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BIOSYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF SILVER NANOPARTICLES USING METHANOLIC EXTRACT OF BULB OF *ZEPHYRANTHES CITRINA*

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Keywords:

Zephyranthes citrina, AgNPs, UV-Vis, FT-IR, SEM, EDAX, XRD, Antimicrobial activity

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ABSTRACT: In the present study, an eco-friendly, cost-efficient, rapid and simple approach was applied to the synthesis of silver nanoparticles by using a methanolic extract of bulb of *Zephyranthes citrina*. The metal salt was reduced to ions by metabolites present in bulb extracts. The plant extracts acts both as a reducing agent as well as a capping agent. The synthesized silver nanoparticles were characterized by various spectral techniques like UV-Visible, FT-IR, SEM, EDAX and XRD analysis. Biosynthesis of silver nanoparticles was confirmed by viewing the colour change of the extract from brown to blackish brown. The functional group which is responsible for the reduction of silver ions in the plant extract was characterized by FT-IR. UV-Visible spectrophotometer showed an absorbance peak at 277 nm for silver nanoparticles. The SEM image depicts the nanosized particles with morphological shape. The elemental composition was determined by EDAX analysis. The XRD results confirmed the crystal structure of the nanoparticles. The spectral data confirm the formation of nanoparticles with its size, morphological structure and chemical composition. The synthesized silver nanoparticles were also investigated for antibacterial activity against both gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermidis*), gram-negative bacteria (*Salmonella typhi*, *Escherichia coli* and *Klebseilla pneumoniae*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum*) activities. The results revealed that silver nanoparticles exhibit good inhibition efficiency against representative microorganisms.

INTRODUCTION: Nanotechnology has become one of the most promising technologies applied in all areas of science. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in the life sciences, especially biomedical devices and biotechnology.

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The term 'nanoparticles (NPs)' are used to describe a particle with size in the range of 1-100 nm [1-2]. NPs may be classified into different types according to their size, morphology, physical and chemical properties. Nanoparticles research is inevitable today not only because of applications and also by way of synthesis [3].

The synthesis of NPs usually involves a chemical reduction reaction where toxic chemicals are used. For that reason, more sustainable methods, known as "Green Synthesis or Biogenic Synthesis" have been proposed. The biosynthesis of metal nanoparticles by biological method involves using the plant extract, fungi, algae, bacteria yeast *etc.* [4]

It is cost-effective, environmentally friendly, a single-step method for the biosynthesis process, and safe for human therapeutic use [5-6]. The plant or plant extract act as reducing and capping agent for the synthesis of NPs, are more advantageous over other biological processes [7-8]. The types of NPs include carbon-based NPs, ceramic NPs metal NPs, semiconductor NPs, polymeric NPs and liquid-based NPs [9]. Among other NPs metal NPs have raised attention over the last few decades because they have a larger surface area per weight or volume and many characteristics; biological, thermal, chemical, dielectric, electrical, physical, mechanical, electronic, magnetic and optical properties make them attractive tools for research work [10]. NPs of gracious metals like Pt, Au, Ag, Cu and Zn have received global attention due to extensive application in biomedical and Physio-chemical fields [11-12]. In this sense, silver nanoparticles (AgNPs) are the most used and have been applied in many consumer products owing to their excellent properties, especially antimicrobial activity and biochemical detection [13].

Silver is a non-toxic, safe inorganic antibacterial agent and capable of killing about 600 types of diseases causing microorganisms [14]. The Noble Silver NPs are striving towards the edge-level utilities in every aspect of science and technology, including the medical fields [15]. Silver NPs are important materials that have been studied extensively; such NPs possess unique electrical, optical as well as biological properties [16]. NPs with antimicrobial activity is very advantageous in reducing acute toxicity, lowering cost and overcoming resistance as compared with other prevalent antibiotics [17]. Several metallic materials, including gold, silver, iron, copper NPs are reported to be antimicrobial agents against various bacterial and fungal pathogens [16, 18].

