RESEARCH ARTICLE

Green Synthesis and Characterization of Silver Nanoparticle from *Calanthe masuca* (D.DON) Lindl. Leaf Extract and Evaluation of Biological Activity - An Endangered Orchid (Orchidaceae)

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Abstract

Researchers looked at the therapeutic benefits and biological properties of silver nanoparticles (AgNPs) and created and tested novel eco-friendly methods to make them using an extract from the *Calanthe masuca* (D. Done) Lindl plant (Orchidaceae). After the *C. masuca* leaf extract was transformed, X-ray diffraction confirmed the existence of crystalline nanoparticles by revealing different peaks. The AgNPs were found to be spherical in shape, with a size ranging from 2 to 50 nm and a diameter of 0.500 nm, according to transmission electron microscopy, energy dispersive X-ray analysis, and scanning electron microscopy. The presence of silver nanoparticles in the synthetic leaf components was confirmed by a 428 nm absorption peak in the UV-visible spectra. Scanning electron microscopy (SEM) confirmed the nanoparticles' size, while FTIR analysis revealed their inclusion of many functional groups. They proved their antibacterial effectiveness by producing silver nanoparticles and tested them against different human pathogenic pathogens. *Staphylococcus aureus* (6.5 ± 1.0 µg/ml), *Bacillus subtilis* (7.6 ± 1.0 µg/ml), *Escherichia coli* (10.4 ± 1.0 µg/ml), *Streptococcus oralis* (7.6 ± 1.0 µg/ml), *Aspergillus niger* (9.3 ± 1.0 µg/ml), and *Candida albicans* 7.5 ± 1.0 µg/ml) and the high zone of inhibition were seen in *Escherchia coli* and *Candida albican*. This study demonstrates the biomaterial's promise for use in the production of silver nanoparticles using green chemistry methodology. An eco-friendly way of generating nanoparticles is highlighted in this paper, which highlights their promising applications in biology and medicine.

Keywords: Silver nanoparticle, XRD, SEM, EDAX, TEM, Antimicrobial endeavor.

Highlights

- *Calanthe masuca* leaf extract utilized for synthesizing silver nanoparticles (AgNPs) *via* eco-friendly methods.
- Characterization through X-ray diffraction confirms crystalline nanoparticle formation, spherical in shape, ranging from 2 to 50 nm.
- Various analytical techniques, including TEM, EDX, SEM, UV-vis spectroscopy, and FTIR analysis, validate AgNPs presence and characteristics.
- AgNPs demonstrate significant antibacterial effectiveness against various human pathogens: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus oralis*, *Aspergillus niger* and *Candida albicans*.
- The eco-friendly synthesis of AgNPs showcases the potential for diverse applications in biology and medicine, emphasizing the promise of green chemistry in addressing biomedical challenges.

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Introduction

The orchid plant is of important commercial significance in India, specifically in the cut flower and container plant trade, and boasts a difference of about 1,300 classes and 180 to 190 genera of orchids across the country. Thailand is conspicuous as the realm's biggest exporter of praise cut flowers among the rich past of the orchid profession on the planet (Kovačová *et al*., 2020). Meanwhile, silvery nanoparticles (AgNPs) have taken far-reaching attention for their different uses, from electronics and photonics to snack electronics and pharmacology (Nokkrut *et al*., 2019). Synthesis of these nanoparticles using organic plants, particularly plant leaf materials, has won celebrity due to its environmental, secure, and persuasive nature distinguished concerning matter and chemically combined alternatives (Ramya *et al*., 2016).

Orchids, flavorful perpetual plants reaching 60 cm in altitude, are a valuable resource in India among their different purple flowers and secret tubers that hold resins, gel, proteins, vigor, and organic compounds composed of carbon and minerals. In addition to their decorative advantage, these tubers have been utilized in usual cures to treat skin afflictions and tuberculosis, but they still symbolize a special to be-consumed component

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(Shabeeb *et al*., 2016; Ajayi and Afolayan, 2017; Zanello *et al*., 2022). Biological arrangements of bearing nanoparticles using plant extracts are deliberately dependable and environmentally friendly and determine an economical way of removing poisonous chemical compounds (Rao and Hussain, 2010; Hussain *et al*., 2019; Ravi *et al* ., 2013).

