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Research

Phytoniosomal gel of acalypha indica: a novel topical delivery system for anti-inflammatory activity

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Abstract

This study aimed to develop and evaluate an anti-inflammatory gel formulation utilizing Acalypha indica phytoniosomes. Acalypha indica, a traditional medicinal plant, possesses potent anti-inflammatory properties attributed to various bioactive compounds. Phytoniosomes are novel drug delivery systems that combine the therapeutic benefits of plant extracts (phytoconstituents) with the advantages of niosomes. They form stable complexes that enhance the bioavailability, stability, and targeting of the phytoconstituents. The study involved formulating a gel base incorporating Acalypha indica phytoniosomes and evaluating its physicochemical properties (pH, viscosity, spreadability) and in vitro release characteristics. The developed gel demonstrated promising anti-inflammatory potential, with sustained release of Acalypha indica phytoconstituents. This research provides a foundation for further investigation into the clinical application of this novel gel formulation for the management of inflammatory skin conditions.

Keywords: niosomes, phytoconstituents, phytoniosomes, delivery systems, sustained release, formulation.

INTRODUCTION

Inflammation is a protective mechanism that involves immune cells, blood vessels, and molecular mediators, which is part of the complex biological response of body tissues to harmful factors like pathogens, damaged cells, and irritants. The primary objectives of anti-inflammatory processes are to eliminate the initial cause of cell damage and remove dead cells and injured tissues while promoting repair. Many medications, including non-steroidal anti-inflammatory drugs and corticosteroids, are utilized to alleviate joint pain and swelling.[1] Anti-inflammatory medications come in various forms including gel ointments and sterile preparations. These medications are often used together because of their unique effects. Synthetic drugs like NSAIDs can lead to side effects such as gastric bleeding, stomach discomfort, and ulcers. [2]

Topical drug administration involves delivering medication directly to specific areas of the body via routes such as ocular, rectal, vaginal, and cutaneous applications. The stratum corneum, which is the outermost layer of the epidermis, poses a significant challenge for skin penetration. For successful transdermal delivery,

drugs should ideally have a low molecular weight (≤500 Da), possess lipophilic properties, and demonstrate efficacy at small dosages.[3]

Niosomes, or non-ionic surfactant vesicles, are tiny lamellar structures formed by combining a non-ionic surfactant from the alkyl or dialkyl polyglycerol ether category with cholesterol (CHO), followed by hydration in water. [4] Niosomes have garnered significant interest due to their benefits in numerous areas, including chemical stability, high purity, consistent content, low expense, easy storage of non-ionic surfactants, and the wide variety of surfactants that can be utilized in niosome design. [5] A possible medicine delivery method is niosomes. Drugs can be encapsulated in niosomes to reduce inactivation and degradation after administration, avoid unwanted side effects, improve drug bioavailability, and target the diseased area. [6]

Due to their ability to dissolve functional compounds and remove the mucus layer, surfactants also serve as penetration enhancers. Based on their amphiphilic nature, the non-ionic surfactants use energy, such as heat and physical agitation, to form a closed bilayer vesicle in aqueous conditions. Their physiological characteristics, including composition, size, charge, lamellarity, and application conditions, have a significant impact on their efficacy. Additionally, niosomes have been investigated as delivery systems for oral vaccines, anti-inflammatory, anti-tubercular, anti-leishmanial, anti-cancer, and hormone medications. [7-10]

Several techniques have been devised to synthesize niosomes.

These methods include-

1. Ether injection method
2. Bubble method
3. Manual shaking method
4. Micro-fluidization method
5. Sonication method
6. Thin film hydration method
7. Multiple membrane extrusion method
8. Transmembrane pH gradient method
9. Reverse phase evaporation method

In this study, based on availability of resources and equipment Reverse phase evaporation method was used to formulate niosomes. Hence, in the present investigation, an attempt is made to formulate niosomal gel loaded formulation of Acalypha indica, using reverse phase evaporation technique.