To the best of our knowledge and understanding, there is no report on the utilization of methanolic extract of bulb of *Zephyranthes citrina* on the synthesis of Ag NPs. Hence, the objective of the present study is an attempt for the first time to synthesis Ag NPs by a green biological route using an extract derived from the bulb of *Zephyranthes citrina*. The synthesized AgNPs were characterized by different techniques such as UV-Visible spectroscopy, Fourier infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy-dispersive X-ray spectroscopy (EDX or EDAX). Furthermore, antimicrobial activity against bacterial and fungal species was also exhibited by biogenically synthesized silver nanoparticles.

MATERIALS AND METHODS:

Materials: Silver nitrate is of analytical grade and was procured from Merck, India. Representative microorganisms of gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Salmonella typhi*, *Escherichia coli* and *Klebseilla pneumonia*), as well as fungal species (*Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum*) were used to evaluate the antimicrobial activity of prepared silver NPs. All the micro-organisms were purchased from Acme Pro-Gen Biotech (India) Private Limited, Salem, Tamil Nadu, India, for antimicrobial investigation. The bacterial strains were maintained on Nutrient Broth (NB) at 37 ºC, and fungi were maintained on Potato Dextrose Agar (PDA) at 28 ºC.

Collection, Authentication and Extraction of Plant Material: The plant was collected from the host area of Institution, Paramathi Velur, Namakkal District, Tamil Nadu, India. The plant was identified and authenticated by Scientist 'D' in the Botanical Survey of India (Voucher No.: BSI/SRC/5/23/2018/Tech/1113), Southern Regional Centre, Agricultural University, Coimbatore. The bulbous plant material was cut into pieces, dried under shade for 15 days, coarsely powdered and stored in airtight containers for further studies. The powdered sample of bulb of *Zephyranthes citrina* was successfully extracted in methanol using a Soxhlet apparatus.

Biosynthesis of Silver Nanoparticles (AgNPs): The synthesis of silver NPs was carried out using a similar procedure reported by Sasikala *et al.*, [19]. 100 ml of 1 mM solution of silver nitrate was freshly prepared and stored in an amber color bottle. Then 1 mM $AgNO_3$ solution was added to the methanol extract of the bulb of *Zephyranthes citrina*; the formation of AgNPs occurs. This setup was kept aside for 24 h without shaking. The reduction of Ag^+ to Ag° was confirmed by the colour change of the solution from brown to

blackish brown and also by UV-Visible spectroscopy.

Characterization of Synthesized AgNPs: The reduction of metal ions was monitored as a function of wavelength using a UV-Visible spectrophotometer (UV-2450, Shimadzu). With the help of FT-IR spectroscopy, the functional group present in the nanoparticles can be identified (Thermo Nicolet-nexus 670 spectrometer of resolution 4 cm^{-1}). The nature of nanoparticles can be studied by X-ray diffraction analysis using a Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with CuKα radiations at 2θ angle. A morphological analysis of the sample was carried out by using a Hitachi S-4500 SEM machine. Elemental analysis of the nanoparticles was performed by the Hitachi S-3400N SEM instrument equipped with thermo EDAX attachments.

Antimicrobial Activity of AgNPs:

Preparation of Inoculums: The growth method is performed as follows: At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture is incubated at 35 ºC until it achieves or exceeds the turbidity of the 0.5 McFarland standards 20 (usually 2 to 6 h).

Well Diffusion Method – Antimicrobial Activity: The antibacterial and antifungal activity of methanolic bulb extracts of *Zephyranthes citrina* was determined by well diffusion method [21]. Prepare Muller - Hinton agar plates, optimally, within 15 min after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculums from the swab. The dried surface of a Muller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two or more times, rotating the plate approximately 60º each time to ensure an even distribution of inoculums. As a final step, the rim of the agar is swabbed. Sterile 6 mm filter paper discs were placed on the plates and immediately the test sample was added which already dissolved in water or DMSO. The plates were left for 30 min at room temperature to allow diffusion and were incubated at 35 ºC for 24 h [22-23].

RESULTS AND DISCUSSION:

UV-Visible Spectroscopy: The biosynthesis of silver nanoparticles has been characterized by various techniques so far [24]. The absorption of crude methanolic extract was observed at 298 nm in **Fig. 1**. The metal nanoparticles have free electrons, which give the absorption band due to the combined vibration of electrons of metal NPs with light waves. The silver nanoparticles were synthesized using silver nitrate as initiate the reaction using a crude methanolic extract of bulb of *Zephyranthes citrina*.

