Short Communication

Investigation of Antimicrobial Activity of Plant-Mediated Green Synthesis of Silver Nanoparticles

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Abstract

For the past couple of years, the synthesis of silver nanoparticles via the green route had gained much attention due to its cost-effectiveness and eco-friendly technology. Here we report for the first time, to the best of our knowledge the synthesis of silver nanoparticles from aqueous leaf extract of Curcuma Caesia plant and investigated its antimicrobial activity. The bio-reduced silver nanoparticles were characterized using UV-Visible spectroscopy, FTIR, XRD, TEM, and SEM. XRD showed the crystalline nature of nanoparticles, observed peaks (111), (220), (200), and (311). The formation of AgNPs is confirmed by recording the UV–vis absorption spectra for surface plasmon resonance (SPR) peak (~446 nm). The antimicrobial examination of the silver nanoparticles shows great antibacterial action because of high zones of hindrance against test microscopic organisms.

Keywords: *Green synthesis, Silver nanoparticles, XRD, TEM, Antimicrobial activity.*

1. INRODUCTION

The green synthesis process includes a myriad of promising approaches for the assembly of metal nanoparticles with desired properties. The utilization of plant material for the production of silver nanoparticles has drawn attention due to its rapid, ecofriendly, and cost-effectiveness.

The traditional Indian medicine system suggests the use of medicinal and aromatic plants to treat various human diseases. Plant extracts containing phytoconstituents that are biologically and pharmacologically active, which are known to be excellent reducing and capping agents that will be custom to synthesize nanoparticles effectively within a short period [1]. As compared with microbial synthesis, phytosynthesis is rapid, as the latter requires no aseptic conditions and leads to the formation of stable nanoparticles [2]. On various fronts owing to their improved properties depending on the size, distribution, and morphology, the application of nanomaterials is also developing rapidly. In natural food, there is a need to build up the ecological cordial methods to stay away from the poisonous synthetic compounds in the union conventions to evade unfriendly impact in clinical applications. Within the last couple of years, the research thirst within the field of metal nanoparticles was emerged owing to their physiological as well as biological properties. To tackle those disease-causing microbes, the development of new antimicrobial agents is necessary. Most of the studies are highlighting the application of metal nanoparticles as an antimicrobial agent in addition they found application as an efficient antimicrobial drug. Nanoparticles are used for all of the above purposes, metal nanoparticles are

considered to be the most promising as they contain significant antimicrobial properties due to their large surface area to volume ratio, which is widely recognized by the research studies. Researches are interested due to the increased resistance of microorganisms against metal ions, antibiotics, and the development of drug-resistant strains [3]. Owing to surface plasmon resonance property, AgNPs found application as sensors such as chemiluminescence sensor, colorimetric sensors, etc [4]. Toxicity studies revealed that AgNPs are less toxic to mammalian cells than other metal nanoparticles [5]. Penetration into the cell via cell membrane is possible since they are relatively smaller in size, hence serving as a potential antimicrobial agent [6]. The nanoparticles are usually synthesized by reducing silver salts with appropriate reducing agents. For green synthesis, the reducing agents are obtained from the extraction of various parts of the plant such as root, leaf, fruit, bark, etc. In this process, the average size of AgNPs is influenced by various factors including extract concentration, temperature, pH, etc [7]. Many research papers reported the synthesis of silver nanoparticles using plant extracts such as Ocimum Sanctum stems and roots [8], leaves [9], Murraya Koenigii [10], Ficus benghalensis [11], Mulberry leaves [12]. However, here we report for the first time, the synthesis of silver nanoparticles using Curcuma Caesia leaf extract in the aqueous solution by introducing the solution of silver nitrate.

The Curcuma species are broadly used for their huge pharmacological advantages. From the previous centuries, the Curcuma species were used in the traditional clinical frameworks to treat respiratory issues, different agonies, stomach related ailments, incendiary sicknesses, wound mending, hypertension, hypercholestero-lemia, microbial contaminations, circulatory irregularities, and even malignant growth (Dosoky and Setzer, 2018 [13]). Curcuma caesia Roxb is a less known and immaculate medication. The variety Curcuma is an individual from the ginger (family Zingiberaceae), which contains more than 70 types of rhizomatous herbs. The plants have loads of potential as far as restorative properties. Writing uncovers its calming, hepatoprotective, blood purifier, cell reinforcement, antiasthmatic, hostile to the tumor, stomachic, and carminative properties. Different plant parts like root, stem, bark, leaf, and blossoms can be utilized for the same. The Curcuma caesia showed the various biological activities, for example, Anthelmintic (Gill Randeep et al.) [14], depressant action (Karmakar I, et al.) [15], antibacterial (Ahmed et al.) [16], anti-inflammatory and cell reinforcement exercises (Angel et al.) [17]. Thus, the current work was planned to synthesize the silver nanoparticles from Curcuma Caesia Roxb leaves employing green course and examining their antimicrobial action against the clinical microorganisms.

