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Research Article

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Improved Antibacterial and Antibiofilm Activity of Plant Mediated Gold Nanoparticles using *Garcinia cambogia*

B. Nithya* and A. Jayachitra

[1]Research Scholar, Research and Development Centre, Bharathiyar University, Tamil Nadu, India

[2]Assistant Professor, Department of Plant Biotechnology, School of Biotechnology

Madurai Kamaraj University, Tamil Nadu, India

*Corresponding Author E-mail: nithya.hannah@gmail.com

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ABSTRACT

Gold nanoparticles biosynthesis is burgeoning day by day making an impact in all biological applications. Green synthesis of nanoparticle has proven to be worthy due to slower kinetics, better manipulation, and stabilization and assuring values in medicinal field due to its environmental friendly approach and also inexpensive techniques. In the present investigation gold nanoparticles, were synthesized using the aqueous leaf extract of Garcinia combogia as the reducing agent. The disposition of gold nano particles were confirmed and characterized by UV–visible spectrum, X-ray diffraction (XRD) spectroscopy, Fourier transform Infra-red spectroscopy (FTIR), Scanning electron microscopy (SEM).Further, the antibacterial activity of the synthesized gold nano particle were screened against Bacillus Subtilis, E. coli, L. Monocytogenes, Proteus vulgaris, Vibryo parahaemolyticus., The gold nanoparticles also showed effective inhibitory activity against the Bacillus licheniformis strain Daph2 with the 20 µl of concentration. Confocal laser scanning microscopic (CLSM) images demonstrated the antibioflim potentials of AuNps concentrations ranging from 20-40µg/ ml against B. licheniformis antibioflim activity.

Key words: *Garcinia cambogia, Nanoparticles, Surface Plasmon resonance, XRD, SEM, CLSM.*

INTRODUCTION

Nanotechnology is a growing field in the modern research technology. Its application is widened in material research, electronic, and medicine. Nanotechnology involves nanoparticles synthesis with the size ranging from 1 to 100 nm[1]. which have remarkable optical, mechanical, electrical and electronic properties that vary from other bulky materials.

The increased environmental concerns have influenced the researchers to create novel methods of synthesizing the nanomaterials in biological systems such as bacteria, fungi and plants, termed as environmentally benign “green chemistry”. Hence the advancement of efficient, environmentally benevolent biological method for synthesizing nanoparticles is significantly considered to be an attribute of prevailing nanotechnology research. Gold nanoparticles are pertaining to have a wide range of applications due to their novel properties and application in biomedical field.

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In the present scenario, large number of novel diseases are emerging due to specific microorganisms that are resistant to multiple antibiotics. Hence the focus of researchers is on the development of new and effective antimicrobial reagents that are cost effective and highly efficient. The bacteriocidal property of the Nano particles, causes cell damage by penetrating into the cell and interacting with the phosphorous and sulfur containing compounds such as DNA and protein[2]. Due to this gold nanoparticles are considered as potent anticancer, antimicrobial and cytotoxic agents. A variety of Gold nanoparticles synthesis has been previously reported such as *Mangifera indica* leaf[3]. *Hibiscus rosasinensis*[4], *Murraya koenigii*[5] and *Ocimum sanctum*[6], *Diospyros kaki* leaf extract[7], phyllanthin[8] have been reported.

In the current study, we have reported on the synthesis of AuNPs from *Garcinia cambogia* belongs to the family *Guttiferae (Clusiaceae).* It is a wild sub tropical and tropical medicinal plant. Phytochemical analysis shows that *G. cambogia* contains phenolic compounds, steroids, xanthins, benzophenone[9] tannins, gutiferrins, and Saponins. Animal and human studies revealed that the extracts of *G.cambogia* exhibit aphrodisiac effects on male subjects[10]. *G. cambogia* extracts have been shown to possess antipyretic, anti-inflammatory, analgesic, antiviral, hepotoprotective[11], antidepressant , antioxidant[12] , antidiabetic and antithrombotic activities. The present study was aimed to synthesis of gold nanoparticles using aqueous leaves extract of *Garcinia cambogia.* The green synthesized GNPs of Garcinia Combogia were Characterized by UV- VIS Spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis for size and shape and scanning electron microscopy (SEM).), The synthesized and well characterized nanoparticles were tested for its antibacterial activity against *Bacillus subtilis, E. coli, L. Monocytogenes, Proteus vulgaris, Vibryo parahaemolyticus.*, and its antibioflim inhibition against *B. licheniformis* strain Daph[2].