The colour of the reaction mixture started changing to reddish-brown after 2 h, indicating the generation of silver nanoparticles, due to reduction of silver ions to silver NPs during exposure to plant extract, similar results were observed in different extracts from plant sources [25]. The color change is due to the excitation of Surface Plasmon Resonance (SPR). A characteristic and well defined SPR band for silver NPs was obtained at 277 nm. These absorption peaks confirm the formation of corresponding metal nanoparticles of methanolic extract of bulb of *Zephyranthes citrina* and the graph obtained was represented in **Fig. 1**.

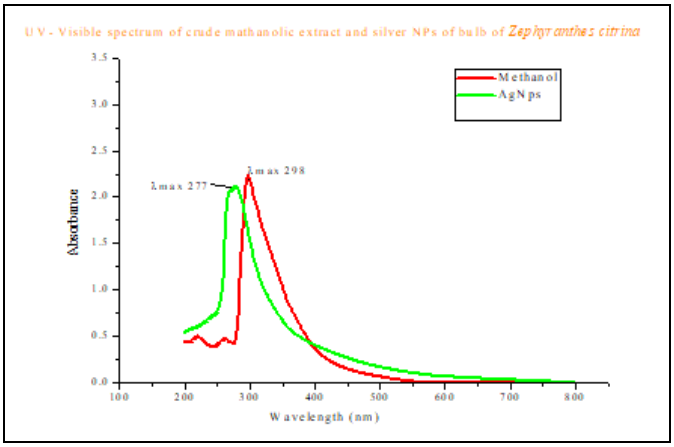

FIG. 1: UV- VISIBLE SPECTRUM OF CRUDE MATHANOLIC EXTRACT AND SILVER NANOPARTICLES OF BULB OF *ZEPHYRANTHES CITRINA*

FT-IR Spectral Analysis: FT-IR measurements were carried out to identify the major functional group present in the methanolic extract of bulb of *Zephyranthes citrina* and their possible involve-

ment in the synthesis and stabilization of silver nanoparticles. FT-IR spectra of crude methanolic extract of bulb of *Zephyranthes citrina* and synthesized silver nanoparticles from plant extract are shown in **Fig. 2**. The spectral data for crude methanolic extract showed several peaks indicating the complex nature of the biological material and also proved that the synthesized nanoparticles are surrounded by plant metabolites [26-27].

The broadband formation at 3417.08 cm^{-1} shows the presence of N-H stretching for silver compounds. The intense peak at 2924.62 cm^{-1} and 2851.50 cm^{-1} are recognized to the C-H asymmetric stretching (methyl group-CH_3) and symmetric stretching (-C-H), respectively. The small band that appeared at 1727.76 cm^{-1} shows the existence of aldehyde (carbonyl group) appropriate to a phytochemical compound present in the extract. Whereas the appearance of peaks at 1459.95 cm^{-1} and 1457.29 cm^{-1} are recognized as the C-H asymmetric bending from the methyl group (CH_3), peak at 1381.08 cm^{-1} is due to O-H bending in the medium aqueous synthesis of particles. The characteristic peak at 1250.32 cm^{-1} due to the presence of Hydroxyl (-OH) group from aryl-O-stretching in aromatic ethers and 1114.50 cm^{-1} show C-H bending (aromatic in plane). The peaks at 1062.80 cm^{-1} characterized due to C-C vibrations (Skeletal) and 824.29 cm^{-1} C-H bending (out of plane) in silver nanoparticles.