2. MATERIALS AND METHODS

2.1. Preparation of Leaf Extract

Fresh leaves of Curcuma Caesia were collected from Lingam Gardens and Herbals, Chennai, India. The fresh leaf extract used for the reduction of silver ions to silver nanoparticles was prepared by taking 20g of thoroughly washed finely cut leaves in 500 ml Erlenmeyer flask with 100 ml of distilled water and allowed for boiling the mixture for about 5 min. before decanting it. In addition, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C for further processes.

2.2. Synthesis of Silver Nanoparticles

The aqueous solution of silver nitrate ($AgNO_3$, Merck, India) of 1mM is prepared in a 100 ml standard flask and the solution is added to the leaf extract solution at room temperature. To 10 ml of Curcuma Caesia, leaf extract was added into 90 ml of the aqueous solution of 1 mM silver nitrate for reduction of Ag^+

ions into silver nanoparticles and incubated for 24 hours.

Figure 1. *Picture of Curcuma Caesia plant, leaves, and rhizomes.*

3. CHARACTERIZATION TECHNIQUES

UV–vis absorption spectra were measured using a Shimadzu UV-1601 spectrophotometer. Crystalline metallic silver nanoparticles were examined using an X-ray diffractometer (Bruker D8 Advance) equipped with a Cu Kα radiation source at a setting of 40 kV/35 mA. All X-ray diffraction (XRD) data were obtained under the experimental conditions in the angular range $3° \leq 2\theta \leq 50°$. Fourier transform infrared (FT-IR) spectra for Curcuma Caesia leaf extract and silver nanoparticles were obtained in the range 4000- 400 cm^{-1} with an IR-Prestige-21 Shimadzu FT-IR spectrophotometer, by KBr pellet method. Scanning electron-Energy Dispersive X-ray Photoelectron spectroscopy (SEM-EDAX) analysis of synthesized silver nanoparticles was done using a JSM-6390 SEM machine. High-Resolution Transmission Electron Microscopy (TEM) analysis of silver nanoparticles was done using JEM 2100.

4. RESULTS AND DISCUSSIONS

4.1. UV-Visible Spectroscopy

The formation and stability of AgNPs in distilled water are confirmed using UV-vis spectrophotometer in a range of wavelengths from 200-800 nm. As soon as, *Curcuma Caesia* leaves extract was mixed in the aqueous solution of silver ion complex, the reduction of pure Ag+ ions to Ag^{o} was confirmed by measuring the UV–vis spectrum of the reaction media. UV–vis spectra were recorded as a function of reaction time, Fig. 2 shows the gradual color change of the solution from yellow to dark brown. Fig.2a shows the UV-Visible spectrum of silver nanoparticles and the surface plasmon resonance at 446nm showed the formation of AgNPs.

4.2. Fourier-Transform Infrared Spectroscopy

To investigate the functional groups of *Curcuma Caesia* leaves extract, an FT-IR study was carried out and the spectra are shown in Figure 3. The *Curcuma caesia* extract displays several absorption peaks, reflecting its complex nature. A peak at 3478cm^{-1} results due to the stretching of the N–H bond of amino groups and is indicative of bonded hydroxyl (-OH) group. The strong absorption peak at 2883cm^{-1} could be assigned to –CH stretching vibrations of –CH3 and –CH2 functional groups. The shoulder peak at 1641 cm-1 was assigned for the C=O group of amide groups. The absorption peaks at 1083 cm −1 could be attributed to the presence of C–O stretching is carboxyl. FTIR study indicates that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of *Curcuma caesia* leaves extract are mainly involved in the reduction of silver ions to silver nanoparticles.

