Check for updates

RESEARCH ARTICLE

Synthesis and characterization of chitosan nanoparticles of *Achillea millefolium* L. and their activities [version 1; peer review: 2 approved with reservations]

Dolly Kain, Suresh Kumar

Medicinal Plant Research Laboratory, Department of Botany, Ramjas College, University of Delhi, Delhi, 110007, India

V1 First published: 04 Nov 2020, 9:1297
<https://doi.org/10.12688/f1000research.26446.1>
 Latest published: 04 Nov 2020, 9:1297
<https://doi.org/10.12688/f1000research.26446.1>

Abstract

Background: *Achillea millefolium* L. is an herbal aromatic plant of family Asteraceae reported to have various medicinal activities in the literature. The current study evaluated the potential of chitosan nanoparticles of *A. millefolium* as an effective strategy for targeted treatment of bacterial diseases and urolithiasis.

Methods: *A. millefolium* was collected from Poonch, Jammu and Kashmir, and its inflorescence extracted in water by maceration. Chitosan nanoparticles of *A. millefolium* (AMCSNPs) were prepared by ionic gelation method using 0.1% chitosan, different concentrations of the cross-linking agent sodium tripolyphosphate (STPP; 0.5%, 1%, 1.5%, 2%) and different concentrations of *A. millefolium* extract (0.5%, 1%, 1.5%, 2%). Characterization of AMCSNPs was done using UV-Vis spectroscopy, Fourier transform-infrared (FT-IR) spectroscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Antibacterial screening of AMCSNPs was performed by well-diffusion method. Antiurolithiatic screening of AMCSNPs was done by nucleation and aggregation assay.

Results: The best chitosan nanoparticles of *A. millefolium* (AMCSNPs) were obtained with 0.1% chitosan, 1% STPP and 20% *A. millefolium*. These AMCSNPs showed maximum zone of inhibition of 30 ± 0.5 mm using the well-diffusion method against both *Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) and maximum antiurolithiatic activity with 68% inhibition shown at aggregation stage.

Conclusions: The current study suggests that AMCSNPs are an excellent strategy for targeted drug delivery for treatment of bacterial diseases and urolithiasis.

Keywords

Achillea millefolium L., AMCSNPs, Drug delivery, Targeted treatment, Antibacterial, Antiurolithiatic

Open Peer Review

Approval Status ? ?

	1	2
version 1	?	?
04 Nov 2020	view	view
1. Alok Arun , Inter American University of Puerto Rico, Barranquitas, USA 2. Dipjyoti Chakraborty , Banasthali Vidyapith, Banasthali, India		

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **Nanoscience & Nanotechnology** gateway.

Corresponding author: Suresh Kumar (suresh.kumar@ramjas.du.ac.in)

Author roles: **Kain D:** Conceptualization, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Kumar S:** Conceptualization, Formal Analysis, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The University Grant Commission, University of Delhi provided financial support.

Copyright: © 2020 Kain D and Kumar S. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Kain D and Kumar S. **Synthesis and characterization of chitosan nanoparticles of *Achillea millefolium* L. and their activities [version 1; peer review: 2 approved with reservations]** F1000Research 2020, 9:1297 <https://doi.org/10.12688/f1000research.26446.1>

First published: 04 Nov 2020, 9:1297 <https://doi.org/10.12688/f1000research.26446.1>

Introduction

Natural biopolymers are attractive products of living organisms as they serve a number of different applications for human health due to their biodegradability, such as vaccine delivery, drug development, and food preservatives¹. Chitosan (CS) is a natural biopolymer and a derivative of chitin. It is obtained from different sources of chitin and differs on the basis of its degree of deacetylation². In the last few years, CS nanoparticles (CSNPs) have drawn much attention due to their biodegradability, biocompatibility, quantum size effects, large surface to volume ratios, and their simple and inexpensive production³⁻⁵. Different biological activities of CSNPs have been reported, such as antimicrobial, antioxidant, anticancer⁶, drug delivery, tissue engineering, carbon nanotube, food preservative, and purification of water⁷. CSNPs successfully used in drug delivery for treatment of various diseases, including ocular drug delivery⁸, per-oral drug delivery⁹, nasal drug delivery¹⁰, pulmonary drug delivery¹¹, mucosal drug delivery¹², gene delivery¹³, buccal drug delivery¹⁴, vaccine delivery¹⁵, vaginal drug delivery¹⁶, and cancer therapy¹⁷ have been reported. *Achillea millefolium* is a perennial herbal aromatic plant belonging to the family Asteraceae with characteristically finely divided leaves and inflorescence in corymbose cluster. It has been reported to have different medicinal activities, including antibacterial and diuretic^{18,19}. The current study was designed to evaluate the potential of CSNPs of *A. millefolium* (AMCSNPs) as an effective alternative of targeted drug delivery and treatment of various diseases, including bacterial infections specifically urolithiasis.

