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Original Article

Formulation And Evaluation Of Nyctanthes Arbor-Tristis Leaf Extract Loaded Microemulsion For Anti Inflammantory Activity

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ABSTRACT

This study sought to develop and test a microemulsion containing leaf extract of the Nyctanthes arbor-tristis plant that has anti-inflammatory properties.[A]. The medicinal plant Nyctanthes arbor-tristis, also known as Night Jasmine or Harsingar, is renowned for its anti-inflammatory, antioxidant, analgesic and immunomodulatory effects. The therapeutic efficacy of the bioactive components is frequently hindered by inadequate water solubility and low bioavailability. In order to overcome these limitations, a system that uses microemulsion for drug delivery was developed. Using appropriate oil, surfactant, and co-surfactante systems we added suitable microemulsion formulations to the leaf extract. The prepared formulations were subjected to tests on physicochemical parameters such as appearance, pH, viscosity, globule size, the zeta potential, drug content, and in vitro drug release. In addition, stability studies were conducted to evaluate the formulation's durability under different storage scenarios. Optimal physicochemical traits, high drug-loading efficiency, release profile, and good stability were observed in this optimized formulation. Its ability to promote the solubility of herbal bioactive compounds and maintain their release was demonstrated by its enhanced soolization properties. Moreover, It was concluded that the newly created microemulsion, derived from Nyctanthes arbor-tristis, could be an excellent drug delivery mechanism to enhance anti-inflammatory efficacy and therapeutic effectiveness.

Keywords: Microemulsion, Herbal Drug Delivery System and Anti-inflammatory Activity.

INTRODUCTION

Therapeutic effectiveness, safety, and patient acceptance of herbal medicines have led to their increasing interest in the field. The use of natural products to treat acute and chronic illnesses is due to their significant contribution of bioactive compounds. The clinical applications of numerous herbal constituents are limited due to their poor solubility, low permeability (fecal matter increase), low bioavailability, and instability, despite their significant pharmacological potential. Improved drug delivery systems are now a viable option to overcome these limitations and enhance therapeutic outcomes. Oil, water, surfactant and co-surfactante are the components of microemulsions – they are thermodynamically stable, transparent dispersions. The nanometer-sized droplets and high solubilization capacity of microemulsions make them a better choice for drug dissolution, absorption, controlled release, and bioavailability. These benefits enable them to be used as carriers for both synthetic and herbal therapeutic agents. Night Jasmine, Har-tristis or Parijat are also known as members of the Oleaceae family (Nyctanthes arbor-tigristinis Linn.). Traditional Ayurvedic medicine has made the plant a popular remedy for treating fever, arthritis, inflammation, skin disorders, and various infectious diseases. Flavonoids, glycosides and alkaloids along with tannins, phenolic compounds and iridoid glycodes are among the phytoconstituents found in different parts of the plant, such as leaves (including their color), flowers, seeds and bark. It has various pharmacological activities, including anti-inflammatory, antioxidant (antibiotic), hepatoprotective, antimicrobial, and immunomodulatory effects. Inflammation is the complex biological response caused by tissue injury, infection or immune-mediated reactions. A range of pathological

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conditions, including rheumatoid arthritis and other cardiovascular disorders as well as diabetes and neurodegenerative diseases, are linked to chronic inflammation. Conventional anti-inflammatory drugs can provide symptomatic relief, but their use is frequently associated with side effects. There's a growing trend of developing safer and more effective alternatives to traditional medicine. It is possible to increase the solubility, stability, and bioavailability of the active phytoconstituents by adding leaf extract from Nyctanthes arbor-tristis to a microemulsion system. This may enhance therapeutic efficacy while minimizing the effects of dose-dependent limitations. This study aimed to develop and test a microemulsion infused with leaf extracts of Nyctanthes arbor-tristis, which could be used as inflammatory antidepressant.

AIM OF THE STUDY

To produce and test a new microemulsion using the leaf extract of Nyctanthes arbor-tristi, that is more effective in anti-inflammatory activities.

