RESEARCH ARTICLE

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Exploiting the antimicrobial potential of an invasive aquatic weed *Eichhornia crassipes* mediated silver nanoparticles on multi drug resistant bacteria

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ABSTRACT

Resistance against commonly used antibiotics is an emerging global threat, mostly due to the evolution of multi-drug-resistant bacterial strains. This necessitates the development of cost effective and eco-friendly alternatives for antibiotics. Green synthesized silver nanoparticles have gained attention due to their unique physicochemical properties and antimicrobial properties. In this study, silver nanoparticles (AgNPs) were synthesized using the aqueous extract of *Eichhornia crassipes* (EC) leaves. EC is an invasive aquatic weed which depletes oxygen levels in freshwater environments thereby contributing to eutrophication. The synthesized EC-AgNPs were characterized using UV-Visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), X-Ray diffraction (XRD), Zeta potential (ZP) analysis, Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) to determine their size, morphology, surface charge and crystalline nature. Bacteria used in this study, viz; *E. coli, K. pneumoniae, S. aureus, and P. mirabilis* were identified as multidrug resistant (MRD) and extended spectrum beta-lactamase (ESBL) producers. *P. aeruginosa* was assessed for its biofilm formation ability by the crystal violet assay. The antibacterial efficacy of the EC-AgNPs at various concentrations was tested against these MRD pathogens by disc diffusion assay and minimum inhibitory concentration was studied. Results demonstrated significant inhibition zones even at very low concentrations highlighting the potential of EC-AgNPs as an effective antimicrobial. EC-AgNPs was found to be a potent biofilm inhibitor on *P. aeruginosa*. The study emphasizes the use of aquatic weeds like *E. crassipes* for medical and pharmaceutical purposes as an effective remedy for their efficient removal from environment and putting to beneficial use.

Key words: water hyacinth, silver nanoparticles, green synthesis, multi-drug-resistant bacteria, ESBL

Introduction

Nanotechnology is an expanding area of research which deals with the synthesis, design, characterization and application of materials with nano dimensions from 1-100 nm (Bayda, 2020). Nanoparticles are widely used in various applications because of their characteristic properties like relatively large surface area to volume, increased reactivity, and enhanced mechanical strength (Ealia et al., 2017). The size, structure and the composition of a newly synthesized nanoparticle determines its efficiency, especially as a therapeutic agent with broad biological properties (Pandit et al., 2022). One of the popular types of nanoparticles are the silver nanoparticles which are known to possess antibacterial, antioxidant properties rendering them useful in biomedical, industrial and household products (Zhang et al., 2016). Although the synthesis of nanoparticles can be achieved by various methods like physical, chemical and biological, the most preferred method is biological or green synthesis (Ying et al., 2022). This method uses plant, microbes etc. as reducing agents for nanoparticles synthesis, instead of metallic ions. Green synthesis can be used to harness harmful whole weeds, or their parts thereby reducing their noxious effect on the ecosystem which in turn ensures a rapid, safe, eco-friendly and economic method for nanoparticle synthesis.

Silver nanoparticles are smaller than microorganisms and they can easily penetrate the cell wall and rupture the cell membrane thereby exerting their antibacterial action (Siddiqui et al., 2018). Rise of antibiotic-resistant bacteria is an emerging concern globally and possess a grave threat resulting in spread of nosocomial infections (Mancuso et al., 2021). Some of these drug-resistant bacteria possess

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Expanded- spectrum β- lactamase (ESBLs) enzymes and have the potency to inactivate β-lactam ring of antibiotics. ESBL producing bacteria usually belong to gut bacteria genus *Enterobacteriaceae* and show resistance to penicillin, cephalosporins, monobactams etc. Therefore, various attempts have been made to study the antibacterial effect of silver nanoparticles on such drug-resistant bacteria and most of these studies have shown promising results leading to the widespread acceptance of silver nanoparticles in biomedical applications (Bruna et al., 2021).

Eichhornia crassipes {*E. crassipes* (abbreviated as EC)} commonly known as water hyacinth, is a monocotyledonous free-floating aquatic macrophyte. It is considered as one of the most aggressive invasive species and one among the worst weeds in the world (Datta et al., 2021). It spreads like a mat on the surface of water preventing the penetration of sunlight, depleting oxygen, nutrients etc. thereby posing a threat to aquatic life.