Notably, Artocarpus skin in the blue and red colors mixed plant shows substantial potential for a combination of silvery nanoparticles through the accelerated decline of white ions. This discovery opens streets for extensive marketing results of biomedical or nanotechnology-related crops (Kalaiarasan, 2017; Paulkumar *et al*., 2014; El-Shahaby *et al*., 2013). Attention to bright nanoparticles has been raised due to their productiveness against an off-course range of microorganisms, discussing growing drug resistance to typical medicines (Fortin & Beveridge, 2000); Srirangam and Parameswara Rao, 2017; Yang *et al*., 2021; Datarun *et al*., 2023).

Within the orchid plant, the *Calanthe* genus, primarily terrestrial, encompasses over 850 species distributed across tropical Southern Africa, Madagascar, the Neotropics, Asia, and Australia, with one species in tropical America. Particularly abundant in India, including the tropical Himalayas and the Deccan Peninsular region, *Calanthe*, found in places like Kolli Hills, Namakkal district of Tamil Nadu is known for its therapeutic uses among tribal communities. The plant leaves benefit treatment of brain and bone diseases, osteoporosis, stem-cell and bone marrow transplantation, and chemotherapy were utilized in the current study to synthesize silver nanoparticles. The characterization of these nanoparticles included XRD, SEM, EDAX, TEM, and an assessment of antimicrobial activity.

Materials And Methods

Plant Materials and Methods

Fresh leaf samples were obtained from the Solakadu region of Kolli Hills, Namakkal District, Tamil Nadu, India (Fig. 1 a, b). Until protected cleanness, the leaves have utterly laundered with running water before being sterilized and accompanying distilled water. Subsequently & the leaves have been separated into narrow pieces and endangered shade drying. The drained parts of the leaves were separately impolitely crushed and stocked in fixedly finished containers for after-lab study.

(a) (b)

Fig. 1: (a) Habitat of Terrestrial, (b) The plant *C. masuca* grows in adaptation of morphology

Plant Authentication

As Head of the Office and Scientist "E" at the Botanical Survey of India (Southern Circle) in Coimbatore, Tamil Nadu, Dr. V. Ravichandhiran substantially facilitated the botanical identification process that validated the plant's validity. The voucher specimen has been formally recorded by the Department of Botany at National College (Autonomous) in Tiruchirappalli, 620 001, Tamil Nadu, India.

Plant description

Domain: Eukarya
Kingdom: Plantae
Division: Streptophyta
Class: Monocots
Order: Asparagales
Family: Orchidaceae
Genus: *Calanthe*
Species: *masuca*

Synthesis of Silver Nanoparticles

The current standard procedure for the biogenesis of silver nanoparticles involves adding 1.5 mL of plant extracts to 30 mL of a solution containing 30 mM $AgNO_3$. For 24 hours, this mixture was heated to 28°C. Afterward, a little sample of the solution was sent for UV-vis spectroscopy, and the plant extract turned out to be highly concentrated in nitrate after an FTIR analysis. After the counterattack, the next 10 minutes were spent centrifuging the consolidation at 10,000 rpm. The correct dosage is prepared by diluting it with a reasonable volume of bifold distilled water. Here we have the postponement, which develops into a box flow, culminating in a slim film, which eventually dies out in a vacant communication kitchen stove (Fig. 2 a, b, and c). Using

(a)Plant fresh leaf cut piece (b) Dry leaf

(c) Leaf powder

Fig. 2: The plant powder preparation leaf cut and leaf powder (a, b, c)

A) Before incubation b) After incubation for 24h

Fig. 3: Nanoparticle synthesized of silver plant leaf extract (A, B)

the plant extract, they achieved remarkable colors ranging from yellow to black, symbolizing the promising incorporation of silver nanoparticles (Fig. 3: a, b) (Umadevi *et al*., 2012).