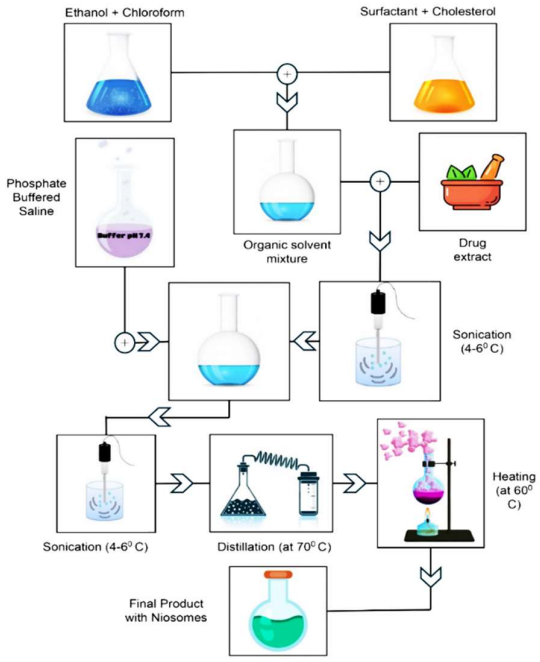


Fig 1: Diagrammatic representation of Reverse phase evaporation technique for niosomal formulation.

MATERIALS & METHODOLOGY

Plant

The plant *Acalypha indica* was collected from Mahabubnagar, Telangana during Dec/2024. The sample was identified by a professional herbalist and a voucher specimen (KF 1356) was also prepared and deposited at the herbarium hyderabadensis of Department of Botany, University college of science, Osmania University. Acalypha indica leaves were manually isolated from the aerial parts, then were dried in a dry and shady place at ambient temperature. The dried parts were grinded in to coarse powder and kept in an air tight and light-resistant container for future experiments.

The materials used in the procedure include

EXCIPIENTS		
S. No.	**Ingredients**	**Manufacturer**
1.	Chloroform	Sd fine chem limited
2.	Ethanol	Generic/Hayman
3.	Surfactant	Oxford laboratory
4.	Cholesterol	Qualigens
5.	Buffer	Molychem
6.	Mannitol	Sd fine chem limited
7.	Propyl Paraben	Molychem

The Method used here to produce niosomes; is Reverse phase evaporation method.

Methodology

Preparation of Drug Extract

- *Acalypha indica* leaves were collected from nearby localities, fields and gardens.
- The leaves are washed neatly and are shade dried for up to 3 days.
- The dried leaves are then subjected to size reduction using a laboratory mixer.
- The leaves powder is allowed to pass through sieve no. 60, to get fine powder of *Acalypha indica* leaves.
- Around 2 gm of this powder is accurately weighed and transferred to a beaker.
- About 25 ml ethanol is added to the beaker and the beaker is subjected to sonication for about half an hour.
- After sonication the solution is filtered and extract is collected.

Preparation of Phytoniosomal Suspension

1) Solvents (Ethanol & Chloroform) were measured in the ratio E:C and transferred in a beaker
2) As per the surfactant to cholesterol (S:C) ratio, accurate amount of surfactant and cholesterol were weighed and transferred to beaker with solvent
3) To this mixture, accurately weighed Mannitol was added (F_2 onwards)
4) Little amount of propyl paraben, was accurately weighed and transferred to above beaker. (F_3 onwards)
5) The contents are thoroughly stirred to dissolve the powders and make a homogenous solution.
6) The mixture is then subjected to sonication for 15 min, at 4^{O} C - 6^{O} C; using a crushed ice bath.
7) After sonication, a little of amount of phosphate buffer (pH 7.4) was added to the mixture and the new mixture is again allowed to undergo sonication, for same time and under same conditions.
8) After 2^{nd} phase sonication, the mixture is transferred to a round bottom flask and subjected to distillation at 70^{O} C to simulate reverse phase evaporation.
9) This is done to get rid of the solvents; ethanol and chloroform as much as possible & to concentrate the drug content.
10) The distillation apparatus is dismantled to add little buffer (around 5 ml); and the mixture is heated to evaporate any remaining solvents to make a suspension.
11) The Suspension is allowed to cool and the product is collected.

Formulation	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**
S:C	1:1	1.5:1	2:1	1:1	1:1	1.5:1	2:1
Surfacntant	Span 40	Span 60	Span 60	Span 60	Span 40	Span 40	Soan 40
E:C				1:2			

Preparation of Buffer

- Accurately weighed Salts Sodium Chloride, Potassium Chloride, Sodium hydrogen orthophosphate, Monopotassium phosphate are transferred to a beaker containing about 200ml water.
- The contents are stirred to dissolve the salts and to make a homogenous solution.
- The solution is then transferred to a 1000 mL volumetric flask, and the volume is made up to 1000mL using distilled water.
- The solution is filtered to make it free from any particles that may have entered the buffer.
- The buffer is then checked for it's pH and adjusted (if any changes required) to pH 7.4.

