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Research Article

**HERBAL EXTRACT ENCAPSULATED IN CHITOSAN
NANOPARTICLE: A NOVEL STRATEGY FOR THE
TREATMENT OF UROLITHIASIS**

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Abstract:

In current study the inhibitory potential of Tridax procumbens leaf extract loading chitosan nanoparticle against calcium oxalate crystallization under in vitro condition.

Kidney stone formation was studied using three assays such as nucleation, aggregation and growth. Nucleation was studied by adding calcium chloride and sodium oxalate solution in the presence and absence of aqueous extracts at 37°C. For aggregation and growth calcium oxalate monohydrate crystals were studied. The effect of extracts on the formation and inhibition of stone forming stages were observed UV-Vis spectrophotometrically. In the present study Tridax procumbens plant extract loaded chitosan nanoparticle were prepared via ionic gelation of tripolyphosphate (TPP) methods synthesized nanoparticle was characterized by Scanning Electron Microscope (SEM) and Fourier Transform infrared (FTIR). The present results showed the optimum encapsulation efficiency (77 %) was obtained by a chitosan concentration of 0.1 mg/mL, chitosan-to-TPP mass ratio of 2 ml and Tridax procumbens plant extract concentration of 0.8 µg/mL. Scanning Electron Microscope (SEM) imaging showed a smooth and homogenous structure for nanoparticles. Fourier Transform infrared (FTIR) spectroscopy confirmed tripolyphosphoric groups of TPP linked with ammonium groups of Chitosan in the Nanoparticle. The present results showed the potential of the plant extract loaded chitosan nanoparticle in our study may provide a suitable alternative to traditional adjuvant systems for urolithiasis.

Key words: *Tridax procumbens, Chitosan, nanoparticle, loading, drug delivery system,*

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INTRODUCTION:

Urolithiasis is a disorder that is recognised throughout the world, although its incidence varies widely amongst countries [1] Stone formation in the kidney is one of the oldest and most wide spread diseases known to man. Urinary stone disease is a common disorder estimated to occur in approximately 12% of the world population, with a recurrence rate of 70-81% in males and 47-60% in females [2]

In India people living in different states utilize different plants for curing urolithiasis [3] Urolithiasis is derived from the Greek words “ouron” (urine) and “lithos” (stone). It is considered as the third most common affliction of the urinary tract [4] Urolithiasis is a complex process that occurs from series of several physicochemical event including supersaturation, nucleation, growth, aggregation and retention within the kidneys [5] Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches, i.e., percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) [6].

Tridax procumbens L. is commonly known as ‘Ghamra’ and in English popularly known as ‘coat buttons’ because of the appearance of its *Tridax* plant is present throughout India and is employed as indigenous medicine for variety of ailments. It has been extensively used in Ayurvedic system of medicine and is dispensed as “Bhringraj” by some practitioners of Ayurveda which is well known medicine for liver disorder. It has been found to possess significant medicinal properties against blood pressure, bronchial catarrh, malaria, dysentery, diarrhea, stomach ache, headache, wound healing, it also prevents hair fall and check hemorrhage from cuts and bruises. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties. The plant also shows various pharmacological activities like Immuno modulatory, Anti-diabetic, Anti hepatotoxic & Anti-oxidant, Anti-inflammatory, Analgesic, and marked depressant action on respiration [7]

Nano-encapsulated compounds have been developed initially for the design of drug delivery systems. The application of nano-encapsulation technology can increase the stability of catechin, thus increase its solubility and bioavailability [8]. Encapsulation of plant extract loaded nanoparticles, liposomes or other pre-formed materials, represents a solution to increase the antioxidant's efficacy of therapy [9] Polymers are very convenient materials for the

manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications [10]. According to National Nanotechnology Initiative, nanotechnology is known as a science that learns the characterization and manipulation of biological and microbiological materials with a size of less than 100 nanometer, including the unique phenomenon and a novel functional property that will emerge. Nanoparticle colloidal system using a non-toxic biopolymer material is expected to be able to protect functional properties of certain bioactive compound like polyphenols. Mixtures of chitosan and sodium tripolyphosphate (Na-TPP) as carrier for plant extract should be protect, stabilize, or control the release of the core. Chitosan as hydrophilic polymer can easily cross-link with counter poly anions like Na-TPP to control the release of the drug [11]. In this case, composition of chitosan and Na-TPP is the key parameter in controlling the properties and the structure of the system, the present research aims to *Tridax procumbens* leaf extract loading Chitosan Nanoparticle against calcium oxalate crystallization under *in vitro* condition and loaded nanoparticles were characterized using Scanning electron microscope SEM and Fourier transform infrared spectroscopy (FTIR).

