

Antimicrobial and Anti-inflammatory Efficacy of an Optimized *Achyranthes aspera* Extract-Loaded Nanogel.

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ABSTRACT

Background: While *Achyranthes aspera* possesses well-documented ethnomedicinal uses, its clinical application is often limited because its raw phytoconstituents do not work well on the skin.

Methods: To overcome topical delivery obstacles, an extract-loaded nanogel was developed utilizing Hyaluronic Acid (HA) and Polyvinyl Alcohol (PVA) through chemical cross-linking. The formulation was statistically improved using a 3² factorial design. The optimized formulation underwent rigorous physicochemical characterization, a 6-month accelerated ICH stability study, *in vitro* antimicrobial screening against pathogenic strains, and *in vivo* anti-inflammatory evaluation using a Croton oil-induced ear edema model utilizing Swiss albino mice.

Results: The optimized batch (AA-4, comprising 1.0% w/v HA and 2.5% w/v PVA) exhibited optimal physicochemical properties: a minimum particle size of 201 ± 13 nm, a

high entrapment efficiency of $90.93 \pm 0.93\%$, an optimal viscosity of 761 cP, and excellent stability over 6 months under accelerated conditions. Pharmacological screening showed that the AA-4 nanogel possessed much stronger antifungal and antibacterial activity than the plain extract. Furthermore, *in vivo* studies demonstrated significant anti-inflammatory therapeutic superiority, effectively limiting localized edema weight and thickness variations to 4.424% and 6.666%, respectively.

Conclusion: The statistically optimized *A. aspera* nanogel is a highly stable, effective and viable alternative for the targeted treatment of localized dermal inflammation and microbial infections.

Keywords: Nanogel Formulation, Hyaluronic Acid, 3^2 Factorial Design, Topical Drug Delivery, Anti-inflammatory Activity, Croton Oil Ear Edema Model.

1. INTRODUCTION

Topical drug delivery remains a highly desirable route for treating localized dermatological conditions, but the efficacy of herbal medicines depends heavily on their penetration and absorption [1]. Maximum herbaceous drugs have a high systemic clearance and low absorption due to their insoluble components and restricted skin absorption [2]. *Achyranthes aspera* is a widely recognized medicinal plant containing a robust profile of bioactive secondary metabolites that exhibit potent antimicrobial and anti-inflammatory properties [3]. Despite these proven *in vitro* benefits, the clinical translation of raw *A. aspera* extracts into viable topical dermatological treatments is hindered by their inability to efficiently permeate and retain within the dermal layers. The rising global prevalence of multidrug-resistant microbial strains, alongside the detrimental cellular damage caused by infection-induced oxidative stress, has necessitated the exploration of such natural, plant-derived alternatives [4]. Consequently, extensive research has recently focused on the phytochemical screening of

medicinal plants to validate their potent antibacterial, antifungal, and anti-inflammatory efficacies [5-7].

Nanogel drug delivery systems represent a sophisticated solution to these pharmaceutical challenges. Nanogels are nanoscale cross-linked polymer systems, typically ranging from 20 to 200 nanometers, with a high capacity to absorb water [8]. Its nanoscale structure has been demonstrated to increase drug bioavailability by providing controlled drug release at the target site [9]. By incorporating biopolymers such as Hyaluronic Acid (HA)—which possesses intrinsic biocompatibility—and Polyvinyl Alcohol (PVA), it is possible to create an optimal topical matrix [10].

The present study aimed to develop, statistically optimize via a 3^2 full factorial design, and evaluate a novel Hyaluronic Acid-Polyvinyl Alcohol nanogel loaded with a standardized *A. aspera* extract. Through comprehensive *in vitro* and *in vivo* pharmacological screening, this research seeks to validate a highly efficacious delivery vehicle for treating localized inflammation and microbial infections.

2. MATERIALS AND METHODS

2.1 Materials

Hyaluronic Acid (HA) and Polyvinyl Alcohol (PVA) were procured as laboratory-grade reagents. A standardized methanolic extract of *A. aspera* leaves was prepared in prior experimental stages.

2.2 Preparation and Statistical Optimization (3^2 Factorial Design)

A 3^2 randomized full factorial design was employed to statistically optimize the nanogel formulation variables [11]. The design evaluated two independent factors:

- HA Concentration (% w/v): 0.5%, 1.0%, 1.5%

- PVA Concentration (% w/v): 2.5%, 5.0%, 7.5%

To prepare the nanogel, 100 mg of the herbal extract was dissolved in 10 mL of distilled water. The HA and PVA solutions were mixed, and the polymer network was synthesized using a chemical cross-linking gel method, utilizing glutaraldehyde (25% w/v) as the cross-linker and hydrochloric acid (6% v/v) as a catalyst [12].