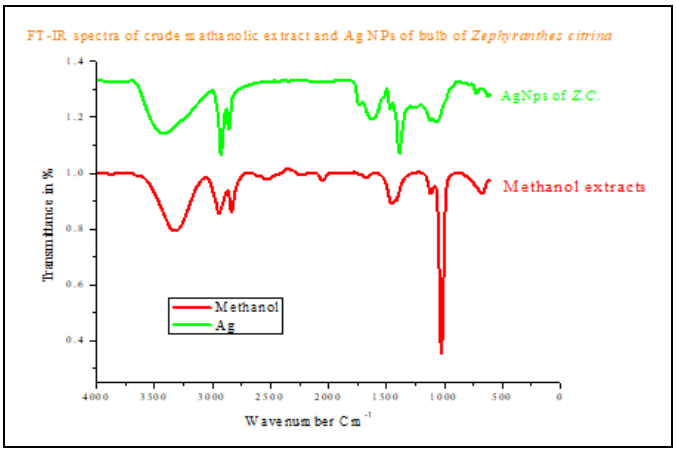

FIG. 2: FT-IR SPECTRA OF CRUDE MATHANOLIC EXTRACT AND SILVER NANOPARTICLES OF BULB OF *ZEPHYRANTHES CITRINA*

XRD Analysis: X-ray diffraction (XRD) is one of the most significant and the easiest tool to determine the crystallite characteristics for any compound. The XRD pattern of Ag nanoparticles is illustrated in **Fig. 3**. The d-spacing (interplanar spacing between the atoms) was calculated using Bragg's equation, $n\lambda = 2d \sin\theta$. The average crystallite size (D) was calculated by Debye - Scherrer formula and was found to be 18 nm for the most intensive planes of AgNPs.

$$D = K\lambda / \beta \cos\theta$$

Where K is a numerical constant factor (0.89), λ is the wavelength of X-ray used (1.50406 × 10-10 m), β is the full width at half maximum and θ is the angle of diffraction.

The spectrum is perfectly matched to that of clean Ag with a monoclinic system. The diffraction pattern of a silver nanoparticle is observed at 2θ values of 28.36°, 32.92°, 46.80°, 55.28° and 77.14°. Lattice cell parameters are a = 3.578, b = 9.20, c = 5.677 for primitive and sharp peaks observed respects to special planes (111), (121), (132), (221) and (143) confirms the formation of Ag nanoparticles. These peaks are perfectly matched with JCPDS Card No.: 84-1261.

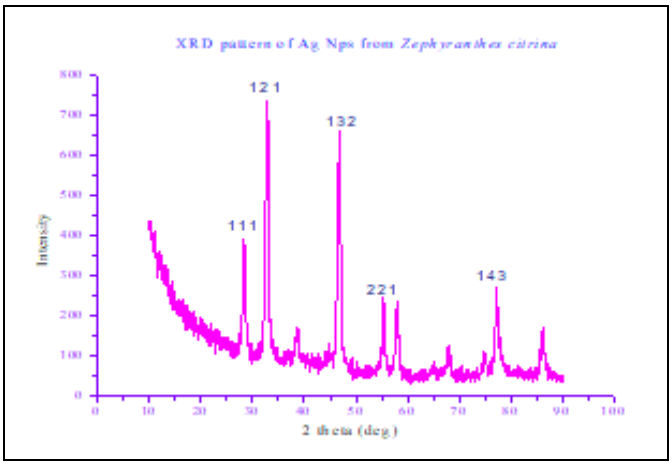

FIG. 3: XRD SPECTRA OF SILVER NANOPARTICLES

SEM-EDAX Analysis: SEM provided further insight into the morphology and size details of the nanoparticles. SEM images of Ag nanoparticles are as shown in **Fig. 4**, which is obtained from the methanolic extract of bulb of *Zephyranthes citrina*.

The formation of silver nanoparticles, as well as their morphological structure, was observed by SEM. The average particle size of silver nano-particle is 15-45 nm calculated by pixel ruler software. The shapes of Ag nanoparticles were identified as spherical.