The present study involves the green synthesis of silver nanoparticles (AgNPs) from aqueous extract of leaves of E. crassipes, followed by its physico-chemical characterization. The nanoparticles are abbreviated as EC-AgNPs. The antimicrobial activity of these nanoparticles on ESBL producing MDR bacteria was studied. The antibiofilm activity of these nanoparticles was also assessed to evaluate their potential in inhibiting biofilm formation. The significance of this study lies in offering a better option for the development of silver nanoparticles in combating multidrug resistant pathogenic bacteria.

Materials and Methods

Chemicals, Reagents and Microorganisms

Silver nitrate was purchased from LABOGENS, India and Sodium hydroxide from HiMedia, India. The chemicals required for antibacterial activity i.e., Muller Hinton agar (MHA), Muller Hinton broth (MHB), Luria-Bertani (LB) broth and sterile antibiotic discs were purchased from HiMedia, India. 96 well plates from Maxome lab sciences, Bengaluru, India, Resazurin from Sisco laboratories pvt Ltd, Mumbai, India. The clinical isolates of *E. coli, S. aureus, P. mirabilis, K. pneumonia and P. aeuroginosa* were gifted by a laboratory.

Plant Material Collection

E. crassipes is a floating aquatic weed which grows in shallow freshwater ponds, lakes and rivers. Fresh leaves of *E. crassipes* were gathered from Vellayani lake, Thiruvananthapuram, Kerala. The lake is infested with the weeds and forms a mat like structure which floats over water surface. The collected leaves were cleaned with distilled water to remove dirt which may contaminate the extract and then they were dried in shade for seven days. Then the dried leaves were crushed using a mixer grinder and kept in an airtight container. This powder was used as the source of crude plant extract as well as for the green synthesis of silver nanoparticles as mentioned below.

Preparation of leaf extract

About 3g of powdered *E. crassipes* was weighed in a 500 mL beaker and to this, 100 mL of distilled water was added and double boiled in a water bath, with continuous stirring for 40 minutes. The mixture was then allowed to cool down and the aqueous extract was filtered out by a gauze cloth. Around 40 mL of green coloured aqueous extract was obtained after this procedure.

Green synthesis of silver nanoparticles

The silver nanoparticles were synthesized using the aqueous extract of *E. crassipes*, according to a previously reported protocol with slight modifications (Hublikar et al., 2021). To the filtered aqueous extract taken in a 250 mL conical flask, about 2 mL of 1N NaOH solution was added. To this, 3 mM silver nitrate was added and mixed well by stirring in a magnetic stirrer for 20 minutes. The reduction of silver nitrate into Ag^+ was confirmed by the appearance of a dark brown coloured sample.

The silver nanoparticle containing aqueous extract of *E. crassipes* was then centrifuged for 5 minutes at 5000 rpm. The supernatant was discarded, and the resulting pellet was dried overnight at 60°C. The dried pellet was scraped out and further ground using a mortar and pestle to get a fine powder. Then the samples were stored in an airtight micro centrifuge tube covered with aluminum foil to avoid the exposure of AgNPs to sunlight.

Characterization of EC-AgNPs

The formation of EC-AgNPs was visually observed by a colour change from yellow to reddish-brown and were characterised for the formation, size, morphology, surface charge etc. using various techniques like Uv-Visible spectroscopy, TEM, SEM, XRD, FTIR, and zeta potential (Zhang et al., 2016; Almatroudi et al., 2020). UV-Visible spectroscopy is used for the primary characterization of nanoparticles and helps to know the SPR of nanoparticles thereby confirming the formation of the same (Alim-Al-Razy et al., 2020) usually at a range of 200-820 nm. TEM analysis was performed to study the morphology and particle size of silver nanoparticles and measurements of EC-AgNPs were obtained using TEM (JEOL JEM-1010, Japan). The measurements using SEM (Zeiss EVO 18, Germany) provided the surface morphology of the nanoparticles. The

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crystalline nature of nanoparticles was analyzed using XRD (Bruker D8 ADVANCE with DAVINCI design) and the powdered pellet was used for analysis (Holder & Schaak, 2019). To look for the presence of phytochemicals in the plant extract responsible for the reduction of silver and capping of nanoparticles, FTIR was done using the FTIR instrument (Nicolet iS50, Thermo Scientific, USA) by using powdered pellets of EC-AgNPs. Zeta potential measurement was also performed to measure the surface charge of nanoparticles by light scattering method using Zetasizer by Malvern Panalytical, UK. It provides information on the aggregation status of nanoparticle solutions (Metreveli et al., 2016) and also stability of the solutions.

