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A PHYTOCHEMICAL SYNTHESIS OF SILVER NANOPARTICLES USING CURCUMA LONGA AND THEIR APPLICATIONS IN ANTIBACTERIAL AND DYE DEGRADATION STUDIES

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ABSTRACT
Biogenic silver nanoparticles (AgNPs) synthesized by *Curcuma longa* tube extract and characterized by UV–vis spectroscopy, XRD, TEM and FT- IR, Formation of AgNPs is initially confirmed by UV–vis spectroscopic studies with the specific band at **413 nm**. TEM and XRD reports revealed that AgNPs are highly crystalline and rod shaped with average particle size of 30 nm. FT-IR studies indicated the presence of different functional groups on the surface of the AgNPs indicating the stability of AgNPs by capping of biomolecules onto the surface. Further, the bio-synthesized T-SNPs show significant anti-bacterial action on tested pathogenic microorganisms Further catalytic efficiency synthesized AgNPs were evaluated for the reduction/degradation of various organic dyes such as 4methylene blue (MB) and methyl orange (MO) using NaBH4 as reducing agent in the presence of catalytic amount of AgNPs. It was found that degradation was faster.

INTRODUCTION
NANOPARTICLES
The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology [1,2]. There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements[3,4]. New applications of nanoparticles and nanomaterials are increasing rapidly. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) materials for the development of science [5,6,7]. It deals with the materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nano scaled size [7,8]. Because of their size, nanoparticles have a larger surface area than macro-sized materials. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology. Nanoparticles, because of their small size, have distinct properties compared to the bulk form of the same material, thus offering many new developments in the fields of biosensors, biomedicine, and bio nanotechnology [9, 10]. Hence, the present study, to be synthesize silver nanoparticles using *Curcuma longa* (Turmeric) powder aqueous extract, to evaluate the antibacterial activity of synthesized AgNPs against *Staphylococcus aureus* and *E.coli* pathogens. And their catalytic activity in degradation of textile effluent dyes such as methyl orange and methylene blue[11].

MATERIALS
Curcuma longa (Turmeric) dried powder, Silver nitrate ($AgNO_3$), Methyl orange Methylene blue,Sodium borohydride ($NaBH_4$), Bacterial cultures (S. *aureus* , *E.coli*), Muller Hinton Agar, Double distilled Water (H_2O).

Experimental Methods
Preparation of aqueous extract of Curcuma longa
The dried curcuma longa tubes were collected and further the collected tubes were crushed into fine powder and used [12]. Ten gram of the grinded powder was taken and then the powder were boiled with 250mL deionized water at 60 °C for 15min and cooled. The extract was filtered with Whatman filter paper No.1and stored in a refrigerator at 4 °C for further use **[Figure 1].**

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Figure.1 Formation of colloidal AgNPs

Synthesis of silver nanoparticles by fungi:

The mechanism of silver nanoparticle production by fungi is said to follow the following steps: trapping of Ag+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system[13,14]. The extracellular enzymes like naphthoquinones and anthraquinones are said to facilitate the reduction. Considering the example of F. oxysporum, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation. Though the exact mechanism involved in silver nanoparticle production by fungi is not fully deciphered, it is believed that the abovementioned phenomenon is responsible for the process. A major drawback of using microbes to synthesize silver nanoparticles is that it is a very slow process when in comparison with plant extracts. Hence, the use of plant extracts to synthesize silver nanoparticles becomes an option that is feasible.

Antibacterial activity of silver nanoparticles

To study the antimicrobial activity of AgNPs, two bacterial strains were selected for minimum inhibitory concentration (MIC) determination: S. aureus (***Staphylococcus aureus*** is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose,respiratory tract, and on the skin) and E. coli (***Escherichia coli*** also known as ***E. coli***) is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recallsdue to food contamination) (Gram-negative) Muller Hinton Agar plates were used and swabbed with pa-thogenic organisms from fresh cultures(105–106 CFU/mL) using a sterile cotton swab . Using a micropipette, the disc were poured with 20 µL of the AgNps, silver nitrate, turmeric powder extract in concentrations of 50 µg/mL . And the discs were kept at the center of the plates. The zone of inhibition was measured after the plates were incubated at 37 °C for 24h for bacteria and 25 °C for 24h.