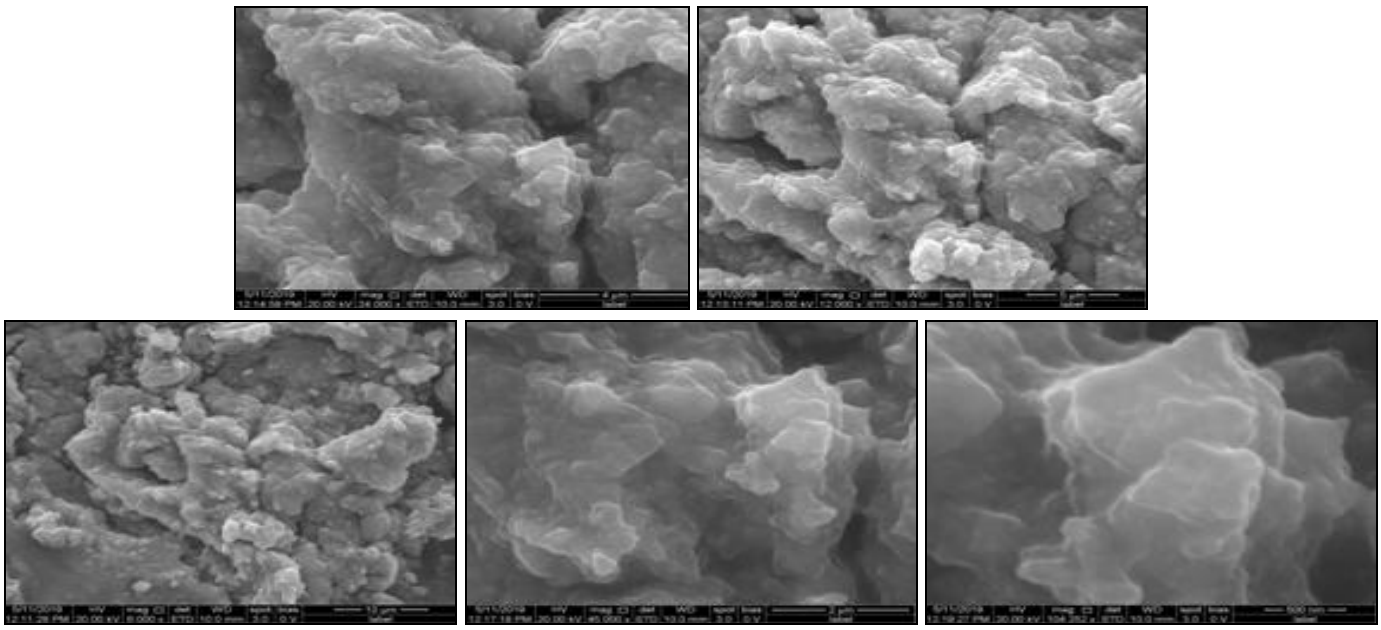

FIG. 4: SEM IMAGES OF SILVER NANOPARTICLES FROM METHANOLIC EXTRACT OF BULB OF *ZEPHYRANTHES CITRINA*

EDAX spectrum of nanoparticles helps to identify the elemental composition of the sample. EDAX spectra for synthesized Ag NPs from the methanolic extract of bulb of *Zephyranthes citrina* are shown in **Fig. 5**. The report provides information about the presence of S, C, N, Cl and Ag from silver nanoparticles with different percentages. The EDAX spectrum of silver nanoparticles does not contain any oxygen element, indicating that formed nanoparticle is metallic nanoparticles that are silver nanoparticles. From the spectral information, we conclude that the silver nanoparticles are reduced by *Zephyranthes citrina*. The weight percentage of silver is 45.33%. The presence of carbon content was obtained from phytochemicals, which acts as a stabilizing agent.

