
Biosynthesis, Characterization and Application of Chitin Nanoparticle with *Cassia auriculata*

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Abstract: Prawns are a common food for humans all around the World. Particularly the prawn exists naturally in south East Asia. Prawn shells contain ash, fibre, minerals and proteins. Prawns like a Shrimps. It's a Dendrobranchiata family. Prawn shells were collected from Chindaripet market. Deproteinization followed by demineralisation was carried out to obtain the final product chitin. *Cassia auriculata* is a legume tree in the subfamily Caesalpinioideae. It contains antioxidants, cardiac Glycosides, anthraquinones, tannins, carbohydrates, alkaloids, phenols, saponin and flavonoids. *Cassia auriculata* flower has anti- bacterial, anti- diabetic property, the flower taste is stringent, pungent and bitter. *Cassia auriculata* flowers were dried under room temperature for 2 to 3 weeks flowers and grounded with mechanical blender. Then chitin and silver nanoparticles were prepared along with *Cassia auriculata* flower extract which was extracted with both ethanol and water. Scanning Electron Microscope (SEM) was done by chitin nanoparticles to identify the particle size and morphology. Fourier Transform Infrared Spectroscopy (FTIR) was done to identify the active compounds. Chitin nanoparticles were used widely in various applications that evaluates anti-microbial activity using *E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus mutans*, *Enterococcus* spp, then anti-inflammatory activity gives better inhibition, anti-larvae activity using mosquito larvae. *Cassia auriculata* (fresh, ethanol) nanoparticles killed all larvae, anti-coagulant and thrombolytic activity were observed.

Keywords: Prawn Shell, *Cassia Auriculata*, Chitin, Nano Particles, SEM, FTIR

1. Introduction

Prawns are similar to shrimp but it's different in the gill structure. Prawn shells contain chitin (C₈H₁₃O₅N)_n which is a long chain polymer [1, 11]. Chitin is made up of glucose monosaccharides. *Cassia auriculata* is commonly called Matura tea tree, Ranawara, Avaram which is grown in black soils on stony hillsand along the road side [2-5]. It's a perennial shrub. The height of the tree is from 30 to 60 cm. The flower is yellow in colour and leaves yellowish green in colour. The taste of *Cassia auriculata* flower is bitter, astringent, and pungent [7-10]. This plant has different medicinal properties. *Cassia auriculata* contains anti-oxidants and cardiac glycosides. This plant, flower powder were traditionally used in the treatment of diabetics. It's also used for urinary problems, kidney disorders and skin problem [12-16].

2. Materials and Methods

2.1. Collection and Extraction of Samples

The prawn shells were collected from Chindaripet fish market, Chennai. Shells were washed thoroughly and dried under the sunlight and stored in an air tight container. The fresh flower *Cassia auriculata* were collected from Parry's Corner, Chennai. It was dried at room temperature for 2 to 3 weeks. Then the flower was powdered and stored in an air tight container. The fresh flowers were collected and dried under room temperature. Then it was grounded by using the mixer. Ethanol and water were used for extraction (both fresh and dry).

2.1.1. Extraction of Chitin

The prawn shells were collected and washed thoroughly with distilled water. The shells were dried under the sun light

for 1 week. Prawn shells were soaked in 4% of boiling sodium hydroxide solution for 1 hour. This process is called Deproteinization. The samples were removed and then allowed to cool at room temperature for 30 minutes and then washed with distilled water for 3 to 4 times until it reaches the neutral pH. It was then crushed into small pieces around 0.5 – 5mm. Again the sample was soaked in 1% hydrochloric acid. This process is called Demineralization.

