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Green Synthesis of Chitosan-Curcumin Nanoparticle using Rhizome Extract of *Curcuma Longa* and Evaluation of their Antibacterial Activity

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ABSTRACT

To develop a novel approach for the biological synthesis of curcumin nanoparticles using water extract of rhizome in *Curcuma longa* which has been proven to be active against human pathogenic bacteria such as *Streptococcus pyogenes*, *Staphylococcus aereus*, *Escherichia coli* and *Klebsiella pneumonia*. Characterization of Chitosan- curcumin nanoparticles was determined by using Ultra Violet-Visible (UV-Vis) spectrometry, Scanning Electron Microscopy (SEM), FTIR respectively. The synthesis of Chitosan nanoparticles was confirmed by the change of colour pale green to reddish brown. Further, a peak between 200nm to 2500nm wavelength was obtained on UV-VIS spectrometer which confirmed the biosynthesis of silver nanoparticles. SEM showed the formation of Cucumin nanoparticles with an average size of 25.71 nm-37.96 nm. It concluded that present study of *Curcuma longa* can be good source for biological synthesis of Cucumin nanoparticles which shows a good antibacterial activity of human pathogenic bacteria. The outcome of the study will be the development of new anticancer drugs from the synthesized nanoparticle of the *Curcuma longa* for biomedical and other industrial applications.

KEYWORDS: *Curcuma longa*, Scanning electron microscopy**,** Chitosan Nanoparticles, santibacterial activity.

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INTRODUCTION

The field of nanotechnology is one of the most dynamic areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly[1]. Nano crystalline silver particles have found tremendous applications in the field of high sensitivity bimolecular detection and diagnostics [2], antimicrobials and therapeutics [3, 4], Catalysis [5] and micro-electronics [6]. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles.

An eco-biological approach to the synthesis of silver nanoparticles is an eco-friendly and cost effective method as compared to the other chemical and physical methods. Several antibiotics use silver compounds such as metallic silver, silver nitrate, silver sulfadizine for treatment of burns, wounds and several bacterial infections [7]. Since nanoparticles of noble metals such as silver, gold, and platinum are widely applied to human contacting areas, there is a growing need to develop environment friendly processes for the synthesis of nanoparticles, that do not use toxic chemicals [8]. Among the above forms, silver nanoparticles play a significant role in the field of biology and medicine [9, 10].

MATERIALS AND METHODS

Collection of Plant Material

The plant samples of *Curcuma longa* were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Biotechnology, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Preparation of the Extract

The whole plant of *Curcuma longa* was used to make an aqueous extract. The plant materials weighing 20g were thoroughly washed in distilled water and filtered through Whatmann No.1 filter paper (Pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filters.

Preparation of Curcumin Nanoparticles

Chitosans (Cs) is dissolve in 2% (w/v) acetic acid and Curcumin soluble in mixed solution of anhydrous ethanol and Tween-80 (1:1 v/v) and homogenized with the Chitosans solution and Curcumin up to fully dissolving. The mixture will be add drop wise into agitated Sodium Tripolyphosphate (TPP) solution (0.3% w/v) and the suspension will be stirred at room temperature

for 30 min. After preparation of Chitosan- Curcumin nanoparticle (CCN) complex stored at 4°C for further research. The blank CNs will be prepared similarly without adding Curcumin [11].

Encapsulation Efficiency (Ee) And Confirmation Test

To know the encapsulation efficiency Chitosans- Curcumin nanoparticle (CCN) will be analysed by using HPLC and fusion will be confirmed by using Ultra Violet (UV), Fourier Transform Infrared (FT-IR), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), X-ray diffraction (XRD).

CHARACTERIZATION OF CHITOSAN NANOPARTICLES

UV-VIS Spectra Analysis

The reduction of pure chitosan ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 10-12 hours. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV- 2450 (Shimadzu).

Scanning Electron Microscope

Scanning Electron Microscope (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper 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 films on the SEM grid were allowed to dry by putting (placing) them under a mercury lamp for 5 minutes.