2.3 Physicochemical Characterization

- **Viscosity and pH:** Measured using a digital rotational viscometer (at 50 rpm) and a calibrated digital pH meter at 25°C.
- **Vesicle Size, PDI, and Zeta Potential:** Evaluated using Dynamic Light Scattering (DLS) at a scattering angle of 90° [13].
- **Entrapment Efficiency (EE %):** Determined by separating untrapped extract using a cooling centrifuge at 15,000 rpm and analyzing the supernatant spectrophotometrically.

2.4 Stability Study

A stability study was conducted on the optimized formulation (Batch AA-4) in accordance with International Council for Harmonization (ICH) guidelines [14]. Samples were stored at 25°C ± 2°C and 75% ± 5% Relative Humidity (RH) for six months and evaluated periodically.

2.5 *In Vitro* Antimicrobial Screening

Antimicrobial properties were evaluated against *S. aureus*, *B. subtilis*, *E. coli*, *C. albicans*, and *A. niger* using the agar well diffusion and 96-well microdilution methods to determine Minimum Inhibitory Concentration (MIC) [15]. Ciprofloxacin and Fluconazole served as standard controls.

2.6 *In Vivo* Anti-Inflammatory Evaluation

Anti-inflammatory efficacy was evaluated using a Croton oil-induced ear edema model in Swiss albino mice [16]. Edema was induced by applying 20 µl of 2.5% croton oil to the right ear. The groups (n=6) received Placebo, Standard (0.1% Dexamethasone), Plain Extract (200 mg/kg), and Optimized Nanogel (AA-4, 2% w/w). The percentage reduction in edema weight and thickness was calculated after 9 days.

3. RESULTS AND DISCUSSION

3.1 Formulation Optimization via Factorial Design

The 3² factorial design generated nine distinct batches (AA-1 to AA-9). The analysis revealed that formulation AA-4 (1.0% HA and 2.5% PVA) exhibited the most favorable physicochemical characteristics. AA-4 achieved the smallest particle size (201 ± 13 nm), the highest extract entrapment efficiency (90.93 ± 0.93%), and an optimal gelling time of 5.2 ± 0.25 minutes.

Table 1: Matrix of the 3² Factorial Design for Formulation Optimization (Batches AA-1 to AA-9)

Batch No.	HA (%w/v)	PVA (%w/v)	Gelling time (min)	Entrapment Efficiency (%)	Particle Size (nm)
AA-1	0.5	2.5	5.3 ± 0.63	66.64 ± 0.83	301 ± 13
AA-2	0.5	5.0	6.1 ± 0.20	70.73 ± 1.73	311 ± 22
AA-3	0.5	7.5	6.3 ± 0.56	77.63 ± 1.07	360 ± 16

AA-4*	1.0	2.5	5.2 ± 0.25	90.93 ± 0.93	201 ± 13
AA-5	1.0	5.0	5.7 ± 0.17	87.43 ± 1.22	231 ± 19
AA-6	1.0	7.5	5.7 ± 0.36	84.48 ± 0.76	215 ± 11
AA-7	1.5	2.5	6.3 ± 0.07	81.75 ± 0.73	291 ± 14
AA-8	1.5	5.0	6.7 ± 0.18	76.17 ± 0.32	315 ± 14
AA-9	1.5	7.5	7.1 ± 0.32	73.83 ± 0.19	370 ± 26

3.2 Characterization and Stability of Optimized Batch AA-4

Batch AA-4 was transparent green, homogeneous, and clear. It demonstrated an optimal viscosity of 761 cP, making it highly ideal for topical spreadability. The zeta potential was recorded at -24.3 ± 0.08 mV, indicating strong electrostatic repulsion and confirming excellent colloidal stability. Over the 6-month ICH accelerated stability study, no major changes were observed in physicochemical parameters or drug entrapment, confirming a robust shelf life.

Table 2: Characteristic properties of AAE-loaded nanogels

Sr. No.	Batches	Viscosity (cP)	pH	Zeta potential (mV)	PDI Mean ± SD
1	AA-1	685	6.8	-23.5 ± 0.12	0.194 ± 0.035
2	AA-2	702	6.9	-23.9 ± 0.13	0.213 ± 0.038
3	AA-3	743	7.1	-24.3 ± 0.12	0.232 ± 0.027
4	AA-4*	761	7.0	-24.3 ± 0.08	0.239 ± 0.008
5	AA-5	784	7.1	-24.3 ± 0.16	0.249 ± 0.008

6	AA-6	795	7.4	-25.3 ± 0.15	0.250 ± 0.013
7	AA-7	827	7.0	-25.9 ± 0.09	0.270 ± 0.039
8	AA-8	833	7.1	-25.3 ± 0.10	0.279 ± 0.026
9	AA-9	856	7.8	-24.8 ± 0.13	0.290 ± 0.022

