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Original article

Inhibition of multi-drug resistant microbial pathogens using an eco-friendly root extract of *Furcraea foetida* mediated silver nanoparticles

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ABSTRACT

Objectives: Advancement in the biological process of nanoparticles synthesis is an important area of nanotechnology. The aim of this research is to utilize the root extract of *Furcraea foetida* as reduction and stabilization agents to biosynthesize. Silver nanoparticles (AgNPs) from AgNO₃ via a simple green method. **Methods:** By utilizing UV–VIS spectroscopy, FTIR, XRD, Zeta potential, and HR-TEM techniques, the nanoparticles have been characterized. The anti-*Candida* properties of AgNPs were tested using the well-diffusion method in agar plates against five *Candida* species.

Results: With an average diameter of 15 nm, the produced AgNPs are spherical. They were found to be stable and non-aggregated. The synthesized nanoparticles were active against all the tested organisms and showed better efficacy compared to Amphotericin B in most cases. The highest level of inhibition was found against *C. albicans* at 100 µg concentration.

Conclusion: The results prove that *F. foetida* is a very good bioreductant and can be used for one-pot synthesis of stable AgNPs with good anti-*Candida* activity.

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1. Introduction

Candida species especially *Candida albicans*, is a commonly found infectious yeast in medical devices which provides adhesion sites for other fungal and bacterial pathogens and leads to mixed biofilm formation which presents a major threat to public health

Abbreviations: AgNPs, Silver nanoparticles; FFR AgNPs, Silver nanoparticles synthesized using *Furcraea foetida* root extract.

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(Boucherit-Otmani et al., 2019). Immuno-compromised hospital patients get opportunistic bloodstream infections caused by different *Candida* spp. which leads to a high level of morbidity and mortality rate (Wiwing and Cucunawangsih, 2020). Silver is known worldwide as an excellent antimicrobial agent for many years. It shows low toxicity in humans whereas has numerous uses *in vitro* and *in vivo* (Farooqui et al., 2010). Silver nanoparticles (AgNPs) and silver are the most extensively utilized and recognized uses in the pharmaceutical sector (Becker, 1999; Arokiyaraj et al., 2015). Silver-based topical dressings are commonly utilized for the treatment of infections in burns, chronic ulcers as well as open wounds. The recent advances in research on metallic nanoparticles led to special attention on AgNPs as a possible antimicrobial agent (Baker et al., 2005; Firdhouse and Lalitha, 2013; Al-Dhabi et al., 2018). Silver has an inhibitory effect on a large number of bacterial strains and other microorganisms that are common to industrial and medical procedures (Jiang et al., 2004). The specific surface

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area of nanoparticles is directly related to their biological efficacy when surface energy is increased (Willems, 2005). Compared to other types of nanomaterials, AgNPs have proved to show great promise in terms of biomedical applications and are the most effective antimicrobial agents due to their high-volume surface area ratio (Bhattacharya and Mukherjee, 2008; Hirst et al., 2009).

Green nanoparticles are advantageous in comparison to other biological processes through the use of plants because it avoids the tedious cell culture maintenance procedure (Shankar et al., 2004; Judy et al., 2021). Biologically synthesized nanoparticles show impressive antibacterial, antifungal properties (Surendra et al., 2016; Arasu et al., 2019a; Al-Dhabi et al., 2019; Valsalam et al., 2019a; Roopan et al., 2019; Gurusamy et al., 2019), antioxidant (Arasu et al., 2019b) and anticancer activities (Magdalane et al., 2018; Valsalam et al., 2019b). To the best of our knowledge, there is no information on the AgNPs synthesis using *Furcraea foetida* (L.) Haw., which is a monocot plant of the Asparagaceae family. This plant is commonly known as the giant cabuaya, Mauritius hemp, and green-aloe. It is native to Northern South America and the Caribbean. Many cultivars are used as ornamental plants in different parts of the world. It is also cultivated in many countries for fibre extraction. To understand the biological synthesis process and to discover novel eco-friendly methods of prefabricating nanomaterials, it is essential to investigate the formation of nanoparticles utilizing *F. foetida* root extract. This work centers on one-pot green synthesis of AgNPs utilizing the root extract of the plant *F. foetida* and analyzing its anti-fungal activity.