Methods

Collection, identification, and extraction of *A. millefolium*

A. millefolium was collected from Pathanteer Village, Mendhar Tehsil, Poonch District (Jammu and Kashmir, India; GPS coordinates 33° 39' 40" N- 74° 11' 11" E) and identified by Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), Pusa with reference IDNISCAIR/RHMD/consult/2018/3293-94. 5g powder of inflorescence of *A. millefolium* was extracted in 50 ml of water by maceration at 90° C using water bath.

Crude extract of the plant was evaporated using rotary evaporator (Khera KI- 102), which resulted in the semi solid form of extract. This was then weighed and dissolved in a known amount of solvent for making a stock concentration of the plant extract. Different concentrations were made by serial dilution.

Bacterial strains and chemicals

Strains of *Bacillus subtilis* (MTCC 441) and *Pseudomonas aeruginosa* (MTCC 1688) were procured from MTCC Chandigarh. All the chemicals used (chitosan, acetic acid, sodium tripolyphosphate (STPP), Tween-80, calcium chloride, sodium oxalate, tris buffer and NaCl) were of good quality and purchased from Fisher Scientific International, Inc.

Synthesis of chitosan nanoparticles of *A. millefolium*

CSNPs were prepared by ionic gelation method. 10ml 0.1% chitosan solution was made in 1% acetic acid with different percentages of the cross-linking agent STPP (0.5%, 1%, 1.5%

and 2%). 5ml of STPP was added drop wise to the chitosan-acetic acid solution, which was magnetically stirred at room temperature. An opalescent color was observed, and stirring was continued for 60 min.

To obtain AMCSNPs, variable concentrations of plant extract (5%, 10%, 15% and 20%) were added to the 10 ml chitosan solution by magnetic stirring prior to adding the 5 ml STPP drop wise. This solution was stirred for a further 2 h followed by centrifugation at 10000g for 10 min and then the AMCSNPs were washed three times with distilled water. The pH of the nanoparticles was maintained at 4.8, and 1–2 drops of 1% Tween-80 was used to prevent agglomeration.

Percentage encapsulation efficiency of each concentration of extract was determined using the following formula²⁰⁻²²:

Encapsulation efficiency (%) = (total amount – free amount/ total amount) *100.

Characterization of chitosan nanoparticles of *A. millefolium*

UV-Vis spectroscopy using SPUV-1000 spectrophotometer attached to Mwave professional software 2.0 (or any software used to obtain the UV- Vis absorption spectra) and spectrum between 200 nm-700 nm was obtained for determining the main absorbing region. FTIR (Fourier Transform-Infrared) Spectroscopy using spectrometer (Brukers) in the range of 1000 cm⁻¹–3500 cm⁻¹ to identify the peaks of main functional groups, DLS (Dynamic Light Scattering) in the range between 0 nm to 1000 nm using zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) at room temperature for particle size distribution and TEM (Transmission electron microscopy) at an accelerating voltage of 200 kV using Tecnai G2 30U-twin kV Ultra-twin microscope to study the morphology.