MATERIAL AND METHODS:

Chemicals

Chemicals all chemicals used in this investigation were of analytical grade and were obtained from himedia, india, india sea food, cochin. tris, sodium chloride, calcium chloride, sodium oxalate, acetic acid, cross linking agent sodium tri polyphosphate, chitosan all the chemicals used were of analytical grade and used with further purification.

Preparation of sample

The leaves were cleaned and cut into small pieces and shade dried. The dried plant were powdered and passed through the coarse sieve (0.2mm). The extract was evaporated in a water bath at 60 °C. The residue was stored in an airtight container in a refrigerator.

Nucleation assay

The stone formation begins with the occurrence of nuclei, therefore we chose the classical model for the study of oxalate crystallization with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared separately at a final concentration of 3mM/L and 0.5mM/L respectively in a buffer containing Tris 0.5mM/L and NaCl 0.15mM/L of pH 6.5. Both the solutions were filtered thrice. For the assay, 950µl of calcium chloride and

varying concentration of corm extracts (final volume of 100 μ l) were pipetted out against a reagent blank (without extract). To this added 950 μ l of sodium oxalate and shook well. The absorbance was measured at 620nm.

Growth assay

4mM calcium chloride and 4mM sodium oxalate of 1ml each were added to a 1.5ml of solution containing NaCl (10mM) buffered with Tris (10mM) at pH 7.2. To this 30 μ l of calcium oxalate monohydrate crystal slurry (1.5mg/ml acetate buffer) was added. Consumption of oxalate begins immediately after calcium oxalate monohydrate crystal slurry addition and was monitored for 600 seconds for the disappearance of absorbance at 214 nm. When plant extract was added into this solution, depletion of free oxalate ions will decrease if the extract inhibits calcium oxalate crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30 seconds incubation with or without the extract.

Preparation of calcium oxalate monohydrate (COM) crystal

Preparation of COM seed crystals calcium oxalate monohydrate (COM) seed crystals were prepared by mixing equal volumes of 0.01 M CaCl₂ and 0.01 M sodium oxalate by drop wise addition of sodium oxalate solution to calcium chloride solution with constant stirring for 1 week at 4°C. The solution was centrifuged at 2,000g for 10 min at room temperature. The crystal pellet was washed with distilled water followed by methanol and these crystals were air dried before their use for further studies

Aggregation assay

'Seed' CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The Crystals were harvested by centrifugation 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 0.15mol/L at pH 6.5. then experiments were conducted at 37°C in the presence or absence of plant extract after stopping the stirring. The rate of aggregation was estimated by comparing the slopes of turbidity in presence of extract with that obtain in control.

Chitosan nanoparticle preparation

The ionic gelation method was followed for the preparation of chitosan nanoparticle chitosan (0.1 % w/v) was dissolved in 1 % acetic acid and cross linking agent sodium tri polyphosphate 2 % (w/v) was added drop wise in the solution stirring condition at room temperature. Opalescent colour was observed and stirring was continued for 60 min. loading of plant extract to chitosan nanoparticle was performed by dissolving variable concentrations of plant extract and distilled water. The solution was added drop wise in 10 ml chitosan solution in magnetic stirring. It was further stirred for 2 h followed by centrifugation the absorbance was measured at 327.14 nm for the supernatant.

Drug Load in chitosan nanoparticle

Loading of plant extract to chitosan nanoparticle was performed by variable concentrations of plant extract 0.2 – 0.8 μ g / mL in 2.5 % of distilled water the solution was added drop wise in 10 ml of chitosan solution in magnetic stirring. It was further stirred for 2 h followed by centrifugation for 10 min at 1000 rpm. Supernatant was discarded and pellet was resuspended in PBS. The nanoparticle were collected by centrifugation at 20,000 rpm for 10 min and then washed three times with distilled water [12-15]

RESULTS AND DISCUSSION:

Nucleation, growth and aggregation assay

In vitro studies as follows as evaluate the medicinal important plant *T. procumbens* extract loaded chitosan nanoparticle for the efficacy of *in-vitro* studies, containing calcium oxalate assay, nucleation assay, growth assay and aggregation assay for lithiasis.