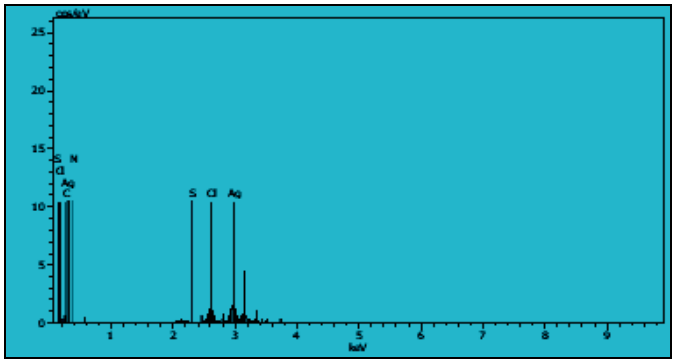

FIG. 5: EDAX SPECTRA OF SILVER NANOPARTICLES

Antibacterial Activity of Synthesized AgNPs: The antibacterial activities of silver nanoparticles were investigated with pathogens of Gram-positive *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and Gram-negative *Salmonella typhi*, *Escherichia coli*, *Klebseilla pneumonia* bacteria by measuring the zone of inhibition [28-29]. **Fig. 6** shows the zone of inhibition on bacteria against AgNPs at different concentrations. The present studies provide the zone of inhibition measured in millimeter with different concentrations (25 µl, 50 µl, 75 µl, 100 µl) of synthesized nanoparticles. The nanoparticle inhibition activities measured by the radial diameter of zones are shown in **Table 1**. Methanol is used as a negative control and Tetracyclin (10 mg/ml) as standard. The results revealed that increasing the concentrations of silver nano-particles, the zone of inhibition also increased against the test bacteria. Several main mechanisms lie behind the bacterial properties of AgNPs against representative microorganisms. First, the NPs attach to the negatively charged cell surface, alter the physical and chemical properties of the cell membranes and cell wall and disturb the important functions [30]. Some carboxyl and amines group of compounds react on the cell surface of gram-positive and gram-negative bacteria and consequently merge to nanoparticles [31].

The synthesized silver nanoparticles show good antibacterial activity with efficient inhibition against the *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Salmonella*

typhi, *Escherichia coli* and *Klebseilla pneumonia*. The nanoparticles released the silver ion (Ag^+) and it reacts with the enzyme system of bacterial cell wall, which helps to restrict the growth of bacteria [32]. *S. typhi*, *S. aureus* and *S. epidermidis* showed higher activity than others. *Bacillus subtilis* showed the least activity compared to all pathogens studied. Based on the result obtained, we concluded that silver nanoparticles synthesized from the methanolic extract of bulb of *Zephyranthes citrina* exhibit good inhibition activity against gram-negative and gram-positive bacteria on the culture plates and the graph are shown in **Fig. 7**.