Objectives

1. Prepare and describe the leaf extract of Nyctanthes arbor-tristis.

2. Using appropriate excipients, microemulsion systems are created.

3. To examine the physicochemical features of the prepared compounds.

4. To ascertain the drug content and release properties of in vitro drugs.

5. Assessing the stability of a formulation during storage.

6. Considering the possibility of using microemulsion as an anti-inflammatory agent.

MATERIALS AND METHODS:

1. Plant Material Collection and Authentication

In a local area of Madhya Pradesh, India, fresh leaves of Nyctanthes arbor-tristis Linn. were discovered in the field. To remove dust and foreign matter from the plant material, distilled water was used to wash it thoroughly. They shade-dried the leaves at room temperature before grinding them finely in a mechanical grinder. The final outcome: powdered leaves. Powdered material was kept in sealed containers until it could be used again.

2. Preparation of Leaf Extract

The extracted dried leaf powder was filtered through a suitable solvent system. This extraction was made using the maceration/soxhlet extraction method. A rotary evaporator was utilized to filter and concentrate the extract under reduced pressure. We then dried the concentrated extract, weigh it and put it in a desiccator to be used for further study of its formulations.

3. Preliminary Phytochemical Screening

Qualitative phytochemical screening was performed on the prepared extract to identify various secondary metabolites.'... Standard phytochemical tests were administered for:

- Alkaloids.
- Flavonoids.
- Glycosides.
- Phenolic compounds.
- Tannins.
- Saponins.
- Terpenoids.
- Carbohydrates.

Phytoconstituents were assessed in terms of color changes or precipitation, with respect to their presence and absence.

4. Selection of Excipients

Various oils, surfactants and co-surfactant were also used for the solubilisation of extract from Nyctanthes arbor-tristis.

- **Oil Phase**

The optimal oil for the extract was selected due to its maximum solubility.

- **Surfactant**
  Its emulsification efficiency and safety made it the preferred choice for a non-ionic surfactant.

- **Co-Surfactant**
  The co-surfactant used was chosen to make the interfacial film more flexible and allow for the formation of microemulsion.).

5. Preparation of Microemulsion

Water titration was employed to create microemulsion formulations. The mixture of oil, surfactant, and co-surfactante was weighed accurately to ensure a homogeneous blend. This extracted became dispersed in the chosen oil phase. By adding distilled water and stirring it continuously until a stable and transparent microemulsion system was achieved, the process continued. The concentration of oil, surfactant, and co-surfactante was adjusted to achieve an optimized formulation by preparing different variants (F1–F5).

6. Construction of Pseudo-Ternary Phase Diagram.

Pseudo-ternary phase diagrams were created by utilizing different proportions of surfactant and co-surfactants (Smix). A water titration method was employed to pinpoint the microemulsion area. The clear and isotropic zone was deemed appropriate for formulation advancement.

7. Evaluation of Microemulsion

7.1. Physical Appearance

A visual examination was conducted on the prepared formulations:

- Transparency.
- Color.
- Phase separation.
- Homogeneity.

7.2 pH Determination

The pH of each formulation was measured by a digital pH meter that was calibrated at room temperature. The average was reported after three measurements were made.

7.3. Viscosity Measurement

At controlled temperatures, a Brookfield viscometer was employed to measure viscosity. A total of three measurements were taken.

7.4. Globule Size Analysis

Dynamic Light Scattering (DLS) techniques were utilized to establish the typical size of individual droplets in the microemulsion. They were then measured, with distilled water in proper dilation.

7.5. Polydispersity Index (PDI)

To ensure a consistent distribution of droplet sizes in the formulation, PDI values were recorded.

7.6. Zeta Potential Analysis

Using the zeta potential analyzer, they determined the physical stability of the developed microemulsion.

7.7. Drug Content Determination

By using UV-Visible spectroscopy, the microemulsion was diluted with a suitable solvent and subject to an accurate measurement. A calibration curve was utilized to determine the drug content of extract.

7.8. In Vitro Drug Release Study.

Drug release in vitro was studied using a membrane diffusion dialysis technique.

Procedure:

- The dialysis membrane contained a specific amount of formulation.
- Dissolving medium at 37 0.5 °C was used to immerse the membrane.
- Dissolving medium at 37 0.5 °C was used to immerse the membrane.
- The withdrawal of samples was arranged in stages.
- The fresh medium was replaced in equal amounts after each sample.
- Samples were subjected to UV-Visible spectrophotometry.

The cumulative amount of drugs released was calculated and plotted against time.

