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DEVELOPMENT OF *SYZYGIUM CUMINI* LEAF PHYTOCHEMICAL-LOADED STIMULI-RESPONSIVE POLYMERIC NANOGELS FOR INFLAMMATION-TARGETED DRUG DELIVERY IN DYSMENORRHEA: FORMULATION OPTIMIZATION AND *IN VITRO* EVALUATION

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ABSTRACT

Background: Primary dysmenorrhea is associated with prostaglandin-mediated uterine contractions and menstrual pain. *Syzygium cumini* leaves contain phenolic compounds and flavonoids with reported biological activities. However, herbal extracts exhibit compositional variability, which may complicate formulation development and reproducibility. Stimuli-responsive polymeric nanogels offer a potential approach to controlling the release of encapsulated phytochemicals. **Objective:** This study aims to develop, optimize and characterize *Syzygium cumini* leaf phytochemical-loaded stimuli-responsive polymeric nanogels and investigate their *in vitro* anti-inflammatory potential relevant to dysmenorrhea. **Methods:** Authenticated *Syzygium cumini* leaves will be extracted using a standardized procedure. Phytochemical characterization will include chromatographic fingerprinting and quantification of selected marker compounds. A suitable biocompatible polymer network will be selected according to the intended stimulus. Formulation variables will be optimized using design of experiments. Particle size, polydispersity index, zeta potential, morphology, encapsulation efficiency, loading capacity, stability and marker release under reference and stimulus conditions will be evaluated. Biological studies will compare the loaded formulation with free extract, blank nanogels and appropriate controls. Cell viability and selected inflammatory endpoints, including prostaglandin E2 where justified, will be assessed using validated methods. **Expected contribution:** The investigation will establish whether the formulation can be prepared reproducibly, demonstrate stimulus-dependent release and provide evidence regarding its comparative biological performance. **Conclusion:** This study provides an experimental framework for evaluating a responsive herbal nanogel as an early-stage drug-delivery candidate. Actual results must establish its formulation performance and biological value. Clinical effectiveness in dysmenorrhea cannot be inferred without further preclinical and clinical investigation.

KEYWORDS

Syzygium cumini, Jamun, Herbal phytochemicals, Polymeric nanogels, Stimuli-responsive drug delivery, Dysmenorrhea, Controlled release and Inflammation.

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INTRODUCTION

Background

Primary dysmenorrhea is characterized by painful menstrual cramps without identifiable pelvic pathology. Its pathophysiology is associated with increased endometrial prostaglandin production, particularly prostaglandin F2α and prostaglandin

E2. These mediators contribute to uterine contractions, vasoconstriction, reduced uterine perfusion and pain. Nonsteroidal anti-inflammatory drugs are commonly used because they inhibit cyclooxygenase-mediated prostaglandin synthesis[1,2]. Although established treatments are available, their suitability and tolerability differ between individuals. Investigating alternative phytochemical delivery systems may therefore provide useful pharmaceutical research opportunities, provided their benefits are demonstrated experimentally. *Syzygium cumini* (L.) Skeels, commonly known as Jamun, belongs to the family Myrtaceae. Its leaves contain several classes of phytochemicals, including phenolic acids, flavonoids, tannins, and other secondary metabolites. Compounds reported in this species include gallic acid, ellagic acid, quercetin, myricetin, and related phenolic constituents. The precise composition depends on plant part, geographical origin, extraction method, and analytical conditions[3,4].

Herbal phytochemicals and drug delivery

Plant-derived extracts contain multiple constituents with different physicochemical properties. Their composition can vary between batches, and some constituents may have limited solubility, stability, or membrane permeability. Chemical standardization is therefore important when developing a reproducible herbal formulation. Chromatographic fingerprinting and marker-compound quantification can help establish extract consistency. The selected markers should be confirmed in the actual extract rather than assumed to be present at a particular concentration.

Stimuli-responsive polymeric nanogels

Nanogels are crosslinked polymer networks with nanoscale dimensions and the capacity to absorb water. Depending on polymer chemistry and network structure, they can exhibit changes in swelling, structure, or release behavior when exposed to selected environmental stimuli. Potential stimuli include pH, temperature, redox conditions, and specific biological molecules. Such systems have been investigated for controlled drug release and other biomedical applications[5,6]. However, stimulus responsiveness does not automatically establish targeted delivery. A formulation responding to a laboratory pH or oxidant concentration may not exhibit the same behavior in the human uterus. The trigger must be scientifically justified, and the formulation's response must be validated under defined experimental conditions.

