulation.

Table 7: Particle Size and Polydispersibility Index of F1

Sr. No.	Formulation Code	Mean Particle Size (nm)	Polydispersibility Index
1	F1	193.4	0.624
2	F2	131.0	0.446
3	F3	176.2	0.454
4	F4	115.5	0.365

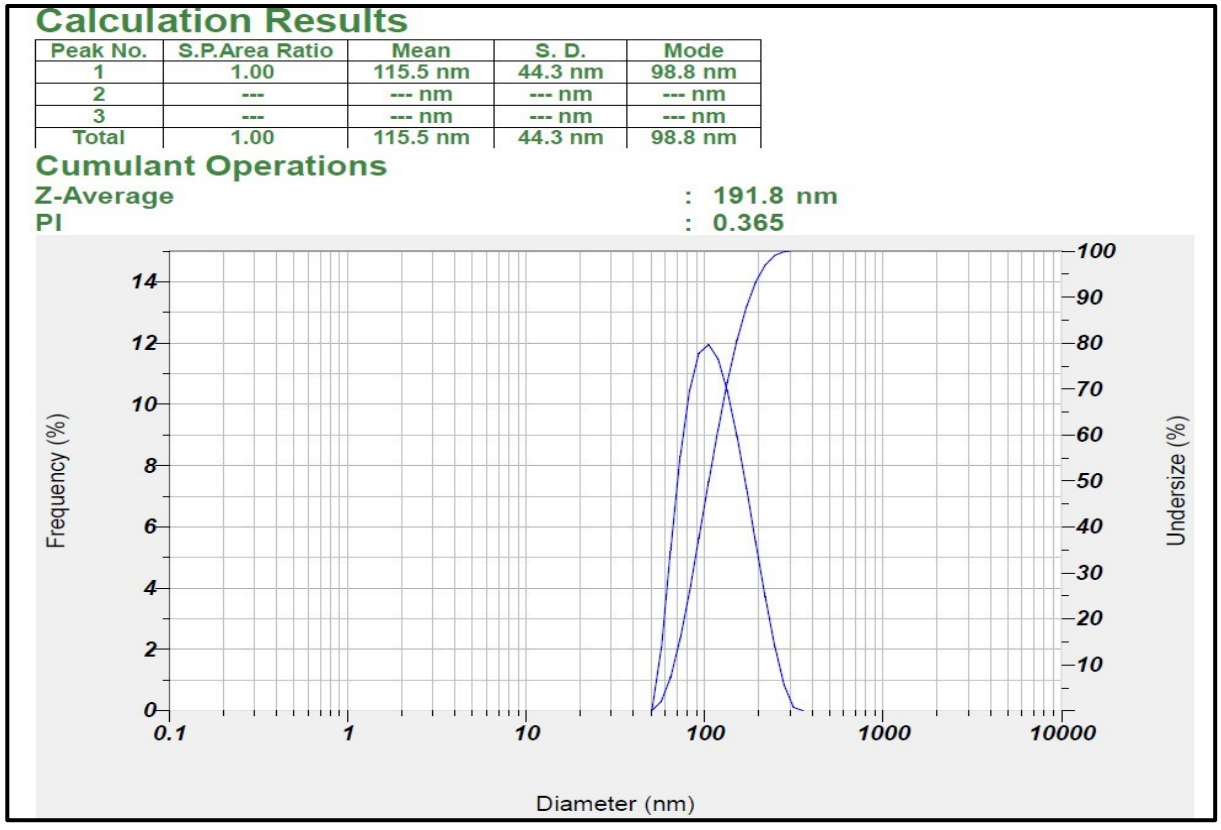


Fig 4: Particle Size and Polydispersibility Index of F1

Zeta Potential

The measurement range for the Zeta Potential was -28.60 mV to -44.80 mV. The Zeta Potential value is a useful indicator of the stability of the nanostructured lipid carrier, and it should be within the range of +30 mV to -30 mV. The results for each formulation are shown below:

Table 8: Zeta Potential

Sr. No.	Formulation Code	Zeta Potential (mV)
1	F1	-44.8
2	F2	-39.6
3	F3	-40.9
4	F4	-28.6