2. Materials and methods

2.1. Chemicals

All analytical reactive utilized in the analysis were analytic and were acquired from Sigma-Aldrich, India. PDA (Potato Dextrose Agar) & Amphotericin B have been bought from Hi-Media, India.

2.2. Microorganisms

The assessment of anti-*Candida* activity was carried out using five different strains. The following organisms have been acquired from MTCC ("Microbial Type Culture Collection and Gene Bank"): *Candida albicans* (183), *C. tropicalis* (184), *C. glabrata* (3019), *C. parapsilosis* (7043), and *C. krusei* (9215).

2.3. Collection and processing of plant samples

The plant *F. foetida* was collected from Kodaikanal, Dindigul district, Tamil Nadu. It was washed three times with tap water and then cleaned using distilled water to remove external debris. The roots of the plant were separated, chopped into small pieces, and dried in a hot air oven at 40 °C. They were pulverized using an electric grinder and stored in airtight containers for further use.

2.4. Extract preparation

Aqueous extract of the roots of *F. foetida* was made with 1 g of powdered plant sample and 50 ml double-distilled water are mixed in a conical flask. The mixture was heated at 80 °C for 30 min to denature the enzymes in the extract. Filter the solution using Whatman No. 1 for eliminating residues.

2.5. Silver nanoparticles Biosynthesis

In the synthesis of nanoparticles, 15 ml of the aqueous extract has been combined in a 300 ml Erlenmeyer beaker of 1 mM AgNO₃

solution and left 24 h to react at room temperature. The nanoparticles were separated by centrifugation at 10,000 rpm for 10 min. The pellets were then redispersed three times in deionized water for removing water-soluble biomolecules including secondary metabolites and proteins (Raut et al., 2010). The pellets have been freeze-dried using a lyophilizer and stored at room temperature until further analysis. The freeze-dried AgNPs were re-suspended in distilled water for all characterization assays as well as procedures.

2.6. AgNPs characterization

Different methods characterized the synthesized AgNPs. UV–VIS spectroscopy is an essential technique and is one of the simplest ways to confirm the formation of nanoparticles compared to other methods. Reaction medium readings were obtained at distinct periods to check the reduction process. UV–Vis spectroscopic studies were carried out using a Shimadzu –UV 260 spectrophotometer in a range between 200 nm and 800 nm. FT-IR analysis was carried out with a scan range between 400 and 4000 cm⁻¹ using an FTIR spectroscope (Shimadzu, IR Affinity 1, Japan). This method was used to identify the possible bio-molecules and metal ions interaction responsible for AgNPs' formation and stabilization. The distinct vibration modes have been discovered and allocated to identify the different functional groups in the AgNPs.

An XRD method was used to investigate the development of a single-phase compound. The XRD data have been obtained by X-ray diffractometer (XPRT-PRO, PW3050/60) at room temperature, with CuK_α radiation ($\lambda = 1.5406 \text{ \AA}$) between 20° & 90°. The XY data from this experiment (2 θ vs intensity) are generated with the Origin Pro program and the angular sites of the peaks are also acquired with this. Using Henry's equation, Zeta potential is to be determined. A titrator MPT-2a and Zetasizer Nano ZS (Malvern) were used to conduct the analysis. It is an essential characterization of the stability of the synthesized AgNPs.

HR-TEM is one of the most commonly used techniques for the characterization of nanomaterials. It is used to obtain quantitative measures of particle size, morphology, and size distribution. HR-TEM analysis was performed on a JEOL JEM-2100 (HT) electron microscope, with an accelerating voltage of 200 kV. The freeze-dried pellet was redispersed in deionized water and a drop of it was put on Cu grids pre-coated with carbon films.