Antibacterial screening of chitosan nanoparticles of *A. millefolium*

Primary culture of bacteria was obtained from lyophilized culture by inoculating in LB broth, which was incubated in an incubator shaker at 120 rpm and 37°C for 12–16 h. Pure colonies of each bacterium were obtained from primary culture by streak plate method using LB agar plates, which were inoculated in LB broth, incubated in an incubator shaker at 120 rpm 37°C for 12–16 h. Absorption of bacterial culture was adjusted to 0.1±0.02 at 600nm using SP-UV1000 spectrophotometer to reach the concentration of 10⁸ CFU/ml for final use, which is equal to 0.5 McFarland standards, as previously performed in the literature²³⁻²⁵ to obtain a similar concentration of each bacterium. Each reading was taken thrice.

Antibacterial screening of AMCSNPs was done using well-diffusion method³¹. 1.5% LB agar plates were used and a 5mm cork-borer made four wells in each plate. 20 µl of *B. subtilis* and *P. aeruginosa* culture was added to the plates and spread using a glass spreader. 100 µl of AMCSNPs was poured in the wells. Plates were sealed with parafilm, incubated at 28° C for 12–16 h and the zone of inhibition (ZOI) recorded.

Antirolithiatic screening of chitosan nanoparticles of *A. millefolium*

Nucleation and aggregation assays were used to determine the antirolithiatic potential of AMCSNPs.

Nucleation assay: The method of Hennequin *et al.*²⁷ was used with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared at a final concentration of 3mmol/l and 0.5mmol/l, respectively, in a buffer containing Tris 0.05mol/l and NaCl 0.15mol/l at pH 5.5. A total of 1.9 ml of calcium chloride solution mixed with 200 µl of AMCSNPs was incubated for 30 minutes in a 37° C water bath. Crystallization was started by adding 1.9 ml of sodium oxalate solution. Equal volume of water has used for a control instead of AMCSNPs. The optical density of the solution was recorded at 620 nm for 420 sec using spectrophotometer SPUV- 1000.

$$\% \text{ Inhibition} = \{(\text{Abs. Control} - \text{Abs. Sample}) / \text{Abs. Control}\} * 100$$

Aggregation assay: The method of Hess *et al.*²⁸ was used with some minor modifications. `Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50mmol/l. Both the solutions were equilibrated in a 60° C water bath for 1h and then cooled at 37° C overnight. The crystals were harvested by centrifugation at 10000g and then evaporated at 37° C. COM crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/l and NaCl 0.15 mol/l at pH 5.7. A total of 1 ml of AMCSNPs were added in a test tube to 3 ml COM crystal solution and incubated at 37°C. Equal volume of water was used for a control instead of AMCSNPs. Absorption at 620 nm was recorded at different time intervals (30 min, 60 min, 90 min, and 120 min).

$$\% \text{ Inhibition} = \{(\text{Slope Control} - \text{Slope Sample}) / \text{Slope Control}\} * 100$$

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2007. One-way ANOVA was used followed by t-test to determine

the significant difference of antibacterial activity between different samples and regression analysis was used to plot graphs of nucleation and aggregation assays.

Results

Synthesis of chitosan nanoparticles of *A. millefolium*

On addition of STPP to chitosan solution, an opalescent color was observed, which indicates the formation of CSNPs. Different concentrations of STPP (0.5%, 1%, 1.5%, and 2%) were used for nanoparticle preparation and 1.0% STPP was found to be most suitable with sharpest peak shown by UV spectroscopy, indicating the most CSNPs made (Figure 1). Therefore, 1.0% STPP was used to obtain AMCSNPs. Different percentages of *A. millefolium* water extract (5%, 10%, 15% and 20%) in 0.1% of chitosan solution were used to make AMCSNPs and excellent loading efficiency was observed, i.e. 94%, 94.7%, 94.7%, and 95.2% for 5%, 10%, 15% and 20% *A. millefolium* respectively. A standard graph for absorbance of *A. millefolium* extract at 417 nm (maximum absorption verses concentration of extract) was obtained. The amount of loaded extract was determined using the standard graph as a decrease in the absorption values of the supernatant of AMCSNPs indicated the loading of extract of the nanoparticles. Loading efficiency was calculated using the above encapsulation efficiency formula for each concentration. Hence 20% AMCSNPs have been used for further analysis.