The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The O.D. was monitored at 620 nm after 30 minutes. The turbidity of solution in the presence of herb extract was lower in comparison to the control, showing that oxalate crystallization was less in the presence of extract (Fig. 1 and 3) showed the percentage inhibition of the crystallization of calcium oxalate (CaOx) with plant extract concentrations, Thus the absorbance increases with increased dissolution of CaOx crystals. Plant extracts loaded chitosan nanoparticle were inhibiting the growth of calcium oxalate crystals, at various concentrations the results of the *in vitro* assays

performed clearly indicate that *T.procumbens* extracts could readily prevent crystal nucleation, growth and aggregation. The maximum effect was mediated by *T.procumbens* extract can be given to reduce the stone formation in individuals. An extract of *Tribulus terrestris* promote the nucleation of calcium oxalate crystals, increasing their number but decreasing their size. It also promotes the formation of octahedral crystals, despite the presence of COM crystals. Plant extracts loaded chitosan nanoparticle was inhibiting

the growth of calcium oxalate crystals, at various concentrations. *A. lanata* extracts (1600 µg/ml) shows high inhibitory percentage compared to *Tridax procumbens* extracts [16], discovered the *Phyllanthus niruri* extract did not inhibit calcium oxalate nucleation, but inhibit crystal growth [17] have suggested that studies using certain plant extract may contain substances that inhibit the growth of COM crystals.

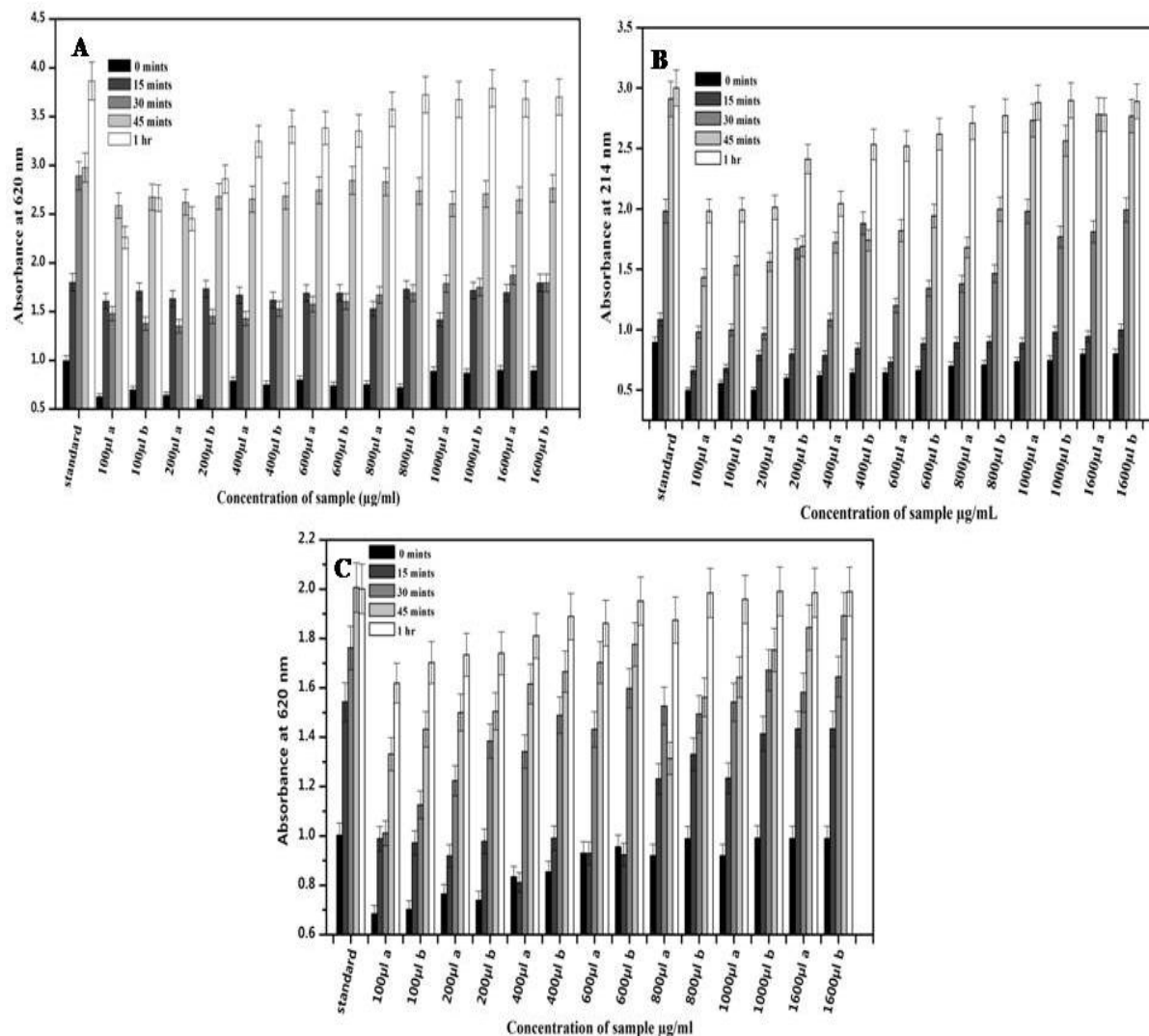


Fig.1: Effect of plant extract and loaded Nanoparticle on A) Nucleation B) Growth C) Aggregation of CaOx Encapsulation