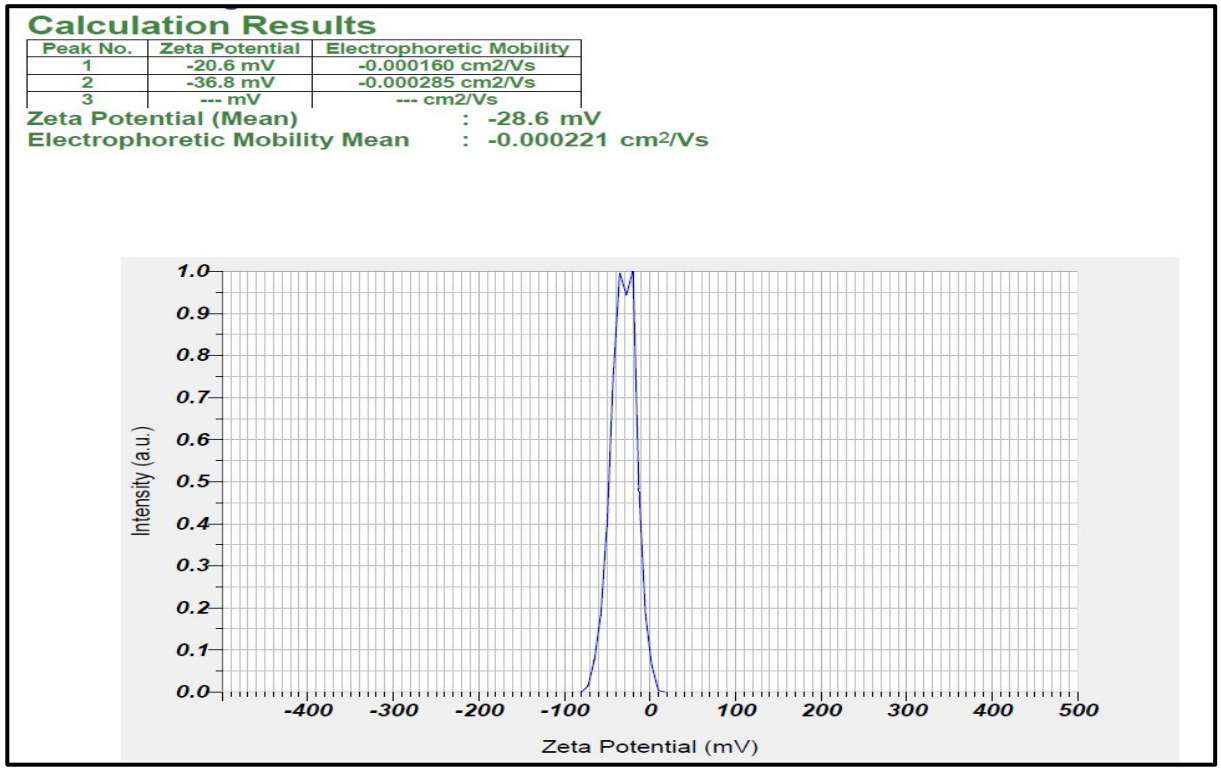


Fig 5: Zeta Potential of F1

Scanning Electron Microscopy (SEM)

According to SEM analyses of Polyherb-loaded NLC, Polyherb is entirely dissolved in the NLC. The conclusion that the particles are spherical and highly separated is supported by the SEM picture of the NLC loaded with polyherb.