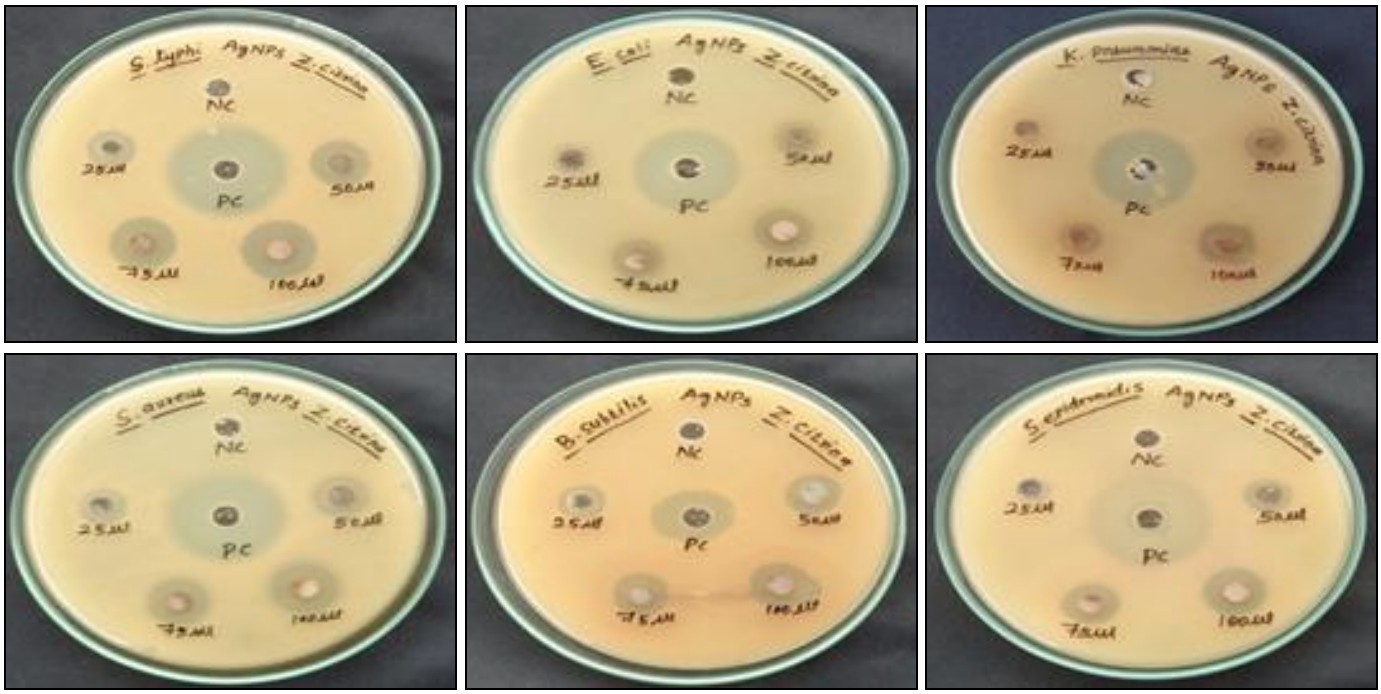

FIG. 6: THE INHIBITION ZONE OF SILVER NANOPARTICLES WITH DIFFERENT CONCENTRATIONS AGAINST BACTERIA

TABLE 1: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM *ZEPHYRANTHES CITRINA*

S. no.	Test organisms	Zone of inhibition(mm) in different concentrations					
		Control	25 µl	50 µl	75 µl	100 µl	Standard (10 mg/ml)
1	*S. typhi*	NA	6	7	8	9	15
2	*E. coli*	NA	5	6	7	8	12
3	*K. pneumoniae*	NA	0	5	6	7	12
4	*S. aureus*	NA	5	6	8	9	14
5	*B. subtilis*	NA	6	7	7	9	10
6	*S.epidermidis*	NA	4	5	8	8	14

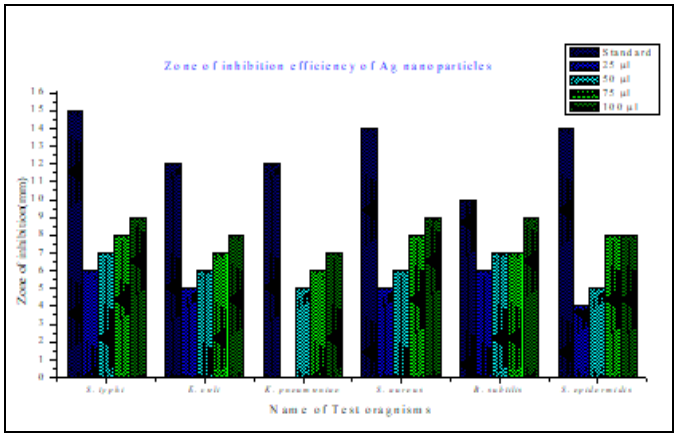

FIG. 7: ZONE OF INHIBITION EFFICIENCY OF SILVER NANOPARTICLES

Antifungal Activity of Synthesized AgNPs: The fungal activities of Ag were investigated with *Aspergillus niger*, *Candida albicans* and *Fusarium oxysporum*. Antifungal action of ionic or silver nanoparticles has a huge prospective for use in controlling spore-producing fungal plant pathogens [33]. Silver is less toxic to humans and animals than other synthetic fungicides. Silver nanoparticles exhibit considerable antifungal activity in a petri dish assays [34]. Silver nanoparticles can directly connect and go through the cell membrane to destroy spores, even though the diffusion of silver nanoparticles into cell membranes.

In the present study, we have reported the antifungal activity of the AgNPs synthesized from methanolic extract of bulb of *Zephyranthes citrina*. The inhibition efficiency of nanoparticles is shown in **Fig. 8** and the activities are reported in **Table 2**. The antifungal activities of the synthesized metal nanoparticles were measured at different concentrations (25 µl, 50 µl, 75 µl and 100 µl). Methanol was used as a negative control and Kanamycin used as standard. Antifungal activities of silver nanoparticles are illustrated in the graphical **Fig. 9**. From the results obtained, we concluded that the *Candida albicans* showed the highest inhibition efficiency, whereas *Fusarium oxysporum* exhibit high inhibition than *Aspergillus niger*.