FTIR Analysis of Dried Biomass after Bio Reduction

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

IN–VITRO Antibacterial Activity

In the present study antibacterial activity of sample was determined by Well Diffusion method as described by [12].To perform antimicrobial activity, various bacterial species were selected viz., Escherichia coli, *Staphylococcus aeureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus species* as bacterial cultures respectively.

Media and Culture Condition

Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani (LB) were used throughout the study for determining the antibacterial assay. The media was adjusted to the pH and autoclaved at 121°c for 15 minutes.

Preparation of the Bacterial Inoculum

Stock cultures were maintained at 4 degree C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50ml nutrient broth bacterial cultures were incubated with agitation for 24hours and at 37 degree C on shaking incubator. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar. Bacterial cultures then incubated at 37degree C for 24 hours. A single colony was transferred to nutrient agar media slants were incubated at 37°c for 24 hours and potato dextrose slant were incubated at 27 degree C for 3-5 days. These stock cultures were kept at 4degree C. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37 degree C for 18-20 hours for bacterial culture.

Well Diffusion Method

MHA plates were prepared by pouring 20ml of molten media into sterile petriplates. After solidification of media, 20-25µl suspension of bacterial inoculums was swabbed uniformly. The sterile paper discs were dipped into required solvents then placed in agar plates. Wells were put into the agar medium using sterile forceps. Then 10-40 µl of sample was poured into the wells and Chloramphenicol antibiotic was used as standard. After that, the plates were incubated at 37degree C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm.

Statistical Analysis

All analyses were carried out in triplicate and the data were reported as mean ± SD. Where there was significance of the difference between means was determined by Duncan's multiple range test ($P<0.05$) using ANOVA.

RESULTS AND DISCUSSION

Uv-Vis Spectra Analysis

In the present study, *Curcuma longa* extract were used as reducing agent for the Chitosan Nanoparticles. Curcumin Nanoparticles exhibit yellowish brown colour aqueous (Water extract)

solution due to excitation of surface Plasmon vibration in silver nanoparticles (Fig 1). The surface plasma absorption band occurred a maximum of 200-800 nm indicating the presence of spherical Ag nanoparticles. The size and shape of the nanoparticles was characterized by SEM. The synthesized nanoparticles of *Curcuma longa* compared to the standard silver oxide material and the result showed same colour. The present study clearly confirmed that the water extract *of Curcuma longa* exhibit the presence of silver nanoparticles.

Fig 1. Photographs showing colour changes after adding chitosan- curcumin before reaction (a) and after reaction time of 10-12 h.

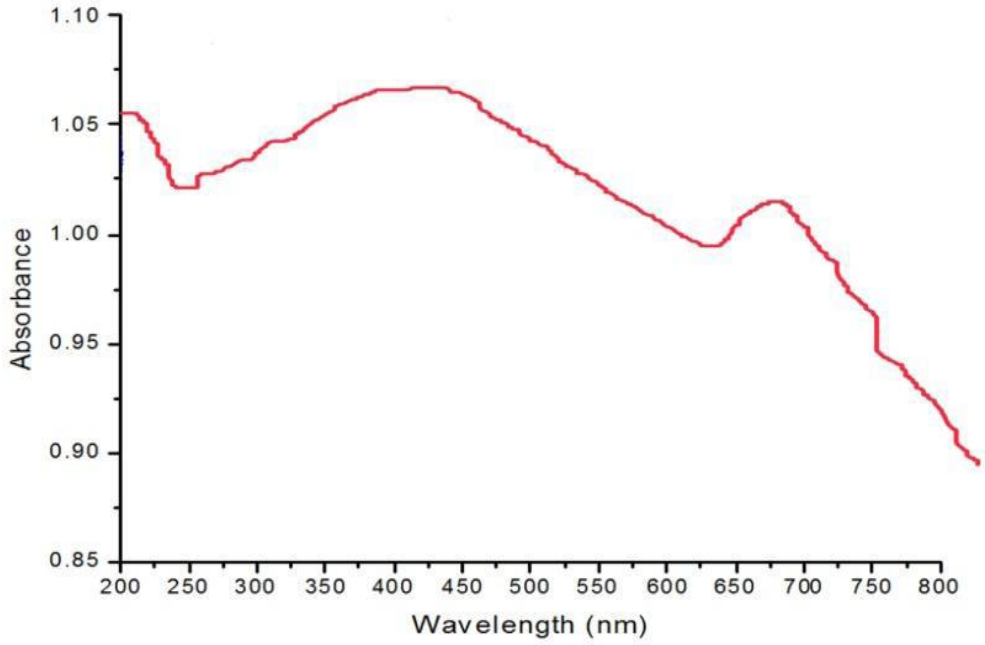

Fig 2. UV spectrometry.