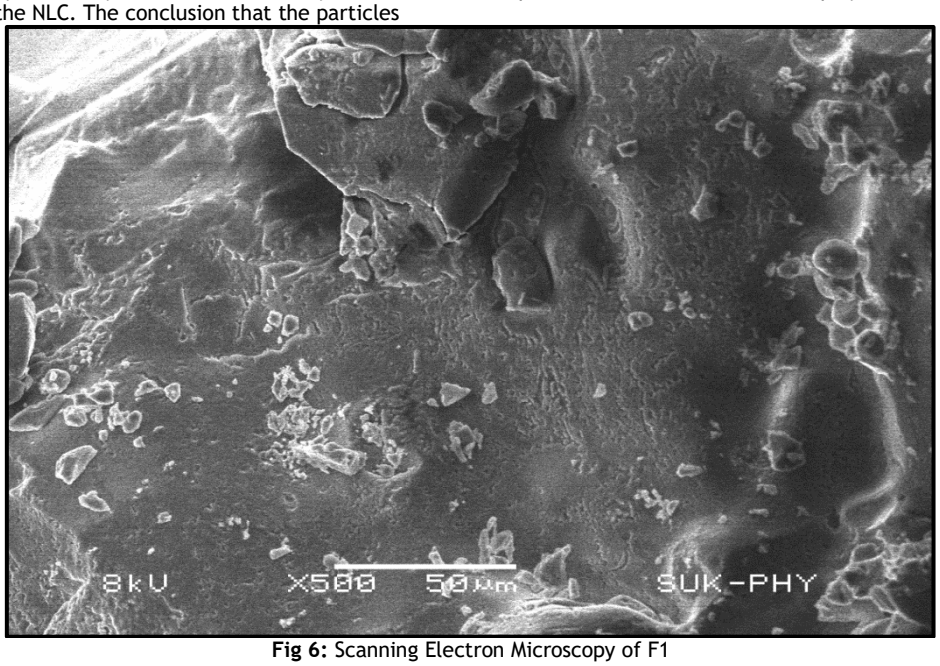


Fig 6: Scanning Electron Microscopy of F1

***In-vitro* Study**

Polyherb-loaded NLC formulations were reported to have a percent cumulative drug release of 81.98%, 79.25%, 78.19%, and 87.32%, respectively, after 12 hours for formulations F1, F2, F3, and F4. Polyherb release was determined to be 87.32%. Significant improvement was seen in the drug release of polyherb from nanostructured lipid carriers.

Table 8: In-vitro Release

Sr. No.	Time (Hr)	In-Vitro Release			
		F1	F2	F3	F4
1	0	0	0	0	0
2	1	15.84	15.18	14.94	17.35
3	2	26.05	28.19	29.47	36.56
4	3	33.81	37.68	36.22	44.18
5	4	46.09	45.15	47.87	50.63
6	5	56.87	55.46	58.45	58.87
7	6	59.51	60.02	60.52	62.89
8	7	64.57	62.13	62.09	68.12
9	8	71.36	66.29	63.13	72.27
10	9	74.58	68.94	69.74	79.25
11	10	77.28	72.51	71.24	82.35
12	11	79.45	75.04	75.26	85.22
13	12	81.98	79.25	78.19	87.32