2.7. Anti-*Candida* activity

Anti-*Candida* activity of the synthesized AgNPs has been tested using the well-diffusion method. 20 ml of molten as well as cooled PDA media has been added into sterilized Petri-plates. Wells are loaded with 25, 50, 75, and 100 μg of AgNPs suspended in 100 μl of sterile double distilled water. For 24 h the plates have been incubated at a temperature of 37 °C and then examined for the presence of zones of inhibition. A meter ruler was used to measure the diameter of zones of inhibition. The mean values of each organism were noted and presented in millimeters (mm). The antibiotic Amphotericin B (20 mcg) was employed as a positive control as well as wells loaded with sterile water were used as a negative control. All procedures were done in triplicates.

3. Results and discussion

3.1. Synthesis of Silver nanoparticles

The usage of plant extracts for the synthesis of nanoparticles is suited for therapeutic applications as it is free of toxic contaminants (Mittal et al., 2013). The resultant color change from trans-

parent liquid to reddish-brown colloid indicates that AgNPs synthesis has been effective.

3.2. UV-Vis spectroscopy

This analysis showed sharp absorbance at around 440 nm for AgNPs synthesized from the root extract (Fig. 1). The greatest absorption is seen at 440 nm and the intensity gradually rises as a function of the reaction time. At 60 mins the absorption intensity was 1.5a.u. A well-defined absorption peak of 420–450 nm shows the existence of SPR (“Surface Plasmon Resonance”) of metallic AgNPs (Henglein, 1993). The biosynthesized FFR AgNPs showed a sharp peak within this range. The concentrations of AgNPs could be monitored quantitatively as there was no shift in the peak wavelength of the reaction (Song and Kim, 2009).

3.3. Fourier transforms infrared spectroscopy (FT-IR)

Biosynthesized AgNPs were measured by FTIR to determine the interaction between the bio-organics of the nanoparticle and root extract. The absorption peaks of FFR AgNPs in the FTIR spectrum at 444.57, 618.09, 1109.07, 1415.91, 1556.99, 1634.38, and 2092.30 cm^{-1} (Fig. 2). This shows the presence of alkynes, aliphatic amines, primary amines, nitro compounds, and aromatic groups of compounds.

3.4. X-ray powder diffraction investigations

XRD (“X-Ray Diffraction”) patterns of the AgNPs show that the AgNPs structure is FCC (face-centered cubic). Furthermore, at 2 Å the diffraction peaks values of 38, 44, 64, and 78 of FFR AgNPs could be attributed to (1 1 1), (2 0 0), (2 2 0), (3 1 1) planes of centrosymmetric cubic Ag (Fig. 3). According to the JCPDS (Joint committee on powder diffraction standards), the resulted value is acquired with 01-071-3762 file no.

3.5. Zeta potential analysis

The zeta potential of the biosynthesized AgNPs root extract was found as a sharp peak at -10.6 mV (Fig. 4). It indicates that the nanoparticles’ surface is negatively charged as well as dispersed into the media. The negative value of zeta potential proves that they are very stable and confirms the repulsion among the parti-

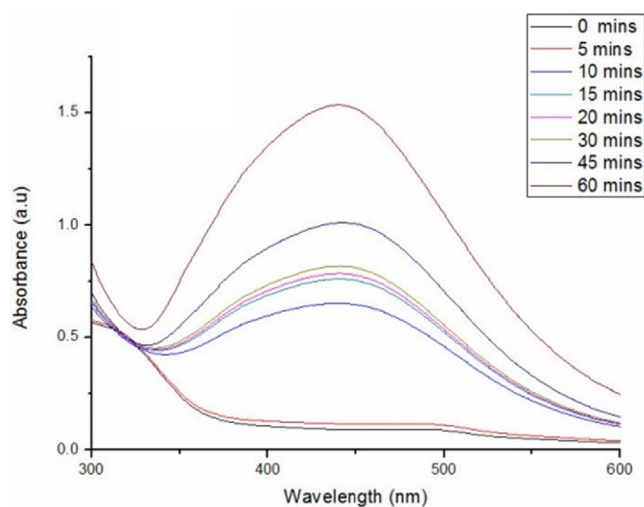


Fig. 1. UV-vis spectra for AgNPs synthesized from root extract of *F. foetida*.