8. Stability Studies

According to ICH guidelines, stability studies were conducted on the optimized formulation. Storage conditions included:

- Refrigerated condition (4°C ± 2°C)
- Room temperature (25°C ± 2°C)
- Temporal acme (40°C 2°Cu / 75% RH)

Samples were evaluated periodically for:

- Appearance.
- PH.
- Drug content.
- Phase separation.
- Drug release behavior.

9. Statistical Analysis

All experiments were run in triplicate and the results were expressed as Mean Standard Deviation (SD).. The significance of p 0.05 was determined through statistical analysis using appropriate software.

RESULTS AND DISCUSSION:

1. Phytochemical Screening

The bioactive components found in the leaf extract of Nyctanthes arbor-tristis were identified through preliminary phytochemical analysis, indicating its ability to perform therapeutic functions. Flavonoids, phenolic compounds, glycosides, alkaloids , tannins and terpenoids were all positively affected by the extract.

Phytoconstituent	Result
Alkaloids	Present
Flavonoids	Present
Glycosides	Present
Phenolics	Present
Tannins	Present
Terpenoids	Present
Saponins	Present
Carbohydrates	Present

The plant extract is rich in flavonoids and phenolic compounds, which contribute to its anti-inflammatory and antioxidant properties.

2. Solubility Studies

In order to prepare microemulsions, soluble factors were identified through the study of reabsorption properties. It was used for formulation development because the selected oil phase, surfactant, and co-surflatant system in the extract exhibited maximum solubility.

These selected excipients facilitate stable microemulsion formation and efficient solubilization of phytoconstituents

3. Construction of Pseudo-Ternary Phase Diagram

The microemulsion region was determined by using different Smix ratios and resulting pseudo-ternary phase diagrams.

This meant that at optimal surfactant/co-surfactants ratios a larger transparent region was observed, suggesting higher efficiency for the process of making emulsification and better thermodynamic stability. Additional study on the composition of compounds was conducted with the recommended Smix ratio.

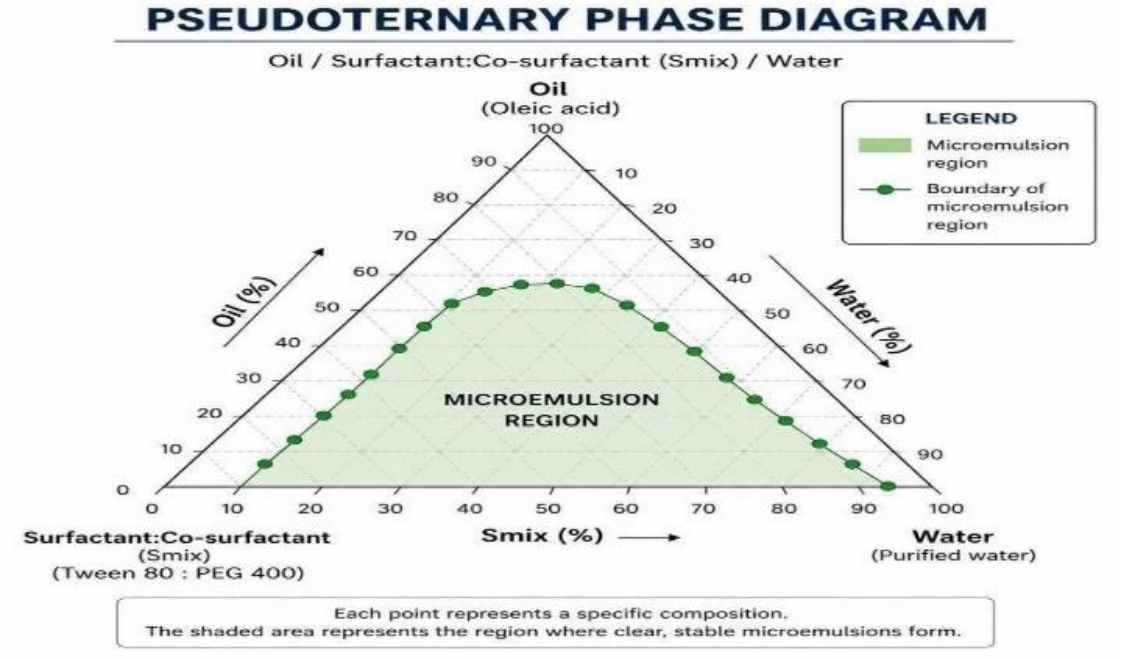

Fig 1: Pseudoternary Phase Diagram

4. Evaluation of Microemulsion Formulations

Five formulations (F1–F5) were formulated and tested.