Table 3: Stability Data over 6 Months

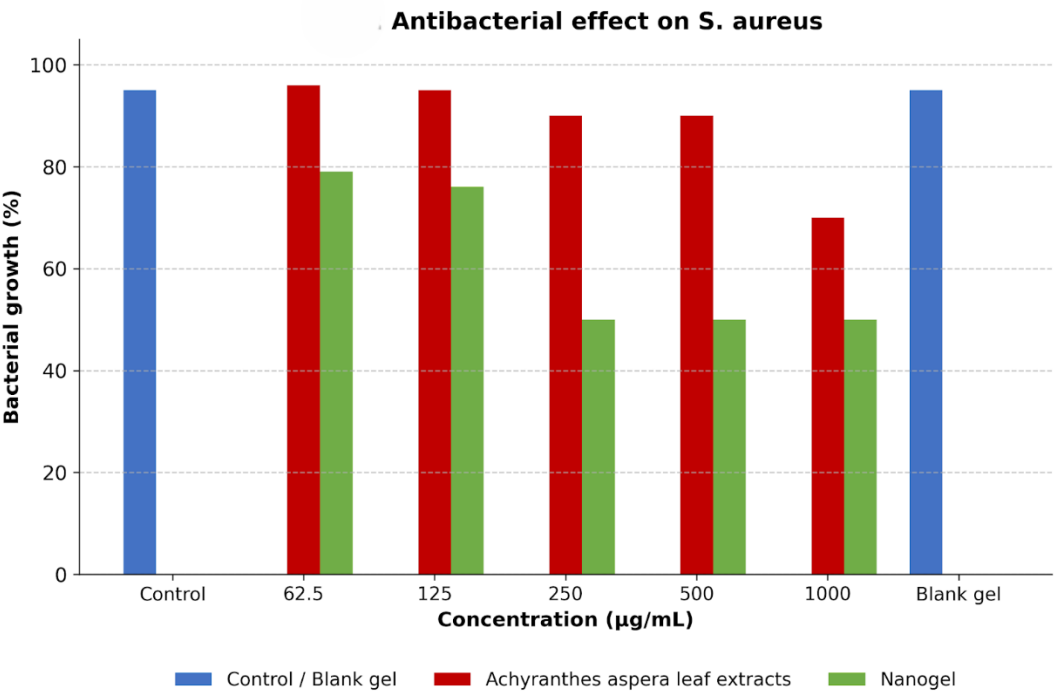
Parameters	25°C $\pm$ 2°C Temperature and 75% $\pm$ 5% R.H.				
	Initial Values	1 Month	2 Month	3 Month	6 Month
Appearance	Clear Transparent	Clear Transparent	Clear Transparent	Clear Transparent	Clear Transparent
pH	6.8	6.8	6.8	7.0	7.0
Particle Size (nm)	201 ± 13	204 ± 22	206 ± 35	210 ± 53	216 ± 70
% Drug entrapment	90.93 ± 0.93	90.46 ± 0.15	89.60 ± 0.69	88.74 ± 0.14	87.18 ± 0.26
Viscosity	761	765	763	771	782

3.3 Enhanced *In Vitro* Antimicrobial Activity

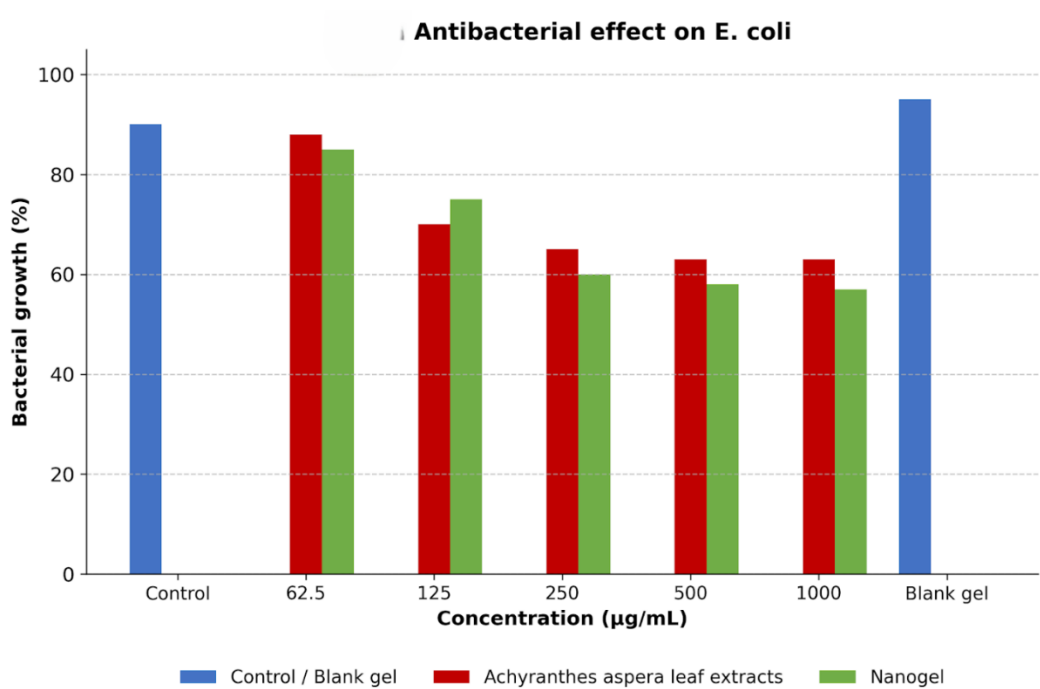
The AA-4 nanogel exhibited significantly enhanced antimicrobial efficacy compared to the plain extract across all tested strains. The nanogel demonstrated significantly higher efficacy against *E. coli* at concentrations of 500 and 1000 $\mu\text{g/mL}$ (p-values of 0.0433 and 0.0157, respectively) compared to the unformulated extract. Similar dose-dependent superiority was observed against *S. aureus* (p-value < 0.001).