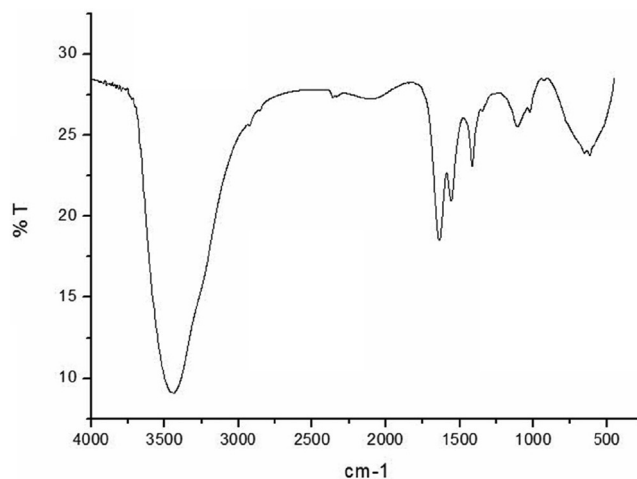


Fig. 2. FTIR spectrum of AgNPs synthesized using *F. foetida* root extract.

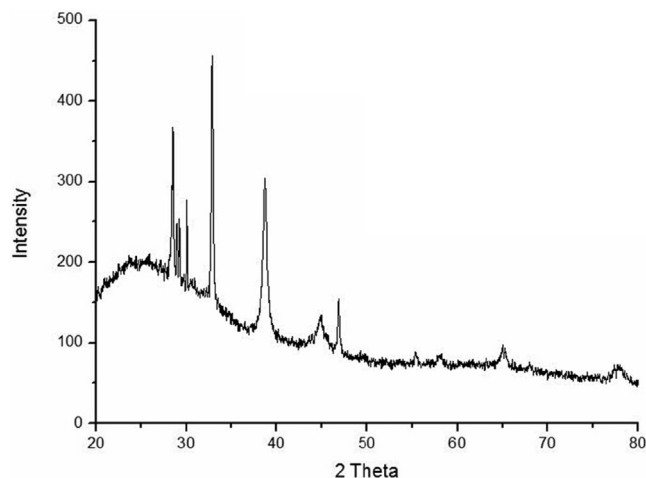


Fig. 3. XRD spectra of AgNPs synthesized from *F. foetida* root extract.

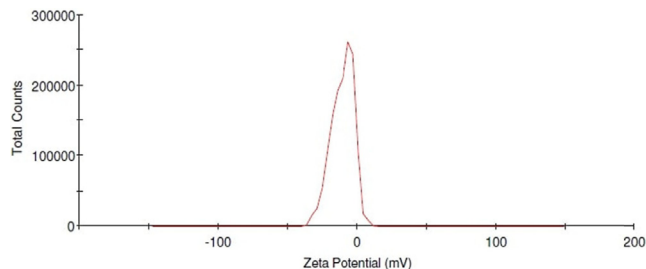


Fig. 4. Zeta potential analysis of AgNPs synthesized from *F. foetida* root extract.

cles preventing agglomeration. The AgNPs were spherical as seen in TEM micrographs.

3.6. HR-TEM analysis

HR-TEM (“High Resolution-Transmission Electron Microscopy”) analysis has been performed to understand the morphology as well as the size of the AgNPs. FFR AgNPs demonstrated the synthesis of poly-dispersed high-density spherical AgNPs, with moderate variation in size from 12 to 19 nm. Most of the nanoparticles were scattered and aggregation was rare as observed from the micrographs (Fig. 5). SAED (Selected Area Electron Diffraction) patterns