Antibiotic susceptibility test

The most simple and well standardized method for antibiotic susceptibility test is Disk diffusion method (Loo et al., 2018). The log phase culture of the bacteria in MHB, with approximately 1.5 X 10^8 CFU/mL which is equivalent to about 0.5 McFarland standard was prepared. The inoculum from these was spread plated on the surface of MHA plate for checking their antibiotic susceptibility. Disks of antibiotics namely Cefixime (CFM), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CRO) Cefepime (CFP), Cefpodoxime (CPD), Tetracycline (TE) were placed on the inoculated agar surface. Then the plates were incubated at 37°C for 18-20 hours. After incubation, the zones of growth inhibition around each disc were measured to the nearest mm. The diameter of the zone is proportional to the susceptibility of the isolate to the antibiotic added and depends on the diffusion rate of antibiotic through agar medium. The zone diameter was interpreted using standards published by CLSI for each antibiotic and results classified accordingly as resistant, intermediate or susceptible.

Modified Double-disc synergy test for studying ESBL production

Modified Double Disc Synergy Test (MDDST) is the most widely used test designed specifically to detect ESBL production (Kaur et al., 2013). The inoculums were prepared as described above and was spread onto MH Agar using a sterile cotton swab. A disc of Augmentin {(AMC - 20 µg and Amoxicillin -10 µg (CLA)} was placed on the surface of MHA; then the discs of CPD (30 µg), CAZ (30 µg) and CTX (30 µg) were kept around it in such a way that each disc was at distance ranging between 16 and 20 mm from the Augmentin disc (centre to centre). The plate was incubated at 37°C overnight for 18-20 hours. Distances between the discs were required to be suitably adjusted for each strain, in order to accurately detect the synergy. The organisms were considered to be producing ESBL when the zone of inhibition around any of the antibiotics tested.

Antibacterial activity study of EC-AgNPs and EC plant extract against clinical isolates

The powdered sample of EC-AgNPs and crude plant extract of EC were prepared in various concentrations in distilled water and subjected to see if it possessed any antibacterial activity against the clinical isolates using agar disc diffusion assay. The disc diffusion method is among the most flexible susceptibility testing methods to evaluate the antibacterial activity of silver nanoparticles (Cunha et al., 2016). The antibacterial activity of EC-AgNPs were carried out using protocol described in section 2.6. Sterile discs were placed on the MHA plate and 20 µL of EC-AgNPs in varying concentrations namely 10 µg, 50 µg, 100 µg and 200 µg were added onto each disc. Sterile molecular biology grade water which was used to suspend the nanoparticles was also added to one of the discs to serve as the negative control and Gentamicin disc was used as positive control. To see if the plant extract used to prepare AgNPs possessed any antimicrobial activity, the same concentration of EC aqueous plant extract was also added to sterile discs and placed on a different MHA plate with the bacteria. Then all these agar plates were incubated at 37°C for around 18-20 hours. If the extract or nanoparticle possesses an antibacterial activity, it is likely to inhibit the growth of bacteria tested after diffusing into the medium (Balouiri et al, 2016).

Resazurin-based 96 well plate broth dilution method for the determination of minimum inhibitory concentration (MIC) of EC-AgNPs

MIC (Minimum Inhibitory Concentration) is defined as the lowest concentration of an antimicrobial agent that is bactericidal (Loo et al., 2018). MICs are used to evaluate antimicrobial efficiency of various compounds by measuring the effect of decreasing concentration of antibiotic over a defined period, in terms of inhibition of microbial population growth (Kowalska-Krochmal & Dudek-Wicher., 2021).