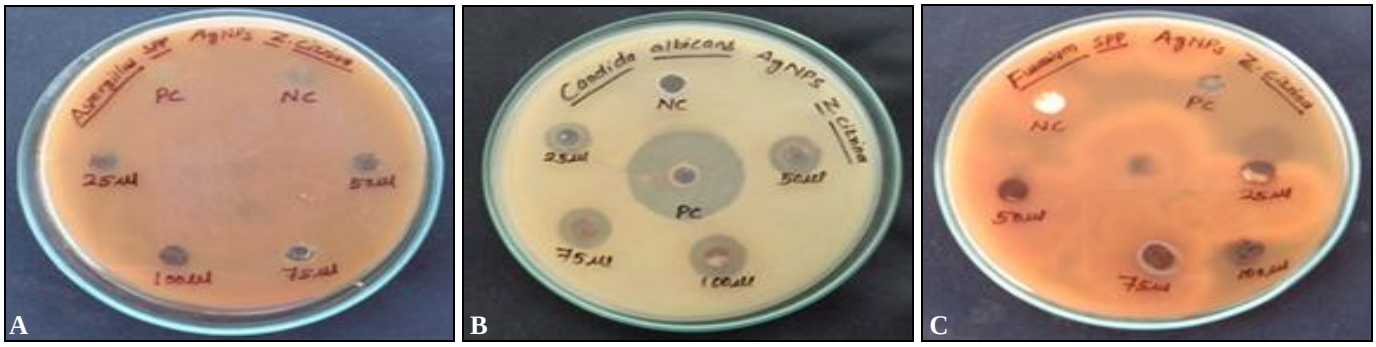

FIG. 8: THE INHIBITION ZONE OF SILVER NANOPARTICLES WITH DIFFERENT CONCENTRATIONS AGAINST FUNGI

TABLE 2: ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES FROM *ZEPHYRANTHES CITRINA*

S. no.	Test organisms	Zone of inhibition in (mm) different concentration					
		Control	25 µl	50 µl	75 µl	100 µl	Standard Kanamycin
1	*Aspergillus niger*	NA	NA	NA	4	5	8
2	*Candida albicans*	NA	5	6	7	8	14
3	*Fusarium oxysporum*	NA	4	5	6	7	8

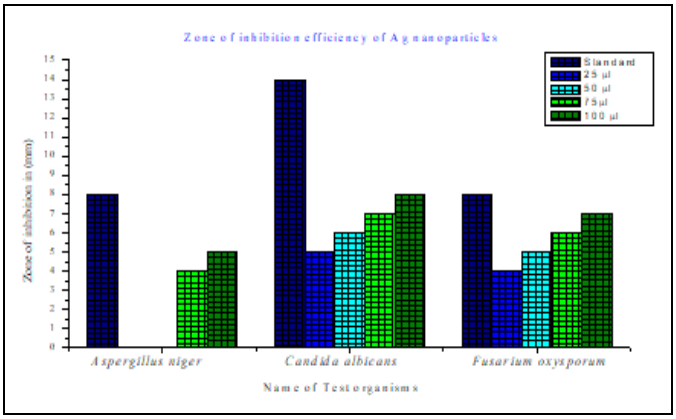

FIG. 9: ZONE OF INHIBITION EFFICIENCY OF SILVER NANOPARTICLES

CONCLUSION: The methanolic extract of bulb of *Zephyranthes citrina* was successfully utilized for the biosynthesis of silver nanoparticles. The secondary metabolites present in the plant extract act as reducing agents as well as capping agents. The biosynthesized AgNPs from plant material was characterized by various spectral techniques and confirmed that AgNPs is monoclinic in nature. The particles were identified as the spherical shape with high stability. The biosynthesized nanoparticles were investigated by antibacterial activity for Gram-positive and Gram-negative bacteria and antifungal activity. The results revealed that the synthesized AgNPs show good antimicrobial activity against the selected microorganisms.

This biosynthesis approach appears to be a cost-effective, non-toxic, eco-friendly and alternative to conventional microbiological, physical and chemical methods. Hence, the silver nanoparticles may be used as prospective for a biological mediator on the microorganism.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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