Determination of Encapsulation Efficiency (EE) and Loading Efficiency (LE). The encapsulation efficiency, defined as the percentage of plant extract encapsulated in the suspended Chitosan nanoparticle, was estimated as below. After the centrifugation mentioned in the nanoparticle preparation sections, both the precipitate was collected.

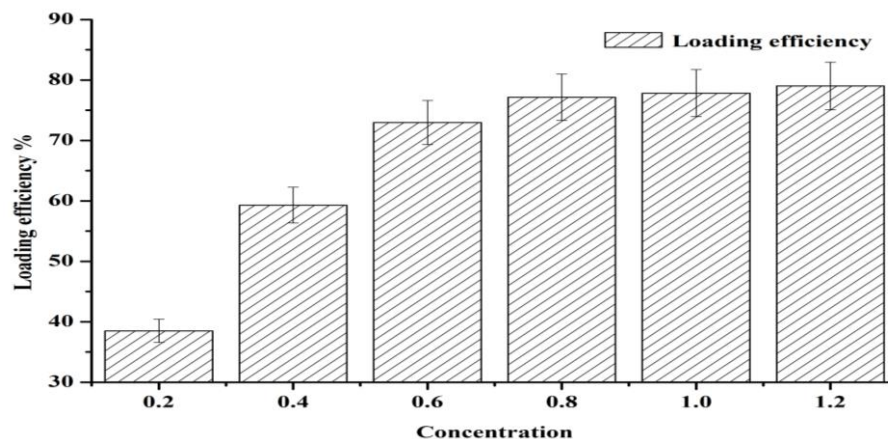


Fig.2: Shows Percentage loading efficiency of various concentration of plant extract with Chitosan Nanoparticle

The suspension was centrifuged at 1000g and 20 °C for 10 min to remove plant extract aggregates. The resultant supernatant was subjected to UV-vis spectrophotometric analysis at 350 nm with a UV/vis spectrophotometer. The absorbance was converted to Scanning electron microscope analysis

plant extract concentration based on the established standard curve ($R^2 = 0.991$). The EE of the samples were calculated with the following equation: After the aforementioned centrifugation, the nonpermeable part of the dispersion was collected and lyophilized.

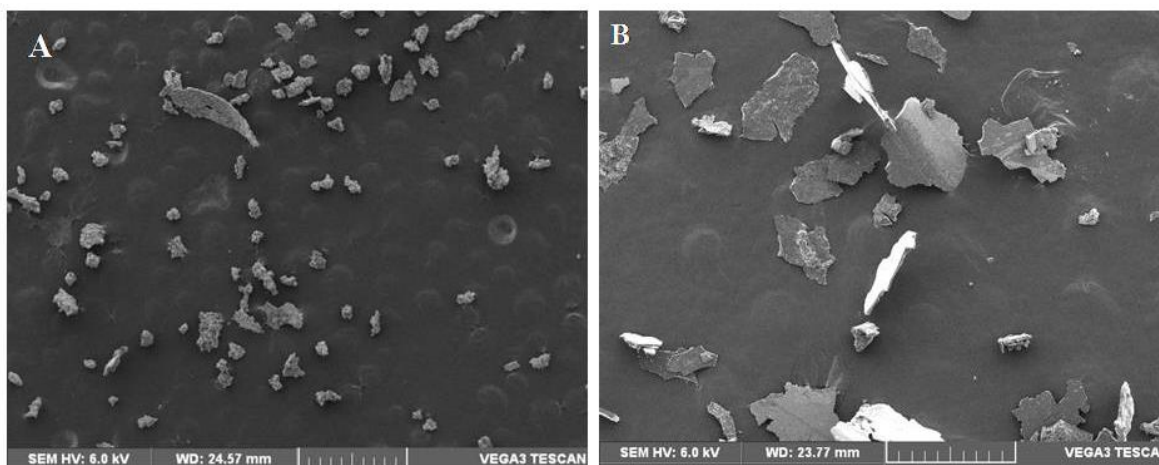


Fig.3: SEM images (A) plant extract loaded Nanoparticle (B) Chitosan nanoparticle

