GREEN SYNTHESIS OF SILVER NANOPARTICLES USING RHIZOME DERIVED CALLUS EXTRACT OF *CURCUMA AMADA* (MANGO GINGER) ROXB

[2]Suman Polaki and [1]Gopi Mamidi*
[1]Department of Chemistry, Govt. Degree College, Baruva, Dr.B.R.Ambedkar University, Atcherla, Andhra Pradesh.
[2]Department of Biotechnology, Dr VS Krishna Govt. Degree College, Visakhapatnam, Andhra University, Andhra Pradesh. =

Abstract: Plant mediated synthesis of nanoparticles are an increasing economical demand due to the wide applications in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. In the current study, green synthesis of silver nanoparticles by using root derived callus extracts of Curcuma amada (Mango Ginger) Roxburgh. The extract incubated with $AgNO_3$ showed gradual change in the extract color from greenish to reddish brown it indicates the synthesis of silver nanoparticles. UV-Vis absorption spectroscopy color change observed was due to excitation of surface Plasmon vibration in the silver nanoparticles. The surface plasmon resonance of AgNPs of *Curcuma amada* rhizome was found to be 410 nm. The shape of the SNPs synthesized by root derived callus extract was spherical and was found to be in the range of 75 nm by AFM. FTIR absorption spectra results conclude that the compounds attached with silver nanoparticles could be polyphenols with aromatic ring and bound amide region. SEM analysis results revealed that the synthesized silver nanoparticles were spherical in shape. The TEM and XRD analysis also results revealed that the size of the silver nanoparticles for *Curcuma amada* was found to be 31nm. The formed novel silver nanoparticles by the action of plant extract exhibited a tremendous antibacterial activity and it showed the maximum activity against bioflim forming bacteria such as *Escherichia coli* (10.1 mm), *Vibrio paraheamolyticus* (10.1 mm), *Pseudomonas aeruginosa* (8 mm), *Proteus vulgaris* (9 mm) and *Listeria monocytogens* (8 mm) and also observed that it showed no activity against *Proteus mirabilis, Salmonella enteritidis* and *Staphylococcus aureus.*

Index Terms: **Green synthesis, UV-Vis spectroscopy, FTIR, SEM, TEM, XRD, Silver nanoparticles, Curcuma amada, Polyphenols.**

I. INTRODUCTION

Curcuma amada commonly called as Mango ginger belongs to the family of Zingiberaceae is a unique spice having morphological resemblance with ginger but imparts raw mango flavour. It has huge importance in Ayurveda and oldest system of medicine in Indian medicinal systems and used as an alexteric, antipyretic, aphrodisiac, diuretic, expectorant and laxative and cure itching, skin diseases, bronchitis, asthma, hiccough and inflammation due to injuries treating mamilities, jaundice and urinary disease[1]. *Curcuma amada* is mainly cultivated for its fresh edible rhizomes have the flavour and colour of mango and seeds which are rich in oil and proteins[2-3]. Nanoparticles usually referred as particles with a size up to 100 nm [4]. It is also used in biliousness, itching, skin diseases, bronchitis, asthma, hiccough and inflammation due to injuries [5-7]. According to the Unani system of medicine, it is a diuretic, maturant, emollient, expectorant, antipyretic and appetizer. It is useful against inflammation of the mouth, ear and gleet, ulcers on the male genitalia, scabies, lumbago and stomatitis [6-8]. *Curcuma amada* has pharmacological significance for a variety of ailments for example activity. Of the 130 bioactive compounds of *Curcuma*, *C. amada* rhizomes reported to have 121 bioactive compounds including curcuminoids[9] effective in skin allergies, effects on blood cholesterol and possess antioxidant properties as well as antibacterial Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Specific surface is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. As specific surface area of nanoparticles is increased, their biological effectiveness can increase in surface energy[10]. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and micro organisms commonly present in medical and industrial processes[11]. The most widely used and known applications of silver and silver nanoparticles are in medical industry. These include topical ointments and creams containing silver to prevent infection of burns and open wounds[12]. Production of nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production of nanoparticles. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using micro organisms[13], enzyme[14], and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticles can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture [15]. It can also suitably scaled up for large-scale synthesis of nanoparticles. The synthesis of pure metallic nanoparticles of silver by the reduction of Ag^+ and Au^{3+} ions using Neem (*Azadirachta indica)* leaf broth[16]. However, little has been carried out about engineering approaches such as rapid nanoparticles synthesis using plant leaf extracts and size control of the synthesized nanoparticles. The times required for more than 90% reduction of Ag^+ and Au^{3+} ions using Neem leaf broth were about 4 and 2 h, respectively. If biological synthesis of nanoparticles can compete with chemical methods, there is a need to achieve faster synthesis rates. The exact mechanism of silver nanoparticles synthesis by plant extracts is not yet fully understood. Only participation of phenolics, proteins and reducing agents in their synthesis has been speculated. In the present study, we screened coastal sand dune species *Curcuma amada* leaf extracts for extracellular nanoparticles synthesis, characterized by using UV- visible spectroscopy, SEM, AFM and FT-IR.