Dye degradation studies

Catalytic activity of synthesized AgNPs was performed by degrading different organic dyes such as methyl orange and methylene blue. Briefly, for reduction of organic dyes different concentrations of purified AgNPs (10 mg/mL) were added to the reaction tube containing organic dye (10^{-4} M) and 0.1 M $NaBH_4$. The reduction was monitored by recording spectra using UV–vis spectrometer at regular time intervals. A blank experiment was carried out without AgNPs and was monitored by UV–vis spectrometer at regular time intervals.

RESULTS AND DISCUSSIONS

UV spectroscopic studies

The recorded UV-Vis spectrum of the biosynthesized AgNPs is presented in Fig.3. It was observed that the silver surface plasmon resonance (Surface plasmon resonance (SPR) is the resonant oscillation of conduction electrons at the interface between a negative and positive permittivity material stimulated by incident light. The interaction of light with noble metal nanoparticles produces a collective oscillation of conduction band electrons known as the localized surface plasmon resonance (LSPR). Only materials with a negative real and small positive imaginary dielectric constant are capable of supporting surface plasmons.

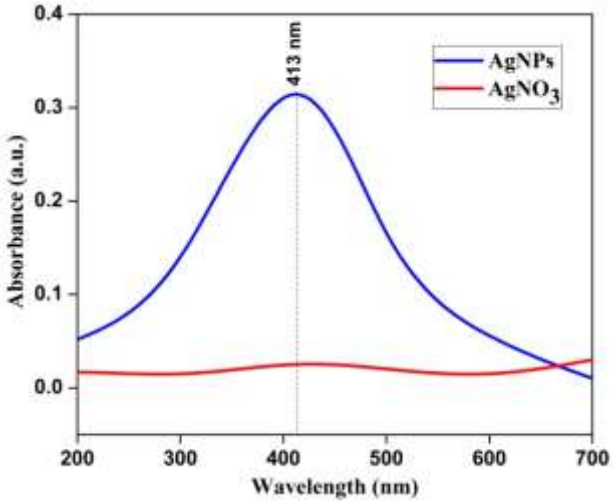


Figure.3 UV spectra for synthesized T-AgNPs

X-Ray diffraction studies

The crystal structure of the biosynthesized AgNPs was determined by using X-ray diffraction Fig.4 shows the XRD pattern of the silver nanoparticles synthesized by turmeric powder extract. Four major diffraction peaks at 2Θ values of 38.1°, 44.3°, 64.5° and 77.3° corresponding to the (111), (200), (220) and (311) planes of face centered cubic silver were observed. The XRD profile also revealed the presence of some insignificant additional peaks which appeared at 2Θ which might correspond to the bioorganic compounds present in the turmeric powder extract.