Figure 2. *(A) Silver Nitrate Solution (AgNO3), (B) Curcuma Caesia Leaf Extract, (C) AgNO3 + Leaf Extract, (D) AgNO3 + Leaf Extract After 60 min.*

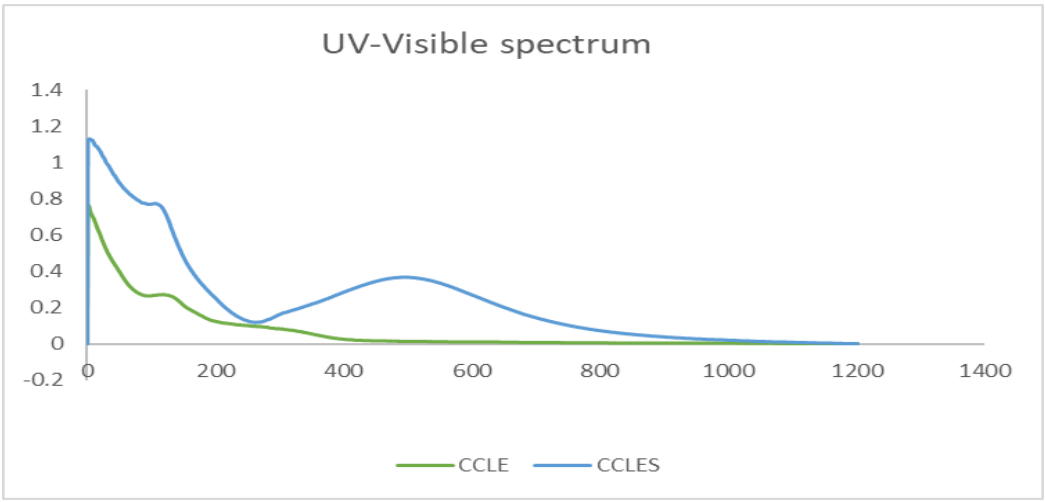


Figure 2a. *UV-visible spectrum of CCLE (leaf Extract) and CCLES (AgNPs).*

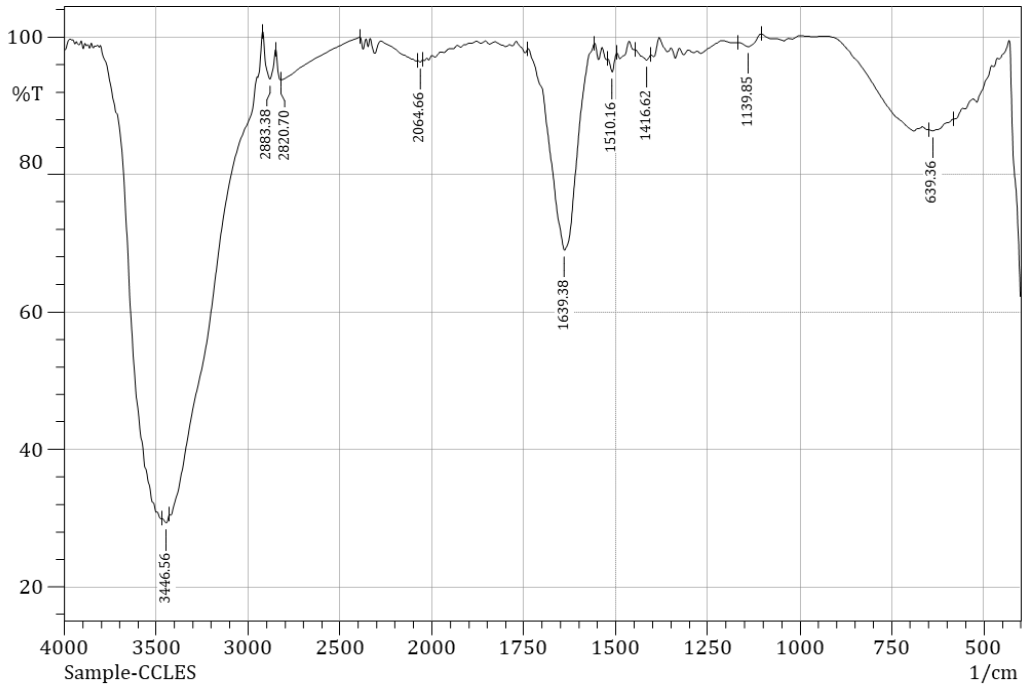


Figure 3. *FTIR Spectrum of Leaf Extract.*

4.3. Scanning Electron Microscopy-Energy Dispersive Xray Photoelectron Spectroscopy

The suspended silver nanoparticles in sterile distilled water were used for scan electron microscope analysis by fabricating a drop of suspension onto clean electric stubs and allowing water to completely evaporate. The SEM image of silver nanoparticles, Fig. 4 showed a flake-like structure and relatively uniform shape of nanoparticle formation with a diameter range of 200-350 nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. Elemental composition and atomic % was obtained from EDAX.