Characterization of chitosan nanoparticles of *A. millefolium*

A broad absorption band between 200 to 300 nm was shown for the UV spectrum of AMCSNPs (Figure 2). FTIR spectra of CS showed peaks at 3324.15, 2153.28, 1638.72 and 1279.29; FTIR spectra of CSNPs showed peaks at 3317.48, 2139.29 and 1638.46; and FTIR spectrum of AMCSNPs showed peaks at 3281.73, 2163.36 and 1636.78 (Figure 3). DLS revealed the size range of nanoparticles with Z average of 118nm having characteristic peaks at 10 nm, 122 nm and 712 nm, and highest intensity was recorded at size 10nm (Figure 4). TEM was used to study the morphology of the nanoparticles, which revealed a spherical shape with smooth surface. TEM also revealed the size of AMCSNPs: <100 nm with smallest size of 4.15 nm (Figure 5).

Figure 1. UV-Vis spectrum of chitosan (CS) and chitosan nanoparticles prepared with different concentrations of sodium tripolyphosphate (0.5%, 1%, 1.5% and 2%).

Figure 2. UV absorption spectrum of *Achillea millefolium* chitosan nanoparticles (0.1% chitosan, 1% sodium tripolyphosphate and 20% *A. millefolium*).

Figure 3. Fourier Transform-Infrared graph of chitosan (A), chitosan nanoparticles, (B) and *Achillea millefolium* chitosan nanoparticles (C).

Figure 4. Particle size distribution of *Achillea millefolium* chitosan nanoparticles, as revealed by Dynamic Light Scattering.

Figure 5. Transmission Electron Microscopy showing *Achillea millefolium* chitosan nanoparticles.

Antibacterial screening of chitosan nanoparticles of *A. millefolium*

AMCSNPs exhibited excellent antibacterial activity against both Gram-positive *B. subtilis* and Gram-negative *P. aeruginosa*. AMCSNPs showed three-times the increase in antibacterial activity as compared with *A. millefolium* extract only (control); ZOI increased from 10 mm to 30mm against both *B. subtilis* and *P. aeruginosa* with a statistically significant difference between *A. millefolium*, CSNPs and AMCSNPs (Figure 6).

Antiuro lithiatic screening of chitosan nanoparticles of *A. millefolium*

AMCSNPs showed significant antiuro lithiatic activity with 68% inhibition in the aggregation assay and 51.26% inhibition

in the nucleation assay as compared to 55.132% and 9.09% inhibition by *A. millefolium* extract (control). In the nucleation assay, the % inhibition is nearly equal in the case of *A. millefolium* and AMCSNPs, but CSNPs did not show any inhibition. In the aggregation assay there is a significant increase in % inhibition with 9.09%, 63.63% and 68% inhibition by *A. millefolium*, CSNPs and AMCSNPs, respectively, (Figure 7).

Discussion

For characterization of nanoparticles, different techniques used in the literature including UV- Vis spectroscopy, FTIR spectroscopy, DLS and TEM^{29,30}. In our study, a UV-Vis absorption band for AMCSNPs of 200-300 nm indicates the presence of a CO group in the CSNPs, as reported by Vaezifer *et al.*³¹. A shift of

Figure 6. Antibacterial activity of *Achillea millefolium* chitosan nanoparticles. CSNP's= Chitosan nanoparticles, AM= *A. millefolium* and W= water.

Figure 7. Antiurrolithiatic activity of *Achillea millefolium* chitosan nanoparticles (AMCSNP's) as shown by a nucleation assay (A) and aggregation assay (B). AM= *A. millefolium*, CSNP's= chitosan nanoparticles.

FTIR peaks from 3317.48, 2139.29 and 1638.46 for CSNPs to 3281.73, 2163.36 and 1636.78 for AMCSNPs indicates that the loading of *A. millefolium* into the CSNPs, as reported by Khan *et al.*³². Our DLS results are comparable to the average size of CSNPs reported in literature, i.e. 189 and 197 nm by Khan *et al.*³², 216 nm by Agarwal *et al.*³³, and size range of 135–729 nm by Rasae *et al.*³⁴ and 6.5–1331.2 nm by Iswanti *et al.*³⁵. TEM of AMCSNPs in our study revealed a spherical shape with a smooth surface, which was also reported by Da Silva *et al.*²¹.