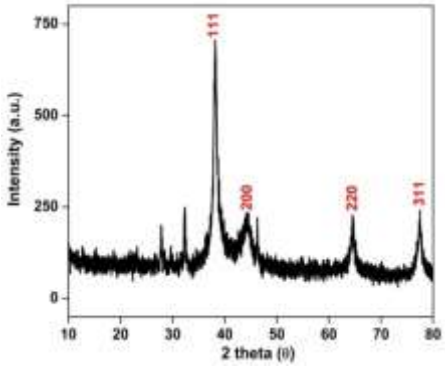


Figure 4 XRD pattern

The FT-IR spectrum **[Figure 5]** of aqueous extract of turmeric powder shows the presence of O-H containing groups in their extract which corresponds to the broad peak at 3410 cm^{-1} and the peaks at 1633, 1412,1007 and 847 cm^{-1} corresponds to the presence of C-O, C-N, -C-O-C and C=C respectively . These results indicates the presence of alkaloids, polyphenols, terpenoids and flavonoids in the turmeric extract which acts as a naturally reducing agents by donating free electrons for the reduction Ag^{+} to Ag^{0}. The IR spectra of the formed Ag NPs shows all other characteristic peaks which resembles to the extract with a new peak at 1938 cm^{-1} which attributed to the Ag…O vibration. This information confirms the formation of AgNPs with a weak bonding the oxide ions present in the O-H and C-O groups

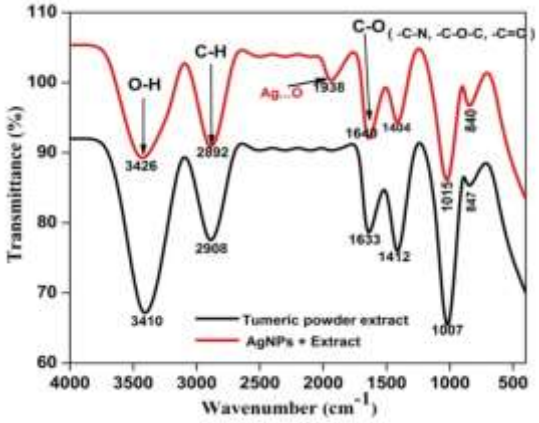


Figure.5 IR spectra turmeric powder and T-AgNPs

TEM studies

The morphology and particle size was determined by transmission electron microscopy. The TEM image [**Figure 6]** revealed the obtaining of even size of silver nanoparticles with rod shape. The particles were found to have sizes ranging from30 to 50 nm in diameter. The average particle size as obtained from TEM analysis was found to be 26 nm. The TEM images clearly revealed the presence of a faint thin layer on the surface of AgNPs prepared using the aqueous extract of C.longa which may be due to the organic molecules from the extract who also act as capping and stabilizing agents of the silver nanoparticles.