Research gap

Existing literature describes the phytochemical and pharmacological properties of *Syzygium cumini* and the potential of responsive polymeric nanogels. Nevertheless, the proposed study requires a specific investigation of the selected formulation, its stimulus-dependent release and its biological performance relative to suitable controls. The research gap will be considered established only after searching relevant scientific databases and patent records for the same plant extract, polymer chemistry, stimulus and therapeutic application.

Aim

To develop, optimize and characterize *Syzygium cumini* leaf phytochemical-loaded stimuli-responsive polymeric nanogels and evaluate their *in vitro* anti-inflammatory potential relevant to dysmenorrhea.

Objectives

To authenticate *Syzygium cumini* leaves and develop a reproducible extraction procedure.

To characterize the extract using phytochemical screening and chromatographic fingerprinting.

To select a suitable polymer network with a defined stimulus-response mechanism.

To optimize formulation variables using design of experiments.

To characterize particle size, polydispersity index, zeta potential, morphology, encapsulation efficiency, and loading capacity.

To investigate stimulus-dependent release and formulation stability.

To evaluate cytocompatibility and selected inflammatory endpoints using appropriate *in vitro* models.

To compare the optimized formulation with free extract, blank nanogels, and a nonresponsive comparator where feasible.

Research hypothesis

A standardized *Syzygium cumini* leaf extract can be incorporated into a responsive polymeric nanogel with reproducible physicochemical properties and measurable stimulus-dependent release. Whether encapsulation improves anti-inflammatory activity will be determined experimentally.

MATERIAL AND METHODS

Study design

The study will consist of five stages: herbal extract preparation and standardization, nanogel formulation, statistical optimization, physicochemical characterization and *in vitro* biological evaluation.

Plant collection and authentication

Healthy *Syzygium cumini* leaves will be collected from a documented source. Botanical identification will be performed by a qualified botanist or pharmacognosist, and a voucher specimen will be deposited in an appropriate institutional collection. The collection date, location, plant condition, drying conditions, and storage details will be recorded. Dried leaves will be pulverized and stored under conditions that minimize moisture exposure and phytochemical degradation.

Preparation of the leaf extract

A suitable extraction solvent will be selected based on the intended phytochemical profile, extraction efficiency, polymer compatibility and subsequent biological testing. Hydroethanolic extraction may be evaluated during preliminary method development.

The following parameters will be documented:

Initial mass of dried plant material.

Solvent composition and volume.

Extraction method, duration and temperature.

Filtration and solvent-removal conditions.

Final mass of dried extract.

Extract storage conditions and batch identifier.

Extraction yield will be calculated as:

Extraction yield (%) = (Mass of dried extract / Mass of starting dried plant material) × 100.

Independent extraction batches will be evaluated to establish reproducibility.

Phytochemical characterization

Qualitative phytochemical screening will investigate relevant classes, including phenolics, flavonoids and tannins. Total phenolic and total flavonoid content may be determined using validated colorimetric assays. HPLC-DAD or HPTLC will be used where available to establish a chromatographic fingerprint and quantify selected markers. Authentic reference standards will be used wherever possible. The analytical procedure will document specificity, calibration performance, precision, recovery and relevant detection limits. Matrix interference from the complex extract will be assessed.

Selection of the responsive polymer

The polymer system will be selected according to the physicochemical characteristics of the extract, intended stimulus, compatibility with the intended route of administration and laboratory resources. The initial study will investigate one principal trigger, such as pH or redox conditions. The selected polymer network must show a measurable and reproducible response under justified test conditions. A matched nonresponsive formulation will be included where feasible to determine whether the stimulus-response mechanism contributes to the observed release behavior.

Nanogel formulation

Nanogels will be prepared using a method appropriate to the selected polymer chemistry, such as controlled ionic gelation, polymer crosslinking, or self-assembly. Formulation development will investigate polymer concentration, extract-to-polymer ratio, crosslinking conditions, mixing parameters, preparation pH, and purification method. Blank nanogels will be prepared without extract. Free extract, loaded nanogels and relevant formulation controls will be retained for subsequent experiments.

Formulation optimization

A design-of-experiments approach will be used to identify influential formulation variables and their interactions. Initial screening will be followed by an appropriate response-surface design if preliminary data support this approach.

Potential independent variables include polymer concentration, extract-to-polymer ratio, and crosslinking conditions. Responses will include particle size, polydispersity index, encapsulation efficiency and marker release.

The final design, factor ranges, number of experiments, and statistical model will be selected based on preliminary feasibility studies. The optimized formulation will be confirmed using independently prepared batches.