MATERIALS AND METHODS

Preparation of the plant extract

The fresh leaves of *G. cambogia* were collected from the Western Ghats of Idukki district. It was washed thoroughly thrice with deionised water. After cleaning the plant was dried in shade at room temperature for one week and was crashed to give powder and stored in airtight amber bottles.

Preliminary Phytochemical screening: About 50 gms of the plant sample was taken in 100 ml of the different solvents and the extracts were filtered through Whatman‟s filter paper. Different qualitative chemical tests were carried out on the aqueous extract of various using standard procedures to identify the phytochemical constituents as described by Trease and Evans[13], Sofawara[14] and Harborne[15,16].

Gold nano particle synthesis:

One gram leaf powder was mixed with 50 ml of sterile distilled water ground in a blender and the mixture was left in a shaking incubator operating at 200 rpm, 25°C for 24 h., filtered through mesh and centrifuged at 10,000 rpm for 10 min at 4°C by REMI cooling centrifuge to remove cell-free debris. The resulting supernatant was then filtered through a 0.2 µm filter paper and employed for the synthesis of gold nanoparticles. 5 ml of leaf extract of Garcinia combogia were mixed with 4 ml aqueous solution of HAuCl4 (1 mM) and incubated at room temperature for 24hrs . The immediate change in color of the solution from pale yellow to violet color indicated the preliminary confirmation for the formation of plant extract mediated synthesis of gold nanoparticles[17].

Characterization of gold nanostructures :

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + Garcinia combogia) were recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored as a function of time of reaction on Elico UV-vis Nanodrop spectrophotometer (Model SL 159) operated at a resolution of 1 nm (300-700 nm.) The absorption peak is assigned to the surface Plasmon resonance band (SPR) nano particles formed by the synthesis of Au^{3+} ions[18].

Fourier transform infrared (FTIR)

The possible functional groups of phytochemicals in plant extract involved in nanoparticles synthesis are identified by FTIR analysis. FTIR spectra were recorded at room temperature on a Bomem MB100 FTIR spectrometer. For FTIR measurements of capped gold nanoparticles, a small amount of gold nanoparticles (0.01g) dried at 60°C for 4 h was mixed with KBr to form a round disk suitable for FTIR measurement. To obtain the FTIR spectrum of the extract, an appropriate amount of the extract was mixed with KBr[19].

X- ray diffraction analysis

Crystalline AuNPs were determined by X-ray diffraction analysis. The biosynthesized gold nanoparticles were laid onto the glass substrates on a Phillips PW 1830 instrument operating at a voltage of 40 kV and a current of 30 mA with CuK_{α} radiation[20].

SEM Analysis: Scanning electron microscopy are used for morphological characterization at the nanometer scale.The Garcinia Combogia gold nanoparticles were characterized using high resolution Scanning Electron Microscope (SEM – Hitachi model – S 3000H). The samples were prepared by coating a single drop of of *Garcinia combogia* gold solution on to an electric clean glass and the solvent is made to evaporate. The samples were left to dry at room temperature.

Bactericidal activity of Garcinia combogia GNPs:

The antimicrobial activity of the *Garcinia combogia* GNPs was tested by agar well diffusion method[21]. Different gram negative and gram positive organisms such as *Bacillus subtilis, E. coli, L. monocytogenes, Proteus vulgaris, Vibryo parahaemolyticus* were grown and diluted using Mueller –H inton broth. Fresh cultures were prepared by growing the bacterial strains to the exponential phase in Mueller-Hinton at 370c for 18 hrs and adjusted to a final density of 108CFU/ml. The anti bacterial activity was tested with 20,40, 60µl of *Garcinia combogia* GNPs in the Agar well. The extract without gold nano particles were considered as control. The plates were incubated at 37° C and the antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well by using metric scale.