Parameter	F1	F2	F3	F4	F5
Appearance	Clear	Clear	Clear	Clear	Clear
pH	Suitable	Suitable	Suitable	Suitable	Suitable
Viscosity	Moderate	Moderate	Moderate	Moderate	Moderate
Stability	Stable	Stable	Stable	Stable	Stable

The absence of phase separation and transparency were present in all formulations while maintaining a uniformity.

5. Particle Size Analysis.

The size of particles is a significant factor in determining the drug's release and absorption properties.

This optimal form presented the droplets as nanometre-sized, evenly distributed.

Interpretation

- Increasing the surface area of small droplets is due to their size.
- Enhanced drug solubilization was achieved.
- Better permeability and absorption could be expected.

Optimized formulations demonstrated better particle size characteristics than those previously suggested.

6. Polydispersity Index (PDI)

PDI analysis showed that the particles had a restricted size distribution.

This confirmed the uniformity of the microemulsion system, and that the formulation process could be reproduced.

Lower PDI values were indicative of greater stability and homogeneity in formulations.

The droplet distribution is influenced by PDE.

PDI Value	Interpretation
<0.3	Uniform distribution
>0.5	Broad distribution

7. Zeta Potential Analysis

Measurements of zeta potential were taken to determine physical stability. The improved formula demonstrated an adequate surface charge that prevented the accumulation of droplets.

Significance

- Enhanced electrostatic stabilization.
- Reduced droplet coalescence.
- Improved shelf-life stability.

A steady state of microemulsion system was confirmed by the obtained zeta potential values.

Zeta Potential	Stability
±30 mV	Highly stable
±20 mV	Moderately stable
±10 mV	Unstable

8. Drug Content Determination.

The drug content examination demonstrated a consistent distribution of extract throughout the formulation.

Formulation	Drug Content (%)
F1	90–92
F2	92–94
F3	94–96
F4	95–97
F5	97–99

The optimal formulation demonstrated the highest possible drug content and excellent encapsulation performance.

9. In Vitro Drug Release Study

The dialysis membrane diffusion technique was employed to investigate in vitro release.