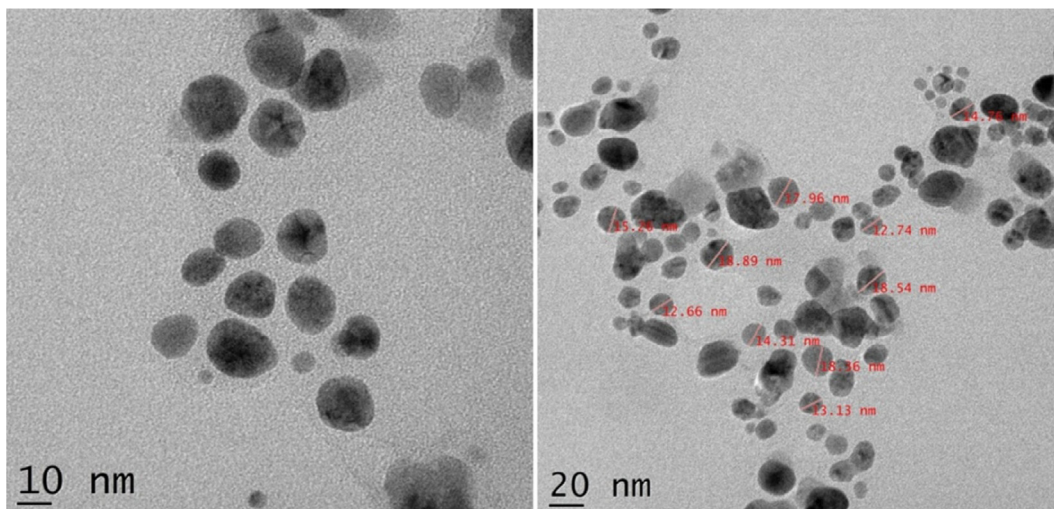


Fig. 5. HR-TEM micrographs of FFR AgNPs.

for the sample have been produced to validate the nature of the nanoparticles. The ring-like diffraction pattern in SAED shows that FFR AgNPs are entirely crystalline and may be indexed based on the silver FCC structure (Fig. 6). This supports the results obtained by XRD analysis.

Previously spherical AgNPs have been successfully synthesized using plant extracts of *Acalypha indica* (20–30 nm) (Krishnaraj et al., 2010), *Allium sativum* (4–22 nm) (Ahamed et al., 2011), *Citrus sinensis* peel (35 nm) (Kaviyaet al., 2011), *Rhododendron dauricam* (25–40 nm) (Mittal et al., 2012), *Sesuvium portulacastrum* (5–20 nm) (Nabikhan et al., 2010) and *Syzygium cumini* (29–92 nm) (Kumar et al, 2010; Banerjee and Narendhirakannan, 2011).

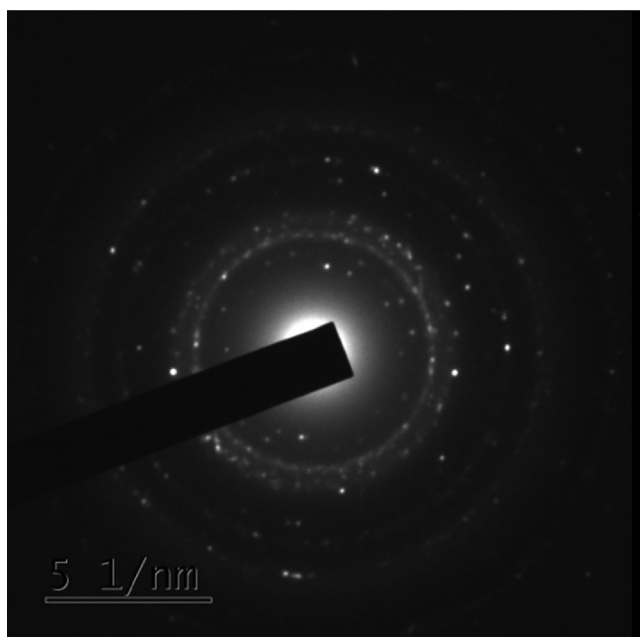


Fig. 6. SAED pattern of FFR AgNPs.

