



FORMULATION AND EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF HERBAL NANO GEL BY USING LEAF EXTRACT OF *ACHYRANTHUS ASPERA* LINN

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Article Received on 04 Feb. 2026,
Article Revised on 24 Feb. 2026,
Article Published on 01 March 2026,

<https://doi.org/10.5281/zenodo.18811794>

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How to cite this Article: Nandhini S.^{1*}, Sakthival M.², Mohamed Halith S.³, Rajkumar S.⁴, Ramkumar R.⁴, Reshina Parveen A.⁴, Sadhana S.⁴, Saki R.⁴. (2026). Formulation And Evaluation Of Anti Inflammatory Activity Of Herbal Nanogel By Using Leaf Extract Of *Achyranthus Aspera* Linn. World Journal of Pharmacy and Pharmaceutical Sciences, 15(3), 871–883.

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ABSTRACT

Nanogels are nano-sized hydrogel systems that combine the advantages of nanoparticles and hydrogels, offering improved drug loading, controlled release, and enhanced skin permeation. *Achyranthes aspera* Linn. is a well-known medicinal plant widely used in traditional medicine for its anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties, attributed to the presence of bioactive phytoconstituents such as alkaloids, saponins, tannins, flavonoids, and phenolic compounds. The present study aimed to formulate and evaluate a topical herbal nanogel using *Achyranthes aspera* leaf extract for anti-inflammatory activity. The leaves were collected, dried, and extracted using suitable solvents. Preliminary phytochemical screening was performed to identify the presence of major secondary metabolites. The herbal nanogel was formulated using appropriate polymers and excipients and evaluated for physicochemical parameters including appearance, pH, viscosity, spreadability, particle size, drug

content, and in-vitro drug diffusion. The optimized nanogel formulation showed acceptable pH, good spreadability, uniform drug content, nano-sized particle distribution, and satisfactory stability. The anti-inflammatory activity of the formulation demonstrated significant therapeutic potential when compared with conventional formulations. The results indicate that *Achyranthes aspera*-loaded nanogel is a safe, effective, and promising topical drug delivery system for the treatment of inflammatory conditions, offering enhanced efficacy and improved patient compliance.

KEYWORDS: Anti inflammatory activity, Herbal Nanogel, *Achyranthes Aspera*.

INTRODUCTION

Nanogels are highly cross-linked, nanosized hydrogel particles typically ranging from 20–200 nm. They exhibit unique properties such as high water content, biocompatibility, high drug-loading capacity, tunable size, and stimuli responsiveness. Nanogels can be synthesized from natural or synthetic polymers and may incorporate various functional groups to control swelling, degradation, and drug release behavior. Due to their three-dimensional polymeric network, nanogels can encapsulate both hydrophilic and hydrophobic drugs, protect them from degradation, and enable controlled or targeted delivery. Their small size allows efficient cellular uptake and prolonged circulation time. These advantages make nanogels promising carriers for drug delivery, imaging, and therapeutic applications, particularly in overcoming limitations associated with conventional delivery systems.

Achyranthes aspera (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The present article gives an account of updated information on its phytochemical and pharmacological properties.

It is widely used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain. The seeds are employed as an emetic, purgative, and cathartic, in gonorrhoea, for insect bite and in hydrophobia cough including whooping cough as an anti-asthmatic.

MATERIALS AND METHODOLOGY

Table 1: LIST OF CHEMICALS.

S.no	Chemical name	Vendor name
1	Ethanol	S. D. Finechem Ltd
2	Chitosan	S. D. Finechem Ltd
3	Propylene Glycol	S. D. Finechem Ltd
4	Carbapol-940	Prakarti Aroma Care
5	Triethanolamine	S. D. Finechem Ltd

COLLECTION OF PLANT MATERIALS

Achyranthes aspera was collected from agricultural fields in perambalur, Tamil Nadu, India in March.

PREPARATION OF PLANT POWDER

The collected material were dried from 7 to 8 days, after drying the collected material were crushed using grinder and passed through sieve no 60 and stored in an air tight container.

