

Development and Physicochemical Evaluation of *Aerva lanata* Extract-Loaded Pullulan Nanoparticle Herbal Syrup for Antiurolithiatic Applications

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Abstract

Urolithiasis remains a recurrent urinary tract disorder for which plant-derived formulations are of continuing interest. The present study developed an oral herbal syrup containing pullulan nanoparticles loaded with ethanolic extract of *Aerva lanata* and evaluated the formulation using phytochemical, spectroscopic, nanoparticle, antioxidant and physicochemical parameters. Shade-dried plant material (50 g) was macerated with 500 mL ethanol for 7 days. The extract was incorporated into pullulan nanoparticles by nanoprecipitation using Tween 80, followed by magnetic stirring, centrifugation and membrane filtration. Three 100 mL syrup formulations (F1-F3) containing 200 mg of *Aerva lanata*-loaded nanoparticles were prepared with graded concentrations of sucrose, xanthan gum, propylene glycol and citric acid. Qualitative screening showed flavonoids, alkaloids, saponins, tannins and terpenoids. Total flavonoid content was 15.71 $\pm$ 0.25 mg quercetin equivalents/g extract. FTIR showed bands at 3423, 2924/2853, 1736, 1629 and 1035 cm^{-1} . Dynamic light scattering gave a Z-average particle size of 163.0 nm and a polydispersity index of 0.436, with intensity peaks at 824.7 and 16.06 nm. The reported drug content was 92%, while entrapment efficiency was 92%, 95% and 93% for F1, F2 and F3, respectively. DPPH radical-scavenging response increased with concentration, reaching 42.24 $\pm$ 0.81 for the extract/sample at the highest tested concentration, compared with 76.54 $\pm$ 0.60 for ascorbic acid. The syrups showed densities of 1.21-1.22 g/mL, viscosities of 2.66-4.20 cP, pH values of 4.84-5.71 and specific gravities of 1.21-1.23. F2 showed the best overall balance of entrapment efficiency and syrup characteristics. The study supports the feasibility of formulating *Aerva lanata* extract as a nanoparticle-loaded oral syrup. Direct antiurolithiatic efficacy, long-term stability and in-vitro drug-release testing were not established in the present dataset and should be evaluated before therapeutic claims are made.

Keywords: *Aerva lanata*; pullulan nanoparticles; nanoprecipitation; herbal syrup; urolithiasis; flavonoids; DPPH; entrapment efficiency

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1. Introduction

Urolithiasis is characterized by the formation of calculi within the urinary tract and is associated with recurrence, pain, obstruction and potential renal complications. Calcium oxalate is a major component of urinary calculi, and strategies that can reduce crystal formation or support renal protection continue to attract pharmacological interest. *Aerva lanata* (L.) Juss. ex Schult. has a long history of use in traditional medicine for urinary disorders and has been reported to contain flavonoids, phenolics, alkaloids and other secondary metabolites relevant to antioxidant, nephroprotective and antiurolithiatic activity [1-6].

Herbal extracts, however, can present formulation challenges related to solubility, stability, taste and reproducible delivery of multiple phytoconstituents. Nanoparticulate systems may improve dispersion and protect plant-derived constituents, while oral syrups offer a convenient and palatable dosage form. Nanoprecipitation is a simple solvent-displacement approach that has been widely used to prepare polymeric nanoparticles [7-9]. Pullulan, a water-soluble polysaccharide, is attractive as a pharmaceutical carrier because of its hydrophilicity and biocompatibility.

Accordingly, the present work aimed to formulate *Aerva lanata* extract-loaded pullulan nanoparticles, incorporate the nanoparticles into an oral herbal syrup, and evaluate the resulting system through phytochemical screening, total flavonoid content, FTIR, particle-size analysis, drug content, entrapment efficiency, DPPH radical-scavenging activity and physicochemical testing of three syrup formulations. The study is primarily a formulation and characterization investigation; no direct calcium oxalate crystallization or in-vivo antiurolithiatic efficacy experiment was included.