2.1.2. Preparation of Nanoparticles

Prawn shells were collected and it was cleaned properly with distilled water. Prawn shells were treated with sodium hydroxide and hydrochloric acid to prepare chitin. 0.05g of silver nitrate and 0.25% of glucose were added to 10 ml of distilled water. It was autoclaved at 121°C for 20 minutes. It was cooled at room temperature and centrifuged at 3000 rpm for 20 minutes. The supernatant was stored at 4°C for overnight. This solution was taken and thawed at room temperature. 1ml of plant extract and chitins at various concentrations (10mg, 20mg, 30mg, and 40mg) were added to 1ml of supernatant. This mixture was centrifuged at 6000 rpm for 10 minutes. Then it was placed in water bath for 2 hours. Nanoparticles were obtained. [17]

2.2. Scanning Electron Microscopy (SEM), UV Spectroscopy and FTIR of Nanoparticle

Scanning electron microscope analysis was done by using S-3400 SEM machine. Thin film of sample were prepared on the grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry putting it under the mercury lamp for 5 minutes. Finally SEM analysis was determined to identify the size, shape, and the morphology of the shape. To prepared a liquid sample for IR analysis, a drop of liquid was placed on the face of a highly polished salt plate such as (NaCl, AgCl, or KBr), then placed a second plate on top of the first plate so as to spread the liquid in the thin layer between the plates, and clamps the plates together. The sandwiched plate was mounted on to the sample holder. Absorption of light in the ultra violet region (400-800nm) was checked

2.3. Phytochemical Analysis

The different qualitative chemical tests were performed for establishing profile of given extracts for it is chemical composition. The extracts were subjected to different tests to detect various phytoconstituents present in them. [8]

2.4. Anti- Microbial Activity

The agar well diffusion method was used to enumerate the antimicrobial activity of the test organisms by measuring the zone of inhibition. Different types of Fungi and Bacteria such as *E. coli*, *Klebsiella* spp, *Salmonella* spp and *Aspergillus* spp were used. [2]

2.5. Thrombolytic Activity

Whole blood was drawn from healthy human volunteers without a history of contraceptives or anticoagulant therapy. 1 ml of blood was transferred to the sterile Eppendorf tubes and was allowed to form clots. 1g of clotted blood was taken and added 1 ml nanoparticle samples. Then tubes incubated at 45 minutes. The Eppendorf tubes were incubated at 37°C for 45 minutes. The nanoparticles were added to the clot to check the time to break the clot. [9]

2.6. Anti-Coagulant Activity

The sample of blood was obtained from the healthy volunteers, using a disposable polypropylene syringe, and then anti – coagulated using 3.8% tri – sodium citrate in a polypropylene container. Blood sample were centrifuged at 3000rpm for 15 minutes to separate blood cell and plasma. The plasma was separated and used for determination of pro thrombin time test. The freshly prepared plasma was stored at 4°C.

GROUP 1: Negative control group 0.2 ml plasma+ 0.1 ml 0.9% saline + 0.3 ml of 25 ml calcium chloride.

GROUP 2: Positive control group 0.2 ml plasma + 0.1 ml 50mg/ml EDTA + 0.3 ml calcium chloride (0.5 g/ml)

SAMPLE 1: 0.2 ml plasma + 200 µl *Adhatoda vasica* (dry) ethanol + 0.3 ml calcium chloride

SAMPLE 2: 0.2 ml plasma + 200 µl *Adhatoda vasica* (fresh) ethanol + 0.3 ml calcium chloride

SAMPLE 3: 0.2 ml plasma + 200 µl *Adhatoda vasica* (dry) water + 0.3 ml calcium chloride

SAMPLE 4: 0.2 ml plasma + 200 µl *Adhatoda vasica* (fresh) water + 0.3 ml calcium chloride

SAMPLE 5: 0.2 ml plasma + 200 µl *Cassia auriculata* (dry) ethanol + 0.3 ml calcium chloride

SAMPLE 6: 0.2 ml plasma +200 µl *Cassia auriculata* (fresh) ethanol + 0.3 ml calcium chloride

SAMPLE 7: 0.2 ml plasma + 200 µl *Cassia auriculata* (dry) water + 0.3 ml calcium chloride

SAMPLE8: 0.2 ml plasma + 200 µl *Cassia auriculata* (fresh) water + 0.3 ml calcium chloride

Incubated at room temperature for 15 minutes. All the tubes were fitted at an angle of 45°C for every 30 seconds to measure the clotting time. Stop watching was used for measuring the clot formation.