3.7. Anti-Candida activity

This study has measured the antifungal impact of FFR AgNPs at 25, 50, 75, and 100 µg concentrations were based on the inhibitory zone. All the tests were done in triplicates. The AgNPs showed potential against five test organisms. The AgNPs effectively inhibited the growth of all five *Candida* species. At 100 µg concentration, they showed a higher level of inhibition compared to that of Amphotericin B against all organisms except *C. glabrata* (Fig. 7). The greatest impact of nanoparticles on *C. albicans* was the 16 ± 2 nm inhibitory zone (Table 1).

Only a few studies on the antifungal effect of AgNPs have been published as the topic has received only marginal attention (Zeng et al., 2007; Falletta et al., 2008; Roe et al., 2008). In 2008 a study dealing more specifically with the activity of AgNPs against dermatophytes was published (Kim et al., 2008). AgNPs were again reported to be able to suppress the pathogenic yeasts *Candida krusei* & *Candida albicans* in 2018 (Ahmed et al., 2018). The anti-*Candida* activity of stable AgNPs of size 12.5 nm against *C. tropicalis* & *C. albicans* have also been assessed and AgNPs were concluded to be an option for the treatment of fungal infection (Mallmann et al., 2015).

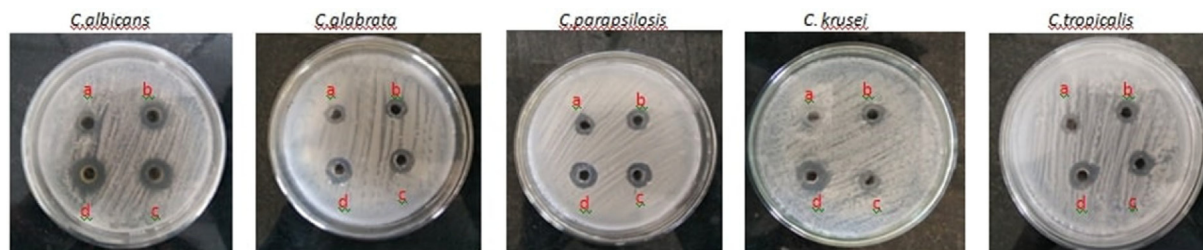


Fig. 7. Anti-Candida activity of FFR AgNPs.

Table 1
Anti-*Candida* activity of AgNP synthesized using *F. foetida*.

Organism	Zone of inhibition (mm)		FFR AgNPs			
	Negative Control	Positive Control				
			25 µg	50 µg	75 µg	100 µg
<i>Candida albicans</i>	Nil	10 ± 2	6 ± 2	9 ± 3	14 ± 3	16 ± 2
<i>C. tropicalis</i>	Nil	8 ± 3	5 ± 2	8 ± 3	10 ± 4	12 ± 2
<i>C. glabrata</i>	Nil	7 ± 2	3 ± 1	4 ± 2	4.5 ± 2	5 ± 1
<i>C. parapsilosis</i>	Nil	10 ± 3	4 ± 2	7 ± 2	9 ± 1	11 ± 2
<i>C. krusei</i>	Nil	9 ± 2	6 ± 3	10 ± 3	12 ± 2	13 ± 4

*Values are mean of triplicate measurements ± standard deviation.

The most commonly studied organism is *Candida albicans* compared to other *Candida* species. AgNP anti-*Candida* activity was measured against pathogenic *Candida* species using an MFC (minimum fungicidal concentration) as well as MIC (minimum inhibitory concentration) determination and it was found that the MICs of stabilized AgNPs were comparable or better than the MICs of conventional antifungal agents (Panáček et al., 2009). The FFR AgNPs exhibited greater efficacy against most *Candida* species in comparison to Amphotericin B which is in support of the study.

4. Conclusion

F. foetida is an effective reducing agent to successfully synthesize and stabilize AgNPs. The average size of the synthesized AgNPs was less than 15 nm. The characterization techniques show that the nanoparticles are stable and crystalline in nature. Pathogenic fungi such as *Candida* continue to develop resistance to many antibiotics thus alternative methods are needed. The synthesized AgNPs show good activity against all the fungal species used in the study. The present study showed a simple, rapid, and eco-friendly route to synthesize stable AgNPs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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