The MIC of EC-AgNPs against the bacteria namely E. *coli*, *K. pneumoniae*, *S. aureus* and *P. mirabilis* were tested by Resazurin based 96 well plate broth dilution method (Elsheikh et al., 2016). The concentration of bacteria used in the assay was about 0.5×10^8 CFU/mL. To all the wells except the first well, 50 µL MHB was added. To the first well 100 µL of EC-AgNPs was added, in such a way that the concentration was 5 µg/mL. Subsequently serial dilutions were performed in such a way that the concentration gradients of EC-AgNPs were obtained ranging from 5 µg/mL to 0.0195 µg/mL. A growth control (with bacterial culture and without EC-AgNPs), a negative control and, a positive

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control with gentamicin was also maintained. The contents of the wells were mixed thoroughly and incubated at 37°C for 20-22 hours. The Resazurin was prepared at 0.015% and the assay was performed (Elsheikh et al., 2016). A colour change from blue to pink would indicate the presence of viable cells.

Antibiofilm activity of EC-AgNPs by Microtiter plate assay method

Bacterial communities often attach in surfaces to form a film complex encapsulated by polymer matrix with strong adherent properties and persistent phenotype (D'Cruz et al., 2018). In this study, we wanted to see if our EC-AgNPs were possessing any antibiofilm activity. A microtiter plate assay with crystal violet was performed to quantify the anti-biofilm property, if any (O'Toole, 2011). 96 -well microtiter plate was used to determine the antibiofilm activity of EC-AgNPs.

Results and Discussion

Preparation of silver nanoparticles using E. crassipes aqueous leaf extract

The powder obtained from the dried leaves of *E. crassipes* was used to make the aqueous extract. The green-coloured aqueous extract thus obtained was used to synthesize silver nanoparticle using silver nitrate. The synthesis of nanoparticle was visually confirmed by the colour change of the extract, upon addition of silver nitrate. The colour changed from pale yellow to dark yellow and then to dark brown indicated the reduction of silver ions by the leaf extract as shown in Figure 1a.

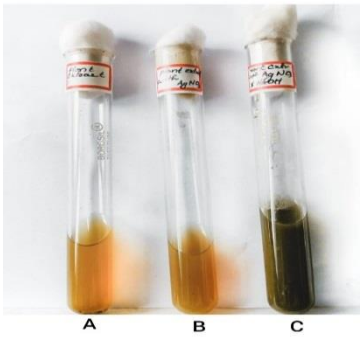

Figure 1a. *Shows the formation of EC-AgNPs as indicated by the colour change of extract from pale yellow to dark brown after incubation at room temperature 37℃. Tubes marked A contain aqueous extract of E. crassipes, tube B shows the colour change of extract frompale yellow to dark yellow in reaction with addition of NaOH and tube C shows the colour change to dark brown after the addition of silver nitrate to the extract.*

Characterization of EC-AgNPs

UV-visible spectroscopy

UV-visible spectroscopy technique is generally used for the analysis of structural and optical characteristics of silver nanoparticles. The SPR of the synthesized EC-AgNPs were measured using visible spectroscopy. The synthesis of nanoparticles was observed by the changes in colour of the sample semi-colon, these colour variations were due to optical properties exhibited by the silver nanoparticle. A well-defined SPR band obtained around 400 nm as shown in the absorption spectrum Figure 1b confirmed the formation of the synthesized EC-AgNPs. The absorbance spectrum range was between 300-400 nm confirmed the formation of silver nanoparticles.

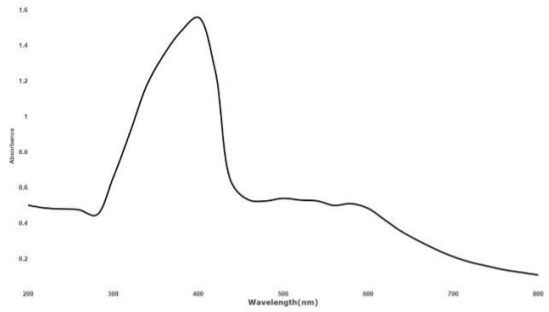

Figure 1b. *Shows the absorption spectrum of EC-AgNPs in the Uv-Visible range.*

Morphological analysis (TEM and SEM)

