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Mycosynthesis of Silver Nanoparticles from Endophytic *Fusarium moniliforme* and its Biological Activities

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Endophytic fungi are a rich source of bioactive compounds with diverse pharmacological potentials. *Fusarium moniliforme*, an endophytic fungal strain isolated from the leaves of *Catharanthus pusillus*, was employed in the present study for the green synthesis of silver nanoparticles and evaluation of their biological activities. The AgNPs were characterized using UV-vis spectroscopy, FTIR, AFM and XRD. The UV-visible spectra analysis confirmed the formation of AgNPs with a surface plasmon resonance peak at 443nm. FTIR analysis revealed the involvement of fungal metabolites in the reduction and stabilization of AgNPs, while AFM analysis showed nanoscale morphology with particle sizes ranging from 2.8 to 4.5nm. XRD analysis confirmed the highly crystalline nature of the synthesized nanoparticles. The highest inhibition zone was found to be 21mm against tested bacteria and fungi. Antioxidant assays showed high free radical scavenging activity of 91.58% and EC 50 of 975 µg/mL. 201 µmol TE/g was obtained from FRAP assay when compared to crude extract. The AgNPs also showed notable anti-inflammatory activity, exhibiting 70.58% inhibition of albumin denaturation and 92% proteinase inhibition. Cytotoxicity studies revealed moderate anticancer activity against HeLa cells, with an IC50 value of 53.58 µg/mL when compared to podophyllotoxin. Mycosynthesized AgNPs from *F. moniliforme* demonstrated broad-spectrum antimicrobial, strong antioxidant, anti-inflammatory and moderate anticancer activities. This green synthesis approach offers a sustainable route for developing multifunctional nanomaterials with biomedical potential.

Keywords : *Catharanthus pusillus*, endophytes, *in-vitro* assays. mycosynthesis, nanoparticle characterization;

INTRODUCTION

Nanoparticles have found extensive applications in the biomedical and pharmaceutical sectors, particularly in the treatment of human cancers. Nanoparticle-based research has attracted intense scientific interest owing to its wide range of potential applications, especially in the development of innovative drug-delivery systems that may effectively overcome several limitations associated with conventional cancer therapies. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have gained considerable attention due to their broad-spectrum biological activities, high surface to volume ratio, enhanced bioavailability, and ability to induce reactive oxygen species mediated cytotoxicity in cancer cells. AgNPs can be synthesized using different approaches, including chemical, radiational, electrochemical, and photochemical methods. However, these methods often involve toxic chemicals and high energy requirements, limiting their biomedical applicability. In recent years, biological synthesis methods have emerged as promising alternatives due to their cost-effectiveness, environmental compatibility, and use of non-toxic reagents. Biological synthesis primarily involves microorganisms capable of producing nanoparticles either intracellularly or extracellularly, depending on the site of nanoparticle formation. Biomolecules such as proteins, enzymes, phenolics, and other secondary metabolites act as reducing and stabilizing agents during nanoparticle biosynthesis, thereby improving stability and biological functionality. Among biological approaches, mycosynthesis refers to the biosynthesis of nanoparticles using fungi and is

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considered an eco-friendly and economically viable technique due to the inherent advantages offered by fungal systems, such as high metal tolerance, secretion of large amounts of extracellular enzymes, and ease of large-scale cultivation.

Medicinal plants serve as unique ecological niches for endophytes, and host–microbial interactions often stimulate the production of several novel metabolites by endophytic microbes, in addition to compounds naturally synthesized by the host plants (Samanta *et al*. 2021, Kusari *et al*. 2012). Endophytes remain relatively underexplored and represent promising sources of structurally diverse natural products with potential applications in medicine, agriculture, and the pharmaceutical industry. Factors such as chemo diversity, traditional medicinal usage of the host plant, and the geographical origin of the plant are considered critical criteria for the successful isolation of endophytes. The increasing demand for novel compounds with potent anticancer and antioxidant activities has further intensified interest in endophytic research. In certain instances, endophytes have also been reported to induce enhanced production of secondary metabolites in host plants (Tiwari *et al*. 2010). Although microbial inoculants derived from rhizospheric soils have been previously shown to improve secondary metabolite content in medicinal plants (Awasthi *et al*. 2011), limited information is available on the role of endophytes in enhancing the biosynthetic potential of medicinal plants for the sustainable production of therapeutically valuable phytomolecules.