Scanning Electron Microscope

SEM technique was employed to visualize the size and shape of silver nanoparticles. The synthesis of nanoparticles of *Curcuma longa* extracts SEM images were obtained in (Fig 3). The

SEM (JEOL-MODEL 6390) used SEM grids which were prepared by placing a small amount of sample powder on a copper coated grid and drying under lamp. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 21.57 nm-38.16 nm with interring particle distance. The shape of the silver nanoparticles proved to be spherical shaped.

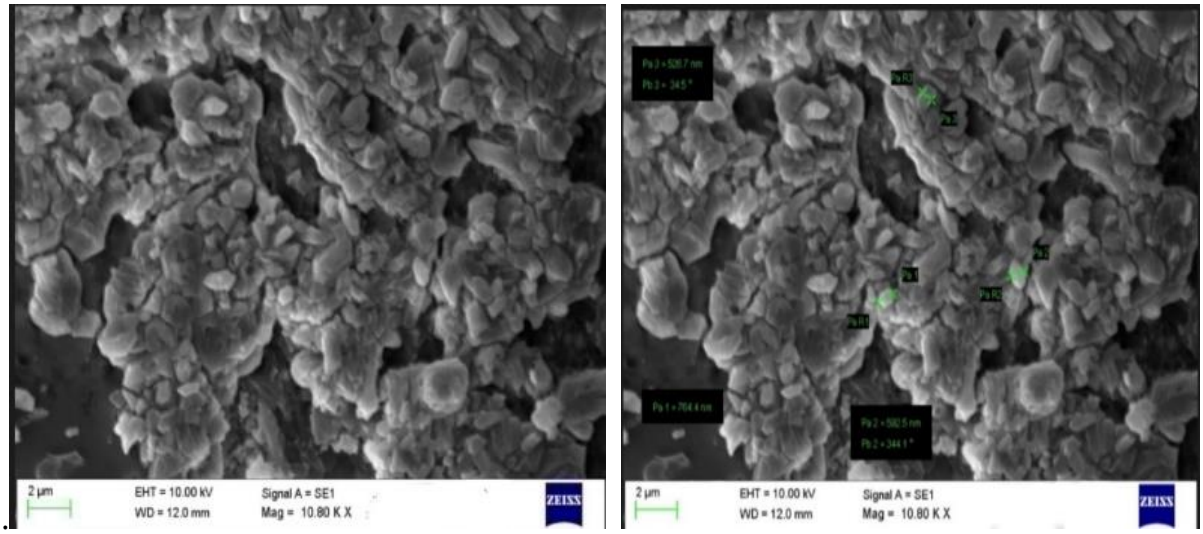

Fig 3. Scanning electron microscope image of *Curcuma longa*

FTIR

FTIR analysis was used for the characterization of the extract and the resulting Chitosan nanoparticles (Table 1; Fig 4). FTIR absorption spectra of water soluble extract before and after reduction of Ag ions are shown in Fig. 6. Absorbance bands in (before bio reduction) are observed in the region of 500–2000 cm-1 are 1697, 1618, 1514, 1332, 1226 cm-1. These absorbance bands are known to be associated with the stretching vibrations for –C C–C O, –C C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols), respectively17. In particular, the 1226 cm-1 band arises most probably from the C–O group of polyols such as hydroxyflavones and catechins.

Table: 1 FTIR analysis of *Curcuma longa*.