PREPARATION OF EXTRACT

- To prepare *achyranthes aspera* leaves extract, fresh leaves were rinsed multiple times using double distilled water and then dried in sunlight for few days.
- To create a homogeneous fine powder, the dried leaves were Milled in a mixer grinder and sieved through 60# size.
- About 40gm of dry powdered leaves are taken with 250ml of distilled water and agitated at 80°C for 30 to 40 minutes.
- Then after cooling down at room temperature, and it was filtered using Whatmann No:1 filter paper.
- The filtrate was evaporated to dryness at a temperature not exceeding 40°C for 2 hrs and it was used as a reducing agent for the preparation of chitosan nanoparticles.

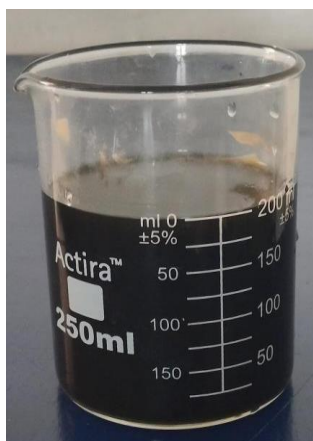


Fig. 1: Extract.

PREPARATION OF CHITOSAN NANOPARTICLES

Chitosan nanoparticles (CS) were prepared by ionic gelation method. The Chitosan nanoparticles were obtained by inducing gelation of a Chitosan solution with Sodium tripolyphosphate (TPP) chitosan was dissolved in 1% acetic acid aqueous solutions and make up 90 ml with distilled water under magnetic stirring at room temperature until a clear solution was obtained. Add 1 gm of extract to the chitosan solution and make up to 100 ml.

PREPARATION OF TPP SOLUTION

Sodium tripolyphosphate solution of 0.1% was prepared by dissolving 10mg of TPP in 10ml of deionised water. TPP solution was added drop wise with a syringe to chitosan solution under magnetic stirring at 800 rpm at room temperature. The prepared solution was centrifuged at 12000 rpm.

PREPARATION OF NANO GEL

The nanogel is prepared from modified emulsion solvent diffusion method. It is having 4 steps. The first step accurately weighed quantity of extract is dissolved in ethanol and propylene glycol with stirring (organic phase).

The second step aqueous phase is prepared by using Carbopol -940 dissolved in water with continuous stirring and heat for a 20min in a magnetic stirring. And the drug phase (extract) is sonicated under Ultrasonic Bath Sonicator for 10min. The drug phase (extract) is added drop by drop into aqueous phase during high-speed homogenization for 30 min at 6000rpm to form emulsion. The emulsion is converted into nano droplet by homogenizer results in o/w emulsion formed. Step 4 in this step o/w emulsion is homogenized for 1 hour at 8000rpm and triethanolamine is added with continues.



Fig. 2: Nanogel.

Table 2: Formulation of herbal nanogel.

S. NO	Ingredients	Formulation 1	Formulation 2
1.	Extract	2gm	2gm
2.	Carbopol 940	0.5gm	1gm
3.	Ethanol	10ml	10ml
4.	Propylene glycol	4ml	4ml
5.	Triethanolamine	4ml	4ml
6.	Water	Q.s	Q.s

EVALUATION OF NANO GEL

a) Appearance

The prepared nanogel were inspected visually for clarity, colour and the presence of any particles.

b) pH

The pH of the all nanogel was determined using digital pH meter about 1 gm of nanogel was stirred in distilled water till a uniform suspension effected. The volume was made upto 50 ml and pH of the solution was measured.

c) Spreadability

Spreadability is determined by apparatus suggested by Multimer. It consists of wooden block, which is provided by a pulley at one end.

By this method, spreadability is measured on the basis of “Slip” and “Drag”. A ground glass slide is fixed on this block. A sample of 0.1 g of nanogel under study is placed on this ground slide. The gel is fixed on the beach formula was pressed between two slides and a 1 kg weight is placed on the top of two slides and left for about 5 min to expel air and to provide a uniform film of the nanogel between two slides. Excess of the gel is scrapped from edges.

The top plate is then subjected to pull the weight. With help of string attaches to the hook and the time required by top slide to cover the distance is noted. A shorter interval indicates better spreadability, spreadability was calculated by using the formula,

$$S = M.L/T,$$

Where, S=spreadability, L=Length of glass slide, M=weight tied to upper slide, T=Time taken to separate the slides.

d) Extrudability

Slug's test is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting plug flow.

The method adopted for evaluating nanogel formulation for extrudability is based upon the quantity in percentage of nanogel and nanogel extruded from lacquered aluminium collapsible tube on application of weight in grams required at least 0.5cm ribbon of nanogel in 10 sec. The measurement of extrudability of each formulation shows the triplicate and averages value is presented.