II. Experimental

Plant material and preparation of the extract

Fresh *Curcuma amada* plants with rhizome were collected from the Araku hills, Eastern Ghats of Andhra Pradesh, India. The specimen was certified by Department of Botany, Andhra University, Visakhapatnam and documented in the department Herbaria, college of science and technology, Andhra University, India, during 2018. The experimental chemicals were purchased from Sigma Chemicals (Chennai).

Sample preparation for synthesis of Silver Nanoparticles

One month old compact, hard greenish white callus derived from root explants was used to obtain the callus extract in our lab [17]. The callus was washed twice with sterile distilled water to remove medium components before grinding. Approximate 20 g of callus was crushed in 100 ml of sterile distilled water in mortar and pestle. The resulting extract was filtered through filter paper (Whatmann No.1) and used for the synthesis of silver nanoparticles. 10 ml suspension of callus culture was added to 90 ml aqueous solution of silver nitrate (1mM) solution separately for reduction in to Ag^+ ions and incubated at room temperature (35° C) for about 24 hours. The primary detection of synthesized silver nanoparticles was carried out in the reaction mixture by observing the colour change of the medium from greenish to dark brown. After 5h of incubation the silver nanoparticles were isolated and concentrated by repeated (4-5 times) centrifugation of the reaction mixture at 10,000 ×**g** for 10 min. The supernatant was replaced by distilled each time and suspension stored as lyophilized powder for the optical measurements [18].

Atomic Force Microscope

Purified SNP in suspension was also characterized their morphology using a VEeco diNanoscope 3D AFM (Atomic Force Microscope). A small volume of sample was spread on a well-cleaned glass cover slip surface mounted on the AFM stub, and was dried with nitrogen flow at room temperature. Images were obtained in tapping mode using a silicon probe cantilever of 125 µm length, resonance frequency 209-286 kHz, spring constant 20-80 nm^{-1} minimum of five images for each sample were obtained with AFM and analyzed to ensure reproducible results.

Fourier Transform Infra Red Spectroscope

To identify Silver nanoparticles associated biomolecules, the Fourier transform infra red spectra of washed and purified Silver nanoparticles powder were recorded on the Nicolet Avatar 660 FT-IR Spectroscopy (Nicolet, USA) using KBr pellets. To obtain good signal to noise ratio, 256 scans of Silver nanoparticles were taken in the range of 400-4000 cm^{-1} and the resolution was kept as 4 cm^{-1}

Isolation of Biofilm Bacteria from Boat Hull

The present study samples were collected from the Visakhapatnam port harbour (Meghadri creek, Fishing harbour, Lat 11º26'N; Log 79º46' E) during the period of August - October 2018. The bottom of the boat was gently swabbed with a sterile cotton swab, placed in tubes containing 10 mL sterile water. Then they were inoculated in specific media for the isolation of microbes. The bioflilm bacterial strains used in the antibacterial assay were isolated by the pour plate technique [19].