Preparation of Phytoniosomal Gel

- Around 2gm of Carbopol is weighed and transferred to a beaker.
- To this beaker around 200ml water are added to make the Carbopol concentration 1%, and the beaker is kept undisturbed for 24 hours to allow the hydration of gel.
- 2 gm of Phytiniosomal suspension is weighed and transferred to the viscous hydrated gel
- Triethanolamine is added drop by drop, with continuous stirring to the hydrated gel, to adjust the viscosity, until favourable consistency occurs.

Evaluation methods

Spreadability

The test usually involves placing a known weight on a gel sample sandwiched between two glass slides and measuring the time it takes for the slides to separate, with the calculated spreadability value being a function of this time and the applied weight.

Good spreadability: A value within the 4.5 - 6.9 g.cm/sec range indicates the gel can spread smoothly and evenly on the skin.

pH test

Take 3g of gel and dissolve in 30ml making a 10% gel solution, measure pH using pH meter and also with a filter paper.

Normal range: The pH of these formulations can range from 4.58 to 7.96.

Viscosity

Place the sample in the viscometer. Select the correct spindle and speed for the sample. Rotate the spindle. Measure the torque required to rotate the spindle. The torque is proportional to the viscosity of the fluid.

Anti-microbial test

Spread plate method is used to grow B.subtilis and E.coli. To test the antimicrobial activity of a gel, disk diffusion test is used where a sample of the gel is placed on a sterile agar plate inoculated with the target bacteria, and the zone of inhibition around the gel disc is measured to determine its antimicrobial effectiveness.

Permeation study using Franz diffusion method

Prepare the skin sample (pre-soaked nitrocellulose) by mounting it onto the Franz cell. Apply the test formulation to the skin surface. Maintain a constant temperature and stirring in the receptor compartment to maintain a "sink condition" (low concentration of the test substance). Regularly withdraw samples from the receptor compartment at predetermined time points to analyse the concentration of the permeated substance using analytical techniques like HPLC or UV spectrophotometry.

In-vitro Anti-inflammatory Egg Albumin Denaturation

The primary aim of the egg albumin denaturation assay is to assess the ability of various agents or compounds to prevent or mitigate the denaturation of egg albumin under specific conditions. Denaturation refers to the alteration of a protein's structure, resulting in a loss of its biological function. In this experiment, egg albumin serves as a model protein, and denaturation is induced by exposing it to extreme temperatures, pH levels, or other denaturing agents. This process disrupts the original conformation of egg albumin, altering its physical properties and leading to a loss of functional activity. The assay evaluates the effectiveness of a drug or compound in inhibiting or reducing egg albumin denaturation, thereby assessing its potential anti-inflammatory effects. The underlying principle of the egg albumin denaturation assay is that substances with anti-inflammatory properties may stabilize protein structures and prevent denaturation, which is often associated with inflammation and tissue damage. Consequently, agents or compounds that significantly reduce egg albumin denaturation in this assay may exhibit potential anti-inflammatory activity. Protein denaturation is believed to be a contributing factor to inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) not only prevent protein denaturation but also inhibit the COX enzyme. Various concentrations of the test sample can be incubated with the egg albumin solution under controlled experimental conditions, allowing reactions to occur, followed by measuring absorbance to calculate the percentage of inhibition.

RESULTS AND DISCUSSIONS

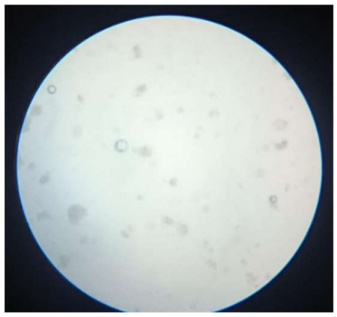
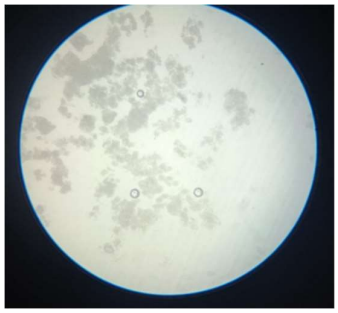
Identification tests for flavonoids in Acalypha indica (Quercetin)

S.no	Test	Observation	inference
1.	**SHINODA TEST**: Dissolve the substance in ethanol. Add a small piece of magnesium turnings. Add a few drops of concentrated hydrochloric acid. Observe the color change.	Crimson red colour was observed. 	Indicates presence of flavonoids.
2.	**SODIUM HYDROXIDE TEST:** Add a few drops of dilute sodium hydroxide to a small amount of plant extract. Observe for a yellow color Add a few drops of dilute hydrochloric acid. Observe if the color changes back to colorless.	Yellow colour was observed.  Solution becomes colourless after adding hydrochloric acid.	Indicates presence of flavonoids.