Particle size and polydispersity index

Hydrodynamic particle size and polydispersity index will be measured using dynamic light scattering. Sample dilution, dispersion medium, temperature, and instrument settings will be standardized and reported. Measurements will include independent batches and appropriate technical replicates. Results will be reported with suitable measures of variability.

Zeta potential and morphology

Zeta potential will be determined using an appropriate electrophoretic light-scattering method. Particle morphology will be investigated using transmission electron microscopy or another suitable imaging technique where available. Morphology and hydrodynamic particle size will be interpreted together because they measure different properties.

Encapsulation efficiency and loading capacity

A validated analytical method will quantify unencapsulated marker compounds after separating them from the loaded carrier.

Encapsulation efficiency (%) = [(Initial marker amount − Free marker amount) / Initial marker amount] × 100.

Loading capacity (%) = [Marker mass retained in the purified carrier / Mass of recovered loaded carrier] × 100.

Analytical recovery and marker mass balance will be evaluated before these values are interpreted.

Stimulus-dependent release

Release testing will compare a reference condition with the selected stimulus condition. Temperature, buffer composition, ionic strength and other relevant parameters will be controlled. The released marker compounds will be quantified at prespecified time points using a validated method. Sampling corrections, sink conditions, adsorption, and marker stability will be considered. Cumulative release will be plotted against time. Appropriate kinetic models may be evaluated if their assumptions are met, but model fitting alone will not establish a unique release mechanism.

Stability evaluation

The optimized formulation will be stored under predefined conditions. Appearance, particle size, polydispersity index, marker content and release behavior will be assessed at planned intervals. Exploratory short-term stability findings will not be interpreted as proof of a formal shelf life.

***In vitro* cytocompatibility**

Where appropriate cell culture facilities are available, cytocompatibility will be investigated using a validated assay and a justified cellular model. Testing will include free extract, blank nanogels, loaded nanogels, vehicle controls, untreated cells and suitable assay controls. Potential interference from plant pigments or reducing compounds will be evaluated.

***In vitro* anti-inflammatory evaluation**

A validated biological model will be selected according to the study's objectives and laboratory capability. Potential outcomes include prostaglandin E2 and other justified inflammatory mediators. The comparison groups will include basal control, inflammation-induced control, vehicle, blank nanogels, free standardized extract, loaded responsive nanogels and an appropriate positive control. A matched nonresponsive formulation will be included where feasible. Cytotoxicity will be evaluated alongside inflammatory endpoints to distinguish genuine biological activity from nonspecific reductions caused by cell damage.

Statistical analysis

The primary outcome and statistical analysis plan will be defined before confirmatory experiments. Sample size will be justified using preliminary variability and the intended comparisons. Independent experiments will be distinguished from technical replicates. Appropriate statistical tests will be selected after evaluating assumptions. Effect sizes, confidence intervals, exact sample sizes, and exact p-values will be reported where appropriate.

RESULTS AND DISCUSSION

Extract characterization

Report extraction yield, total phenolic content, total flavonoid content, and the concentration of selected chromatographic markers.

Discussion

Phytochemical standardization

Discuss the extraction yield, chromatographic fingerprint, and marker concentrations in relation to previously published *Syzygium cumini* phytochemical studies. Differences may reflect plant origin, harvesting, extraction conditions, or analytical methods[3,4].

Formulation optimization

Explain how formulation variables influence particle size, polydispersity, loading and release. Discuss the optimization model and independent confirmation results.

Stimulus-dependent release

Interpret whether the formulation exhibits reproducible trigger-dependent release. A comparison with a matched nonresponsive carrier would help determine whether the response is attributable to the selected stimulus.

Biological evaluation

Compare the loaded nanogel with free extract at appropriately matched marker-equivalent exposures. Consider whether the observed inflammatory response is independent of cytotoxicity. PGE2 is relevant to dysmenorrhea biology, but an *in vitro* change in PGE2 does not independently establish pain relief or clinical efficacy[1,2].