Chitosan is a positively charged macromolecule, which interacts with the negatively charged microbial membrane, and results in the breakage of intracellular components. Chitosan acts as a chelating agent and limits toxin production and microbial growth^{36–38}. Antibacterial screening of *Ocimum basilicum* CSNPs against *E. coli* and *Bacillus vallismortis* have been reported by Rasae *et al.*³⁴, chitosan-tripolyphosphate nanoparticles against *Staphylococcus aureus* and *P. aeruginosa* have been reported by Bangum *et al.*³⁹, and against phytopathogens of tomato *Xanthomonas* and *Erwinia* strains by Oh *et al.*²⁹. Gallic acid-chitosan conjugates have been reported to inhibit the formation of calcium oxalate crystals by Queiroz *et al.*⁴⁰. Antiuro lithiatic activity of *Aerva lanata* chitosan nanoparticles at 0.8 µg/ml concentration through nucleation assay have been reported by Chandirika *et al.*⁴¹ and *Tridax procumbens* by Chandirika *et al.*⁴². In our study, AMCSNPs showed excellent antibacterial activity against both *B. subtilis* and *P. aeruginosa*, and significant antiuro lithiatic activity at aggregation stage of urolithiasis.

Data availability

Underlying data

Figshare: Chitosan nanoparticles of *Achillea millefolium* L., <https://doi.org/10.6084/m9.figshare.12936293.v3>⁴³.

This project contains the following underlying data:

- Output files of chitosan nanoparticles with different concentrations of STPP
- Raw data for Figures 1, 2 and 4
- Unedited and uncropped FT-IR graphs and TEM images of AMCSNPs
- ZOI of antibacterial activity of AMCSNPs
- Absorption values of antiuro lithiatic activity AMCSNPs

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

The authors are thankful to the Principal, Dr. Manoj K. Khanna, Ramjas College, Prof. S.B. Babbar, Head, Prof. K.S. Rao, and Prof. Veena Agrawal, Department of Botany, University of Delhi, Delhi for providing necessary facilities and encouragement during the course of investigation.

References

- Gomes LP, Paschoalin VMF, Del Aguila EM: **Chitosan nanoparticles: production, physicochemical characteristics and nutraceutical applications.** *Rev Virtual Quim.* 2017; **9**(1): 387–409.
[Reference Source](#)
- Shukla SK, Mishra AK, Arotiba OA, *et al.*: **Chitosan-based nanomaterials: a state-of-the-art review.** *Int J Biol Macromol.* 2013; **59**: 46–58.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ochekpe NA, Olorunfemi PO, Ngwuluka NC: **Nanotechnology and drug delivery part 2: nanostructures for drug delivery.** *Tropical J Pharmaceut Res.* 2009; **8**: 275.
[Publisher Full Text](#)
- He X, Hwang HM: **Nanotechnology in food science: Functionality, applicability, and safety assessment.** *J Food Drug Anal.* 2016; **24**(4): 671–681.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Naskar S, Koutsou K, Sharma S: **Chitosan-based nanoparticles as drug delivery systems: a review on two decades of research.** *J Drug Targeting.* 2018; **27**(4): 379–393.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kean T, Thanou M: **Biodegradation, biodistribution and toxicity of chitosan.** *Adv Drug Deliv Rev.* 2010; **62**(1): 3–11.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gavhane Y, Gurav A, Yadav A: **Chitosan and its applications: a review of literature.** *Inter J Biomed Pharm Sci.* 2013; **4**: 312.
- Gupta H, Velpandian T, Jain S: **Ion- and pH-activated novel in-situ gel system for sustained ocular drug delivery.** *J Drug Target.* 2010; **18**(7): 499–505.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Gao P, Xia G, Bao Z, *et al.*: **Chitosan based nanoparticles as protein carriers for efficient oral antigen delivery.** *Int J Biol Macromol.* 2016; **91**: 716–23.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Shahnaz G, Vetter A, Barthelmes J, *et al.*: **Thiolated chitosan nanoparticles for the nasal administration of leuprolide: bioavailability and pharmacokinetic characterization.** *Int J Pharm.* 2012; **428**(1–2): 164–70.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Islam N, Ferro V: **Recent advances in chitosan-based nanoparticulate pulmonary drug delivery.** *Nanoscale.* 2016; **8**(30): 14341–58.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Martirosyan A, Olesen MJ, Howard KA: **Chitosan-based nanoparticles for mucosal delivery of RNAi therapeutics.** *Adv Genet.* 2014; **88**: 325–52.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Rudzinski WE, Palacios A, Ahmed A, *et al.*: **Targeted delivery of small interfering RNA to colon cancer cells using chitosan and PEGylated chitosan nanoparticles.** *Carbohydr Polym.* 2016; **147**: 323–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mazzarino L, Borsali R, Lemos-Senna E: **Mucoadhesive films containing chitosan-coated nanoparticles: a new strategy for buccal curcumin release.** *J Pharm Sci.* 2014; **103**(11): 3764–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Illum L, Jabbal-Gill I, Hinchcliffe M, *et al.*: **Chitosan as a novel nasal delivery system for vaccines.** *Adv Drug Deliv Rev.* 2001; **51**(1–3): 81–96.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Perinelli DR, Campana R, Skouras A, *et al.*: **Chitosan Loaded into a Hydrogel Delivery System as a Strategy to Treat Vaginal Co-Infection.** *Pharmaceutics.* 2018; **10**(1): 23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lee SJ, Min HS, Ku SH, *et al.*: **Tumor-targeting glycol chitosan nanoparticles as a platform delivery carrier in cancer diagnosis and therapy.** *Nanomedicine (Lond).* 2014; **9**(11): 1697–713.
[PubMed Abstract](#) | [Publisher Full Text](#)