			
3.	**FERRIC CHLORIDE TEST:** Boil a portion of the substance with distilled water and filter. Add a few drops of 10% ferric chloride solution to 2 ml of the filtrate. Observe the color change.	Dark brown colour was observed. 	Indicates presence of flavonoids

Phytoniosome formulations results

Date / Formulation	Short term result	Long term result	Remarks
Placebo 1	Nanoparticles observed	Good overall stability	No remarks
Placebo 2	Nanoparticles observed	Good overall stability	No remarks
Formulation 1	Clusters of Niosomes observed	Good overall stability	Need mannitol to prevent aggregation
Formulation 2	Niosome aggregates	Good overall stability	Bubbling seen during evaporation Missed mannitol
Formulation 3	Individual Niosomes seen	Microbial growth occurred	Need to include a preservative
Formulation 4	Individual Niosomes seen	Good overall stability	No remarks
Formulation 5	Individual Niosomes seen	Good overall stability	No remarks
Formulation 6	Individual Niosomes seen	Good overall stability	No remarks

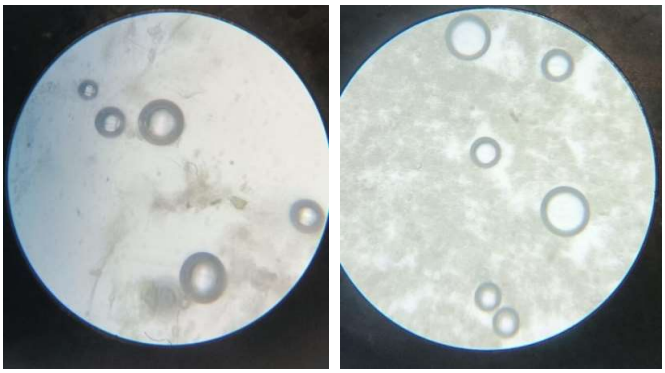


Fig 2: Microscopic view of phytoniosomes

Phytoniosomal gel evaluation

Spreadability

Spreadability was found to be 5.1g.cm/sec, indicating the gel can easily spread on the skin with minimal shear force.

pH

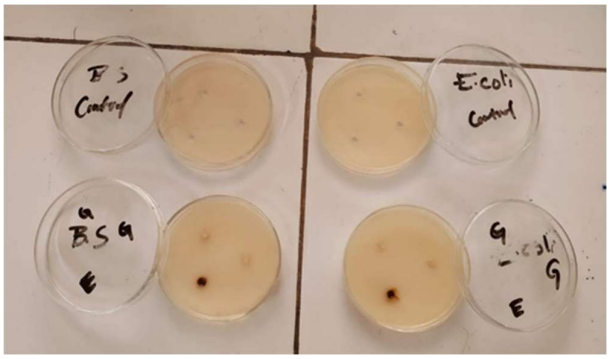
10% gel solution pH was found to be around neutral with a filter paper, and when tested with pH meter it was found to be 6.23 which is within the normal pH range of a topical gel.

Viscosity

Viscosity of 10% gel solution was found to be 25 cP. when the gel was applied on the skin, it was non-greasy, and caused no skin irritation.

Anti-microbial test

Acalypha indica extract shown anti-microbial activity against B.subtilis and E.coli.