The TEM and SEM were employed to analyze the morphology and size of biosynthesized EC-AgNPs. In TEM images, the majority of EC-AgNPs exhibited uniform diameter and a spherical shape. The average particle size of EC-AgNPs was detected between 10 and 15 nm. The SEM analysis revealed the surface morphology and size of biosynthesized EC-AgNPs. SEM images showed noticeable agglomeration of EC-AgNPs which may be attributed to the drying process or high surface energy of the particles. These results provide the evidence of successful synthesis of EC-AgNPs with definite size and shape. Both the TEM and SEM images are shown in Figures 2a and 2b respectively.

XRD

XRD was used to confirm the crystalline amorphous nature of newly synthesized EC-AgNPs. The XRD pattern in figure 3a shows diffraction peaks at 2Θ = 38.28°, 44°, 64.36°, 78° respectively. This shows the pattern of face centered cubic (fcc) and crystalline structure of EC-AgNPs as described earlier (Halder et al., 2022).

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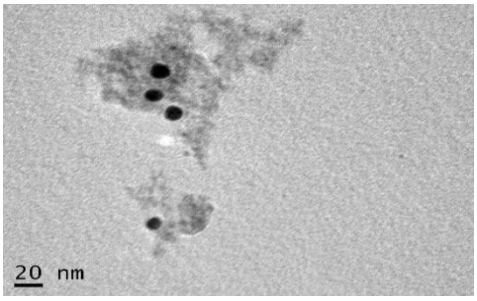

Figure 2a. *Shows the TEM image of EC-AgNPs.*

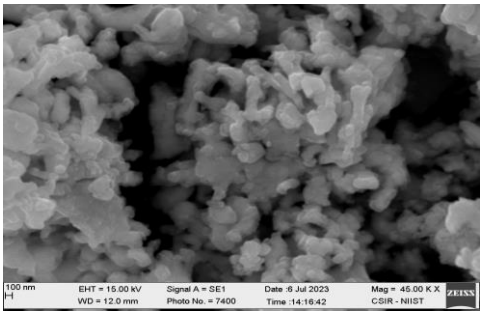

Figure 2b. *Shows the SEM image of EC-AgNPs.*

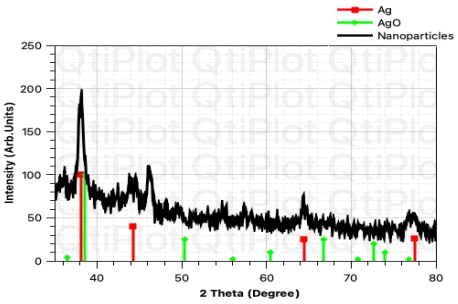

Figure 3a. *Shows the XRD pattern indicating the crystalline nature of EC-AgNPs.*

FTIR

The functional groups of metabolites contained in the leaf extract might be utilized to reduce and cap the silver nanoparticles (Jagathesan et al., 2018) and these moieties can be identified by FTIR. The FTIR spectrum of EC-AgNPs showed the major absorption bands at 3186.40 cm^{-1}, 1600.92 cm^{-1}, 1396.46 $cm^{-1,}$ 1066.64 $cm^{-1,}$ 578 cm^{-1} which corresponds to OH stretch vibration of phenols and alcohols, -C=C- stretching vibration of alkenes, nitro compounds, C-N stretching vibration of aliphatic amines and alkyl halides respectively as shown in figure 3b. The observed results are similar to some studies which previously characterized the green silver nanoparticles (Theivandran et al., 2015).