2.7. Anti - Larvae Activity

Sample was taken in various concentration like 500µl and 1000µl in a microtitre plate. Larvae are poured into the samples and activity was watched continuously in every 5 minutes interval. The time when the larvae are killed noted.

2.8. Anti -Inflammatory Activity

1ml of extract in buffer +1ml of RBC suspension was taken and mixed gently. This was divided into 2 sets. One set was incubate at 54°C for 20 minutes another one set was incubated at 10°C for 20 minutes. Samples were centrifuged

at 3000 rpm/3mins at hemoglobin content in the supernatant was measured in spectrophotometer at 540nm. Percentage inhibition of hemoglobin by the extract was calculated

$$1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \times 100$$

OD₁-absorbance of test sample unheated
 OD₂- absorbance of test sample heated
 OD₃- absorbance of control sample heated

A tablet of disprin was dissolved in 100ml of buffer and 1 ml of this solution with 1 ml of RBC suspension was used as control.

3. Result

3.1. Extraction of Flowers and Chitin

Cassia auriculata flower was extracted using water and ethanol, meanwhile chitin extracted from prawn shell.

3.1.1. Phytochemical Analysis

Dried and fresh flowers were extracted both water and ethanol. This extraction subjected to phytochemical analysis for screening the active compounds.

Table 1. Phytochemical analysis.

S. No	Tests	Cassia auriculata (Dry flower) Ethanol	Cassia auriculata (Dry flower) Water	Cassia auriculata (Fresh flower) Ethanol	Cassia auriculata (fresh flower) water
1	Alkaloids	Positive	Positive	Positive	Positive
2	Phenols	Positive	Positive	Positive	Positive
3	Glycosides	Positive	Negative	Negative	Negative
4	Flavonoids	Positive	Positive	Positive	Positive
5	Tannins	Positive	Positive	Positive	Negative
6	Saponin	Positive	Positive	Positive	Positive
7	Protein	Positive	Positive	Negative	Positive
8	Terpenoids	Negative	Positive	Positive	Positive
9	Sterols	Negative	Negative	Positive	Negative
10	Aminoacid	Negative	Negative	Negative	Positive

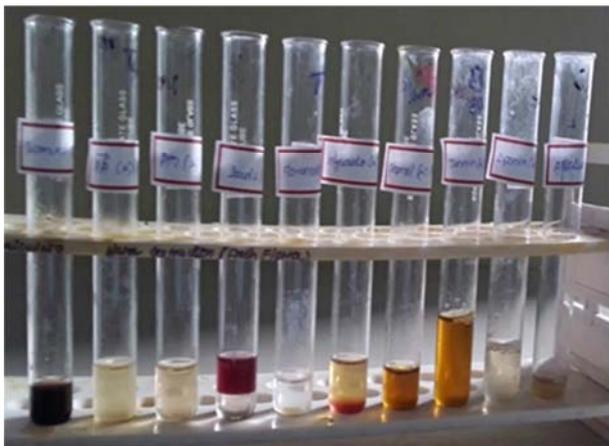


Figure 1. CA (D) Ethanol extraction.



Figure 3. CA(D)Water extraction.

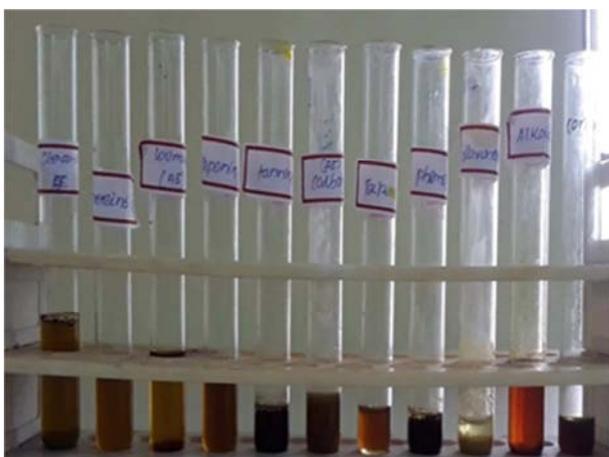


Figure 2. CA (F) Water extraction.



Figure 4. CA (F) Ethanol extraction.