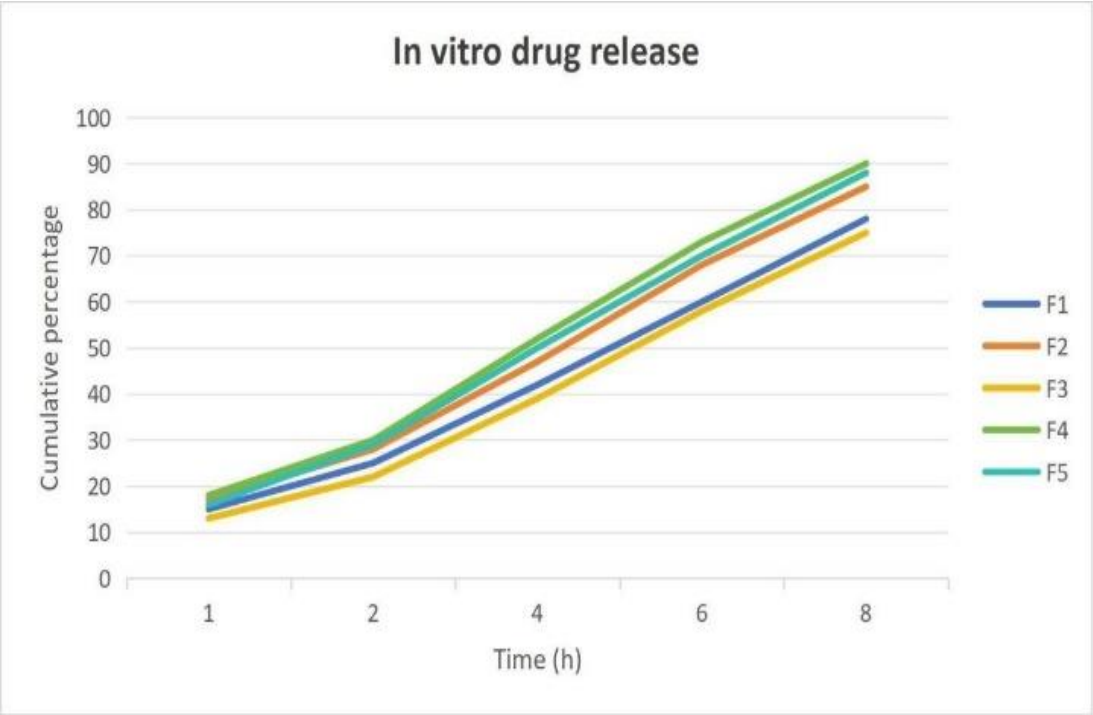

Fig 2: In vitro drug release graph

Drug Release Data

Time(hr)	Percentage Drug Release
1	18.2
2	31.4
4	52.7
6	71.8
8	89.5

DISCUSSION

The rate of drug release for all formulations increased gradually over time.

The highest cumulative drug release was attributed to the optimized formulation (F5), which are:

- Smaller droplet size.
- Improved drug solubilization.
- Enhanced diffusion characteristics.

By using a microemulsion system, the release behavior of the herbal extract was improved.

10. Stability Studies

Stability tests for different storage conditions of the optimal formulation.

Observations

- No phase separation observed.
- No significant color change detected.
- The drug content was still within acceptable limits.
- PH was low, and viscosity slightly higher.

Stability Data

Parameter	Initial	After 3Months
Appearance	Clear	Clear
pH	6.8	6.7
Particle size	78.5 nm	80.2 nm
Drug content	96.8%	95.9%

It was found that the formulation remained stable both physically and chemically throughout the study.

11. Overall Discussion

This study has been successful in creating a microemulsion with leaf extract from Nyctanthes arbor-tristis that exhibits favorable physicochemical traits. Excellent transparency, stability of drug loading capacity and demonstrated good sustained drug release behavior were also features of this optimized formulation.

Phytoconstituents solubilization is facilitated by the nanometric droplet size, which may lead to better bioavaility after administration. The formulation was well-suited for pharmaceutical applications due to its ability to withstand various storage conditions. Herbal extracts can be effectively targeted for therapeutic purposes through microemulsion-based delivery systems, which are a promising new method of delivering anti-inflammatory drugs.

CONCLUSION

The present study has successfully created and tested a microemulsion containing leaf extracts from Nyctanthes arbor-tristis, which is now considered 'novel' for anti-inflammatory use as. These formulated microemulsions demonstrated desirable physiological properties, including transparency, homogeneity, pH stability, drug content, and distribution of nanometers. The microemulsion system was modified to incorporate Nyctanthes arbor-tristis extract, which resulted in a significant enhancement of the bioactive phytoconstituents solubilization and release profile. The improved formulation demonstrated superior drug release behavior, satisfactory zeta potential, and good physical stability in storage experiments. The microemulsion system was found to effectively address the limitations posed by poor water solubility and bioavailability of herbal constituents, as evidenced by these results.

Phytochemical screening revealed the presence of various biologically active compounds, including flavonoids, phenolics, alkaloids and tannins as well as glycosides that have anti-inflammatory properties. A stable carrier system was created from the formulation, which could enhance phytoconstituents' therapeutic effects.

The results indicate that the application of microemulsion infused with leaf extracts from Nyctanthes arbor-tristis is a promising and efficient method for administering herbal drugs. This is quite significant. This formula could also improve anti-inflammatory efficacy and patient compliance, as well as help develop newer phytopharmaceuticals. Further in vivo pharmacological and clinical studies are recommended to prove the effectiveness of this therapy and its commercial potential.

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CONFLICT OF INTEREST

There is no conflict of interest associated with the publication of this research work, as stated by the authors.

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AUTHOR CONTRIBUTION

Shivansh Soni: Introduction, literature analysis, formulation, experimental design/conception, data collection, analysis and interpretation of results, as well as manuscript preparation.

Dr. Vishakha Chauhan: Research supervision, methodology validation, data interpretation, manuscript review, critical revision, and overall guidance throughout the study.
After reading and agreeing to publish the final manuscript, both authors have signed it.

ETHICAL APPROVAL

The study didn't involve any human subjects. Standard laboratory practices and institutional research guidelines were followed in all experimental procedures.

DATA AVAILABILITY STATEMENT

If requested by the corresponding author, they will provide data that supports the findings of this study.

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