Microorganisms

The test organisms such as *S. aureus* (MTCC-902), *B. subtilis* (MTCC-441), *E. coli* (MTCC-443), *S. oralis* (MTCC-902), *C. albicans, and A. niger* were obtained from the Chandigarh culture collection in India. Since.

Characterization of the Synthesized Silver Nanoparticles

We used a battery of analytical tools, including transmission UV-visible spectroscopy, X-ray diffraction, scanning electron microscopy, energy dispersive X-ray spectroscopy, UV-visible spectroscopy, Fourier transform infrared spectroscopy, dynamic light scattering particle size analysis, and UV-visible spectroscopy, to study the plant-derived silver nanoparticles extensively. The optical properties of the synthesized nanoparticles were investigated by means of ultraviolet-visible spectroscopy. A 2 mL solution of deionized water was used to dilute 0.2 mL of the suspension before the periodic sampling could be carried out. The UV-visible spectra were measured in the wavelength range of 190-1100 nm using a Thermo Heyios 2 version spectrophotometer (Chinnappan *et al*., 2018)

Spectroscopy using the Fourier transforms

The solution containing silver nanoparticles was spun at 20,000 rpm for 20 minutes before FTIR measurements were taken in this experiment. About 20 mL of deionized water was used to wash the particle three times after centrifugation. After centrifugation, the pellet was rinsed three times with 20 mL of deionized water. After washing, the materials were dried and analyzed using an infrared prestige-21 (SHIMADZU) spectrometer that has a resolution of 2 cm^{-1} in transmission electron microscopy. Evaluation of antibacterial efficacy using microscopy.

X-ray Diffraction Analysis"

The silver nanoparticle solutions underwent a centrifugation process at 20,000 rpm for a sum of the 20 rpm brief cycles. Subsequently, the resulting pellet underwent three washes, with each wash involving 20 mL of deionized water. Following these purification steps, the silver nanoparticle mixture was collected for further analysis and characterization, particularly focusing on the formation of white nanoparticles. The investigation utilized an X' Pert PRO X-ray diffractometer, operating at 40 kV and 30 mA and employing Cu Kα radiation.

Scanning Electron Microscopy

The study of the silver nanoparticle coating on pure titanium involved the utilization of a Hitachi S-3500 N high-resolution scanning electron microscope (SEM). After preparing the nanoparticles, they were used for SEM analysis in the S-3500 N. SEM grids with the film were allowed to remain undisturbed for 2 minutes after removing the extraction solution with absorbent paper. Afterward, the grids were dried before the measurements were recorded. Using the following parameters: 20,000 x magnifications, 15 mm working distance, operating voltage of 25 kV, objective aperture and condenser lens power set to 50, the Hitachi S-3500 N was used to conduct the scanning electron microscopy experiments.

Energy Dispersive X-ray Spectroscopy

The energy-dispersive X-ray spectrometers use the photon character of light to produce detectable voltage pulses in X-rays. It is possible to get a statistical approximation of the corresponding quantum energy by combining a low-noise system with an ultra-low-noise preamplifier. A low-noise system coupled with an ultra-low-noise preamplifier has statistically represented the associated quantum energy. In order to enhance the overall X-ray spectrum picture at the same time, a multichannel analyzer digitally records and tallies a large number of these pulses. Energy dispersive spectroscopy is now much more reliable thanks to digital quantum computing. This method employs semiconductor materials and processing circuitry to precisely characterize the spectrum to detect and analyse X-rays (Mohammed Fayaz., 2009).

Transmission Electron Microscopy

Silver nanoparticles were produced at TANUVAS in Chennai through drop coating for subsequent examination using transmission electron microscopy (TEM). The nanoparticles were applied onto a TEM grid coated with carbon-coated copper and allowed to sit undisturbed for 280 minutes. After using absorbent paper to carefully remove residual liquids, the grids were air-dried before the measurements were taken. A JEOL 3010 apparatus was used for TEM analysis, using an acceleration voltage of 300 kV (Buhr *et al*., 2009).