2. Materials and Methods

2.1 Plant collection, authentication and extraction

Aerva lanata plant material was collected locally, washed with tap water to remove adhering dirt and shade dried. Botanical identification and authentication were performed at the Department of Botany, Siddha Central Research Institute, Chennai. The dried material was powdered using a mechanical grinder. Fifty grams of powdered plant material were macerated with 500 mL ethanol for 7 days. After maceration, the extract was filtered through Whatman filter paper, concentrated by evaporation to approximately one-fifth of the initial

volume and stored at 4 degrees C in an airtight container until further use.

2.2 Qualitative phytochemical screening

The ethanolic extract was screened for major classes of secondary metabolites. Flavonoids were tested using Shinoda, alkaline reagent and lead acetate tests; alkaloids by Wagner's test; terpenoids by Salkowski test; tannins by ferric chloride test; and saponins by the foam test. Characteristic colour development, precipitate formation or stable foam was used as the qualitative endpoint.

2.3 Total flavonoid content

Total flavonoid content was estimated by an aluminium chloride colorimetric method. One millilitre of extract was mixed with 0.3 mL of 5% sodium nitrite and allowed to stand for 5 min, followed by 0.3 mL of 10% aluminium chloride. Subsequently, 2 mL of 1 M sodium hydroxide was added and the volume was made up to 10 mL with distilled water. Absorbance was measured at 412 nm. Quercetin was used for calibration at 10, 20, 30, 40 and 50 micrograms/mL, and results were expressed as mg quercetin equivalents (QE)/g extract [10,11].

2.4 FTIR analysis

Fourier-transform infrared spectroscopy was used to identify characteristic functional groups in the Aerva lanata extract. The spectrum was interpreted on the basis of the major absorption bands reported in the study.

2.5 Preparation of Aerva lanata extract-loaded pullulan nanoparticles

Nanoparticles were prepared by nanoprecipitation. Pullulan was dissolved in distilled water, after which the Aerva lanata extract was dissolved in the pullulan solution and Tween 80 was added. Ethanol was used as the organic phase. The organic phase was added dropwise to the aqueous phase under magnetic stirring at 1000 rpm at 25 degrees C until the organic solvent had evaporated. The resulting nanosuspension was centrifuged at 10,000 rpm for 30 min at 30 degrees C to separate free extract. The supernatant was removed, and the nanoparticle precipitate was redispersed in distilled water and passed through a 0.22 micrometre membrane filter.

2.6 Formulation of nanoparticle-loaded herbal syrup

Three 100 mL syrup formulations were prepared. In each formulation, sucrose was dissolved in a suitable quantity of distilled water with gentle heating and stirring. Methyl paraben and sodium benzoate were dissolved in the syrup base. Xanthan gum was dispersed in propylene glycol and added slowly with continuous stirring. Aerva lanata-loaded nanoparticles were then incorporated, followed by citric acid and peppermint oil. The final volume was adjusted to 100 mL with distilled water and the syrup was mixed until uniform.

Table 1. Composition of the three nanoparticle-loaded syrup formulations

Ingredient	F1	F2	F3
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Aerva lanata-loaded nanoparticles	200 mg	200 mg	200 mg
Sucrose	60 g	65 g	70 g
Xanthan gum	0.20 g	0.30 g	0.40 g
Propylene glycol	3 mL	5 mL	7 mL
Methyl paraben	0.18 g	0.18 g	0.18 g
Sodium benzoate	0.10 g	0.10 g	0.10 g
Citric acid	0.05 g	0.10 g	0.15 g
Peppermint oil	1-2 drops	1-2 drops	1-2 drops
Distilled water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL

2.7 Characterization and evaluation

Particle size and polydispersity index (PDI) were measured by dynamic light scattering at approximately 25.1 degrees C using water as the dispersant. Drug content was determined spectrophotometrically after dilution and 30 min sonication, using the study calibration curve. Entrapment efficiency was calculated after centrifugation from the difference between total drug and free drug in the supernatant. Antioxidant activity was assessed using the DPPH radical-scavenging assay: 1 mL DPPH solution was added to each extract concentration, incubated in the dark for 30 min and measured at 520 nm, with ascorbic acid as standard [12].

The syrups were examined for colour, odour, taste, visual homogeneity, pH, density, viscosity and specific gravity. pH was measured after dilution of 10 mL syrup to 100 mL with distilled water and 10 min sonication. Density was determined using a 25 mL density bottle, specific gravity using a calibrated pycnometer at 25 degrees C, and viscosity using an Ostwald viscometer. A short-term 72 h stability procedure was described, including observation at 24, 48 and 72 h under room-temperature and elevated-temperature conditions for changes in appearance, turbidity, pH, viscosity, sedimentation, precipitation and phase separation.