3.1.2. Nanoparticle Analysis Using SEM and FTIR

Nanoparticles are screened to Scanning electron microscope for identify the morphology and size of the nanoparticles. Then Fourier Transform Infrared Spectroscopy was performed to identify the active group compounds.

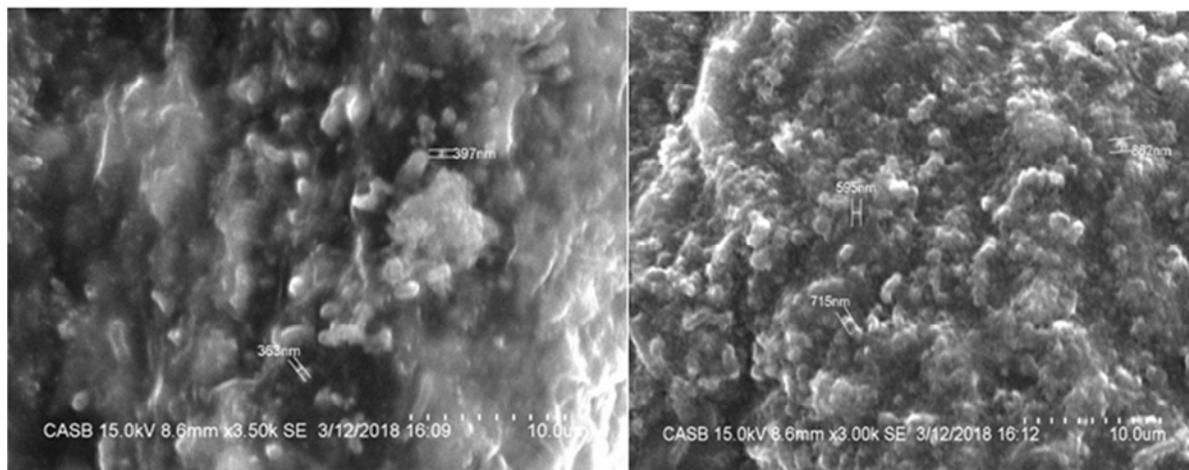


Figure 5. SEM image of *Cassia auriculata* (Fresh) Water Nanoparticles.

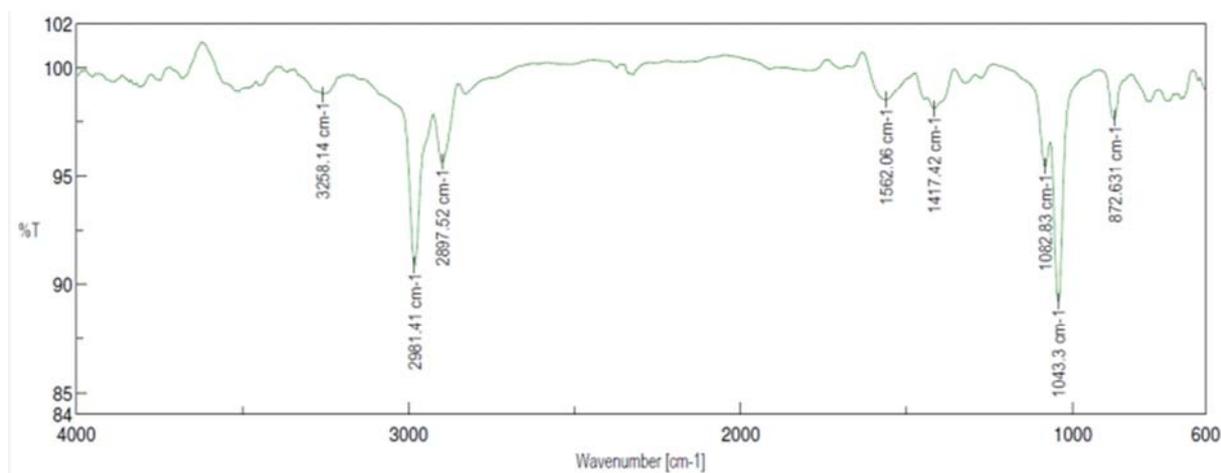


Figure 6. *Cassia auriculata* (Fresh) water Nanoparticles.(FTIR)

Table 2. FTIR Analysis

S.NO	BAND SPECTRA	BONDS
1	872.63	C-H
2	1043.3	CO group
3	1082.83	C-O
4	1417.42	O-H
5	1562.06	N-O
6	2897.52	C-H
7	2881.41	C-H
8	3281.14	C-H

3.2. Anti-Microbial Activity

Antimicrobial activity performed both normal extraction and nanoparticles. In this study used to identify which organisms are effective in this extraction.