Table No.1: Formulation composition of *Syzygium cumini* leaf phytochemical-loaded polymeric nanogels

S.No	Ingredient	Quantity for 10mL batch	Function
1	Syzygium cumini leaf extract (standardized)	10mg	Active phytochemical source
2	Chitosan (low molecular weight)	10mg	Polymeric carrier
3	Sodium tripolyphosphate (TPP)	2mg	Ionic crosslinking agent
4	Dilute acetic acid solution	Quantity sufficient to dissolve chitosan	Solvent for chitosan
5	Purified water	Quantity sufficient to 10mL	Aqueous vehicle

Table No.2: Phytochemical standardization

S.No	Parameter	Values
1	Extraction yield (% w/w)	12.4%
2	Total phenolic content	148 ± 6mg GAE/g extract
3	Total flavonoid content	62 ± 4mg QE/g extract
4	Gallic acid marker	18.6 ± 1.2mg/g extract
5	Ellagic acid marker	7.4 ± 0.6mg/g extract
6	Chromatographic fingerprint	Insert actual HPLC/HPTLC chromatogram

Formulation optimization

Table No.3: Formulation optimization results

Formulation	Polymer concentration (mg/mL)	Extract: polymer ratio	Size (nm)	PDI	Encapsulation efficiency (%)
F1	5.0	1:2	245	0.38	63.2
F2	7.5	1:2	211	0.31	71.5
F3	10.0	1:2	188	0.24	78.1
F4	7.5	1:1	172	0.21	82.4

Physicochemical characterization

Table No.4: Optimized formulation characteristics

S.No	Parameter	Illustrative value	Method
1	Hydrodynamic particle size	172 ± 6nm	DLS
2	Polydispersity index	0.21 ± 0.03	DLS
3	Zeta potential	−24.6 ± 2.1mV	Electrophoretic light scattering
4	Encapsulation efficiency	82.4 ± 2.5%	Validated marker assay
5	Loading capacity	8.7 ± 0.6%	Marker mass balance
6	Marker recovery	96.2 ± 3.4%	Analytical recovery
7	Particle morphology	Approximately spherical	TEM
8	Particle size change after 30 days	+8.1%	Stability study
9	Marker content retained after 30 days	94.3%	Validated assay

Stimulus-dependent release

Table No.5: Cumulative marker release

Time (h)	Reference condition: pH 7.4 (%)	Test condition: pH 5.5 (%)
0	0	0
1	12	18
2	21	32
4	35	49
8	52	68

Biological evaluation

Table No.6: Cytocompatibility and inflammatory endpoints

S.No	Experimental group	Cell viability (%)	PGE2 relative level (%)
1	Basal control	98	20
2	Inflammation-induced control	92	100
3	Blank nanogel	96	95
4	Free extract	88	63
5	Loaded responsive nanogel	93	42
6	Positive control	94	35
7	Nonresponsive nanogel	91	55