Biofilm inhibition using CLSM

The biofilms were monitored under a confocal laser scanning microscopy (CLSM) (Model: LSM 710) (Carl Zeiss, Germany) after washing with PBS and staining with 0.01% acridine orange. The 488-nm Ar laser and a 500– 640 nm band pass emission filter were used to excite and detect the stained cells. CLSM images were obtained from the 24-h old control and treated biofilms and processed using Zen 2009 image software.

RESULTS AND DISCUSSION

Synthesis of AuNP

The immediate change in color of the solution from pale yellow to violet color due to the surface plasmon resource indicated the preliminary confirmation for the formation of plant extract mediated synthesis of gold nanoparticles. The result obtained in this investigation is very interesting in terms of identification of *Garcinia combogia* for synthesizing the Au Nps.

UV-Vis spectra of AuNPs

UV-Vis spectra of AuNPs synthesized by reacting different concentration of *Garcinia combogia* extract with 1Mm HAuCl aqueous solution the Plasmon peak was observed at 512 for 2mm and 535 for 1mM. The absorbance maxima of Gold Surface Plasmon Resonance (SPR) occurred at 550 nm and the intensity increased steadily as a function of time without any shift in the peak wavelength[22].

XRD analysis

XRD Analysis showed three distinct diffraction peaks at 38.1°, 44.1° and 64.1°which indexed the planes 1 1 1, 2 0 0 and 2 2 0 of the cubic face-centered g. The lattice constant calculated from this pattern was a = 4.086Å and the data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The average grain size of the gold nanoparticles formed in the bioreduction process was determined using Scherr's formula $d = (0.9\lambda \times 180^{\circ})/\beta\cos\theta$ where d is the mean diameter of nanoparticles, λ is the wavelength of the X-ray radiation source and β is the angular FWHM of the XRD peak at the diffraction angle θ] by determining the width of the (112) Bragg reflection[23] and the size was estimated as 36 nm (Fig. 3).

FTIR

Measurements were carried out to identify the potential functional groups of the biomolecules in the *G. cambogia* leaf extract responsible for the reduction of the gold ions. FTIR spectra of the green synthesized Garcinia Combogia gold nano particles are shown in the figure. s. The FTIRspectrum of the bio-reduced gold nanoparticles by the phytochemicals had the adsorption peaks located at about 3,965, 3,466 cm-1, 3404, 2678, 2074, 1638, 1368, 1233, 664 cm-1. The majority of the IR bands are characteristic of flavonoids and terpenoids present in the leaf [24]. The weak band at 3,965 cm-1 shows characteristics of O–H stretching of secondary alcohols. The band at 3,466 corresponds to asymmetric stretching vibration of -NH_2- group. The band at 3,404 corresponds to stretching vibration of N-H group. The band at 2678 and 2074 corresponds to O–H stretching vibration of alcohols. The band at 1,638 cm-1 corresponds to C=O stretching of alcohols[25] amide I band and nitro groups. The band at 1,368 corresponds to asymmetric stretching vibration of C-H group[26]. The absorbance peak at 1,233 cm-1 was disappeared in the capped AuNPs indicates to C–N stretching vibrations of aliphatic amines or alcohols or phenols representing the presence of polyphenols may be flavonoids. The band positions from 645 to 686 are due to C\C and C\H phenyl ring substitution as expected for this plant. In this study of FTIR spectrum confirmed the presence of aromatic amine, amide (I) groups, phenolic groups and secondary alcohols may act as reducing agents for the synthesis of AuNPs. Results of FTIR suggested that the AuNPs of *G. cambogia*, act as a good bioreductant for biosynthesis.