Scanning electron microscope (SEM) image shows the morphologies of the *Aerva lanata* plant extract loaded chitosan nanoparticle at different magnifications (Figure 5) The image shows particles are homogenous and mostly spherical in shape, further the particles are around 500 nm in size. SEM analysis of Nanoparticles found to be spherical, rod and triangle. Uniformly distributed poly dispersed plant extracts synthesized is correlated with the

results of chitosan nanoparticles synthesized using Uniformly distributed synthesized chitosan nanoparticles were observed which is obtained by using plant extracts Most of the particles are spherical in shape and monodispersed in nature with size ranging from 50-80 nm. The triangular and twinned nanoparticles relatively are in large size. Similarly, monodispersed chitosan nanoparticle was reported by using the plant extract [18].

Fourier transforms infrared spectroscopy

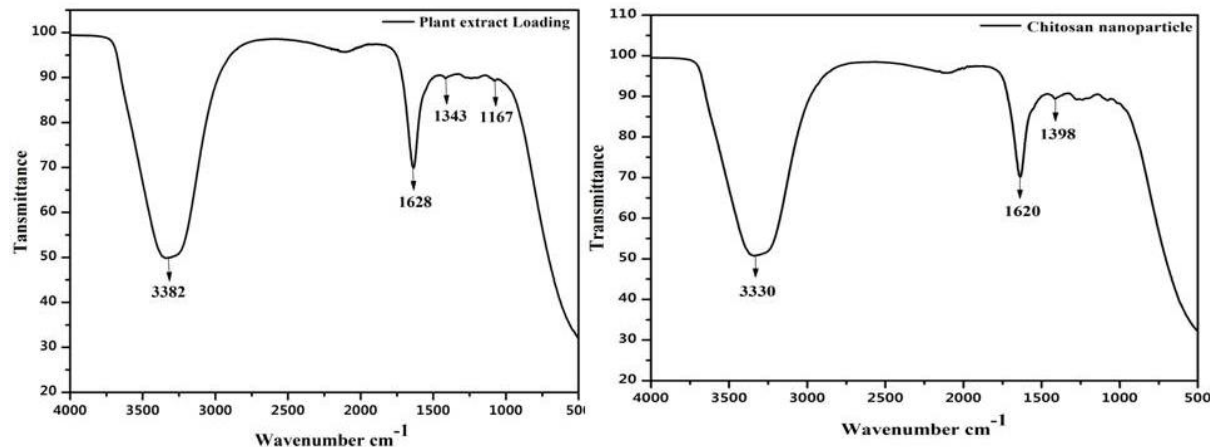


Fig.4: FTIR-spectra of (A) plant extract loaded Nanoparticle (B) Chitosan nanoparticle

The plant extract was observed at 3382 due to the N-H Stretch, 1^o, 2^o, amines, amides in *Tridax procumbens* plant extract the band at 1628 C-Symmetric stretch nitro compound the narrow band was formed at 1040-1038 C-N Stretch aliphatic amines. chitosan Nanoparticle was the broad band was observed between 3349-3219 due to the O-H Stretching and H-bonded alcohols and phenol groups, the band at 1620-1625 indicates presence of C=C Stretch alkenes groups, 1248 shows the C-N Stretch aliphatic amines, a contracted band at 1398 indicates C-O Stretch alcohols, carboxylic acids, esters, ethers. In nanoparticles the peaks for N-H bending vibration of amine I at 1600 cm⁻¹ and the amide II carbonyl stretch at 1650cm⁻¹ shifted to 1630 cm⁻¹, respectively These results have been attributed to the linkage between phosphoric and ammonium ion. The involvement of possible functional groups of chitosan nanoparticle synthesis was loaded plant extract, peak position and corresponding functional groups present in plant extract loaded chitosan nanoparticle synthesized *Tridax procumbens* using Peak position and corresponding functional groups present in chitosan nanoparticle synthesized using *Tridax procumbens*. So we conclude that the tripolyphosphoric groups of TPP are linked with ammonium groups of chitosan. The inter- and intra-molecular actions are enhanced in chitosan nanoparticles.

CONCLUSION:

Based on all experimental results, it can be concluded that Plant extract loaded with chitosan nanoparticle could be suitable for drug delivery of urolithiasis with controlled release properties, making them new potential carriers for local treatment of lithiasis, The preparation procedure used in this study, two-step

process, offers simplicity, reproducibility and stability of the prepared formulations Results from the physicochemical and biopharmaceutical characterization of the prepared particles are in favor of their localization and prolonged residence time, which can reduce the systemic toxic effects and improve the therapeutic efficacy of the drug.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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