Antibacterial Assay

Antibacterial activity of the green synthesis of silver nanoparticles was assessed using the standard agar diffusion method with 6 mm diameter Whatmann No.1 filter paper discs (Becerro *et al.,* 1994). In this method 50 μl of silver nanoparticles prepared from callus extract was mixed in 1 ml of distilled water and applied to sterile paper discs of 6 mm diameter and standard antibiotic disc (ampicillin and tetracycline) used for control. Zobell marine agar was used for the antimicrobial test. Before the antibacterial assay the biofilm forming bacteria such as *Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, Escherichia coli, Listeria monocytogens, Salmonella enteritidis, Staphylococcus aureus and Vibrio paraheamolyticus* were inoculated into the Zobell marine agar plates and incubated at 27º C for 24 hours. Inhibition of zone was measured after 24 - 48 h of inhibition.

III. RESULTS AND DISCUSSION

The callus extract was used for the synthesis of silver nanoparticles. The reaction started with in first hour of the incubation with silver nitrate (1 mM). This was confirmed by the appearance of brown colour in the reaction mixture. UV-Vis absorption spectroscopy is an important technique to monitor the formation and stability of metal nanoparticles in aqueous solution. The absorption spectrum of metal nanoparticles is sensitive to several factors, including particle size, shape, and particle–particle interaction (agglomeration) with the medium [3]. The yellow colour changed to brown colour indicates the synthesis of silver nanoparticle. The color change observed was due to excitation of surface Plasmon vibration in the silver nanoparticles. The surface plasmon resonance of AgNPs of *Curcuma amada* rhizome was found to be 410 nm as shown in fig 1. The shape of the SNPs synthesized by rhizome derived callus extract was spherical and was found to be in the range of 75 nm by AFM as shown in Fig 2. Finally, confirmed the synthesis of spherical green synthesis of silver nanoparticles in the reaction mixture. The larger size of the nanoparticles might be due to the capping of nanoparticles by polyphenols with aromatic ring and bound amide as confirmed from FT-IR analysis as shown in fig 3.