Table 3. Antimicrobial Activity.

S. No	Organisms	CA(D) Ethanol (mm)	CA(D) ater (mm)	CA(D) Ethanol Nanoparticle (mm)	CA(D) Water Nanoparticle (mm)
1	S.mutans	8	11	12	13
2	Klebsiella pneumonia	12	15	18	20
3	Enterococcus spp	5	12	10	9
4	S.aureus	17	12	6	12
5	E.coli	9	17	8	11
6	S.typhi	15	13	9	10

3.3. Thrombolytic and Anticoagulant Activity

Cassia auriculata (fresh flower ethanol) nanoparticles used to perform Thrombolytic activity and also Anticoagulant activity

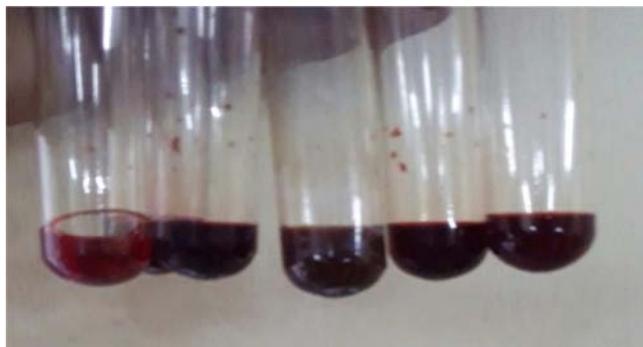


Figure 7. Thrombolytic activity.



Figure 8. Anti-coagulant activity.

3.4. Anti - Larval Activity

Anti-larvae activity test was performed by using mosquito larvae. In this nanoparticle gives good result for anti-larvae activity.

Table 4. Anti-larval activity.

S. No	Conc.	Control	Nano Particles	Time required To kill larvae (minutes)
1	500µl	CA(F) Water	CA(F) Water	35 mins
2	1000µl	CA(F) Water	CA(F) Water	25 mins
3	500µl	CA(D) Ethanol	CA(F) Ethanol	10 mins
4	1000µl	CA(D) Ethanol	CA(F) Ethanol	13 mins

Note: CA- Cassia auriculata F – Fresh, D-Dry

3.5. Anti-Inflammatory Activity

The anti-inflammatory activity of Cassia auriculata(Fresh Ethanol) nanoparticles had good inhibition percentage.

Table 5. Anti-inflammatory activity.

S. NO	Treatment	Concentration	Absorbance (560nm)		Inhibition in %
	Control		Heated	Unheated	
	Sample	µl			
1	CA(F) Ethanol	2	0.072	0.048	91
2	CA(F) Ethanol	4	0.073	0.040	92.5
3	CA(F) Ethanol	8	0.052	0.037	94.6
4	CA(F) Ethanol	16	0.072	0.045	90

Note :CA- Cassia auriculata F - Fresh

4. Discussion

4.1. Extraction of Flowers and Chitin

50g of fresh flower was taken in a mortar and pestle and grounded with 250 ml of ethanol (water) Whatman No. 1 filter paper and it was stored [1]. The same procedure were followed for dried flowers [7]. The deproteinised and demineralised samples were incubated for 24 hours and then washed with distilled water until it reaches the neutral pH. Samples were treated with 50ml of 2% sodium chloride for one hour. It was again washed with distilled water for 2 to 3 times and finally dried at room temperature for 1 week [11].