S.NO	Peak	Bond	Functional group
1	678.94	C=C	cis disubstituted alkene
2	999.13	C=C	Mono substituted alkene
3	1463.97	C=C	aromatic
4	1514.12	N-O	Nitro compound
5	2879.72	C-H	alkane
6	3606.89	O-H	alcohol
7	3726.47	N-H	amine
8	3869.2	N-H	amine

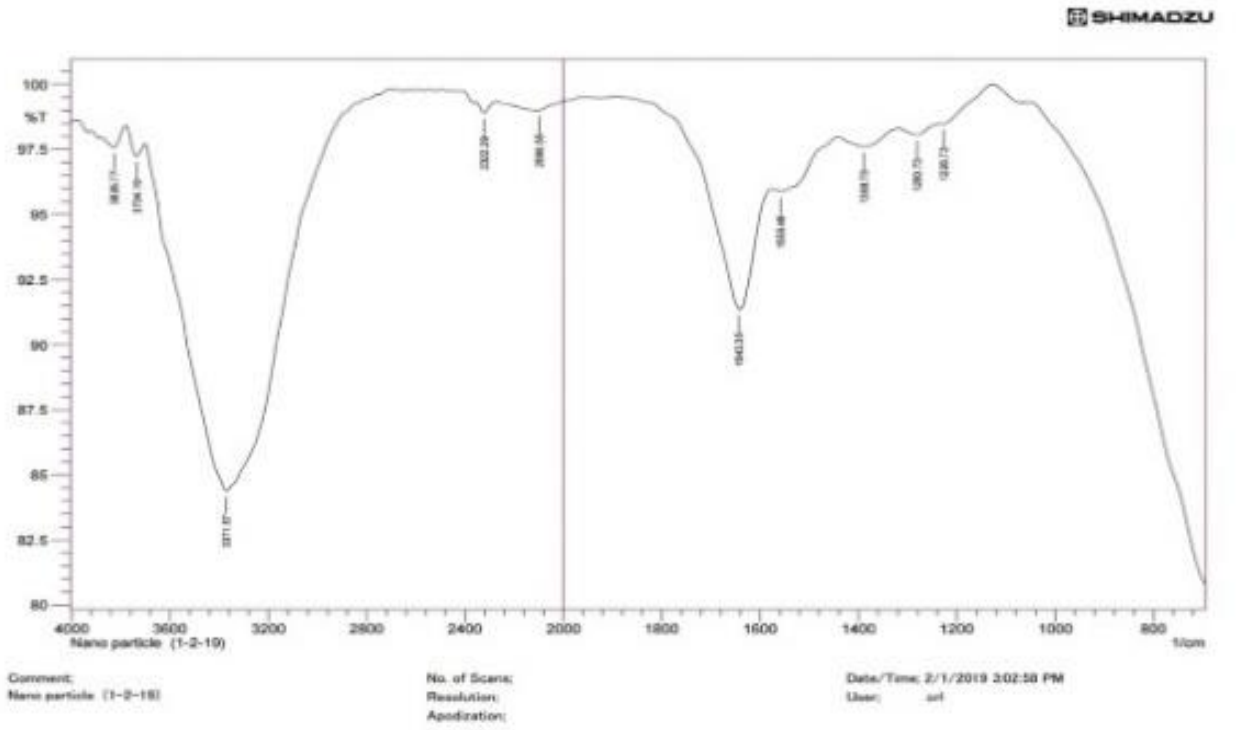

Fig 4. Fourier-transform infrared spectroscopy anbalysis of *Curcuma longa*.

IN-VITRO ANTI-BACTERIAL ACTIVITY

The zone of inhibition of bacterial organisms along with the sample and standard is shown in Table 2 and Fig 5. Sample in four concentrations (10µl, 20µl, 30µl, 40µl) has shown inhibition effect on growth of bacteria. *Proteus species and Pseudomonas aeruginosa* were inhibited more effectively by the sample than *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes,* and *Klebsiella pneumonia* microorganisms. Chloramphenicol antibiotic was used as standard for all tested plates. The mechanism of the bactericidal effect of silver and silver nanoparticles remains to be understood. Several studies propose that silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell [13]. It is also possible that silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the

bacteria. It may be observed that silver nanoparticles have comparatively higher anti-bacterial activity against gram negative organism than gram positive, probably due to thinner peptidoglycan layer and presence of porins. The attachment of either silver ions or nanoparticles to the cell wall caused accumulation of envelope protein precursors, which resulted in dissipation of the proton motive force. Silver nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP [14]. Similarly the silver nanoparticles were obtained from bryophytic plant species of *Anthocerous* showed the antibacterial activity against laboratory pathogens[15] and some bioactive components of *Moringa concanensis* Nimmo shows antibacterial activity against the *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeurginosa* and *Bacillus subtilis*, [16, 17].