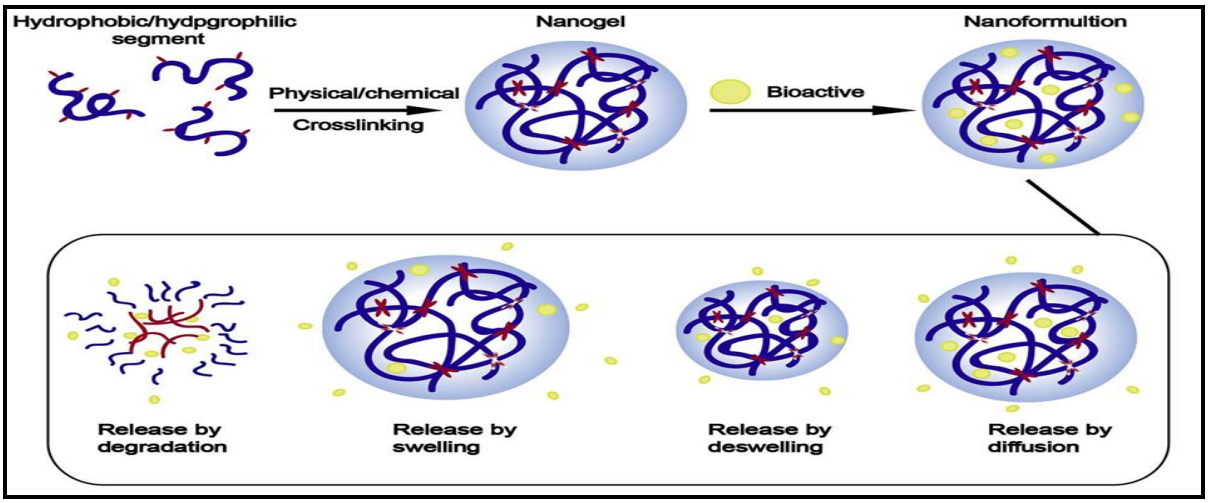


Figure No.1

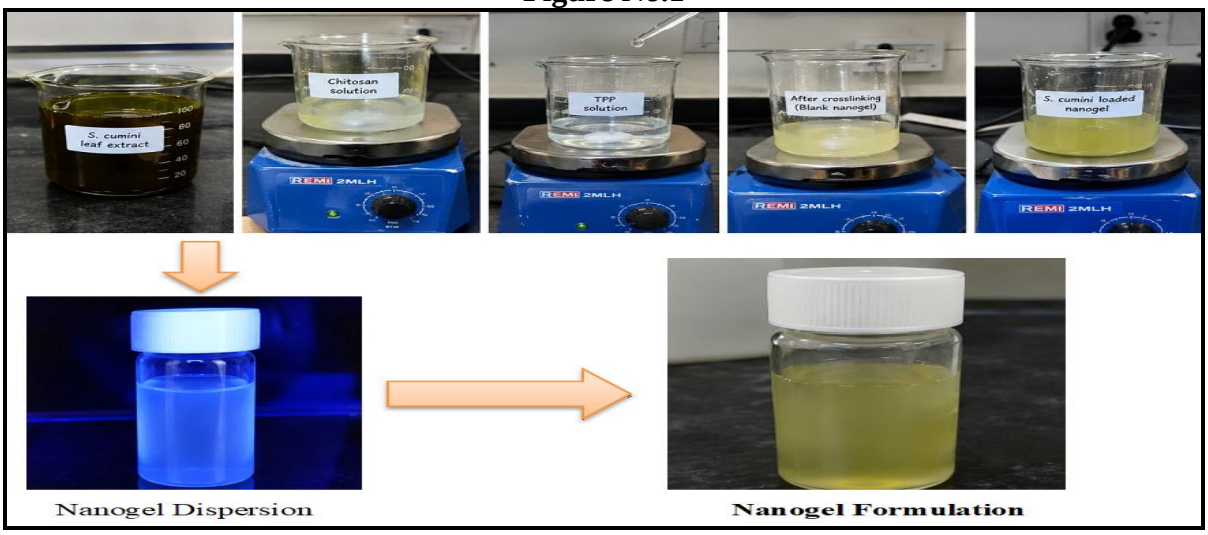


Figure No.2: Formulation and preparation strategy

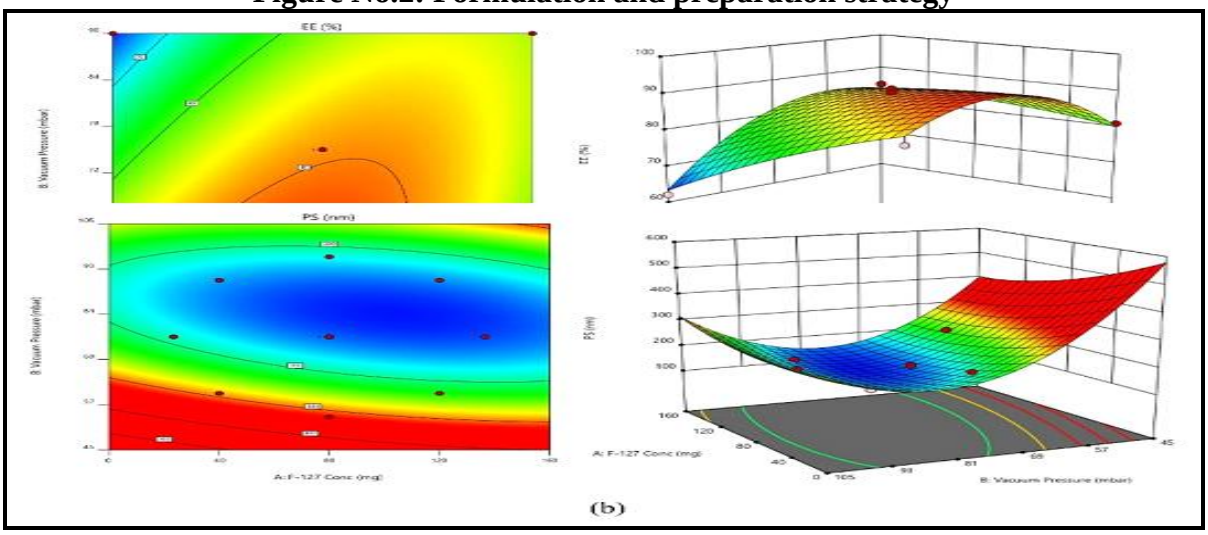