18. Akram M: **Minireview on Achillea millefolium Linn.** *J Membr Biol.* 2013; **246**(9): 661–663.
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Lakshmi T, Geetha RV, Anitha R, *et al.*: **Yarrow (Achillea millefolium Linn.) a herbal medicinal plant with broad therapeutic use - A Review.** *Int J Pharm Sci Rev Res.* 2011; **9**(2): 136–141.
[Reference Source](#)
20. Calvo P, Remunan-Lopez C, Vila-Jata JL, *et al.*: **Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines.** *J Pharm Res.* 1997; **14**(10): 1431–1436.
[PubMed Abstract](#) | [Publisher Full Text](#)
21. Da Silva SB, Amorim M, Pedro F, *et al.*: **Natural extracts into chitosan nanocarriers for rosmarinic acid drug delivery.** *Pharm Biol.* 2015; **53**(5): 642–652.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Servat-Medina L, González-Gómez A, Reyes-Ortega F, *et al.*: **Chitosan-tripolyphosphate nanoparticles as Arrabidaea chica standardized extract carrier: synthesis, characterization, biocompatibility, and antiulcerogenic activity.** *Int J Nanomed.* 2015; **10**: 3897–3909.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Balouri, *et al.*: **2016 and Clinical and laboratory standard institute, USA.**
24. Balouri M, Sadiqi M, Ibsouda SK: **Methods for in vitro evaluating antimicrobial activity: A review.** *J Pharm Anal.* 2016; **6**(2): 71–79.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. LSI: **Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11.** Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA. 2012.
26. Balouri M, Sadiqi M, Ibsouda SK: **Methods for in vitro evaluating antimicrobial activity: A review.** *J Pharm Anal.* 2016; **6**(2): 71–79.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Hennequin C, Lalanne V, Daudon M, *et al.*: **A new approach to studying inhibitors of calcium oxalate crystal growth.** *Urol Res.* 1993; **21**(2): 101–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
28. Hess B, Nakagawa Y, Coe FL: **Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins.** *Am J Physiol.* 1989; **257**(1 Pt 2): 99–106.
[PubMed Abstract](#) | [Publisher Full Text](#)
29. OH JW, Chun Se C, Chandrasekaran M: **Preparation and In Vitro Characterization of Chitosan Nanoparticles and Their Broad-Spectrum Antifungal Action Compared to antibacterial activities against phytopathogens of tomato.** *Agronomy.* 2019; **9**(1): 21.
[Publisher Full Text](#)
30. Arya A, Kumar S, Suryavanshi A, *et al.*: **Biosynthesis of metallic nanoparticles from medicinal plants: A review.** *J Nanosci Technol.* 2019; **5**(5): 827–831.
[Publisher Full Text](#)
31. Vaezifar S, Razavi S, Golozar MA, *et al.*: **Effects of some parameters on particle size distribution of chitosan nanoparticles prepared by ionic gelation method.** *J Clust sci.* 2013; **24**: 891–903.