Extrudability = Applied weight to extrude the nanogel from tube (in gm)/Area (in cm²).

e) Viscosity

Brookfield viscometer was used for the studies. First, the spindle was dipped into the nanogel till the notch on the spindle touched the nanogel surface. 3gm each of nanogel (Stability chamber and Room temperature) was used in the study. The spindle no.61, 63, 64 was selected based on viscosity of nanogel. The dial readings were taken at 50, 100, 150, 250 rpm and viscosity were measured. 2,4,5,6,7.

From the evaluation parameter performed for the all-prototype batches, the result for the batch f- 6 was found to be satisfactory in all attributes and hence selected for trial batches.

f) In vitro Release studies

Using a Franz diffusion cell apparatus, the in-vitro drug release of topical Nanogel from all batches was studied. Prior to its use for diffusion research, the dialysis membrane was soaked in the phosphate buffer of pH 7.4. A weighted amount (0.5 g) of Nanogel was placed in the donor compartment and then pH 7.4 phosphate buffer was filled into the receptor compartment. The dialysis membrane was positioned as a diffusion membrane between the

donor and the receptor compartment and the use of the clamp remained tight.

Using the Teflon coated stirring the bar, the receptor compartment media temperature was held at 37°C ($\pm 0.5^\circ\text{C}$) below 100 rpm. At each prefixed time interval of 1, 2, 3, 4, 5 and 6h, 3ml aliquots were collected and sink condition was preserved over a period of full diffusion analysis. Using UV at wavelength 296 Nm, the collected aliquots were scanned and the percentage of cumulative drug release of Nanogel was calculated.

g) FTIR Studies

Compatibility study was studied by recording the sample using Perkin Elmer Fourier transformation Infrared spectroscope (FT-IR) combined to PC (with spectrum 2000 analysis software) in the range of 4000 cm^{-1} to 400 cm^{-1} . Potassium Bromide Press Pellet was prepared by applying pressure of 5 tons for 5 min in a hydraulic press. The nanogel was placed in light path and the spectra were analysed.

h) Zeta potential

Zetasizer (Malvern Zetasizer) measures the nanogel preparation's zeta potential by placing the formulation in a transparent, single-use zeta cell and measuring the outcome. Using methanol to clean the cuvettes, the sample is added before doing the experiment.

I) U.V. Spectrum

The solution of parts per million (ppm) was prepared. Isolated fraction compound and shimadzu 1800 UV Spectrophotometer with 1cm matched quartz cells was used to obtained U.V Spectrum of isolated fraction compound.

RESULT AND DISCUSSION

A. Organoleptic properties

Physical Appearance	F1	F2
Colour	Brownish	Brownish
Texture	Smooth	Smooth
Homogeneity	Homogenous	Homogenous

B. Ph

Formulation	pH
F1	6.5
F2	7

C. Spreadability

Formulation	Spreadability
F1	56.93
F2	63.25

D. Extrudability

Formulation	Extrudability
F1	Good
F2	Excellent

E. Viscosity

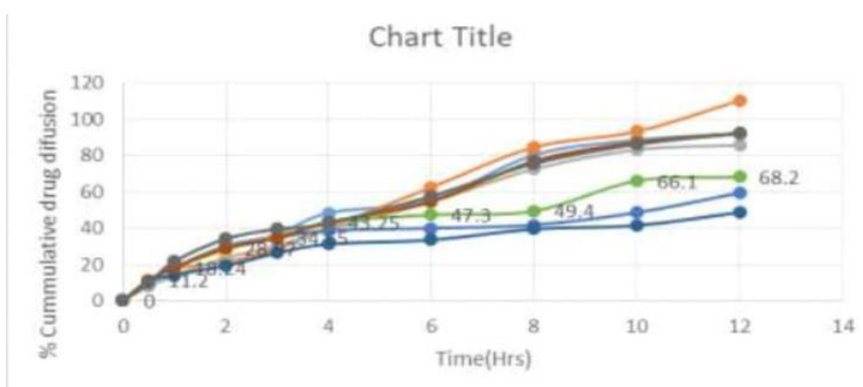
Formulation	Viscosity
F1	0.3821
F2	0.3864