Elements	Line Type	Wt%	Atomic %
O	K series	16.07	50.86
Cl	K series	10.15	14.5
Ag	L series	73.79	34.65

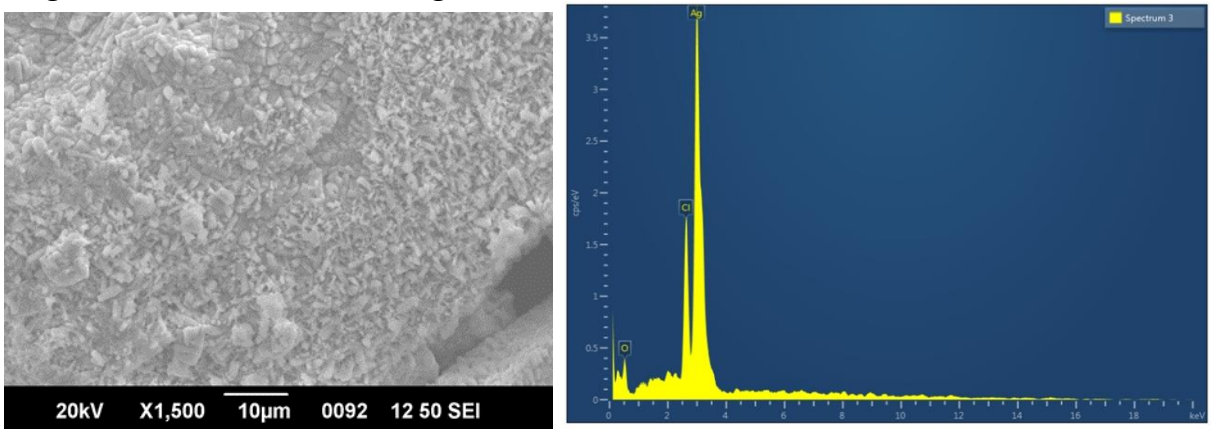
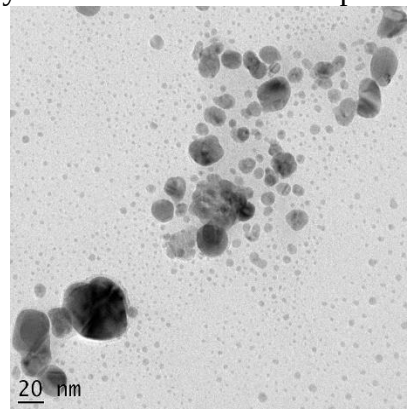
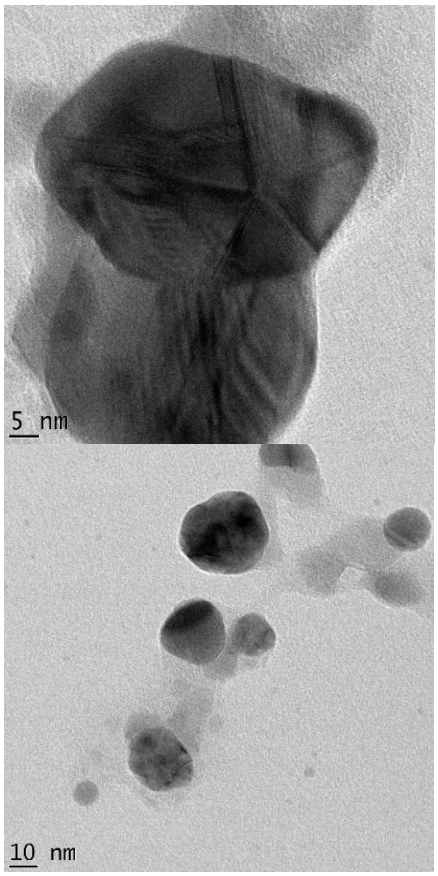


Figure 4. *SEM-EDAX of silver nanoparticles.*

4.4. Transmission Electron Microscopy

Transmission Electron Microscope provides images with high spatial resolution. TEM results show that the synthesized nanoparticles are in nanoscale and uniform. The obtained nanoparticles are mostly spherical and have a diameter in the range of 10-50 nm shown in Fig.5 In addition, the crystalline nature of nanoparticles was revealed using SAED pattern. The results showed a ring pattern with light spots on the dark field describes the crystalline nature of the sample.