[Publisher Full Text](#)
32. Khan MA, Zafaryad M, Mehdi SH, *et al.*: **Characterization and anti-proliferative activity of curcumin loaded chitosan nanoparticles in cervical cancer.** *Int J Biol Macromol.* 2016; **93**(Pt A): 242–253.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Agarwal M, Agarwal MK, Shrivastav N, *et al.*: **Preparation of chitosan nanoparticles and their in-vitro characterization.** *Int J Life Sci Scienti Res.* 2018; **4**(2): 1713–1720.
[Publisher Full Text](#)
34. Rasaei I, Ghannadnia M, Honari H: **Antibacterial properties of biologically formed chitosan nanoparticles using aqueous leaf extract of Ocimum basilicum.** *Nanomed J.* 2016; **3**(4): 240–247.
[Reference Source](#)
35. Iswanti FC, Nurulita I, Djauzi S, *et al.*: **Preparation, characterization and evaluation of chitosan- based nanoparticles as CpG ODN carriers.** *Biotechnol Biotechnol Equip.* 2019; **33**(1): 390–396.
[Publisher Full Text](#)
36. Leuba JL, Stossel P: **Chitosan and other polyamines: Antifungal activity and interaction with biological membranes.** In: *chitin in nature and technolog.* Muzzarelli R, Jeuniaux C, Gooday G, eds. Springer US. 1986; 215–222.
[Publisher Full Text](#)
37. Jung BO, Kim CH, Choi KS, *et al.*: **Preparation of amphiphilic chitosan and their antimicrobial activities.** *J Applied Polym Sci.* 1999; **72**(13): 1713–1719.
[Publisher Full Text](#)
38. Liu H, Du Y, Wang X, *et al.*: **Chitosan kills bacteria through cell membrane damage.** *Int J Food Microbiol.* 2004; **95**(2): 147–55.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Bangum H, Tandiono S, Arianto A: **Preparation and evaluation of chitosan-tripolyphosphate nanoparticles suspension as an antibacterial agent.** *J App Pharm Sci.* 2018; **8**(12): 147–156.
[Publisher Full Text](#)
40. Queiroz MF, Melo KRT, Sabry DA, *et al.*: **Gallic Acid-Chitosan Conjugate Inhibits the Formation of Calcium Oxalate Crystals.** *Molecules.* 2019; **24**(11): 2074.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Chandirika JU, Lakshmi PS, Mathiazhagan A, *et al.*: **Design and development of chitosan nanoparticles in target novel drug delivery systems for Urolithiasis.** *European J Biomed Pharm Sci.* 2015; **2**(4): 528–537.
[Reference Source](#)
42. Chandirika JU, Sindhu R, Selvakumar S, *et al.*: **Herbal extract encapsulated in chitosan nanoparticles: a novel strategy for the treatment of Urolithiasis.** *Indo American J Pharm Sci.* 2018; **5**(3): 1955–1961.
[Publisher Full Text](#)
43. Kain D, Kumar S: **Chitosan nanoparticles of Achillea millefolium L. figshare.** Dataset. 2020.
<http://www.doi.org/10.6084/m9.figshare.12936293.v3>