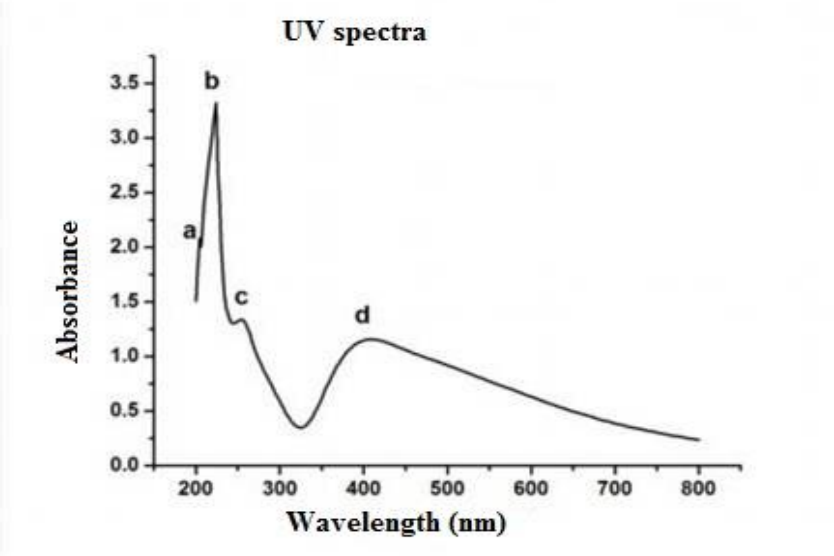

Fig 1: UV- Spectra of silver nanoparticle synthesized by *C. amada*

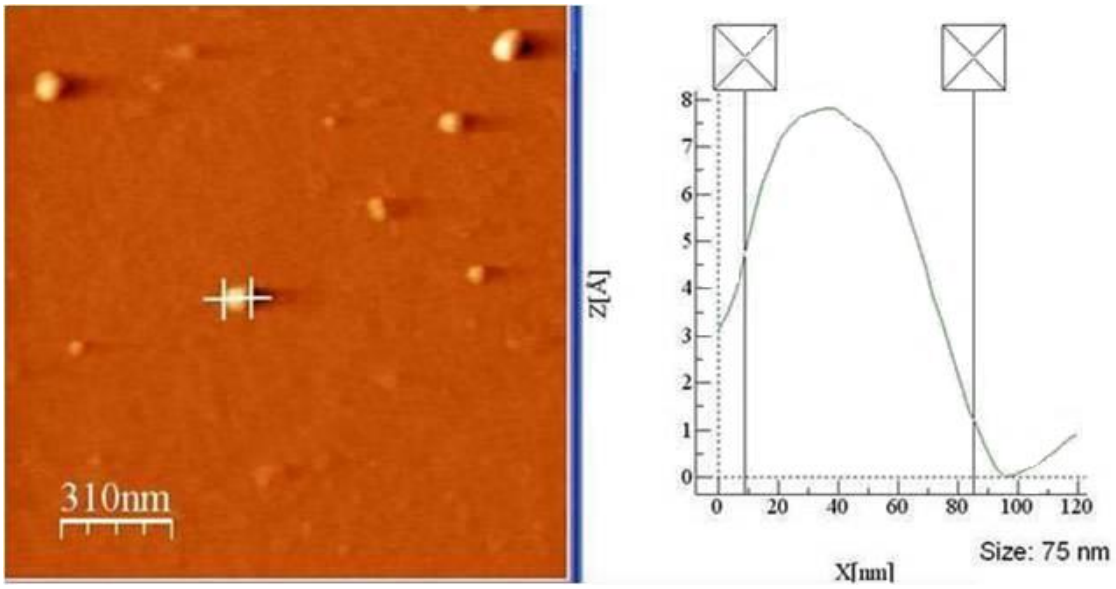

Fig 2: AFM images of silver nanoparticles of *Curcuma amada*.

Representative absorption spectrum of the nanoparticles obtained in the present study is showed in fig 3. Among them, the absorption peak at 1020 cm^{-1} can be assigned as absorption peaks of C-O-C- or -C-O-, also the peak at 1020 - 1091 cm^{-1} corresponds to C-N stretching vibrations of aliphatic amines or to alcohols or phenols representing the presence of polyphenols [20]. The absorbance peak at 1265 and 1384 – 1460 cm^{-1} correspond to the amide III and II group respectively. The peak at 1624 cm^{-1} is associated with stretch vibration of C=C- [21] and is assigned to the amide 1 bonds of proteins. The absorption at about 1384 cm^{-1} is notably enhanced indicating residual amount of NO_3 in the solution [22]. The peak at 1539 cm^{-1} may be assigned to symmetric stretching vibrations of –COO- (carboxyl ate ion) groups of amino acid residues with free carboxyl ate groups in the protein [23]. The peak at 3427 cm^{-1} indicates polyphenolic OH group along with the peak of 882 cm^{-1} which represents the aromatic ring C-H vibrations, indicate the involvement of free catechin [24]. This suggests the attachment of some polyphenolic components on to silver nanoparticles. This means the polyphenols attached to silver nano particles may have at least one aromatic ring. The peaks at 1000-1200 cm^{-1} indicate C-O single bond and peaks at 1620-1636 cm^{-1} represent carbonyl groups (C=O) from polyphenols such as catechin gallate, epicatechin gallate and theaflavin [25]. Result suggests that molecules attached with silver nanoparticles have free and bound amide group. These amide groups may also be in the aromatic rings. This concludes that the compounds attached with silver nanoparticles could be polyphenols with aromatic ring and bound amide region.