SEM

The surface morphology and size of the AuNPs was analyzed by Scanning Electron Microscope. SEM image had shown individual AuNPs as well as number of aggregates (Fig. 5). It illustrates the particles are predominantly spherical in shape and aggregates into larger particles with no well-defined morphology. This aggregation may be due to the presence of secondary metabolites in the leaf extracts. The SEM image shows the size of the AuNPs ranging from 40–50 nm. Similar result of the gold nanoparticles size was reported by using *Aloe vera* extract[27] and by using *Euphorbia hirta* leaves[28.]

Antibacterial activity of gold nanoparticles

The antibacterial activity of gold nanoparticles was studied in Different gram negative and gram positive organisms such as *Bacillus subtilis, E. coli, L. monocytogenes, Proteus vulgaris, Vibryo parahaemolyticus.* The gold nanoparticles demonstrated a zone of inhibition against all the test organisms with maximum inhibition against gram positive organism .

The synthesized gold nanoparticles by green route are highly toxic against *the pathogens* . Fig.6 showed a clear inhibition zone around the gold nanoparticles. The concentration of AuNPs was varied from 10–20 µL. The inhibition of zone increased (upto 6 mm) while increasing the concentration of gold nanoparticles. The mechanism of inhibitory action of nanoparticles on microorganisms is partially known. Nanoparticles have positive charge, it will attach with the negative charged microorganisms by the electrostatic attraction in the cell wall membrane[29] . Nanoparticles closely associated with cell wall of bacteria by forming 'pits' finally it affects the permeability, and cause cell death[30,31] . Most of the nanoparticles were small in size so it easily enters into the bacterial cell and affect the intracellular processes such as DNA, RNA and protein synthesis. The synthesized nanoparticles were binding with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria thus it has more bactericidal activity than the larger sized nanoparticles[31] .

The effect of bacterial extract in *B. licheniformis* biofilm was studied using CLSM. The control biofilms showed a higher surface area and wide range of coverage on the surface of the glass slides (Fig. 7). The effect of *G. cambogia* AuNPs (20 to 40 µl) against *B. licheniformis* biofilm is shown in Fig.7. In the presence of *G. cambogia* AuNPs at the BIC, there was a inhibition was observed in the *B. licheniformis* biofilm formation and a remarkable reduction in the biofilm formation, it might be due to the presence of AuNPs interaction with the bacteria. Even in the 20 µl

of BIC too, *G. cambogia* AuNPs showed good biofilm inhibitory activity as against the observation of some EPS layer formation. Starch stabilized silver nanoparticles showed effective antibiofilm activity against the humans pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*[32] .

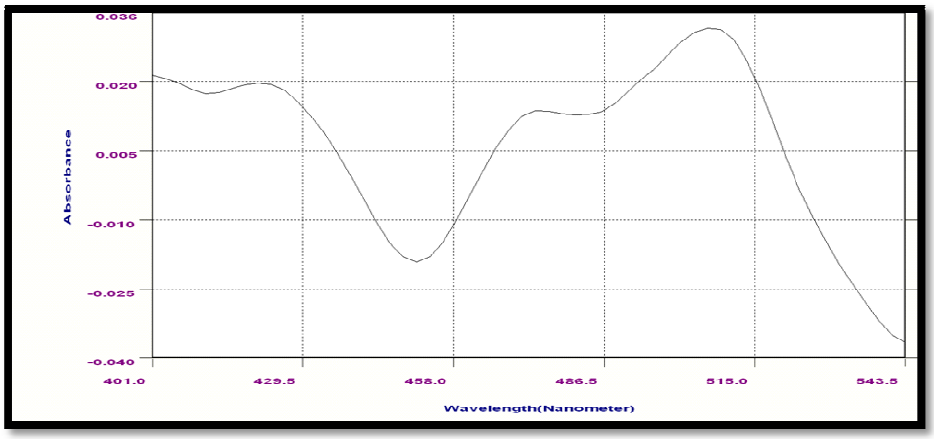
Fig. 1. Digital photograph of *Garinia cambogia* leaves (A) Synthesized AuNPs and its color change (B)