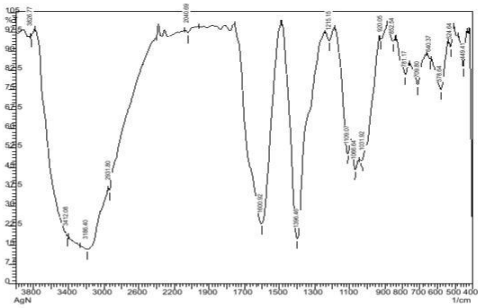

Figure 3b. *Shows the results of FTIR spectrum of EC-AgNPs.*

Zeta potential measurement

Zeta potential measurement was looked for to check the surface charge and stability of nanoparticles in solution. The graph in figure 4 shows the measured zeta potential value as -10.6 mV. A sharp peak at -10.6 mV indicates the negative charge on the surface of EC-AgNPs and the repulsion among the particles in solution. If the Zeta value is more negative than – 30 mV, the nanoparticles are stable as shown in previous studies (Anandalakshmi et al., 2015). So, we can infer that the synthesized EC- AgNPs can be in stable nature.

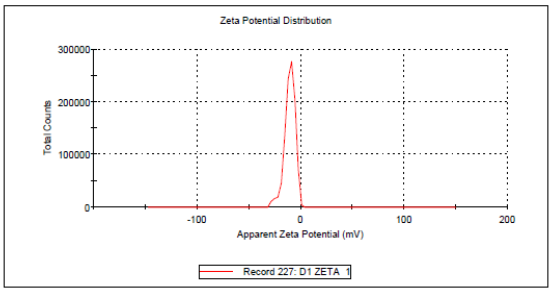

Figure 4. *Shows that zeta potential value of synthesized EC-AgNPs*

Antibiotic susceptibility test

The antibiotic sensitivity of the clinical isolates; *E. coli, K. pneumoniae, S. aureus and P. mirabilis*, was evaluated using disk diffusion assay. The tested antibiotics belonged to β-lactam class specifically cephalosporins and tetracycline which are commonly used to treat infections in both humans and animals (Bush and Bradford., 2016). The various clinical isolates used in the study showed different antibiotic susceptibility patterns. *E. coli* showed resistance to all antibiotics used whereas *P. mirabilis* was resistant to

Cefixime, Tetracycline and Cefotaxime. *K. pneumoniae* was sensitive towards all these antibiotics whereas *S. aureus* was resistant to Cefepime, Ceftriaxone and Cefixime. The results are shown in detail in table no 1.

Table 1. *Shows the antibiotic susceptibility pattern of clinical isolates to the various β-lactam cephalosporins and tetracycline*

Antibiotics	*P. mirabilis*	*E. coli*	*K. pneumoniae*	*S. aureus*
Cefixime (CFM)	11(R)	-(R)	24(S)	-(R)
Ciprofloxacin (CIP)	23(S)	18(R)	28(S)	30(S)
Cefotaxime (CTX)	18(R)	-(R)	25(S)	15(S)
Ceftazidime (CAZ)	24(S)	14(R)	24(S)	14(S)
Ceftriaxone (CRO)	27(S)	9(R)	29(S)	18(R)
Cefepime (CFP)	30(S)	13(R)	27(S)	18(R)
Cefpodoxime (CPD)	19(S)	11(R)	30(S)	26(S)
Tetracycline (TE)	(R)	7(R)	13(S)	23(S)

Modified Double disc synergy test

All the four clinical isolates were tested for ESBL production using the modified double disc synergy test as described. *K. pneumoniae* was sensitive to all the antibiotics tested so it didn't exhibit any synergism. The other clinical isolates: *E. coli, P. mirabilis and S. aureus* showed synergism as shown in the Figure 5. In *E. coli*, the zone can be seen in CPD, CFP, CRO and the zone of CRO was found to be extending towards AMC showing synergism. In *S. aureus* the zone was shown to extend to AMC around three out of four discs, exhibiting synergism and in *P. mirabilis* the zone around all the four antibiotics extends towards AMC indicating it to be a highly positive ESBL producer. Thus, it can be confirmed that the isolates *E. coli*, *S. aureus* and *P. mirabilis* are ESBL producers.