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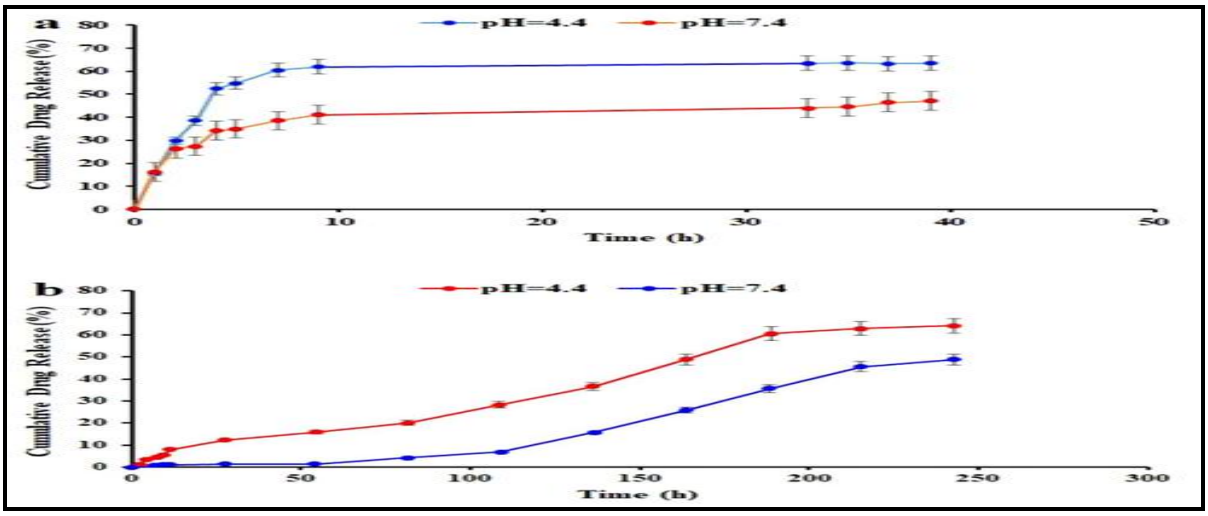


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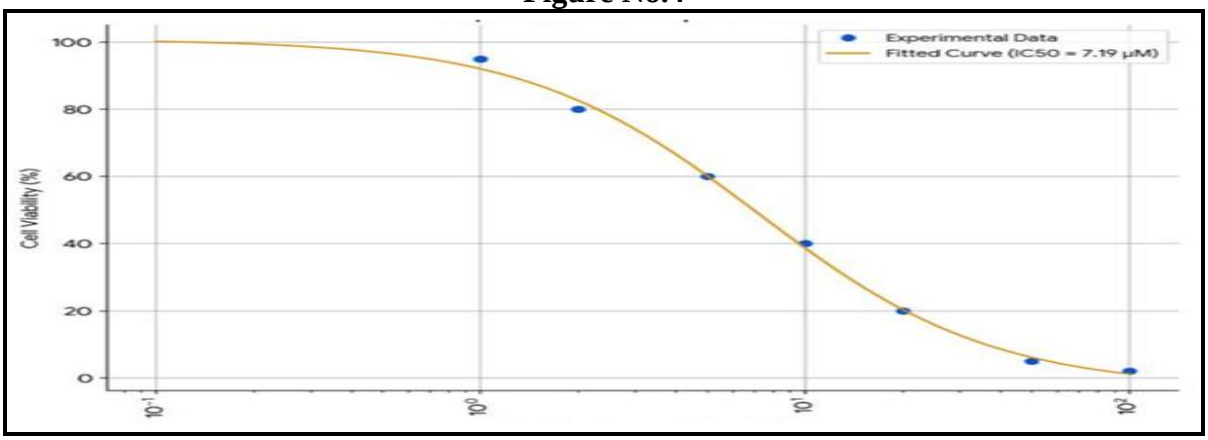