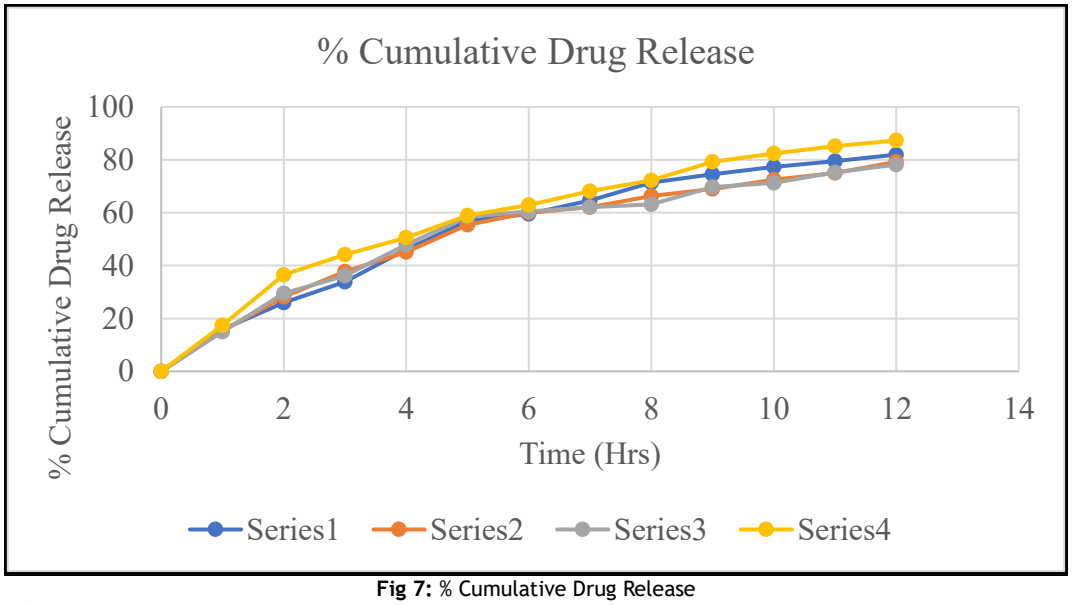


Fig 7: % Cumulative Drug Release

Evaluation of Gel

Six formulations were prepared and evaluated for physicochemical parameters. It is shown in table 2.

Physical Appearance

The physical evaluations of gel such as Appearance, Colour, Odour, Consistency, and Washability were checked. The results of evaluations of the formulations are shown in Table 5

Table 9: Physical Evaluation of Gel

Sr No.	Parameter	F1	F2	F3	F4	F5	F6
1	Appearance	Clear and Transparent	Clear and Transparent	Clear and Transparent	Clear and Transparent	Clear and Transparent	Clear and Transparent
2	Colour	Greenish	Greenish	Greenish	Greenish	Greenish	Greenish
3	Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
4	Consistency	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
5	Washability	Easily Washable	Easily Washable	Easily Washable	Easily Washable	Easily Washable	Easily Washable

Physicochemical Parameter

The physicochemical parameters of gel such as pH, Spreadability, Extrudability, Drug Content, Viscosity, and In vitro release, were checked. The results of the physicochemical parameters of the formulations are shown in Table 6.

Table 10: Physicochemical Parameters of Gel

Sr. No.	Parameters	F1	F2	F3	F4	F5	F6
1	pH	6.34	6.45	6.29	6.41	6.52	6.34
2	Spreadability	20.36	20.18	25.68	21.98	27.86	24.64
3	Extrudability	Good	Excellent	Excellent	Good	Excellent	Good
4	Drug Content	82.31	76.27	79.81	88.44	90.45	89.12
5	Viscosity	3374.1	3499.3	3502.2	3511.9	3523.4	3533.0

