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Green Synthesis and Characterization of Silver Nanoparticles using *Curcuma amada* and Evaluation of their Antimicrobial Activity

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Abstract: *Developing an environment friendly process for the synthesis of nanoparticle is a significant step in the field of nanotechnology. The present study reports an ecofriendly novel method to synthesize silver nanoparticle using rhizome extract of Curcuma amada. The silver nanoparticle formation was observed by the color change of plant extract from light yellow to dark brown. The formation of silver nanoparticles was confirmed by the presence of an absorption peak at 425 nm using UV-visible spectrophotometry. The biosynthesis was further characterized by Scannig electron microscopy (SEM)and X-ray diffraction analysis (XRD).The morphology and size of the silver nanoparticles was monitored by SEM. Crystal structure was obtained by carrying out XRD. Green synthesized silver nanoparticle showed excellent antimicrobial activity and is well demonstrated by considerable zone of inhibition against both gram negative and gram positive bacteria and fungi.*

Key words: *Silver nanoparticle, Curcuma amada, SEM, XRD, Anti bacterial.*

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I. Introduction

Nanotechnology is emerging as rapidly growing field with application in science and technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht, M.A *et al.,* 2009*).* Nanotechnology is a field that is burgeoning day by day making an impact in all spheres of human life (Validyanathan*et al*., 2007). Among the all noble metal nanoparticles, silver nanoparticles are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal activities (Annadurai*et al.*,2003).

Silver is a nontoxic, safe inorganic antibacterial agent that is capable of killing about 650 types of diseases causing microorganisms (S.H.Jeong, S.Y.Yeo, &S.C.Yi, 2005). There is an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display (O.Choi*et al.,* 2008). They are even being projected as future generation antimicrobial agents (M.Rai, A.Yadav, &A.Gade, 2009). Silver nanoparticles are important materials that have been studied extensively, such nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (P.K.Jain*et al.,* 2008 and L.S.Nair and C.T.Laurencin, 2007).

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, ecofriendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The plants or plants extract, which act as reducing and capping agents for nanoparticles synthesis, are more advantageous over other biological processes (Valli&Vaseeharan, 2012), because they eliminate the elaborated process of culturing and maintaining of the cell, and can also be scaled up for large-scale nanoparticle synthesis. Moreover, plant-mediated nanoparticles synthesis is preferred because it is cost-effective, environmentally friendly, a single-step method for biosynthesis process and safe for human therapeutic use (Kumar &Yadav, 2009).

Medicinal plants play a major role in the discovery of new therapeutic agents for drug development. Mango ginger (*Curcuma amada*Roxb.) is a unique perennial herb and its rhizomes have a morphological resemblance to ginger (*Zingiber officinale*) but impart a mango (*Mangifera indica*) flavour. The main use of mango ginger is in the manufacture of pickles, as a source of raw mango flavour for foods and for therapeutic purposes (R S Policegoudra *et al.*, 2010). *C. amada* has pharmacological significance for a variety of ailments for example effective in skin allergies, effects on blood cholesterol and possess antioxidant properties as well as

antibacterial activity. Of the 130 bioactive compounds of *Curcuma, C. amada* rhizomes reported to have 121 bioactive compounds including curcuminoids (Shakeel Ahmad Jatoi*et al.,* 2010).

The present study aims to synthesize silver nanoparticles by a green biological route, using *Curcuma amada* and characterization of the synthesized nanoparticles utilizing UV–visible spectroscopy, scanning electron microscope (SEM), X-ray diffraction (XRD). Besides, their antimicrobial activity against representatives of human pathogenic microorganisms was investigated.

II. Materials and Methods

2.1 Collection of plant material

The Rhizome of *Curcuma amada* was collected from local area, mavoor (Malappuram ,Kerala).They were shade dried after removing the soil dust particle. Then the dried rhizomes were powdered and used for the study.

2.2 Preperation of Plant Extract

10g of rhizome powder was weighed and mixed with 100ml of water. The extraction was carried out in a shaker for 48 hours. The solution was filtered through whatman no:1 filter paper and the extract was used for the synthesis of silver nanoparticle.

2.3 Silver nanoparticle Synthesis

1mMSilvernitrate solution was prepared and used for the synthesis of silver nanoparticles.10 ml of the Rhizome extract was added into 90 ml of aqueous solution of 1mM silver nitrate for the reduction into silver ions. Immediately after the addition of silver nitrate the color change of the rhizome extract from pale yellow to dark brown was noted periodically. Then the extract was incubated at room temperature for further incubation till 48 hrs. The color change to brown indicated the silver nanoparticles were synthesized from rhizome extract and centrifuged at 10000 rpm for 25 minute and the pellet used for SEM analysis, XRD analysis and antimicrobial activity.