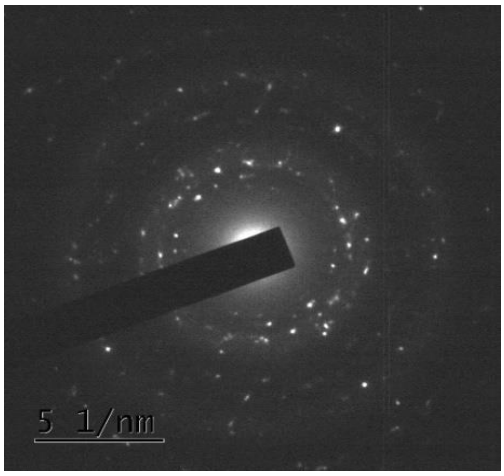


Figure 5. *TEM images of synthesized nanoparticles and the SAED pattern.*

4.5. X-Ray Diffraction

X-ray diffractograms was used to analyze the crystalline structure of the silver nanoparticles. The XRD pattern showed numbers of Bragg reflections that may be indexed based on the face-centered cubic structure of silver. A comparison of our XRD spectrum with the JCPDS file no. 04-0783 confirmed the formation of silver particles in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38. 21°, 46.30°, 67.49°, and 76.84° corresponding to (111), (200), (220), and (311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver. X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of silver ions by the *Curcuma caesia* leaf extract are crystalline. The unassigned peaks at 2θ = 27.90°, 32.31°, and 46.30° in Fig.6 are thought to be related to crystalline and amorphous organic phases. It was found that the average size from XRD data and using the Debye-Scherrer equation was approx-imately 16 nm. The presence of structural peaks in XRD patterns and the average crystalline size around 16 nm clearly illustrate that the AgNPs synthesized by our green route were nanocrystalline. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using the Debye-Scherrer equation [18, 19]:

$$D = 0.9\lambda/\beta \cos\theta,$$

where D is the crystallite size of AgNPs, λ is the wavelength of the X-ray source (0.1540 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle.