Antibacterial activity

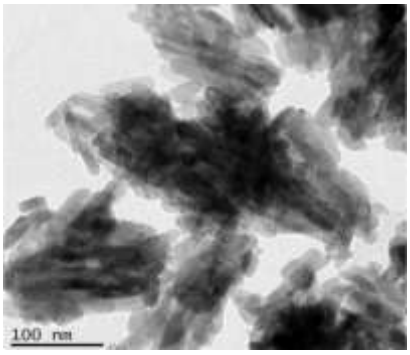


Figure.6 TEM image for synthesized T-AgNPs

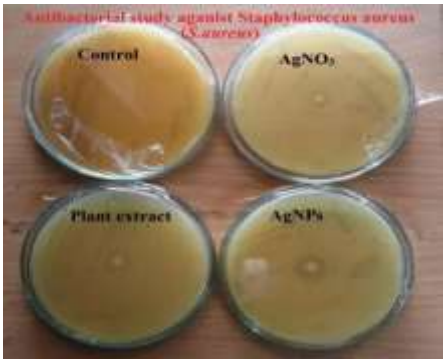


Figure.7 Antibacterial activity against S.aureus

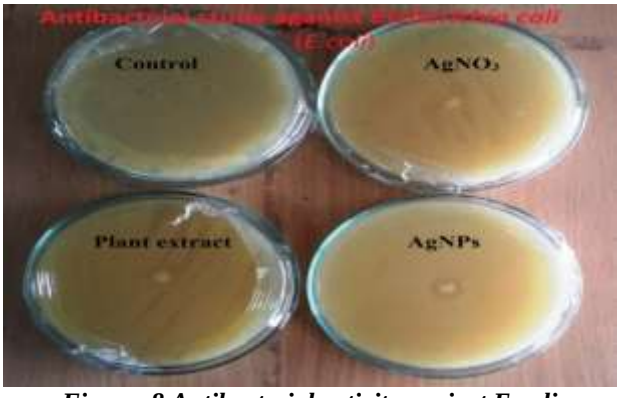


Figure. 8 Antibacterial activity against E.coli

Turmeric powder extract and AgNPs shows anti-bacterial activity against both S.aurues and E.coli, and the results located in Fig.7 & 8. Pristine Turmeric powder extract has less inhibition whereas zone of inhibition increased with T-SNPs which were reduced by of T extract. This clearly indicates that the anti-bacterial activity is increased when associated with T powder extract due to T-SNPs. The exact mechanism for the antibacterial activity of SNPs is not known till now. However, theoretically many studies report that the SNPs could bind to the bacterial membrane, invade the cell and cause appetite of proton motive force which leads to the distraction of bacterial cell by forming pores on the bacterial cell wall.

Dye degradation studies

Methyl orange

Methyl orange is an azo dye which was used in different industrial applications such as dye in textile industries and also used as indicators in various industries. One of its major problem was it was highly toxic and it will pollute the environment and harmful to plants and human beings. So it need in degradation of methyl orange after use . The aqueous solution of methyl orange is orange red in colour. The UV–vis spectrum of aqueous solution shows strong absorption at 454 nm . The λmax at 464 nm is due to the absorption of –N=N– group.

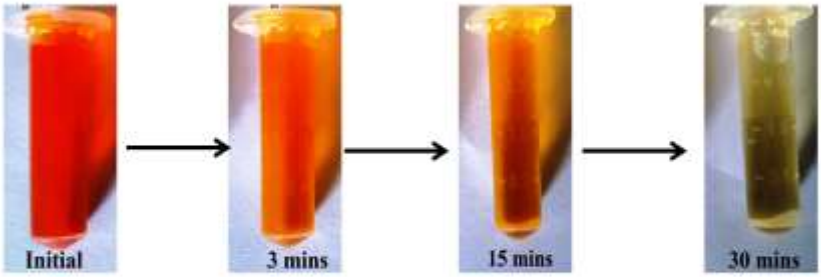


Figure.9 Degradation of methyl orange

The degradation of MO was carried out using C.lomga mediated synthesized AgNPs and shown in Fig.9. The λmax of MO is 454 nm in UV–vis spectrum. Initially, MO shows light orange colour and after addition of NaBH4 it turned to deep orange. After the addition of reluctant $NaBH_4$ to MO no change in absorbance was observed even up to 25 min. Then AgNPs was added to MO solution containing $NaBH_4$ and absorbance was gradually decreased i.e., within 3 min the degradation of MO was completed.

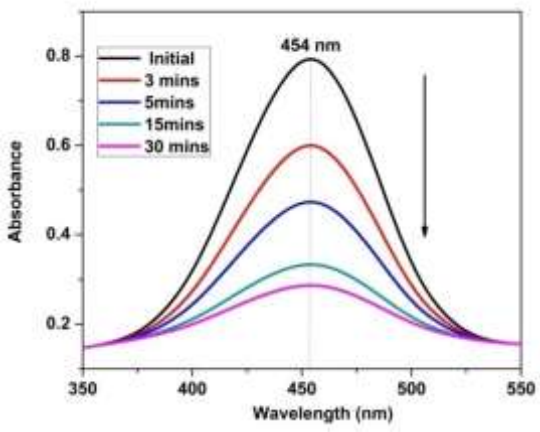
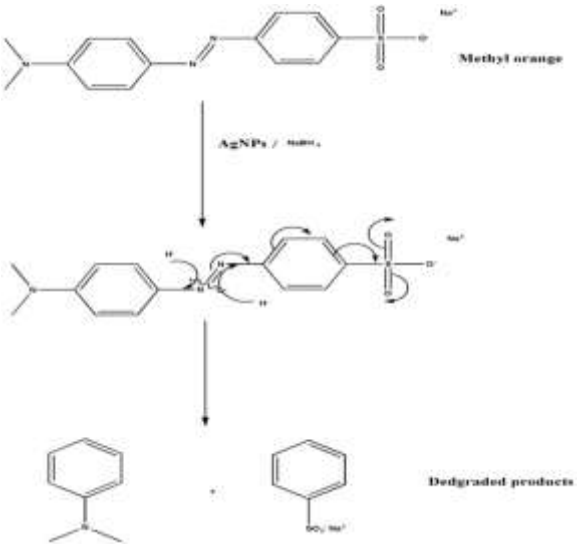