Analysis of Antimicrobial Activity

A variety of microorganisms, including *S. aureus, B. subtilis, E. coli, S. oralis, A. niger, and Candida*, were tested for their antimicrobial effectiveness using the agar well diffusion method. The silver nanoparticles (AgNPs) were synthesised from *C. masuca* leaf extract. To determine whether the AgNPs were bactericidal, 20 µL of each extract was pipetted onto separate sterile 7 mm discs made of Whatman No. 1 paper. We prepared the vitamin agar solution, transferred it to sterile trays, and allowed it to air-dry for one cycle under sterile conditions before letting it set for 30 minutes. Placing the prepared discs on top of the agar followed by covering petri dishes with microbial cultures was the next step. These experiments have gone through three revisions. Next, the nanoparticles were tested for their antibacterial properties by measuring the inhibitory zones after incubating the petri plates at 37°C for 24 hours. Their results demonstrated that AgNPs induced by *C. masuca* leaf extract had potent antibacterial activity, comparable to that of gentamicin medicines, indicating that they might be beneficial against several microorganisms (Jayashri and Ravindra, 2019).

Results And Discussion

This research used UV-visible spectra to further understand the absorption properties of silver nanoparticles made from *Calanthe masuca* leaf extract. Fig. 1, Fig. 4, and Table 1 show that the study, conducted using a SPECORD 50 PLOS UV-vis spectrophotometer, exhibited a strong absorbance peak at 428 nm. Consistent with previous research by Elango *et al*., 2018; Anandalakshmi *et al*., 2015, Kalaiarasan and Chinnappa, and others, the measured absorbance intensity and wavelength provide strong evidence for the effective reduction of silver nanoparticles (2015), Saravanan *et al*., 2023; Sangu *et al*., 2021.

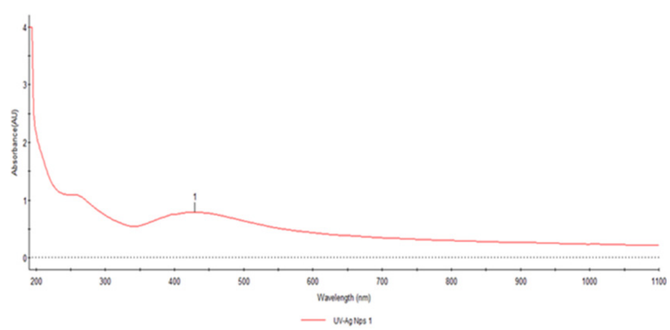


Fig. 4: UV-Visible spectra of Silver Nanoparticles Synthesized of *C. masuca* leaf extract

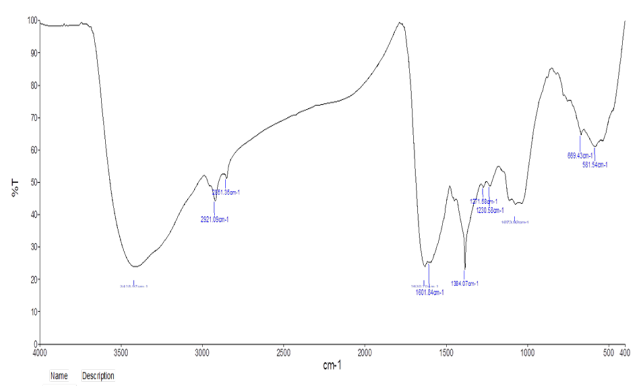


Fig. 5: FT-IR spectra of Silver Nanoparticle of *C. masuca* leaf extract

Table 1: UV-VIS Peak values of leaf extract of *C. masuca*

S. No.	Wavelength (nm)	Absorption Peak (O.D)
1	220.55	4.0000
2	364.10	1.0848
3	397.55	1.3319
4	531.70	0.1693
5	664.50	0.2762

Table 2: FT-IR Spectrum peak value of Silver Nanoparticle of *C. masuca* leaf extract

S. No.	*Peak Value*	*Functional group*	*Functional group Name*	*Vibration type*
1	3416.97	O-H	alcohol	Stretching
2	2921.09	C-H	Alkane	Stretching
3	2851.35	C-H	Carboxylic acids	Stretching
4	1630.70	C=C	Cyclic alkene	Stretching
5	1601.84	C=O	Amide I bond	Stretching
6	1384.07	CH and CH_2	Aliphatic bonding	Stretching
7	1271.58	C-N	Amide III bond	Stretching
8	1230.58	C-N	Amide III bond	Stretching
9	1073.92	O=O	Carbohydrates	bending
10	669.43	C-H	Aromatic group	Stretching
11	581.54	C-H	Aromatic group	Stretching