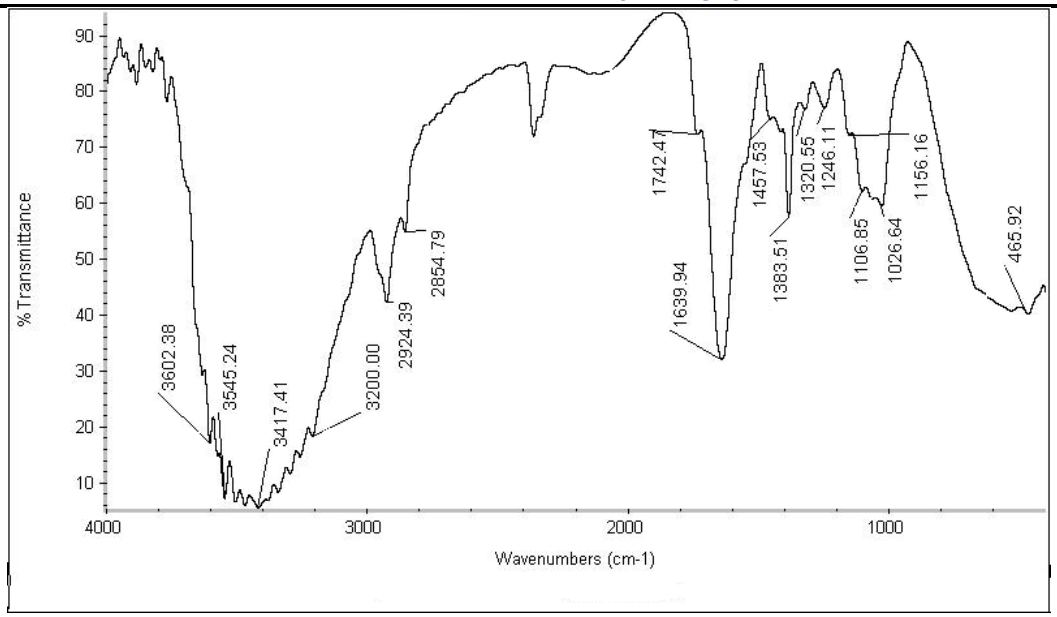

Fig 3: FTIR images of silver nanoparticles.

The metal nanoparticles has free electrons, which gives the surface plasmon resonance absorption bands, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave [26, 27]. SEM analysis results revealed that the synthesized silver nanoparticles were spherical in shape as shown in Fig 4.

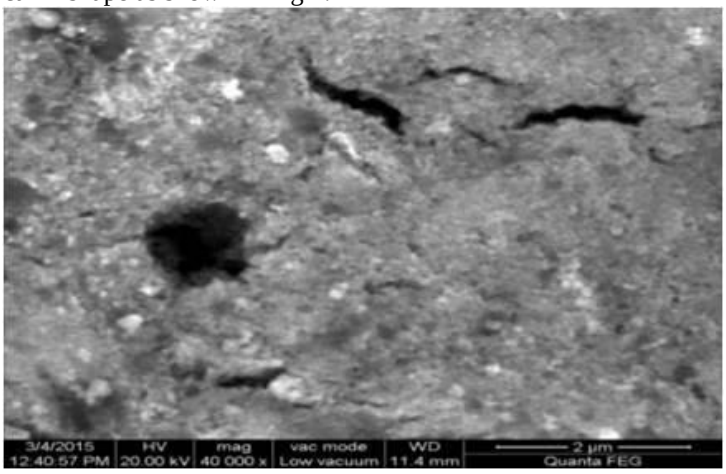

Fig 4: Scanning electron micrograph of the silver nanoparticles of *Curcuma amada*

The morphology of silver nanoparticles was further confirmed by TEM and XRD analysis. Shape is critical parameter which effects cell uptake and the rate of site specific drug delivery from the system. Preferential interaction with specific proteins could be achieved on proper shape selection of nanomaterials. Spherical nanoparticles are good option for drug delivery; however anisotropic structures could be the best option due to their large surface area [28]. This kind of structures can make good seating and binding arrangements for the drug which can be useful for sustained drug delivery. However, sharp edges of anisotropic structures can be responsible for injury of blood vessels. The TEM and XRD analysis also revealed that the size of the silver nanoparticles for *Curcuma amada* was found to be 31nm as shown in Fig 5, 6.