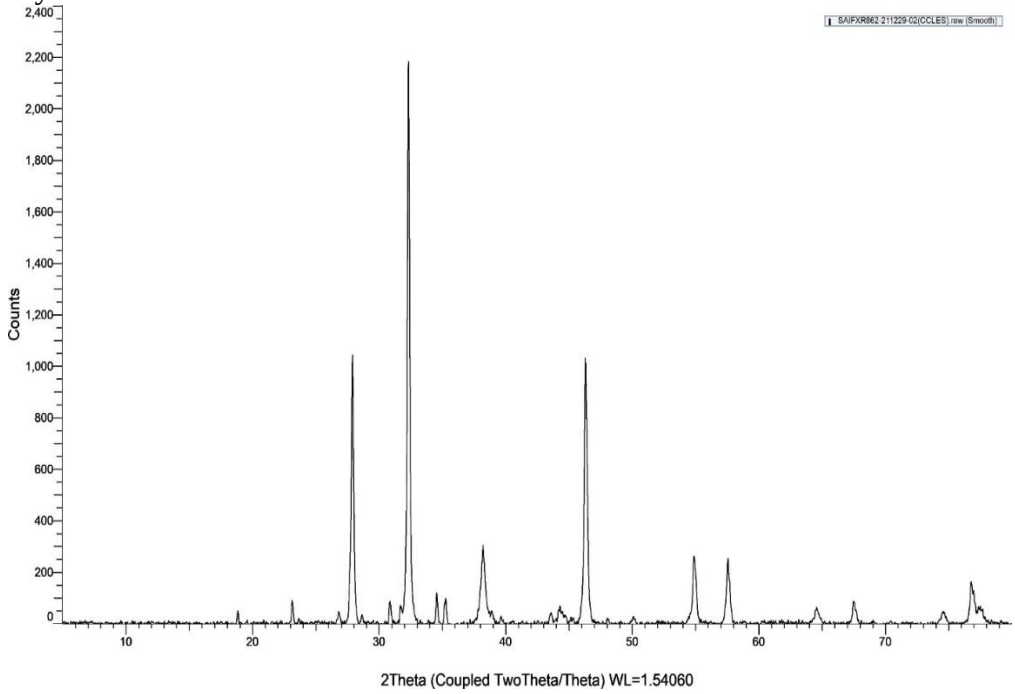


Figure 6. *XRD of synthesized nanoparticles.*

5. ANTIMICROBIAL ASSAY

5.1. Antibacterial Assessment

5.1.1. Inoculum Preparation

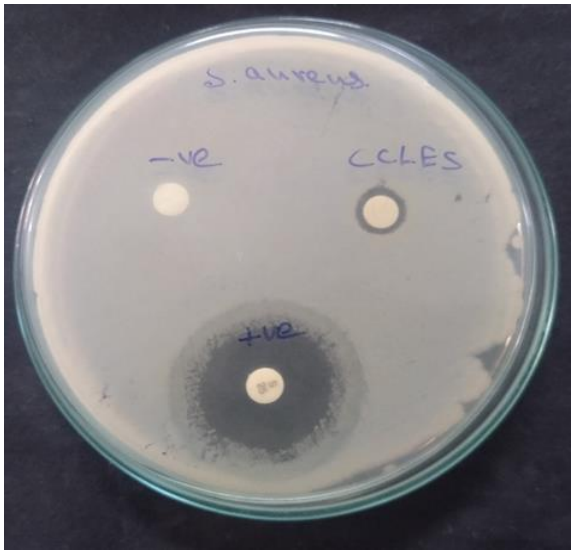
Pure culture from the plate was inoculated into the Nutrient Agar plate and sub-cultured at 37ºC for 24 h. The inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum Used for Antimicrobial test.

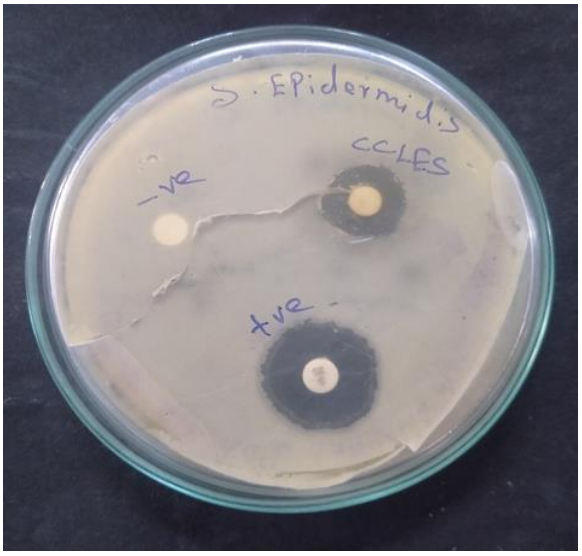
5.1.2. Assessment of Antibacterial Test

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi-Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 1210C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well, and poured Petri plates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis E.coli, Staphylococcus aureus, Klebsiella pneumonia, and Staphylococcus epidermidis. Finally, About 10 µL of the sample (CCLES) was loaded onto the disc then placed on the surface of the Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc (Table.2) and measured with a transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters shown in Fig 7. The absence of zone inhibition was interpreted as the absence of activity (Kohner et al., [20]; Mathabe et al., [21]). The activities are expressed as resistant if the zone of inhibition was less than 7 mm, intermediate (8-10 mm), and sensitive if more than 11 mm (Assam et al., 2010) [22].

Table 1.

Bacterial Strain	**Zone of inhibition in mm CCLES**	**Positive control (Streptomycin)**
Staphylococcus aureus (G+)	10	23
Staphylcoccus epidermidis (G+)	15	20
E.coli (G-)	10	21
Klebsiella pneumoniae (G-)	19	22