A **B**

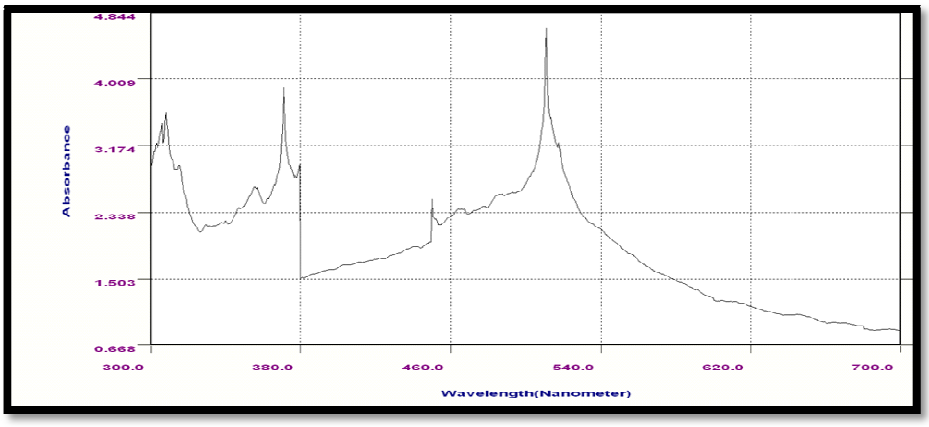
The immediate change in color of the solution from pale yellow to violet color indicated the preliminary confirmation for the formation of plant extract mediated synthesis of gold nanoparticles.