Table 4: Antimicrobial Zone of Inhibition and MIC/MBC Data

Microorganism	Zone of Inhibition of AAE (mm)	Zone of Inhibition of Standard (mm)	MIC (µg/mL)	MBC/MFC (µg/mL)
<i>Staphylococcus aureus</i>	16	26	500	1000
<i>Bacillus subtilis</i>	17	28	500	1000
<i>Escherichia coli</i>	13	24	1000	2000
<i>Candida albicans</i>	19	23	500	1000
<i>Aspergillus niger</i>	15	21	1000	2000



Bar Graph 1: Antibacterial Effect on *S. aureus* (Extract vs. Nanogel)



Bar Graph 2: Antibacterial Effect on *E. coli* (Extract vs. Nanogel)

3.4 *In Vivo* Anti-Inflammatory Superiority

The Croton oil-induced ear edema model provided definitive *in vivo* validation. Group I (Placebo) exhibited severe inflammation with a massive weight difference of $60.952 \pm 2.3\%$. While the plain *A. aspera* extract offered moderate suppression ($23.300 \pm 1.6\%$), the topically applied AA-4 nanogel limited the weight difference to just $4.424 \pm 1.8\%$. Furthermore, the AA-4 nanogel restricted localized tissue swelling to a $6.666 \pm 1.1\%$ difference in ear thickness, performing remarkably close to the standard Dexamethasone treatment.

Table 5: Anti-inflammatory Activity Based on Ear Weight

Groups	Treatment Sample	Dose/ Formulation	Final Weight in mg (After 9 days)		
			Left ear Control (mg)	Right ear (mg)	% Difference in Weight
Group-I	2.5% Croton Oil (Placebo)	2.5% (20 µL)	105 ± 3	169 ± 2	60.952 ± 2.3
Group-II	Dexamethasone (Standard)	0.1% w/v (20 µL)	102 ± 4	103 ± 3	0.980 ± 1.5
Group-III	<i>A. aspera</i> extract plain	200 mg/kg	103 ± 2	127 ± 2	23.300 ± 1.6
Group-IV	AA-4 Nanogel	2% w/w (20 µL)	113 ± 4	118 ± 3	4.424 ± 1.8

Table 6: Anti-inflammatory Activity Based on Ear Thickness

Groups	Treatment Sample	Dose/ Formulation	Final thickness in mm (After 9 days)		
			Left ear Control (mm)	Right ear (mm)	% difference in thickness
Group-I	2.5% Croton Oil (Placebo)	2.5% (20 µL)	1.6 ± 0.03	2.5 ± 0.03	56.250 ± 1.4
Group-	Dexamethasone	0.1% w/v (20	1.7 ± 0.06	1.8 ±	05.882 ± 2.2

II	(Standard)	μL		0.02	
Group-III	<i>A. aspera</i> extract plain	200 mg/kg	1.3 ± 0.05	1.6 ± 0.05	23.076 ± 1.8
Group-IV	AA-4 Nanogel	2% w/w (20 μL)	1.5 ± 0.02	1.6 ± 0.04	06.666 ± 1.1

4. CONCLUSION

The formulation and statistical optimization of the hyaluronic acid-polyvinyl alcohol nanogel demonstrated a highly effective method for the topical administration of *Achyranthes aspera* extract. Using a 3^2 factorial design, Batch AA-4 was developed, exhibiting an ideal nanoscale size (201 nm), high entrapment efficiency (90.93%), and excellent 6-month stability. The nanogel network greatly improved the localized retention of the drugs, leading to markedly superior antibacterial effectiveness and a remarkable *in vivo* anti-inflammatory response. This study effectively validates the AA-4 nanogel as a stable and potent alternative for the treatment of localized dermal inflammation and microbial infections.

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