Our primary objective in conducting this FTIR experiment was to identify any reducing agents present in the *C. masuca* leaf extract that may stabilize and biogenically decrease the silver nanoparticles (AgNPs). Fig. 5 displays the FTIR spectra of the AgNPs, whereas Table 2 displays the same data for the *C. masuca* leaf extract. Proteins, polyphenols, and carbohydrates are all possible candidates for the existence of an OH stretching peak at 3416.97 cm^{-1}. Two nearby bands, at 2921.09 and 2851.97 cm^{-1}, likewise depict the stretching modes of the C-H bonds in aldehydes. A second peak at 1630.70 cm-1 is similarly linked to the carbonyl group's C=O bond. Findings from these studies corroborate those from earlier studies (Sastry *et al*., 2003; Hu *et al*., 2019).

The leaf powder obtained by centrifuging silver nanoparticle solutions at 20,000 rpm for 20 minutes was then used for XRD examination. The biosynthesized nanoparticles were confirmed by detecting unique peaks in XRD tests. The peaks at 2θ values of 32.230, 38.120, 46.220, 57.440, 64.490, 67.440, 74.490, 76.740, and 85.750 were particularly noticeable in the XRD pattern (Otunola *et al*., 2017). The (101), (111), (200), (113), (211), (220), (230), and (311), respectively, are the crystallographic planes that have been attributed to these peaks (222). Following earlier reports by Chartarrayawadee *et al*., the XRD data was used to infer the rhombohedral geometry of the iron oxide nanoparticles (2020). The synthetic form's average grain size was calculated using the Scherer equation and the XRD peak's full width at half maximum (FWHM). A nanometer is the unit of measurement for the resulting size. Notably, the instrument's widening impact is insignificant for particle sizes less than 100 nm. As seen in Fig. 6,

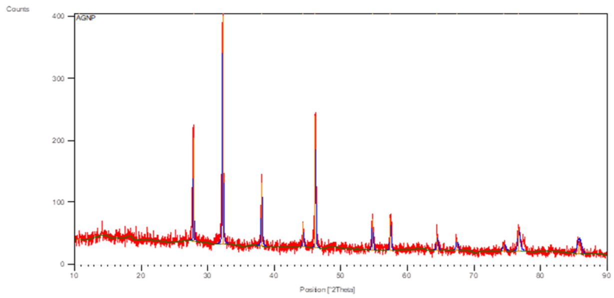


Fig. 6: Shows XRD pattern of silver nanoparticles of *C. masuca* leaf extract of AgNPs