Fig. 2: UV-Visible Spectroscopy Analysis

1mm

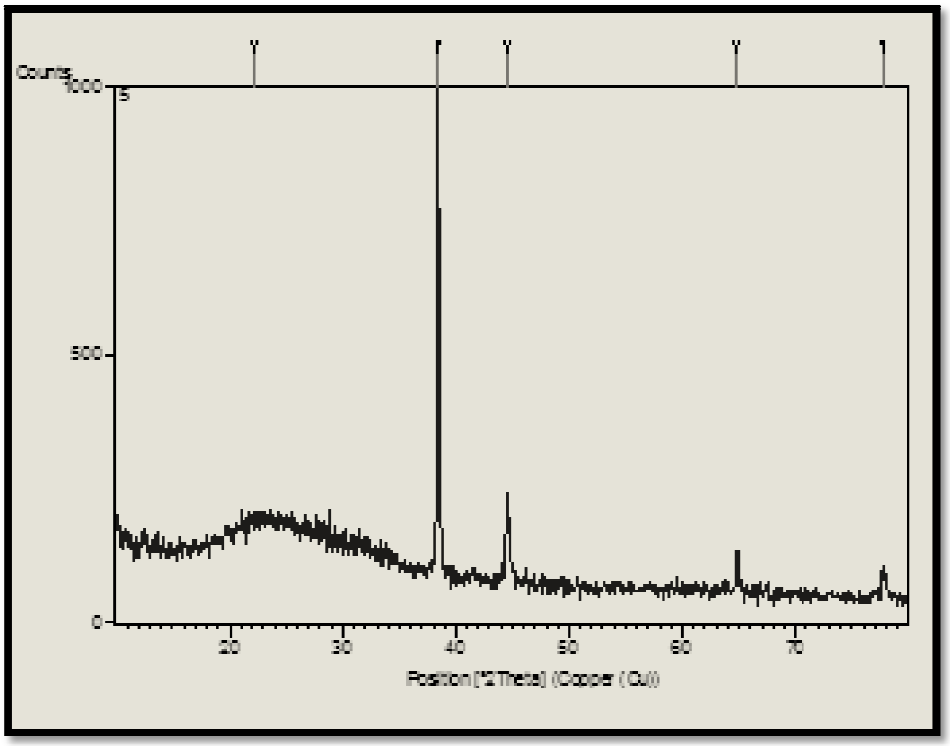


2mm



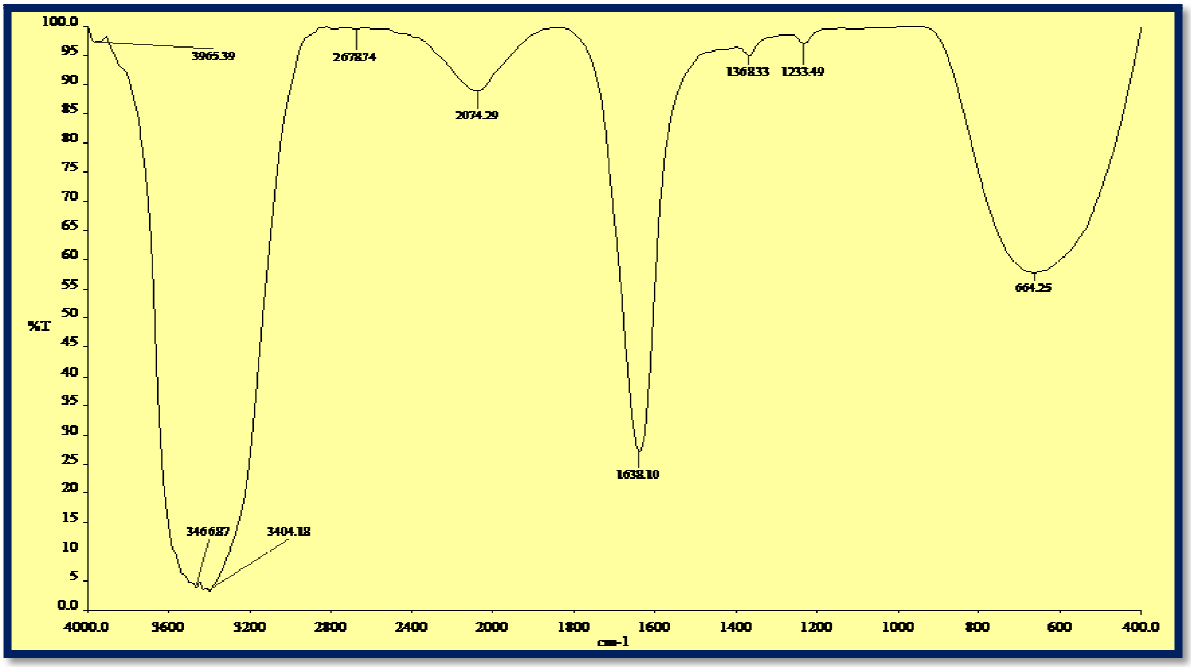
A UV-Vis spectra of AuNPs synthesized by reacting different concentration of *Garcinia combogia.* extract with 1Mm HAuCl aqueous solution the Plasmon peak was observed at 512 for 2mm and 535 for 1mm .

Fig. 3:



XRD Analysis showed three distinct diffraction peaks at 38.1°, 44.1° and 64.1°which indexed the planes 1 1 1, 2 0 0 and 2 2 0 of the cubic face-centered g.

Fig. 4: Fourier transform infrared (FTIR) spectroscopy



The FTIRspectrum of the bio-reduced gold nanoparticles by the phytochemicals had the adsorption peaks located at about 3,965, 3,466 cm-1, 3404, 2678, 2074, 1638, 1368, 1233, 664 cm-1.

Fig. 5: The surface morphology and size of the AuNPs was analyzed by Scanning Electron Microscope