Antifungal Activity

Formulated gel was tested for antifungal activity

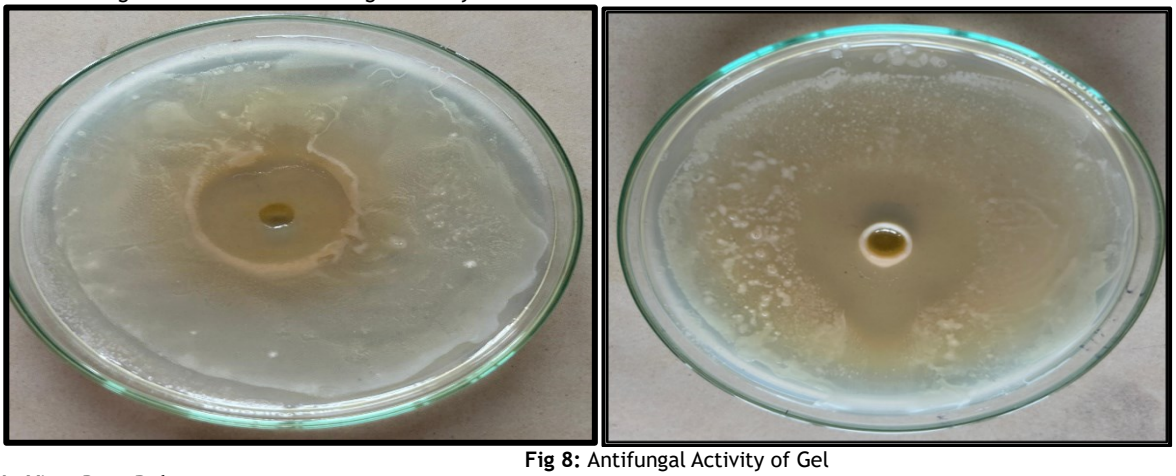


Fig 8: Antifungal Activity of Gel

In Vitro Drug Release

Table 11: % Cumulative Drug Release

Sr. No.	Time (hr)	% Cumulative Drug Release					
		F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0	0
1	1	15.08	16.03	11.79	17.51	19.42	9.88
2	2	25.46	21.95	20.87	25.67	31.14	21.56
3	3	30.34	27.52	28.04	32.02	39.41	30.5
4	4	37.91	33.83	36.47	38.81	48.28	36.11
5	5	43.80	41.52	42.40	47.59	57.33	43.31
6	6	51.45	53.07	55.39	53.95	64.20	50.62
7	7	59.91	61.56	65.98	63.90	74.50	60.54
8	8	67.99	70.78	75.77	73.02	83.96	69.72
9	9	81.64	83.28	86.60	84.28	94.35	82.52

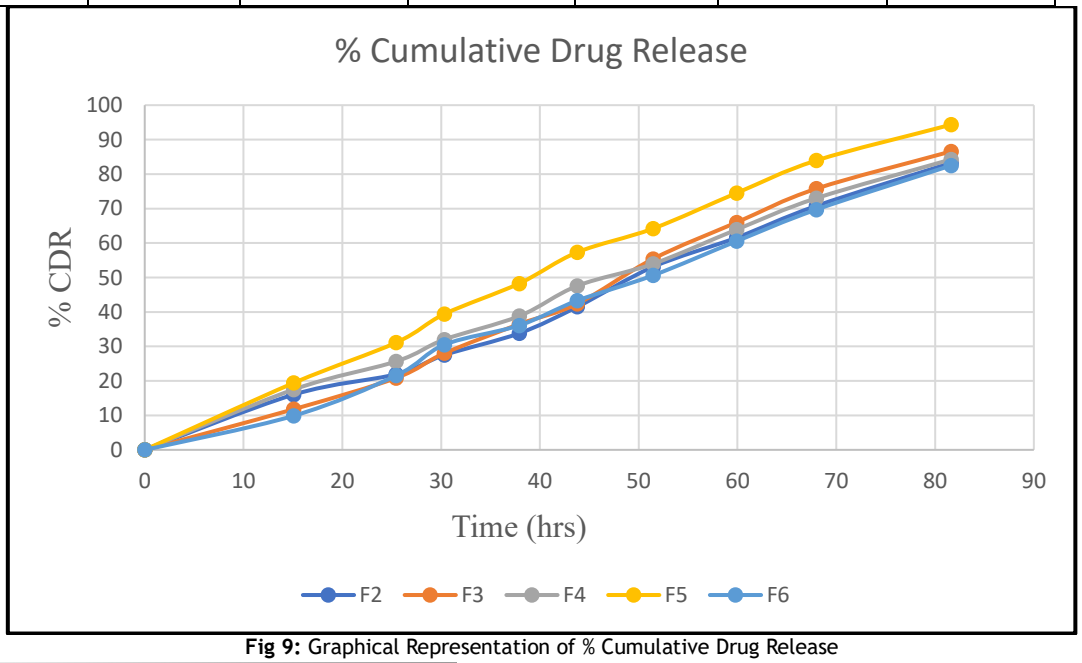


Fig 9: Graphical Representation of % Cumulative Drug Release

CONCLUSION

We deduced from the aforementioned study that herbal gel is ready for application in tropical climates. Demand for herbal formulations is rising globally. Cassia tora leaves extract, Azadirachta indica leaves extract, aerial roots of Ficus benghalensis extract and Hinokitiol are combined to create the polyherbal antifungal gel formulation. The Polyherb-loaded Nanostructured lipid Carriers (NLC) were prepared using the High-Pressure Homogenization method using tween 80 as surfactant. NLC is incorporated into a gel matrix with Sodium Carboxymethyl Cellulose as a gelling agent. The formulations of NLC were characterized by particle size, polydispersibility index, zeta potential, and *In-Vitro* study. The polyherbal gel formulations

were characterized by preliminary studies (Appearance, colour, odour, consistency, and washability) and physicochemical parameters like pH, spreadability, extrudability, drug content, viscosity, and In-vitro release study in phosphate buffer pH 7.4. We can say that this polyherbal formulation is effective in preventing fungal infections.

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