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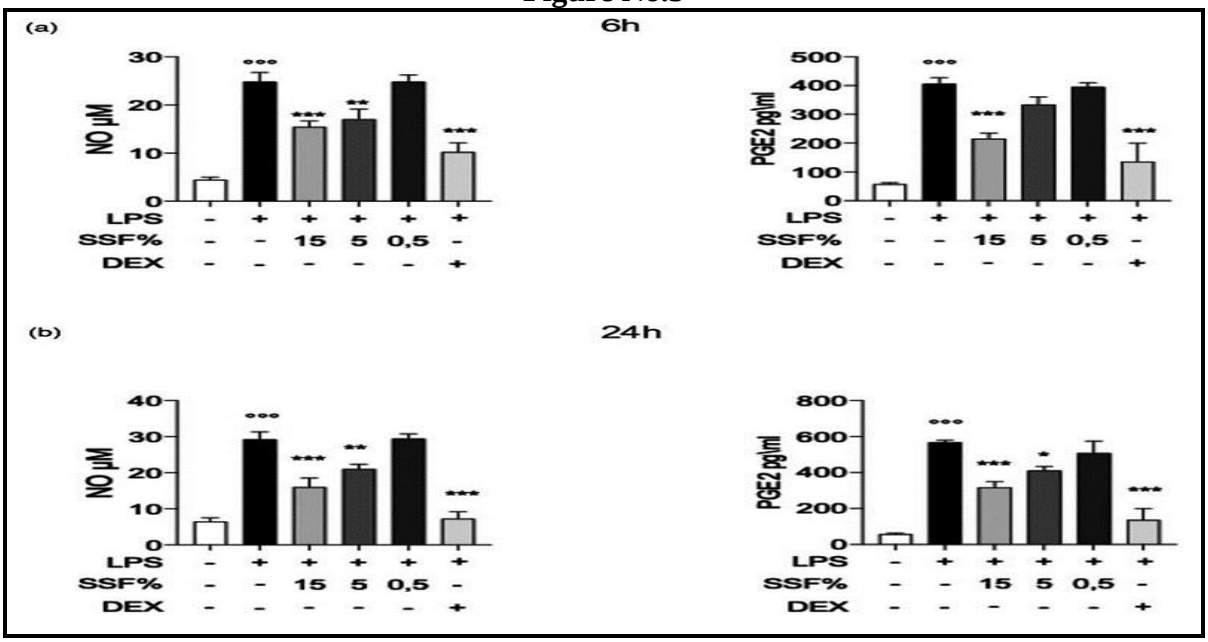


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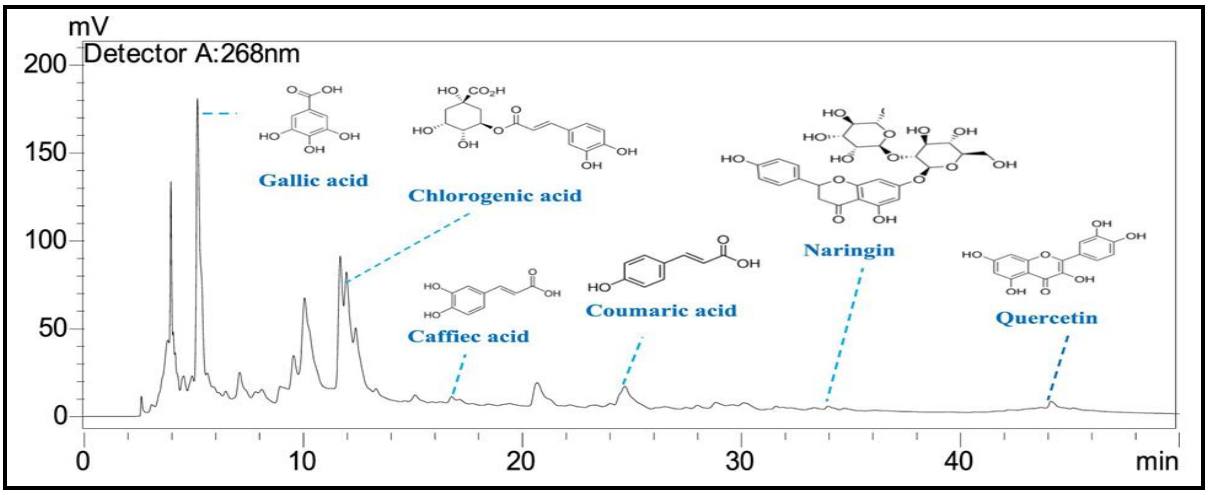