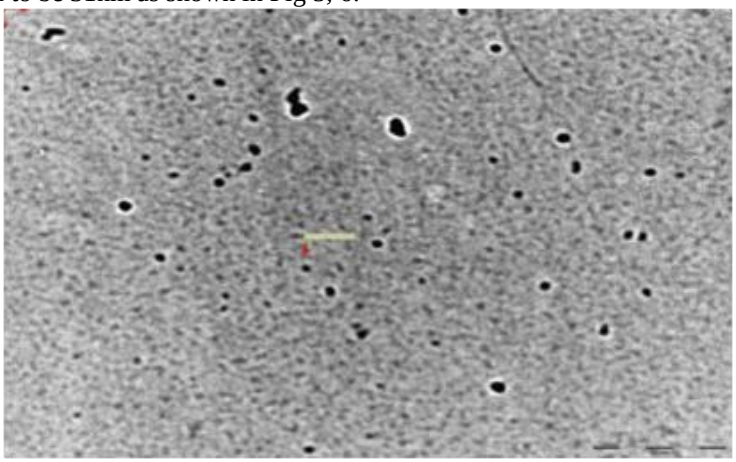

Fig 5: TEM micrographs of Silver nanoparticles synthesized using *Curcuma amada*

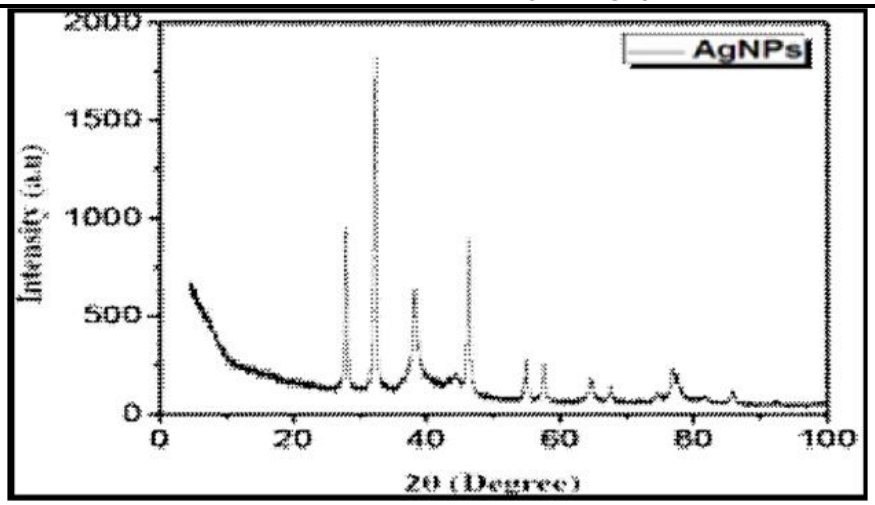

Fig 6: XRD pattern of silver nanoparticles synthesized using *Curcuma amada*

Identification of Biofilm Microorganisms

The incidence of total bacterial population was increased during every month intervals on surface of boat hull. The count varied between 12×106 to 45×106 CFU mL^{1}. The biofilm forming bacteria (*Pseudomonas aeruginosa, Proteus vulgaris, Proteus mirabilis, E.coli, Listeria monocytogens, Salmonella enteritidis, Staphylococcus aureus and Vibrio paraheamolyticus*) were isolated from the boat hull and identified using Bergey's Manual.