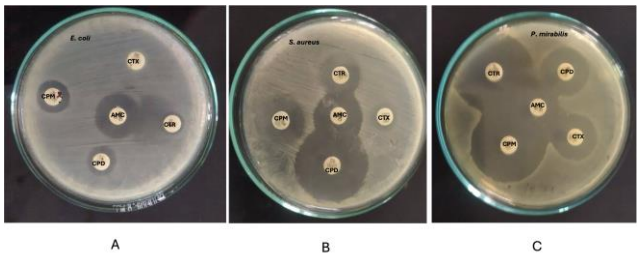

Figure 5. *Shows the results of the modified double disc synergy test of the clinical isolates E. coli, S. aureus, and P. mirabilis indicating ESBL production. The figures A, B, and C shows results of MDDST of E. coli, S. aureus and P. mirabilis respectively.*

Antimicrobial activity of EC-AgNPs and EC plant extract

Disk Diffusion assay

It was imminent to see if the EC-AgNPs and plant extract used for the synthesis possessed any antibacterial activity against these multidrug resistant bacteria. Antibacterial activity of different concentrations of EC-AgNPs i.e., 10 µg, 50 µg, 100 µg and 200 µg were prepared by diluting with distilled water, and their antibacterial activity on antibiotics resistant bacteria was studied using agar well diffusion method as described in section 2.8. EC-AgNPs showed antibacterial activity against all the tested bacteria *E. coli, K. pneumoniae, P. mirabilis* and *S. aureus*, in a concentration dependent manner as depicted in Figure 6a. In some cases, the antibacterial activity was evident at even low concentration i.e., 10 µg. The results show that the isolates were sensitive to EC-AgNPs, and the synthesized nanoparticles possessed good antibacterial activity. There

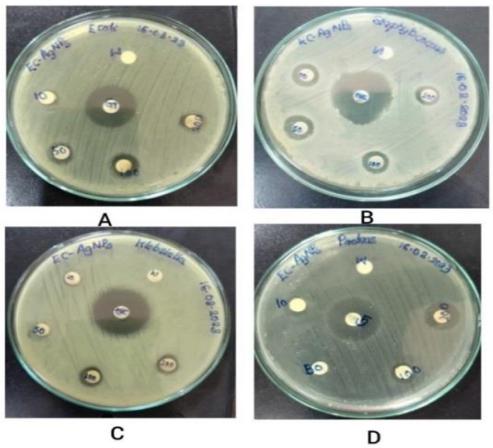

Figure 6a. *Shows the antibacterial activity of EC-AgNPs on multidrug resistant bacteria at various concentrations of 10 µg, 50 µg, 100 µg and 200 µg. The positive control used is gentamicin and negative control is water. As shown in the figures EC-AgNPs have evident effect on multidrug resistant bacteria. The figures A, B, C and D show the antibacterial activity of E. coli, S. aureus, K. pneumonia and P. mirabilis respectively.*

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was no zone of inhibition in negative control and a remarkable zone of inhibition in the positive control. But the plant extract at the same concentrations didn't exhibit any antimicrobial activity as is evident from the Figure 6b.

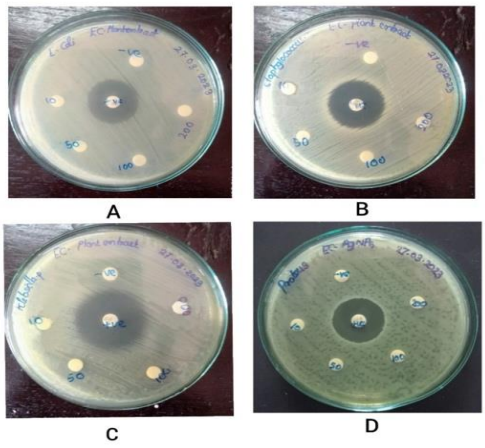

Figure 6b. *Shows the antibacterial activity of plant extract of E. crassipes on clinical isolates at various concentrations of 10 µg, 50 µg, 100 µg, 200 µg. The positive control used is gentamicin and negative control is water. As shown in figure, aqueous extract of E. crassipes does not have no effect on isolates. The figures A, B, C, and D show the absence of antibacterial activity on the aqueous extract of E. crassipes on E. coli, S. aureus, K. pneumonia and P. mirabilis respectively.*

Minimum inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of antibacterial agent that is required to inhibit the growth of bacteria. MIC values of EC-AgNPs were determined for each bacterial strain using Resazurin assay. The oxidoreductase within viable cells reduced the resazurin salt to resorufin and changed the colour from blue/purple non – fluorescent to the pink fluorescent colour (Elsheikh et al., 2016, Chakansin et al., 2022). The results in Figure 7 show that the wells in which EC-AgNPs were present in high concentration i.e., well 1 and 2 with 5 µg/mL and 2.5 µg/mL, the positive control (well 10) and negative control (well 12, with only MH broth) were seen in purple colour, which indicated no bacterial growth. The other wells and the growth control (well 11, with log phase bacterial culture) appeared in pink colour indicating the bacterial growth. This shows that the MIC of Ec-AgNPs is 2.5 µg/mL.