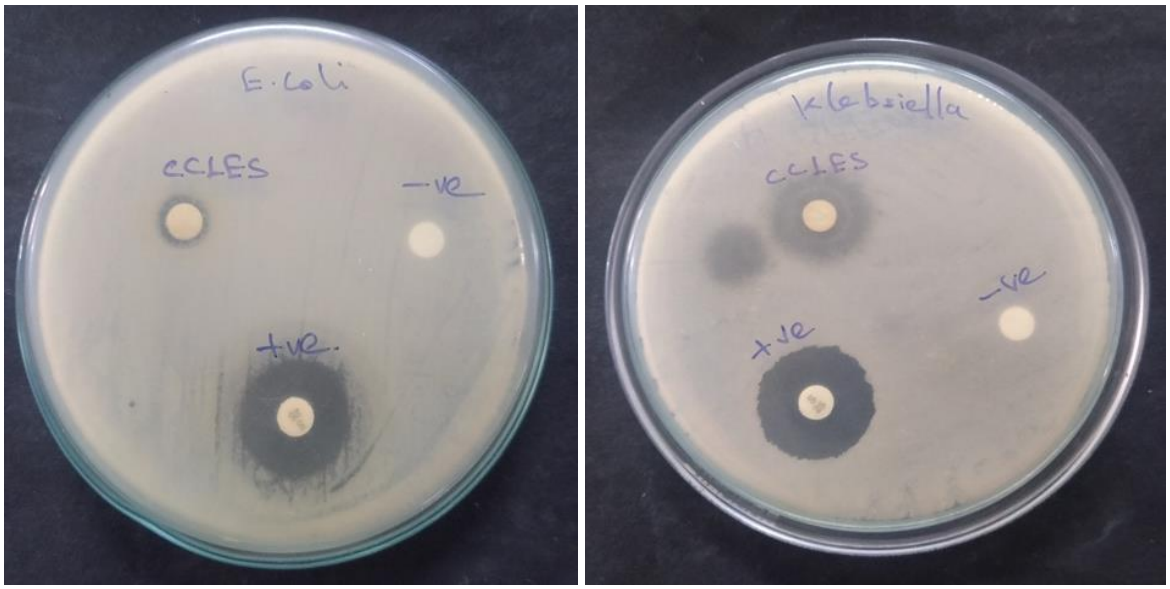


Figure 7. *Anti-bacterial activity of CCLES aqueous extract.*

5.2 Antifungal assessment

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer)[23] method. Fungi strains *Candida tropicalis* and *Candida albicans* were swabbed using sterile cotton swabs in an SDA agar plate. 10 µL of each sample (CCLES) was respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of the SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones (Table.2) were examined around the disc and measured with a transparent ruler in millimeters shown in Fig 8.

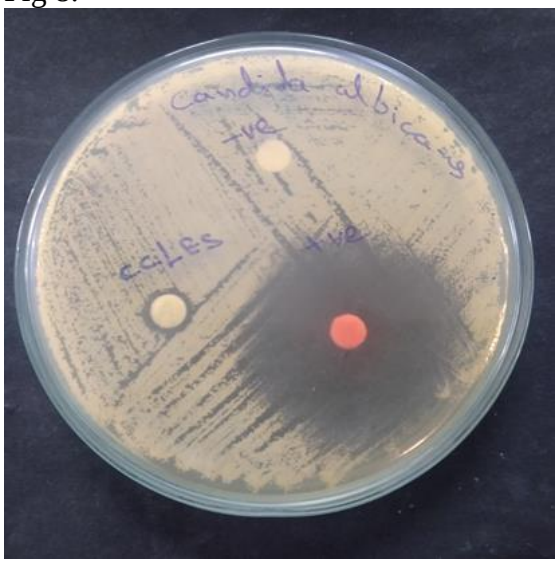


Figure 8. *Anti-fungal activity of CCLES aqueous extract.*

Table 2.

Fungal Starins	**Zone of inhibition CCLES**	**Positive control(Fluconazole)**
Candida tropicalis	8	33
Candida albicans	10	36

CONCLUSION

Nanoparticles are considered as building blocks of nanotechnology. The surface plasmon resonance property of silver

nanoparticles makes them a notch material for developing various biosensors. Here we have reported for the first time, the synthesis of silver nanoparticles using *Curcuma caesia* leaf extract in the aqueous solution at room temperature via a green chemistry approach. The structural characterizations of the samples are performed using SEM-EDAX, TEM, FTIR, and XRD analysis. The UV-visible optical absorption properties are measured and found the shift of SPR wavelengths with the average particle size of the synthesized samples. In addition, the biosynthesized silver nanoparticles using *Curcuma caesia* leaves extract proved to be excellent against pathogenic bacterial and fungi strains. The antimicrobial activity is well demonstrated by the disc diffusion method. Plant extract is being eco-friendly and very cost-effective; the prepared nanoparticles can be used as bactericidal agents, water purification, etc, presented method can be an economic and effective alternative for the large-scale synthesis of silver nanoparticles in nanotechnology processing industries. The excellent antimicrobial activity grants the utilization of silver nanoparticles in antibacterial coating material for implantable devices and medicinal materials to prevent infections and encourage wound healing processes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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