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 24 May 2021

<https://doi.org/10.5256/f1000research.29196.r82696>

© 2021 Chakraborty D. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Dipjyoti Chakraborty

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali, Rajasthan, India

The authors have used crude extracts of *Achillea millefolium*. The possible constituents of the inflorescence used especially with emphasis on secondary metabolites should be discussed with previous references.

Considering seasonal/low availability of flowers, how do the authors justify using inflorescence? Why not any other plant part?

Justify extraction in water at 90°C, are there any previous reports? What was the duration of extraction?

Why were only *Bacillus subtilis*, and *Pseudomonas aeruginosa* used for the study?

The UV spectrum shows absorbance beyond 1 in the Y-axis (Figure-1). It is generally not recommended to have Abs above 1 as it gives erroneous results and the sample is suitably diluted. The authors may consider.

Since the spectrum shows very low or no absorbance other than the uv region, authors may provide another graph - maybe in the inset showing the spectrum only in the UV region for better signature spectrum.

Figure 2 should also be looked into on similar lines.

The authors have used the "well-diffusion method" for antibacterial screening and thus have not been able to calculate MIC. What was the concentration of extract used for the Zone inhibition study and whether a range of concentration was tried?

Discussion section needs a few lines on the possible constituents of *A. millefolium* extract and its implication on the formation of the nanoparticles and or the bioactivity.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Stress Biology, Secondary Metabolite Biotechnology, Proteomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 May 2021

Suresh Kumar, University of Delhi, Delhi, India

I am thankful to the reviewer for critical analysis of work. The point wise response is as follows:

1. Two reasons for use of Inflorescence, firstly its diuretic uses confirmed by the traditional healers of the collection site. Secondly, crude extract of inflorescence has shown maximum antiurolithiatic activity which was determined in my previous study.
2. The temperature of extraction was used as per literature which gives best result at – 10 to 20 °C than B.P. of solvent used). Duration of extraction was 48 hrs.
3. Both bacteria are reported as causal agent for several human diseases/ infections (urinary tract infections, soft tissue infections, bacteremia, gastrointestinal infections, respiratory system infections) and also it cover the two main categories of gram negative (*P. aeruginosa*) and positive bacteria (*B. subtilis*).
4. The sharp peak at particular wavelength indicates the formation of nanoparticles; further dilution will not cover the entire spectrum and absorption pattern.

5. The antibacterial activity of nanoparticles was determined prepared by 1 mg/ml extract and compared with the same crude extract to determine the enhancement in the activity.
6. GC-MS of crude extract of inflorescence of *A. millefolium* reveals the presence of different bioactive compounds including Lupeol, Stigmasterol, alpha.-amyrin, and gamma.-sitosterol.

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 May 2021

<https://doi.org/10.5256/f1000research.29196.r83420>

© 2021 Arun A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Alok Arun

Institute of Sustainable Biotechnology, Department of Science and Technology, Inter American University of Puerto Rico, Barranquitas, Puerto Rico, 00794- 517, USA

The authors assessed the chitosan nanoparticles isolated from a herbal aromatic plant *Achillea millefolium* (here after referred as AMCSNPs) using ionic gelation method after applying various concentrations of plant extract. Further, the authors measured the antiurolithatic and antibacterial activity using two bacterial species and consequently propose AMCSNPs to be an alternative strategy for targeted delivery.

The research seems to be original and will definitely contribute to broadening the fundamental knowledge about AMCSNPs. However, the authors have potential to make this paper stronger by including few minor details/corrections:

1. Why was only inflorescence used for extract isolation? Providing an explanation can highlight the significant of tissue selection for chitosan nanoparticles isolation.
2. Besides herbarium, did others use any other method to ascertain the identity of plant species used in the study?
3. Authors mention the use of Hess *et al.*, for performing the aggregation assay. There is a brief mention of minor modifications in the protocol. Can they highlight the specific modifications that was used in the protocol?
4. The TEM images are not very clear. Can the authors provide the magnification used for these images?

5. The sentence in discussion, "A shift of FTIR peaks from 3317.48, 2139.29 and 1638.46 for CSNPs to 3281.73, 2163.36 and 1636.78 for AMCSNPs indicates that the loading of *A. millefolium* into the CSNPs, as reported by Khan *et al.*" is not clear. Can authors rephrase the sentence and provide clarity?

6. The paper will greatly benefit from an image of the plant and the tissue that was used in the study.

7. The study design may have included one more tissue (for example leaf, stem etc) from the plant for comparative purposes.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant Biotechnology, Genomics, Molecular Genetics, Bioinformatics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research