Anti-inflammatory activity
Effect of AIE on heat induced protein denaturation

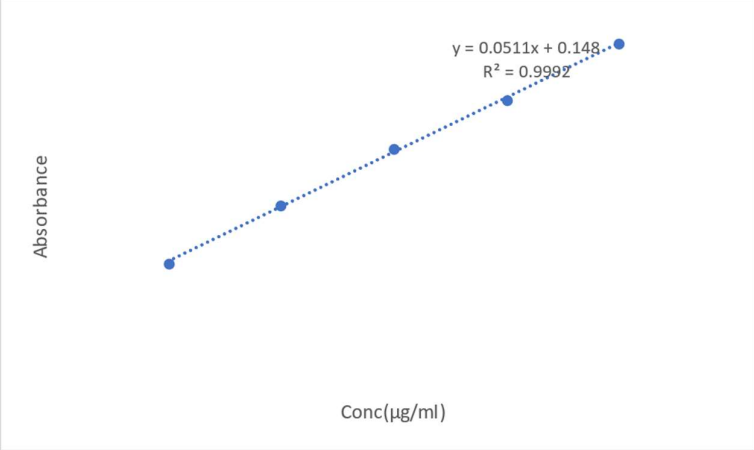
Concentration (µg/ml)	absorbance	% inhibition of protein denaturation
-	0.573	-
2	0.50	12.8%
4	0.47	17.97%
6	0.31	45.89%
8	0.22	61.6%
10	0.14	75.5%

Percentage inhibition = (Abs control -Abs sample)/Abs control×100

Permeation study using franz diffusion method

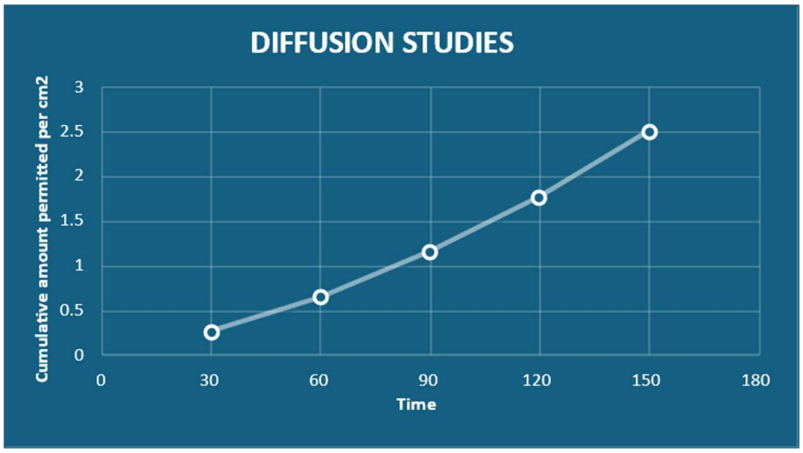
Calibration of Acalypha indica

S.no	concentration	absorbance
1.	2	0.246
2.	4	0.355
3.	6	0.461
4.	8	0.553
5.	10	0.658



Gel permeation

S.no	Time (min)	Absorbance	Conc.	Conc.in receptor compartment	Cummulative amount	Cummulative amount permitted per cm^2
1	30	0.246	4.81	o.48	0.48	0.27
2	60	o.355	6.94	0.69	1.17	0.66
3	90	0.461	9.02	0.90	2.07	1.17
4	120	0.553	10.82	1.08	3.15	1.78
5	150	0.658	12.87	1.28	4.43	2.51



Slope(flux)=0.024333333
Permeation coefficient=flux/concentration
=0.024/5mg
$=0.48\times10^{-2}$

CONCLUSION

Considering all the evaluated parameters, the phytoniosomes we have formulated have given satisfactory results in all tests. The Niosomes have been observed under microscope and the images documented here have shown the clear visibility of the double lamella structure. The niosomes that have been formulated with both Mannitol and preservative have shown individual niosomes with a long shelf life. Formulations with best niosome formation (F1 &F2) were selected and used in gel preparation. The Gel prepared includes 1% Carbopol gel and acceptable amount of Triethanolamine. The Gel has been observed to have satisfactory viscosity, permeability, spreadability, pH etc., The drug extract of Acalypha indica has been observed to have good anti-inflammatory and anti-microbial properties, the constituent which is responsible for these properties is Quercetin flavonoid. Considering all the properties and results determined so far, the gel formulated allows the permeation of the drug due to the incorporation niosomes into the gel, thus accelerating the slow absorption of phytochemicals. The gel can be termed as a good anti-inflammatory and anti-microbial properties, basing from the results from the evaluation tests. The gel has all ideal properties of a pharmaceutical gel. We conclude that preparation of niosomes by reverse phase evaporation technique has been challenging but yet informative and productive. The gel prepared from the niosomes thus prepared can be considered as one of the advanced drug delivery systems, as niosomes can be applied for targeted therapy.

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