2.4 UV-Visible Spectroscopy

The Ag nanoparticles were characterized in a Thermo scientific UV-VIS Spectrophotometer. The scanning range for the sample was 300-500 nm. 1mM silver nitrate was served as a blank reference.

2.5 SEM Analysis

Scanning Electron Microscope (SEM) analysis was done using JEOL model JSM-6390LV SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. (Geethalakshmi and Sarada*et al.,* 2010). The sample for SEM analysis was send to STIC, Cochin.

2.6 XRD Analysis

The phase variety and grain size of synthesized Silver nanoparticles was determined by X-ray diffraction spectroscopy (Bruker AXS D8 Advance). The synthesized silver nanoparticles were studied with CUKα radiation at voltage of 30 kV and current of 20 MA with scan rate of 0.030/s. Different phases present in the synthesized sampes were determined by X′ pert high score software with search and match facility. The particle size of the prepared samples were determined by using Scherrer′s equation $D \approx 0.9\lambda\beta\cos\theta$,Where D is the crystal size, λ is the wavelength of X-ray, Θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians.

2.7 Antibacterial Activity

Silver nanoparticles synthesized using rhizome extract of Curcuma amada, was tested for its potential antimicrobial activity against few human pathogens. The antimicrobial activities of silver nanoparticles were investigated utilizing agar well diffusion assay Nanda and Saravanan (2009). The tested microorganisms were swabbed uniformly on Mueller-Hinton agar plates using sterile cotton swab, then seven wells of 6-mm diameter were made using sterile well borer. Twenty micro liters of silver nanoparticle and pant extract with various concentrations (50,100,150 μg) was poured into corresponding well. DMSO used as a control. The plates were incubated at 37 C for 24 or 48 h for the bacterial and yeast cultures, respectively. The diameter of inhibition zone was measured.

III. Results and Discussion

The formation of silver nanoparticles were confirmed by the color change from yellow to brown (figure: 1). The absorbance spectra of the AgNPs were analyzed using Thermo Scientific spectrophotometer. UV-Vis absorption spectroscopy is an important technique to monitor the formation and stability of metal nanoparticles in aqueous solution. The color change observed was due to excitation of surface Plasmon

vibration in the silver nanoparticles. The surface Plasmon resonance of AgNPs of *C.amada*it was at 424nm (Figure: 2). The metal nanoparticles have free electrons, which give the surface Plasmon resonance absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave (Mostafa M.H *et al.,* 2014 &Priya Banerjee *et al.,* 2014).

The biosynthesized silver nanoparticle from *Curcuma amada* extract was further demonstrated and confirmed by the structural view under the scanning electron microscope. The SEM analyses of extract silver nanoparticles were observed by using JEOL model JSM-6390LV SEM machine. The micrographs of nanoparticles obtained in the filtrate. The SEM analysis showed the particle size between 160nm- 240nm as well the spherical structure of nanoparticles. Figure 3shows the SEM image of the silver nanoparticle formed in rhizome extract of *Curcuma amada.*

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD pattern as shown in figure 4. The five distinct diffraction peaks at 2θ values of 27.61, 32.02, 45.99, 54.58 and 57.24 can be indexed to the (985), (2419), (1343), (380) and (349) reflection planes of face centred cubic structure of silver. These peaks are due to the organic compounds which are present in the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles (Roopan*et al.,* 2013)

The antimicrobial activity of biosynthesized silver nanoparticles and aqueous extract of *curcuma amada* were investigated against both Gram positive (*Staphylococcus aureus, Bacillus cereus*),Gram negative (*Escherichia coli,Pseudomonas aeruginosa,Klebsiella pneumonia*) and fungi *Candida albicans* . various pathogenic bacteria are still unclear and required further investigation (Ahmed, M.J *et al.,* 2015, Bindhu, M *et al*.,2014). AgNPs might have been attached to the surface of the cell membrane of microorganisms, leading to the disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. In general, small nanoparticles have a larger surface area for interaction with bacteria, as compared to that of bigger particles, due to greater antibacterial activity (Bindhu, M *et al*.,2014, Mata. R*et al.*,2015 andNaraginti, S *et al.*,2014). In our results, the Gram-positive bacteria showed the lower zone of inhibition, as compared to Gram-negative bacteria. This could be due to the cell wall of Gram-positive bacteria composed of a rigid thicker multiple layer of peptidoglycan, as it prevented the nanoparticles from entering into cell wall (Ahmed,S*et al.,* 2015).