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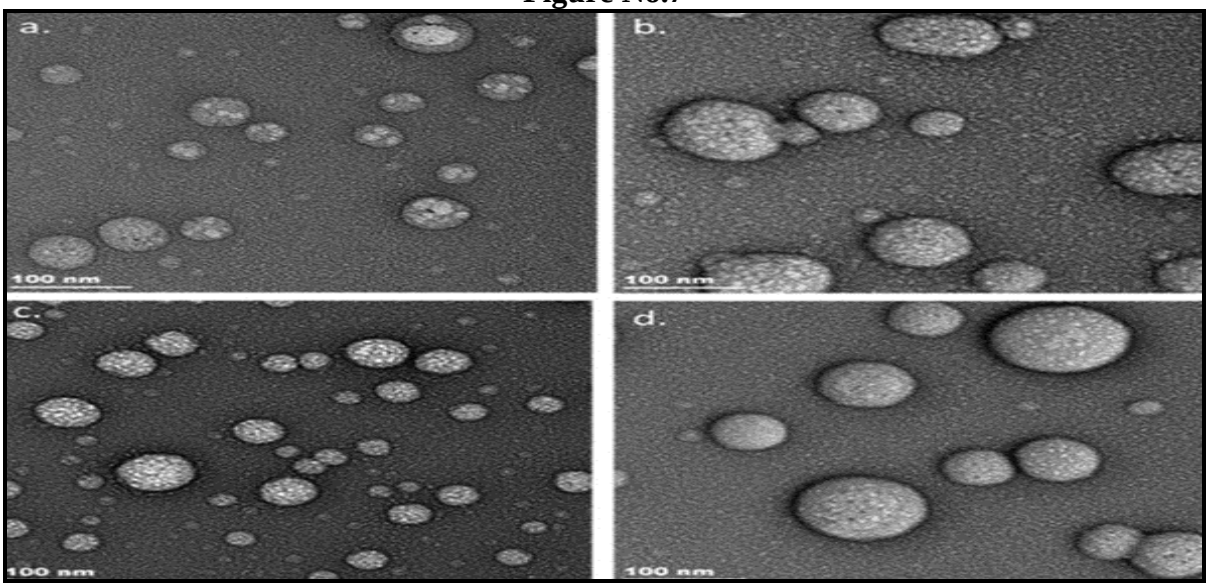


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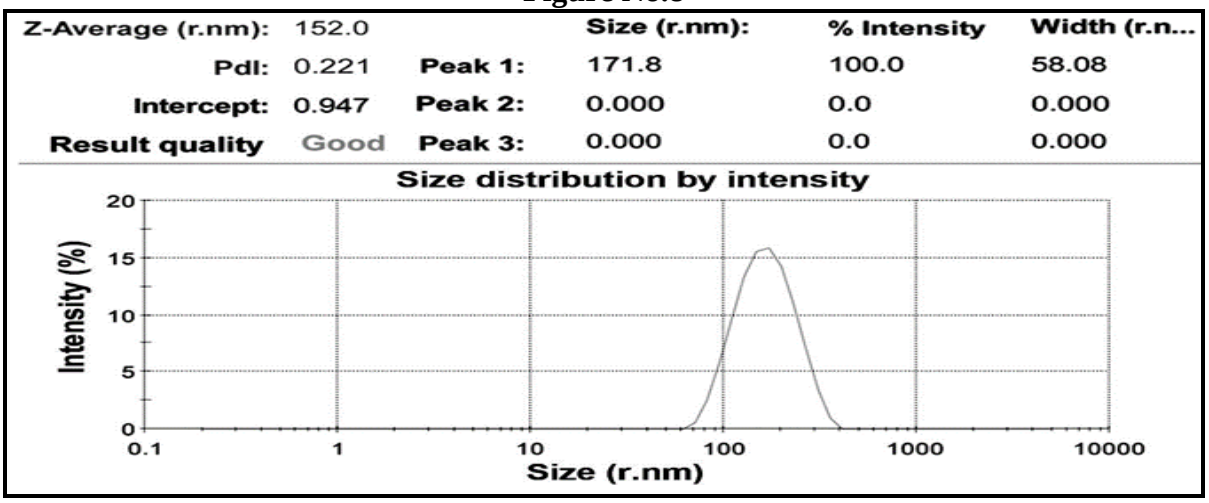


Figure No.9

LIMITATIONS

Potential limitations include variability in plant composition, the complexity of marker quantification, limitations of *in vitro* models and differences between laboratory stimulus conditions and the human biological environment. Further preclinical studies would be needed to establish safety, pharmacokinetics, tissue exposure and analgesic activity.

CONCLUSION

This study proposes a systematic approach to developing *Syzygium cumini* leaf phytochemical-loaded stimuli-responsive polymeric nanogels for investigating dysmenorrhea-associated inflammation. The approach integrates botanical authentication, chemical standardization, formulation optimization, physicochemical characterization, release testing and *in vitro* biological evaluation. The principal scientific contribution will depend on whether experiments demonstrate reproducible formulation properties, stimulus-dependent release and a meaningful advantage over the unformulated extract and appropriate controls. The formulation should be regarded as an early-stage research candidate until its safety and therapeutic relevance are established through further studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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