F. In vitro studies

By using Franz diffusion cell apparatus, drug release of topical Nanogel formulation was observed. The collected aliquots were scanned using UV-Visible spectrophotometer, and % cumulative drug release was calculated. % cumulative drug diffusion was shown

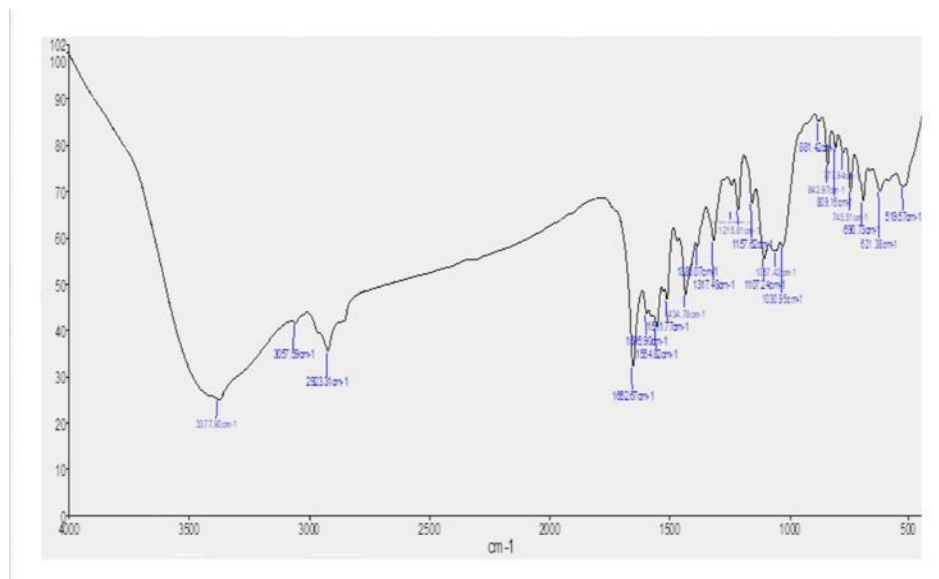
Formulation	% cumulative drug diffusion
F1	92.3
F2	99.9

Time	F1	F2
0	0	0
0.5	11.2	11.02
1	18.24	19.04
2	28.97	27.32
3	34.06	36.01
4	43.25	44.8
6	54.06	55.2
8	76.98	77.6
10	87.32	88.1
12	92.03	99.9



G. FT-IR Studies

From the FTIR spectra of leaves extract and physical mixture of the extract, polymer and other ingredients, it was observed that the peaks of major functional groups of *Achyranthes aspera* leaves extract were also observed in spectrum of physical mixture.



I. Zeta Potential

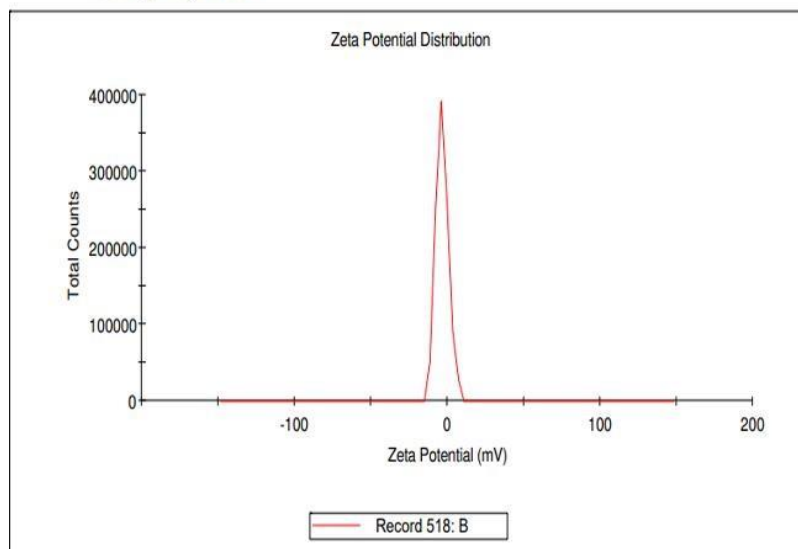
Zeta potential measurement has shown that dispersions with a charge close to zero whether exhibiting a positive or negative charge tend to yield shorter shelf-lives, with an inclination towards coagulation or flocculation of emulsions. Conversely, emulsions with a surface activity greater than.