Figure 1: Color change observed after the addition of silver nitrate

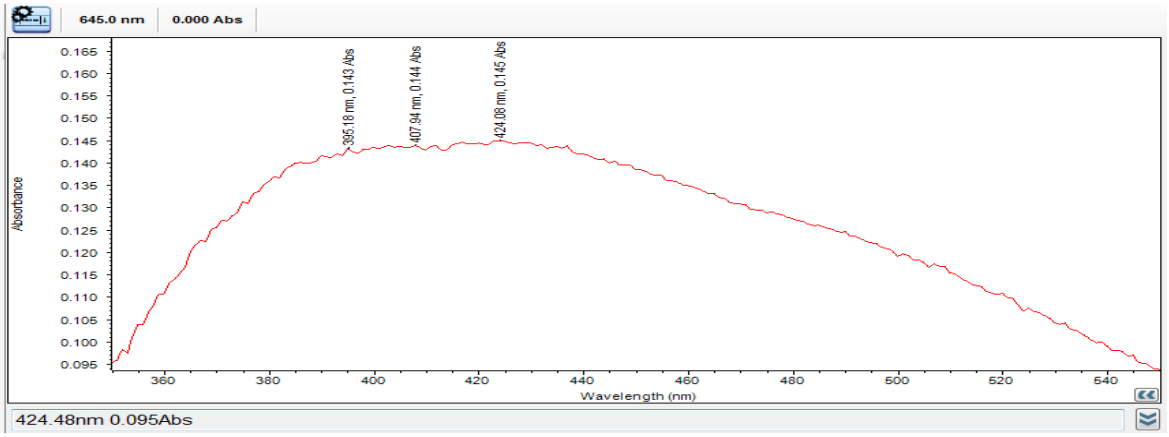

Figure : 2 UV-Vis absorption spectra of *Curcuma amada* silver nanoparticles

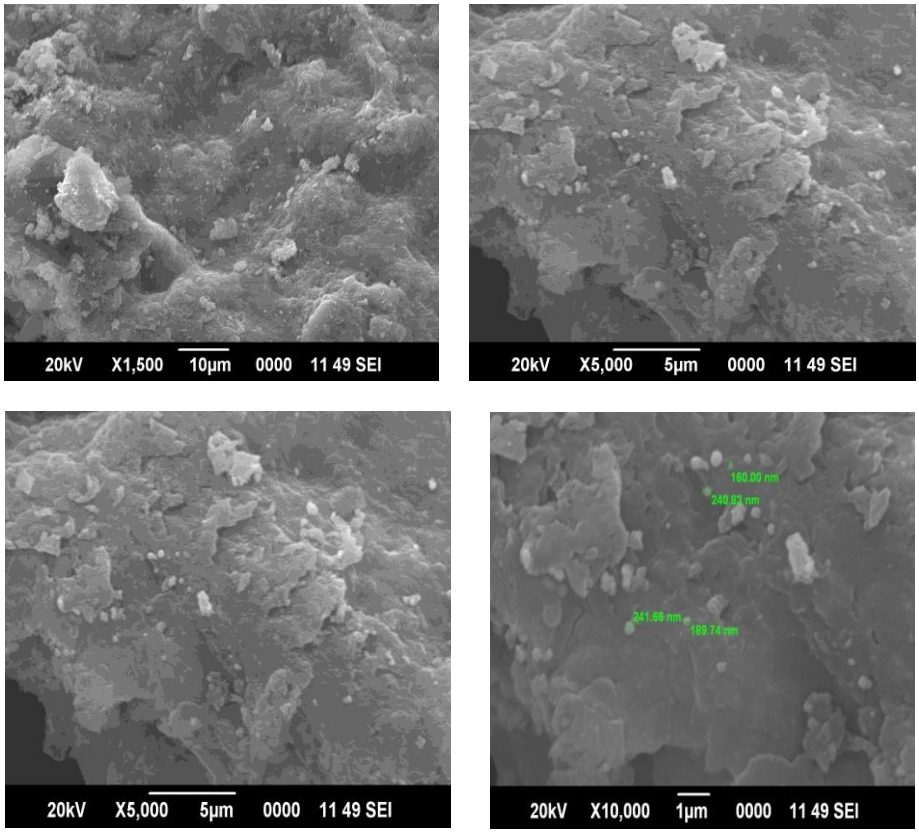

Figure 3: Scanning electron micrograph of *Curcuma amada* silver nanoparticles

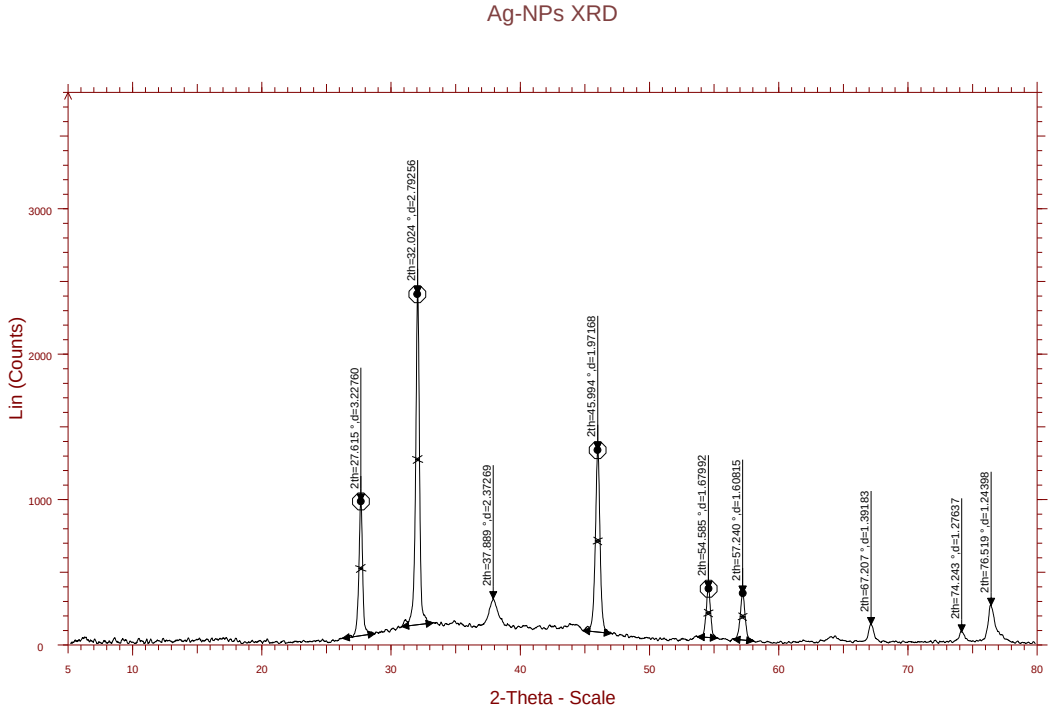

Figure 4: XRD pattern of silver nanoparticles synthesized from *Curcuma amada*

SI.NO	ORGANISMS	ZONE OF INHIBITION(mm)						CONTROL
		SILVERNANOPARTICLE (µg)			PLANT AQUEOUS EXTRACT (µg)			
		50	100	150	50	100	150	
1	*Escherichia coli*	13	14	16	No zone	8	9	No zone
2	*Pseudomonas aeruginosa*	13	15	16	No zone	10	10	No zone
3	*Klebsiella pneumonia*	13	13	15	No zone	No zone	10	No zone
4	*Bacillus cereus*	12	13	14	No zone	No zone	No zone	No zone
5	*Staphylococcus aureus*	10	10	12	No zone	10	11	No zone
6	*C.albicus*	11	12	14	10	10	11	No zone

Table1: Antimicrobial activity of Silver nanoparticles and plant extract of*Curcuma amada*

IV. Conclusion

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. Biosynthesis of silver nanoparticles was carried out by using aqueous rhizome extract of curcuma amada for the reduction of silver ions. The silver nanoparticles formation was confirmed by the color change of plant extract and further confirmed with the help of UV-Vis spectroscopy. The biosynthesis of silver nanoparticles was further characterized by SEM and XRD. SEM image exhibited that the biosynthesized silver nanoparticles were mostly spherical in shape with an average size 240 nm. The synthesized AgNPs were found to have a crystalline structure as investigated by XRD method. Furthermore the silver nanoparticles remarkably inhibited the growth of tested Gram positive and Gram negative bacteria and also fungi Candida albicus.

Nature has elegant and ingenious ways of creating the most efficient miniaturized functional materials. An increasing awareness towards green chemistry and use of green route for synthesis of metal nanoparticles lead a desire to develop environment-friendly techniques. Benefit of synthesis of silver nanoparticles using plant extracts is that it is an economical, energy efficient, cost effective; provide healthier work places and communities, protecting human health and environment leading to lesser waste and safer products.

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