Antibiofilm activity of EC-AgNPs

The antibiofilm activity of EC-AgNPs was evaluated *in vitro* against biofilm forming bacteria *P. aeruginosa*. The bacteria were grown in 96-well microtiter plate for 24h and then 40 µg/µL of EC-AgNPs were added to each well.

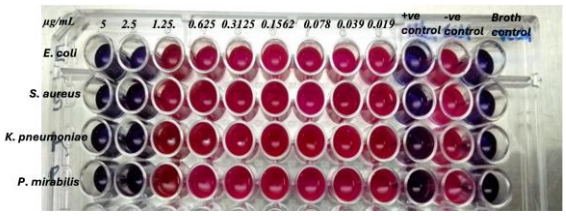

Figure 7. *Shows the resazurin assay to determine the MIC of EC-AgNPs against antibiotic resistant E. coli, S. aureus, K. pneumonia and P. mirabilis. The wells 1 to 9 have bacterial culture with EC-AgNPs serially diluted from 5 µg/mL onwards. Wells 10 has positive control with antibiotic gentamicin, 11 has log phase bacterial culture without any nanoparticles and 12 is plain MH broth serving as broth control.*

Results of the assay revealed that biosynthesized EC-AgNPs highly inhibited biofilm formation by *P. aeruginosa* relative to the negative control. Quantitation analysis of the assay was conducted in triplicate and the results are presented in Figure 8.

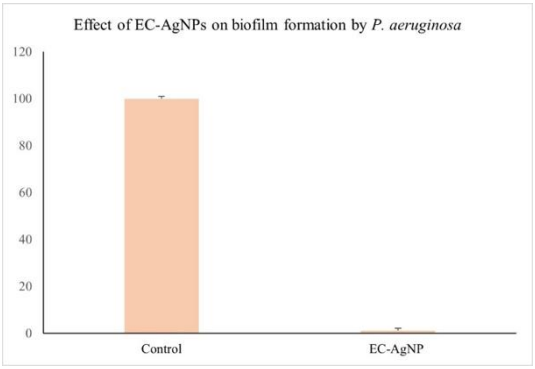

Figure 8. *Shows the quantification of the antibiofilm activity of EC-AgNPs against P. aeruginosa as compared to the control. The graph shows the average with standard deviation obtained from three independent experiments.*

Conclusion

The study successfully demonstrated the synthesis of silver nanoparticle using a noxious aquatic weed *E. crassipes*. The use of this noxious plant for nanoparticle synthesis highlights the concept of waste to value approach. The choice of method for synthesis was green synthesis using aqueous extract of the dried weed. Green synthesis was adopted as it is safe, biocompatible, non-toxic, and highly reproducible method. The synthesized EC-AgNPs were characterized as spherical, crystalline, and highly stable, with an average

diameter of 10-15 nm. The EC-AgNPs exhibited potent antibacterial activity against three MRD bacteria which were ESBL producers i.e., *E. coli, S. aureus, P. mirabilis*. The results showed that EC-AgNPs possessed antimicrobial activity even at low concentrations as 10 µg whereas the plant extract used for the preparation of the nanoparticles didn't exhibit any antimicrobial activity at low concentrations. The MIC of the biosynthesized nanoparticles was found to be 2.5 µg/mL. Furthermore, this silver nanoparticles were also found to exhibit remarkable antibiofilm activity against *P. aeruginosa.* This study is the first to report the antibacterial and antibiofilm efficacy of EC-derived AgNPs against pathogens which are potent ESBL producers and multi-drug resistant. These findings open new possibilities for transforming harmful aquatic weeds into valuable biomedical agents, thereby promoting environmental management and the development of novel antimicrobial strategies.

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