4.1.1. Phytochemical Analysis

The preliminary phytochemical screening of the ethanol and water extract of Cassia auriculata revealed the presence of compounds like Alkaloids, Glycosides, Flavonoids, Tannins, Anthroquinones, Protein, Phenols and Carbohydrates [14, 15]. The result Cassia auriculata showed that presence of bioactive compounds. Phenol, tannin was obtained in Cassia auriculata (dry) both water and ethanol extraction. Phenol, tannin was present in Cassia auriculata (dry) ethanol extraction. Amino acid was absent in both water and ethanol extraction but it was positive result in Cassia auriculata (f) water extraction. However reports absence of amino acid both water and ethanol extraction [12].

4.1.2. Nanoparticle Analysis Using SEM and FTIR

Scanning Electron Microscope (SEM)

In this present study, SEM was performed to study the morphology of the synthesized nanoparticles. The shapes of the nanoparticles were reported to be spherical. Nanoparticle size was reported to be 363nm and 397nm. In *Cassia auriculata* fresh flower, silver nanoparticles showed 876nm size[8]. *Cassia auriculata* (water) fresh extraction nanoparticle size was 363nm and 397nm which showed better result.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR was performed to study the active group compounds of nanoparticles (Table 2). The water extract of *C. auriculata* (fresh) showed characteristic absorption bands at 1043.3 cm⁻¹ and 1082.83(C-O) for a hydroxyl (-OH) group 1417.42 cm⁻¹. 872.63 cm⁻¹, 2897.52 cm⁻¹, 2881.41 cm⁻¹ and 3281.14 cm⁻¹ for (C-H) stretch. and at 1562.06 cm⁻¹ for (N-O) group[13].

4.2. Anti-Microbial Activity

Anti-microbial activity was done using different organisms. *Cassia auriculata* (ethanol) fresh nanoparticles showed maximum zone of inhibition in *E. coli* (20mm) and low inhibition for water extraction 5mm. However low zone of inhibition in *E. coli* for *C. auriculata* water extraction. *Klebsiella pneumonia* was maximum zone of inhibition (20mm, 18mm) in both ethanol and water extract of *C. auriculata* dry flower nanoparticles. In this study methanol extract was low inhibition in *C. auriculata* dry flower [12, 16].

4.3. Thrombolytic and Anticoagulant Activity

Thrombolytic activity test was done by using Nanoparticles. This study proved that nanoparticles had good thrombolytic activity. *Cassia auriculata* (fresh flower) extracted with water had effective thrombolytic activity. Thrombolytic activity test was done by nanoparticles. It was allowed to break the blood clot within 45 minutes blood clot was broken. In this study revealed that similar method was followed by thrombolytic activity [9].

Anti-coagulant activity tests were also done *Cassia auriculata* nanoparticles showed good anti-coagulant activity. *C. auriculata* (ethanol extract) fresh nanoparticles showed better result similar results have reported.

4.4. Anti-larval Activity

Anti-larvae activity was done by using the mosquito larvae. *Cassia auriculata* (Ethanol) nanoparticles killed larvae within 10 minutes. This study shows good anti-larvae activity.

4.5. Anti-Inflammatory Activity

The result shows that the anti-inflammatory activity of *Cassia auriculata* nanoparticles had good inhibition percentage. *Cassia auriculata* (F) ethanol had above 90% inhibition. The result was showed anti-inflammatory activity of nanoparticles though low inhibition for *C. auriculata* water extraction (crude Extract) has been reported[9].

5. Conclusions

The present study aims to synthesize chitin silver nanoparticles with *Cassia auriculata*. The Phytochemical analysis were carried out qualitatively and showed the presence of alkaloids, phenols, tannins, saponins, terpenoids, flavonoids, carbohydrates and sterols. The synthesized nanoparticles were tested for its anti-microbial, anti-coagulant, anti-larvae, anti-oxidant, anti-inflammatory and thrombolytic activity. The *Cassia auriculata*(F) water nanoparticles exhibited the maximum antimicrobial activity. This plant is easily available and thus contributes to future pharmaceutical industry to obtain the active compound present in the *Cassia auriculata* and to prepare chitin with silver nanoparticles which aids in targeted drug delivery of the active compound which can be produced in large scale and used to treat diseases.

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