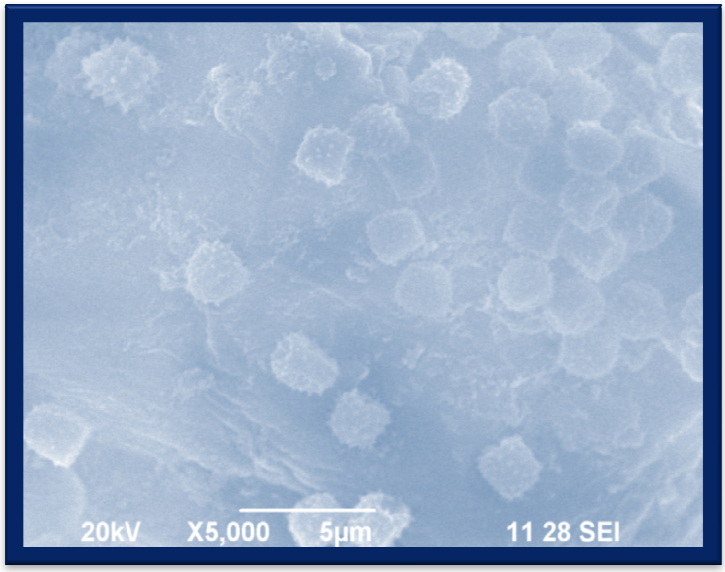
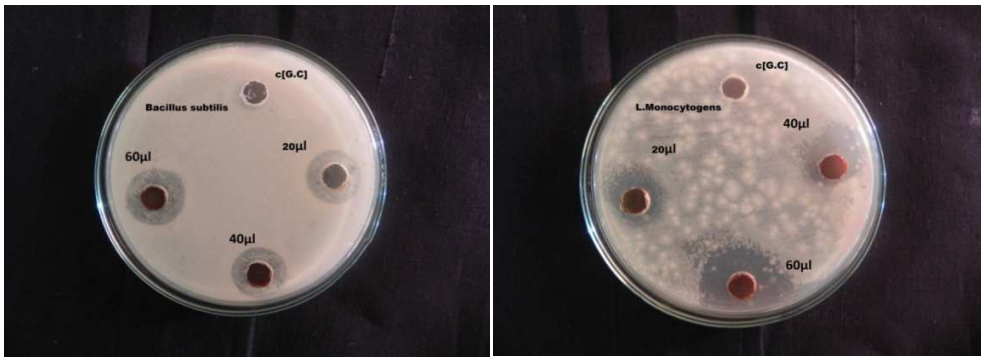


Fig. 6: Antibacterial activity of Gram positive organisms of *Garcinia combogia* Gnps