3. Results and Discussion

3.1 Phytochemical profile of the extract

Qualitative phytochemical screening was positive for flavonoids, alkaloids, saponins, tannins and terpenoids. The concurrent presence of flavonoids and tannins is consistent with the polyphenolic character previously reported for Aerva lanata and is relevant to the plant's antioxidant profile [1,4,5]. Previous experimental studies have also associated Aerva lanata extracts with

modulation of urinary risk factors and calcium oxalate urolithiasis [2,3]. In the present work, however, these constituents were identified only qualitatively and were not individually quantified.

3.2 Total flavonoid content and FTIR findings

The quercetin calibration curve showed good linearity ($y = 0.0136x - 0.0093$; $R^2 = 0.9959$). The total flavonoid content of the extract was 15.71 ± 0.25 mg QE/g extract. This result quantitatively supports the flavonoid-positive qualitative tests and provides a plausible basis for the observed antioxidant response. The use of an aluminium chloride-based flavonoid assay is consistent with established colorimetric approaches [10,11].

FTIR analysis showed a broad O-H stretching band at 3423 cm^{-1} , C-H stretching bands at 2924 and 2853 cm^{-1} , a carbonyl-associated band at 1736 cm^{-1} , an aromatic C=C band at 1629 cm^{-1} and a C-O band at 1035 cm^{-1} . Taken together, these bands are compatible with phenolic/flavonoid hydroxyl groups, aliphatic moieties, carbonyl-containing components and glycosidic structures in the extract. FTIR therefore supported the chemical heterogeneity expected for a phytochemical-rich herbal extract, although it does not by itself identify individual compounds.

3.3 Nanoparticle size distribution

Dynamic light scattering gave a Z-average size of 163.0 nm , confirming formation of a nanoscale population. The PDI was 0.436 , indicating a relatively broad size distribution rather than a highly monodisperse system. The intensity distribution contained major peaks at 824.7 nm and 16.06 nm , suggesting coexistence of larger aggregates/particles and a smaller nanoparticle population. The intercept was 0.933 and the derived count rate was 240.3 kcps . The broad distribution is an important formulation finding: although the Z-average is within the nanoscale range, further optimization of polymer concentration, surfactant level, phase ratio, addition rate or mixing conditions would be needed to narrow the distribution before scale-up [8,9,13].

Table 2. Principal analytical and nanoparticle characterization results

Parameter	Result	Interpretation
Total flavonoid content	15.71 ± 0.25 mg QE/g extract	Quantifiable flavonoid content
Quercetin calibration	$R^2 = 0.9959$	Good linearity
Z-average	163.0 nm	Nanoscale average size

particle size		
PDI	0.436	Broad size distribution
Intensity peak 1	824.7 nm	Larger particle/aggregate population
Intensity peak 2	16.06 nm	Smaller particle population
Derived count rate	240.3 kcps	Adequate scattering signal
Drug content	92%	Reported assay value

3.4 Drug content and entrapment efficiency

The reported drug content of the nanoparticle-loaded formulation was 92% . Entrapment efficiency varied between the three formulations: 92% for F1, 95% for F2 and 93% for F3. F2 therefore showed the highest calculated entrapment efficiency. High entrapment can be advantageous for maintaining the phytoconstituent payload within the carrier system; however, the present data were based on a UV-visible assay of a complex herbal extract rather than quantification of a chemically specific marker compound. For publication, the selected analytical marker and assay specificity should be stated explicitly.

3.5 DPPH radical-scavenging activity

The DPPH response increased progressively with concentration for both the Aerva lanata sample and ascorbic acid. At the highest tested concentration, the reported response was 42.24 ± 0.81 for the sample and 76.54 ± 0.60 for ascorbic acid. This concentration-dependent pattern is compatible with antioxidant activity from polyphenolic constituents and agrees with prior reports of antioxidant properties of Aerva lanata extracts [4,5]. The sample remained less active than ascorbic acid across the tested range. Because the source document labels the concentration units inconsistently between the table and graph, the raw assay sheet should be checked before final journal submission.