Figure.10 Degradation studies of MO via UV spectra



Mechanism of degradation

Methylene blue

The UV–vis absorption spectrum of an aqueous solution of methylene blue shows peaks at 290 and 664 nm with a hump at 612 nm due to π → π* and n → π* transitions. The reduction of methylene blue into its colourless formcan be followed spectrophotometrically bymonitoring the absorption maximum at 664 nm.

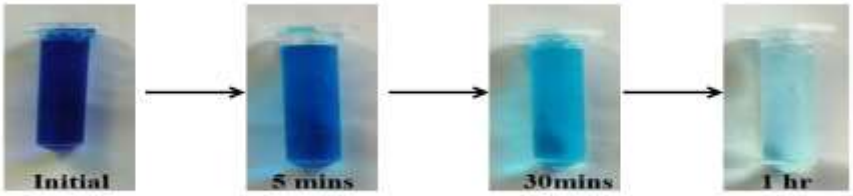


Figure.11 Degradation of methylene blue

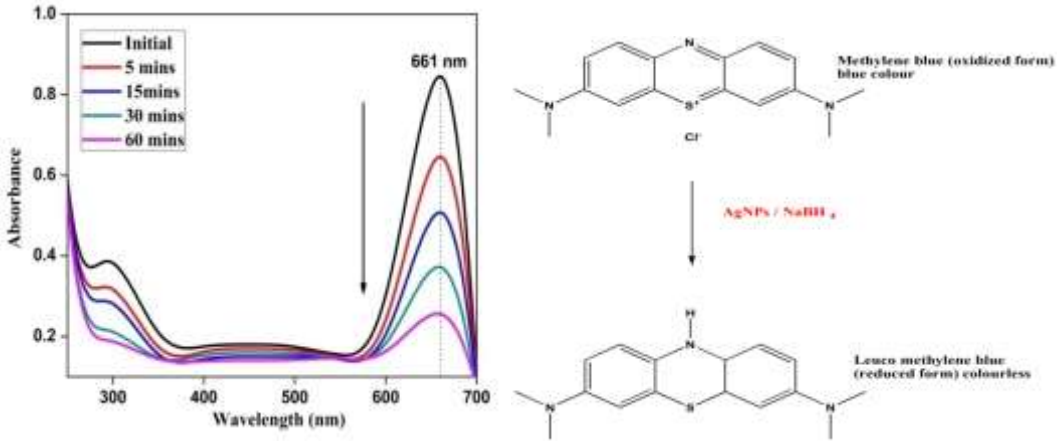


Figure.12 Degradation studies of MB via UV spectra ***Mechanism of degradation***

CONCLUSIONS

We report a simple and efficient way to synthesis stable rod shaped AgNPs using C.longa tube aqueous extract. The synthesized AgNPs were initially confirmed by using UV–vis spectroscopy and TEM study. Crystalline nature of AgNPs was evidenced through XRD studies and multifunctional nature of plant extract was confirmed by FTIR studies. Further, the bio-synthesized T-SNPs show significant anti-bacterial action on tested pathogenic microorganisms. The synthesized AgNPs showed excellent catalytic activity in the reduction/degradation of different organic dyes namely Methylene Blue and Methyl Orange using $NaBH_4$ as reducing agent. Hence this synthesis method is the better alternative of both chemical and other physical methods used in industry for large scale production of Ag NPs.

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