Antibacterial Assay

The callus derived silver nano particles was showed the maximum activity against *E.coli* (10.1 mm), *V. paraheamolyticus* (10.1 mm), *P. aeruginosa* (8 mm), *Proteus vulgaris* (9 mm) and *L. monocytogens* (8 mm) as shown in fig 7 and also observed that it showed no activity against *Proteus mirabilis, Salmonella enteritidis,* and *Staphylococcus aureus.* The antimicrobial properties of silver compounds and silver ions had been historically recognized and applied in a wide range of applications from disinfecting medical devices and home appliances to water treatment [26]. Silver ion and silver based compounds are highly toxic to micro organisms, showing strong biocidal effect against microbial species. The silver nano particles produced by microbes and plant extracts are known to exhibit potent antimicrobial activity. A similar observation has been made with the silver nano particles produced by callus extract to have antimicrobial activity against the biofilm forming bacteria (*E. coli, V. paraheamolyticus, P. aeruginosa, Proteus vulgaris* and *L. monocytogens).*

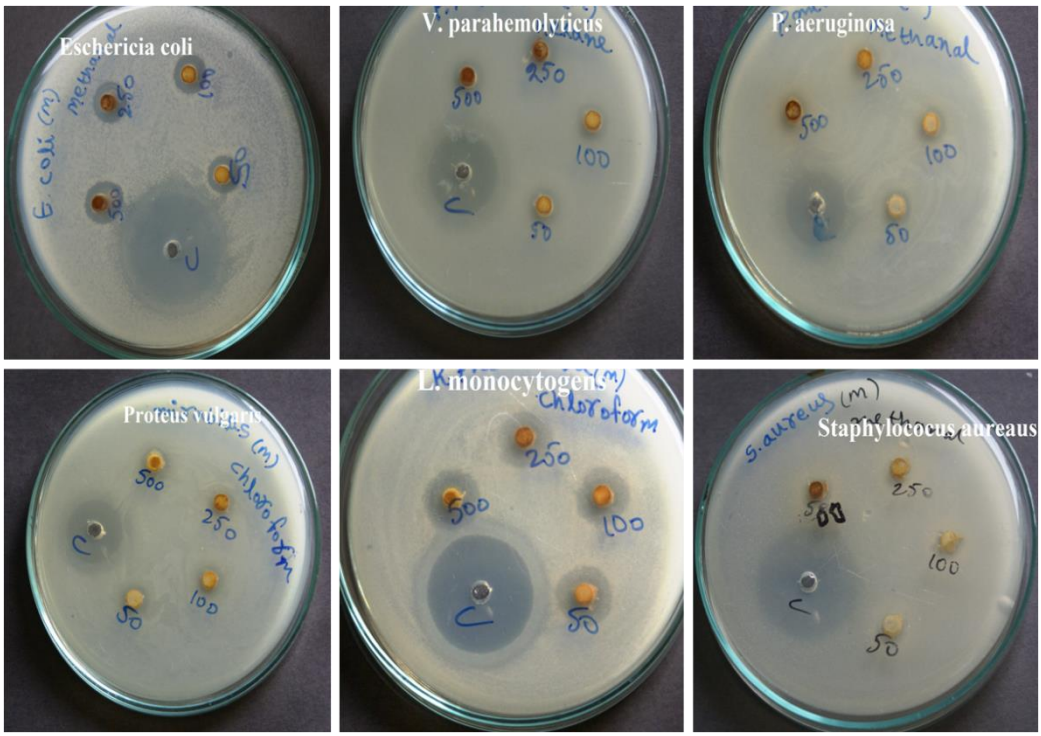

Fig 7: Zone of inhibitions of Silver nanoparticles synthesized using *Curcuma amada* against different pathogens.

IV. CONCLUSION

Our investigation reveals, the bioreduction of aqueous Ag^{+} ions by the callus extract of the *Curcuma amada* has been demonstrated. The reduction of the metal ions through the callus extracts leading to the formation of silver nanoparticles of fairly well – defined dimensions. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages. applications of such eco- friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications make this method potentially exciting for the large- scale synthesis of other inorganic materials (nanoparticles).

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