~ +/- 30mv are inclined towards improved system stability and low aggregation. The zeta potential of formulated nanogel was discussed below: Zeta potential: 4.10 (mv)

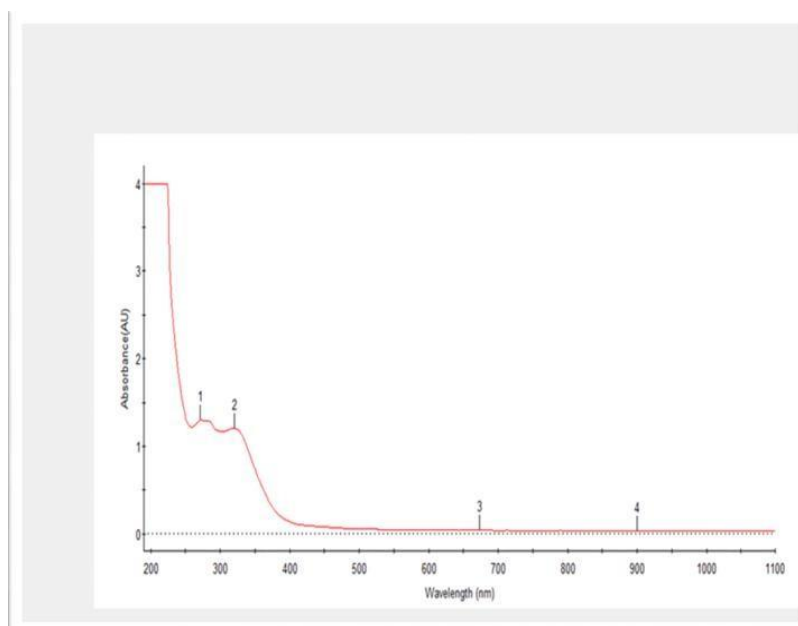
Conductivity: 0.862(ms/cm)

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -3.19	Peak 1: -3.19	100.0	4.10
Zeta Deviation (mV): 4.10	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.862	Peak 3: 0.00	0.0	0.00
Result quality : Good			

**I. Determination of Absorption maximum (λ -max)**

The UV-visible spectroscopy is used to characterize the excitation spectra of the samples, which is useful to prove the presence of nanoparticles. The UV-visible spectra gives an absorption band at 273nm. UV-visible spectroscopy is very useful to identify the formation of nanogel in reaction mixture.



Concentration	Absorbance
0	0
50	0.182
100	0.276
150	0.511
200	0.681
250	0.88

J. In-vitro anti-inflammatory activity Proteinase inhibitory activity

The maximum inhibition was observed from ethanolic extract (97%). The standard aspirin (80%) drug showed the maximum proteinase inhibitory action.

S. NO	Concentration(g/ml)	Inhibition
1	50	72%±0.052
2	100	97%±0.090
3	200	78%±0.056
4	300	86%±0.55
5	500	87%±0.056
6	Standard drug (Aspirin 250µg/ml)	80%±0.050

K. Stability Studies

The optimized formulation is subjected to stability study for the period of 45 days. The sample is stored in described condition as per ICH guidelines. After the stability study sample is subjected to evaluation test and compared to test values just before to that of stability study.

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Brownish	Homogenous	16.93	6.37
F2	Brownish	Homogenous	17.25	6.79

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Brownish	Homogenous	15.32	6.86
F2	Brownish	Homogenous	18.34	7

Formulation	Color	Appearance	Spreadability (g.cm/sec)	pH
F1	Brownish	Homogenous	15.36	6.91
F2	Brownish	Homogenous	17.96	7

CONCLUSION

The present work focused on the formulation and evaluation of a herbal nanogel using *Achyranthes aspera* leaf extract. Nanogels offer an effective topical drug delivery system due to their small particle size, high drug-loading capacity, biocompatibility, and controlled drug

release. The formulated nanogel showed satisfactory physicochemical properties such as suitable pH, good spreadability, and stability for topical application. Phytochemical screening confirmed the presence of bioactive compounds responsible for anti-inflammatory activity. The herbal nanogel provides localized drug delivery with reduced systemic side effects and improved patient compliance. Thus, *Achyranthes aspera*-loaded nanogel can be considered a promising formulation for topical anti-inflammatory therapy. Further in vivo and clinical studies are required to confirm its therapeutic efficacy.

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