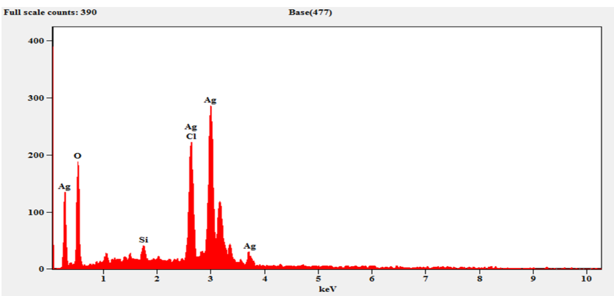
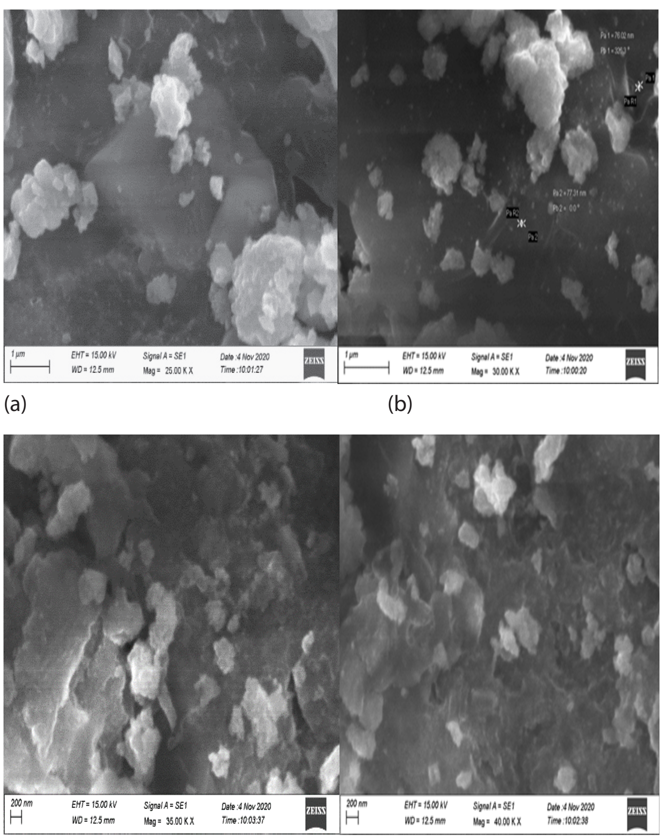
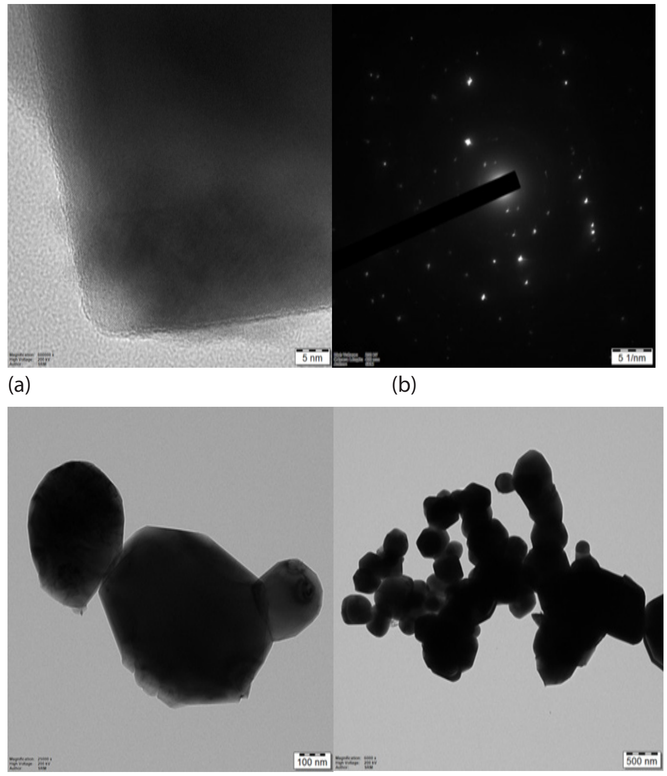


Fig. 7: EDAX analyses of green Synthesized SNPs shows 46.91 weight percentage



(a) (b) (c) (d)

Fig. 8: Scanning electron microscopy of *C. masuca* leaf extract of Ag NPs



(a) (b) (c) (d)

Fig. 9: Transmission Electron Microscopy of *C. masuca* leaf extract of AgNPs

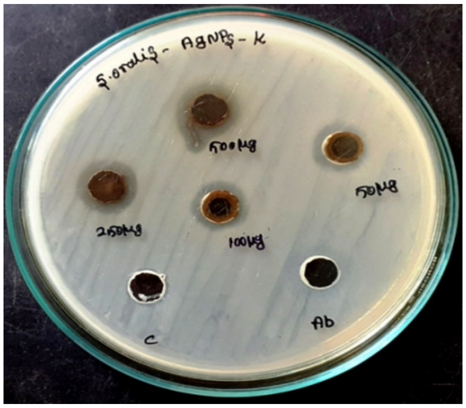
Table 3: EDAX Elemental Micro-Analysis of the Silver Nanoparticles

Element	*Wt %*	*At%*
OK	46.91	80.23
SiK	01.34	01.30
SiL	10.30	07.95
AgL	41.45	10.52
Total	100.00	100.00