Table: 2 *In vitro* antibacterial activity of Curcumin nano particles synthesized of *Curcuma longa*.

Bacteria	10µl	20µl	30µl	40µl	Standard
Escherichia coli	NIL	NIL	1.4±0.3	NIL	1.3±0.1
Pseudomonas aeruginosa	2.4±0.11	2.0±0.1	2.2±1.6	2.1±1.0	3.0±0.2
Staphylococcus aureus	1.6±0.5	1.1±0.2	1.4±0.5	1.2±0.4	2.5±0.4
Streptococcus pyogenes	NIL	NIL	NIL	NIL	1.1±0.2
klebsiella pneumonia	1.0±0.3	1.4±0.4	1.3±0.2	1.1±0.3	1.5±0.6
Proteus species	2.3±0.8	2.6±0.1	3.0±0.2	2.7±0.7	2.5±0.3

Each value represents the mean ± SD

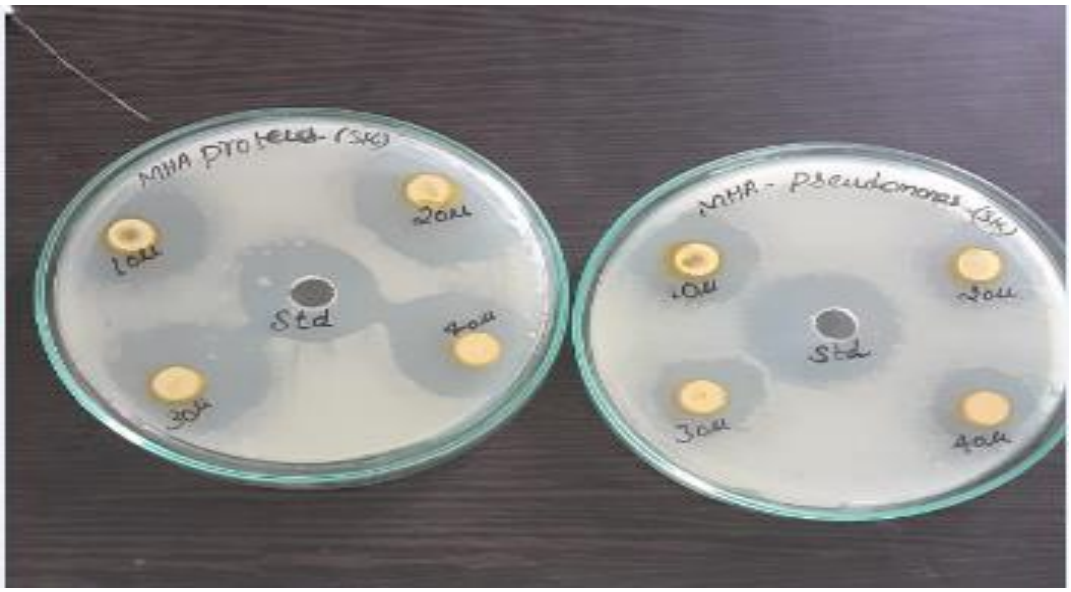

Fig 5. Images showing antibacterial activity of synthesized curcumin nanaoparticles againsst human pathogenic organisms

CONCLUSION

Here, reported for the synthesis of Chitosan *Curcumin* nanoparticles, reducing the curcumin ions present in the solution of Curumin nanoparticles by using the cell free aqueous extract of *Curcuma longa*. We have described a simple environmentally benign method of synthesis of *Curcumin* nanoparticles from a novel primitive plant source. The *curcumin* nanoparticles can be used in various fields of application such as medicine, agriculture, foods, paint industry, pharmaceutical industry. Future therapeutic directions of *Curcumin* nanoparticles could be anticancer, anti-inflammatory agent, antiviral drug, and anti-platelet agent.

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