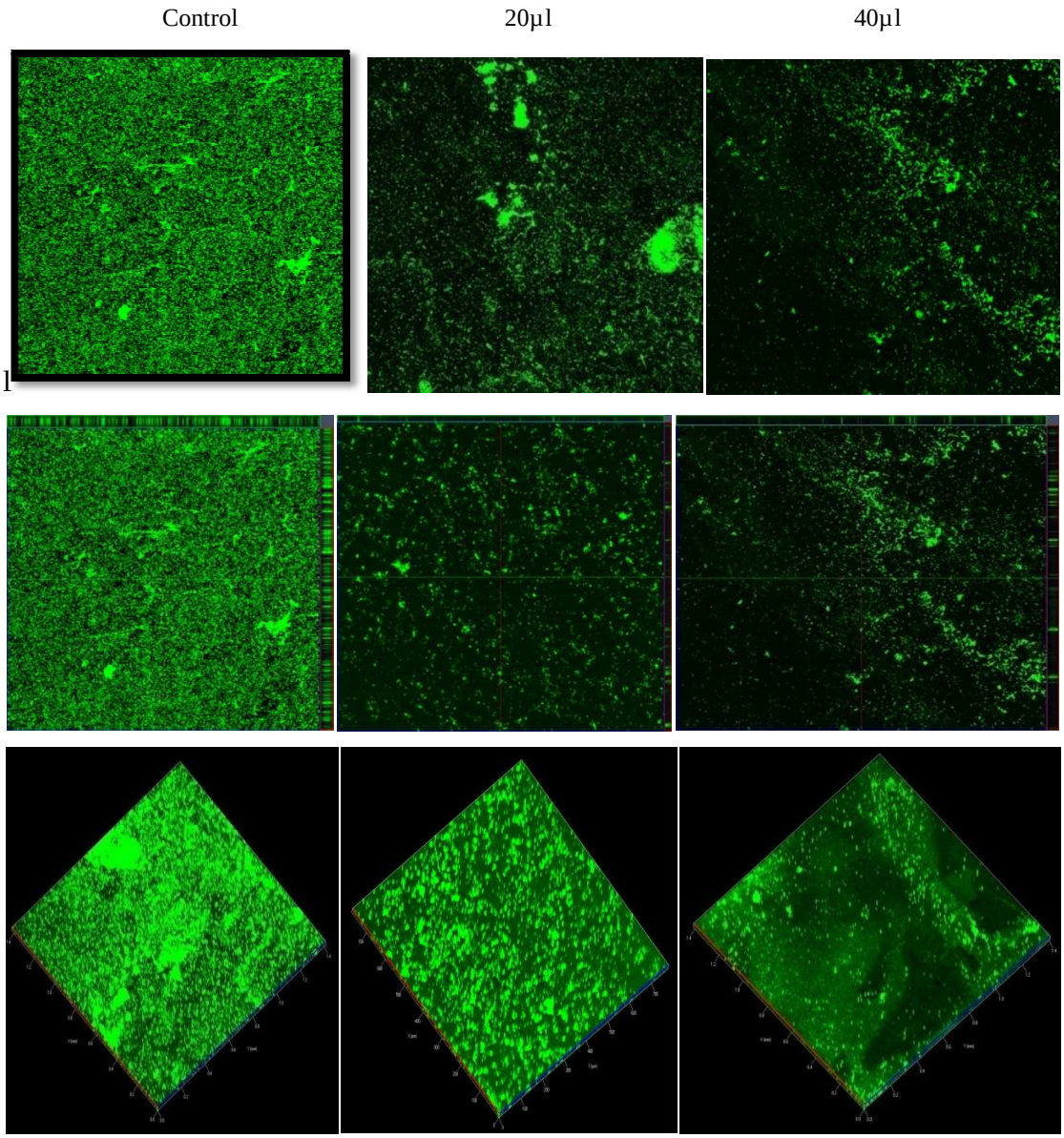
Gram positive organisms such as *Bacillus Subtilis*, *L. Monocytogenes*, showed a clear inhibition zone around the gold nanoparticles. The concentration of AuNPs was varied from 20–60 µL.

Fig.7: Antibacterial activity of Gram negative organisms of *Garcinia combogia* Gnps

Gram Negative organisms such as *E. coli*, *Proteus vulgaris*, *Vibryo parahaemolyticus* showed a clear inhibition zone around the gold nanoparticles.

Confocal laser scanning microscopy

The effect of gold nanoparticles of Garcinia combogia in *B. licheniformis biofilm was studied using CLSM.*



The control biofilms showed a higher surface area and wide range of coverage on the surface of the glass slides. The effect of *G. cambogia* AuNPs (20 to 40 µl) against *B. licheniformis* biofilm shows remarkable reduction in the bioflim formation

CONCLUSION

The eco-friendly green mediated synthesis of gold nanoparticles using *G. cambogia* leaf extract was attained successfully and very rapidly. Gold nanoparticles formation was achieved with in 20 min, which was demonstrated by UV–vis spectroscopy in the absorbance peak at 512 and 535 nm. The synthesized nanoparticles were confirmed by XRD and also average size of the nanoparticles was 36 nm calculated by Debye–Scherrer's equation. The gold nanoparticles size was in the range of 40–50 nm established by SEM. Capping agent/stabilizing agent play the major role in the reduction of gold ion, which was characterized by FTIR. Green synthesized gold nanoparticles had the bactericidal activity *against Bacillus subtilis, E. coli, L. monocytogenes, Proteus vulgaris, Vibryo parahaemolyticus* and *B. licheniformis* was successfully demonstrated by disc diffusion method with zone inhibition on the agar plate.

It also exhibits the antibiofilm activity against *B. licheniformis* in CLSM. Therefore, this green chemistry approach toward the synthesis of gold nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large scale synthesis of other inorganic materials.

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