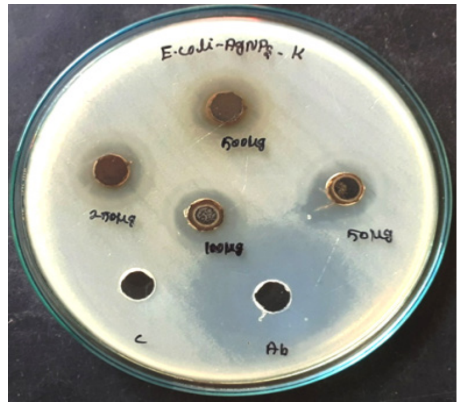
the use of Scherer's formula allowed for the determination of crystal size. This equation is in good agreement with what has been found in previous research by Bykkam *et al.*, 2016; Basin and Jeyachandran, 2015; Holmes *et al.*, 1995; and Ramya and Ramachandra Raja, 2016.

The presence of silver nanoparticles was confirmed by energy-dispersive X-ray (EDX) analysis, which revealed a strong signal in the silver area (Fig. 7). A distinct optical absorption peak at around 3keV, which coincides with the surface plasmon resonance, was used to prove the presence of metallic silver nanocrystals. According to Table 3, more investigation revealed that the nanostructures were completely made of silver (Manoratne *et al.*, 2017; Singh *et al.*, 2015; Doan *et al.*, 2020).

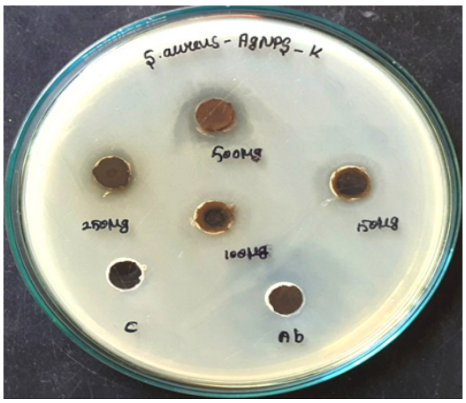
The size and shape of the silver nanoparticles were examined using scanning microscopy. By comparing our experimental data, we were able to determine that the solution-based nanoparticles' diameter ranged from 76.02 nm to 77.31 nm. As a reference, Fig. 9 shows a scan of a plant extract captured by a



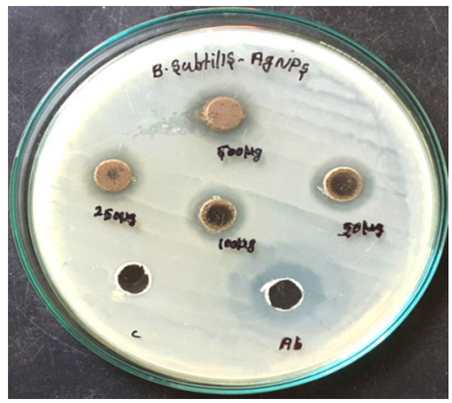
(a) Streptococcus orali



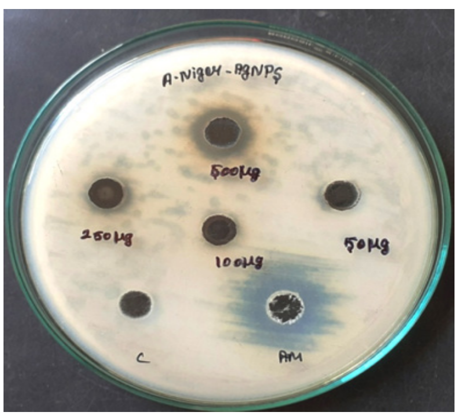
(b) Escherichia coli



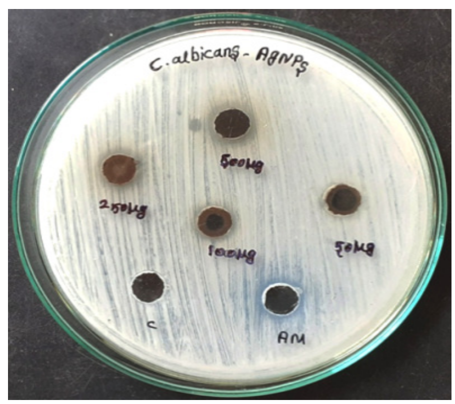
(c) Staphylococcus aureus



(d) Bacillus subtili



(e) Aspergillus niger



(f) Candida albicans

Fig. 10: The zone of inhibition for Antimicrobial activity of AgNPs of *C. masuca* leaf extract