Table 3. DPPH radical-scavenging results reported in the thesis

Concentration*	Ascorbic acid	Aerva lanata sample
0.2	34.36 ± 0.09	25.42 ± 1.60
0.4	47.89 ± 0.71	29.11 ± 0.50

0.6	60.01 +/- 0.24	33.08 +/- 0.32
0.8	69.56 +/- 1.20	37.55 +/- 0.19
1.0	76.54 +/- 0.60	42.24 +/- 0.81

**The source thesis uses inconsistent concentration units (micrograms/mL in one table and mg/mL in the graph). Verify the raw data before submission. Values are reproduced as reported and are interpreted as percentage DPPH inhibition/activity based on the study text and graph.*

3.6 Physicochemical evaluation of the herbal syrup

The syrups were visually homogeneous and were described as yellowish-green/brownish-green, aromatic and bitter-sweet/slightly bitter, with no visible phase separation. Density values were narrowly distributed between 1.21 and 1.22 g/mL. Increasing xanthan gum, sucrose and propylene glycol from F1 to F3 was accompanied by an increase in viscosity from 2.66 to 4.20 cP. F2 showed an intermediate viscosity of 3.51 cP, providing a practical balance between fluidity and consistency. The measured pH values ranged from 4.84 to 5.71, while specific gravity ranged from 1.21 to 1.23. Among the formulations, F2 combined the highest entrapment efficiency (95%) with intermediate viscosity, a density of 1.22 g/mL, pH 5.58 and specific gravity 1.22, supporting its selection as the optimized batch in the thesis.

Table 4. Comparative physicochemical evaluation of F1-F3

Parameter	F1	F2	F3
Entrapment efficiency	92%	95%	93%
Density	1.21 g/mL	1.22 g/mL	1.21 g/mL
Viscosity	2.66 cP	3.51 cP	4.20 cP
pH	5.71	5.58	4.84
Specific gravity	1.21	1.22	1.23

3.7 Stability assessment, significance and limitations

A 72 h short-term stability protocol was described for visual and physicochemical monitoring. The thesis states that the optimized formulation showed acceptable physicochemical characteristics, but it does not provide a complete numerical time-point table for pH, viscosity, turbidity or other parameters at 24, 48 and 72 h. Therefore, the available data support short-term formulation feasibility rather than a formal stability claim. ICH-style long-term and accelerated stability studies would be required for shelf-life assignment.

The formulation concept is scientifically relevant because it combines a phytochemical-rich Aerva lanata extract with a polymeric nanoparticle carrier and an oral liquid dosage form. Nevertheless, two limitations are critical. First, the DLS profile indicates heterogeneity (PDI 0.436 and an 824.7 nm intensity population), so particle-size optimization is warranted. Second, the work did not directly measure inhibition of calcium oxalate nucleation, growth, aggregation or dissolution, nor did it include an animal urolithiasis model. Consequently, the study demonstrates formulation development and characterization, not confirmed antiuro lithiatic efficacy.

4. Conclusion

Aerva lanata ethanolic extract was successfully incorporated into pullulan nanoparticles by nanoprecipitation and subsequently formulated as a herbal syrup. The extract contained flavonoids, alkaloids, saponins, tannins and terpenoids, with a total flavonoid content of 15.71 +/- 0.25 mg QE/g. The nanoparticle system showed a Z-average size of 163.0 nm with a PDI of 0.436. Drug content was reported as 92%, and F2 achieved the highest entrapment efficiency (95%) together with balanced syrup characteristics (density 1.22 g/mL, viscosity 3.51 cP, pH 5.58 and specific gravity 1.22). DPPH activity increased with concentration but remained below that of ascorbic acid. On the basis of the available formulation data, F2 is the most suitable prototype for further development. Future work should include optimization of particle-size distribution, marker-specific assay validation, in-vitro release, calcium oxalate crystallization assays, in-vivo antiuro lithiatic testing and formal long-term stability studies.

Declarations

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Conflict of interest: To be declared by the authors.

Ethics statement: No animal or human experimentation is described in the present formulation study. If subsequent biological experiments are added, relevant ethical approval details should be included.

Data availability: The numerical data in this manuscript were derived from the submitted B.Pharm thesis. Raw analytical files should be retained and made available according to the target journal's policy.

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