Table 4: Antimicrobial Activities of Synthesized AgPNs of *C. masuca* leaf extract

S. No.	*Name of the test organism*	*Name of the test sample*	*Zone of inhibition (mm) SD ± Mean*				
			500μg/ml	*250μg/ml*	*100μg/ml*	*50μg/ml*	*AB*
1.	*Staphylococcus aureus*	AgNPs	6.5±1.0	4.5±0.5	3.2±0.2	2.1±0.1	10.1±1.0
2.	*Bacillus subtilis*		7.6±1.0	5.4±0.4	4.3±0.3	3.2±0.2	11.2±1.0
3.	*Escherichia coli*		10.4±1.0	7.4±0.4	6.3±0.3	4.2±0.2	13.1±1.0
4.	*Streptococcus oralis*		7.6±1.0	5.5±0.5	3.2±0.2	2.1±0.1	7.4±1.0
5.	*Aspergillus niger*		9.3±1.0	5.5±0.5	3.4±0.4	2.3±0.3	11.1±1.0
6.	*Candida albicans*		7.5±1.0	5.4±0.4	3.2±0.2	2.1±0.1	6.3±1.0

scanning electron microscope (incubated with deionized water for 48 hours). In addition, Fig. 8 shows, at varying magnifications, micrographs of silver nanoparticles produced by the suggested bioreduction process. Previous research by Cliff and Lorimer, 1975; Watanabe and Williams, 2006; Gauvin, 2012; Batain *et al*., 2021; Yes *et al*. are very congruent with this observed trend (2021).

The findings indicated distinct inhibition zones, with *E. coli* displaying a prominent zone and *Staphylococcus aureus* showing a comparatively smaller one among the six tested pathogens. Employing *Calanthe masuca* leaf extract for green synthesis, the silver nanoparticles exhibited remarkable antibacterial activity, particularly against drug-resistant strains. This study suggests that these eco-friendly silver nanoparticles hold potential in controlling multidrug-resistant pathogenic bacteria, offering promise for medical applications. Further details are available in Fig. 10 and Table 4. This discovery aligns with prior research by Jayashri and Ravindra, 2019; Sangu *et al*., 2021. Furthermore, our observations suggest that the prepared silver nanoparticles demonstrate enhanced efficacy compared to standard references (Otunola *et al*., 2017; Sarma and Dutta, 2019; Doan *et al*., 2020).

Conclusion

The orchid plant has effectively demonstrated the biosynthesis of silver nanomolecules using an extract from the leaves of *C. masuca*, a plant renowned for its therapeutic medicinal properties. The synthesized silver nanoparticles underwent thorough characterization of molecular morphology through XRD, SEM, EDAX, and TEM analyses. The green synthesis approach exhibited significant antibacterial activity against a range of disease-causing pathogens. In the antimicrobial studies, six pathogens were examined, with *Escherichia coli and* Staphylococcus aureus displaying a substantial zone of inhibition, while Staphylococcus aureus exhibited a comparatively lower zone of inhibition. This research underscores the potential of *C. masuca* in combating bacterial infections. Furthermore, the plant holds cultural significance as tribal communities have traditionally used it to treat various ailments. It is actively cultivated by local populations in Indian reserve forests for commercial export, contributing to the production of a considerable number of plants. The study, conducted in Kolli Hills, Tamil Nadu, India, offers fresh insights into the medicinal compounds present in *C. masuca*, serving as a valuable resource for future pharmaceutical research and aiding researchers in further exploring the plant's therapeutic potential.

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Conflict Of Interest

The authors affirm that they do not have any potential conflicts of interest related to their pursuits.

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