Endophytes offer several advantages over free-living microorganisms, including metabolic stability, the ability to synthesize unique bioactive compounds arising from long-term host association, and reduced ecological impact. Despite increasing reports on biologically synthesized nanoparticles, comprehensive studies integrating endophyte-mediated nanoparticle synthesis, detailed physicochemical characterization, and evaluation of biomedical efficacy remain limited. Moreover, mechanistic insights into endophyte-assisted nanoparticle formation and the contribution of capping biomolecules to biological activity are still poorly understood, highlighting a significant research gap.

Catharanthus pusillus (Murray) G. Don, commonly known as tiny periwinkle, belongs to the family Apocynaceae and is native to India and Sri Lanka (Ankad *et al*. 2016). The plants and seeds of *C. pusillus* are reported to be toxic to cattle and sheep, causing temporary blindness, neurological disturbances, and skin rashes (Srivastava, 2018). Despite its toxicity, the plant has been traditionally used in herbal medicine for the treatment of various ailments, including fever, joint and muscle pain, skin and liver disorders, leprosy, dysentery, intestinal worms, ulcers, tumours, and earache (Balakrishnan *et al*. 2013). Additionally, leaf powder mixed with coconut oil has been traditionally used for antidandruff treatment and for eliminating lice (Nithya *et al*. 2013). Recent research has increasingly focused on the endophytic microbial community associated with *C. pusillus*, which may significantly contribute to its pharmacological potential. Preliminary studies have demonstrated that endophytic *Fusarium moniliforme* can synthesize bioactive metabolites exhibiting antimicrobial, anticancer, and antioxidant activities (Suryanarayan *et al*. 2009; Verma *et al*. 2021; Krishnamurthy *et al*. 2025). Metabolites produced by endophytes not only support host plant fitness but also offer substantial potential for pharmaceutical development, agriculture, and industrial bioprocesses.

In this context, the present study involves the isolation of endophytic fungi from *Catharanthus pusillus*, their application in the green synthesis of silver nanoparticles, comprehensive physicochemical characterization of the synthesized nanoparticles, and evaluation of their antimicrobial, anticancer, and antioxidant potential, thereby contributing to sustainable and eco-friendly nanotechnological approaches for biomedical applications.

MATERIALS AND METHODS

Collection of Plant material and endophyte isolation

Catharanthus pusillus plants were collected from a village called Hudem, Kudligi Taluk, Vijayanagara

District, Karnataka, India. The collected plant material was washed in tap water for 3-4 times to remove dust and soil particles. Roots, stem and leaves are separated, cut into small segments and were subjected for surface sterilization. Surface sterilization was done using 70% alcohol and 0.1% sodium hypochlorite solution for 2 minutes each. The surface sterilised plant segments were placed on PDA culture plates and incubated. Fungal colonies were isolated, sub cultured for purity and scaled up. Aqueous extracts of *Fusarium moniliforme* were prepared and stored in refrigerator for biological activity assays (Barnett and Hunter, 1998).

Mycosynthesis of AgNPs

Aqueous Silver nitrate ($AgNO_3$) solution is prepared and mixed with endophytic fungal extract of *Fusarium moniliforme* in 1:1 ratio. The mixture was stirred for proper mixing and incubated at room temperature or slightly elevated temperature for a few hours. A colour change typically yellow to brown indicates the formation of silver nanoparticles. The nanoparticles are then by centrifugation, washed with ethanol and dried overnight at 80^{o} C and preserved for further studies (Kaviya *et al.* 2011).

Characterization of silver nanoparticles

Mycosynthesized nanoparticles were characterised by conducting multiple advanced analytical techniques to confirm their formation, structure and properties. UV- Visible spectroscopy was done to monitor the surface plasmon resonance, indicated successful synthesis. Fourier Transform Infrared Spectroscopy (FT-IR) helped to identify functional group involved in the reduction and stabilization of nanoparticles. Atomic Force Microscopy (AFM) provided detailed information on the surface topography and morphology of nanoparticles. X-ray Diffraction (XRD) analysis was performed to determine the crystalline nature and average particle size of the synthesized silver nanoparticles (Shekar *et al*. 2025).

Antimicrobial activity of AgNPs

The antimicrobial properties of biosynthesized nanoparticles were examined using the disc diffusion method (Radhakrishnan *et al.* 2018; Thejaswini *et al*. 2024) against selected human pathogenic bacteria such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (MTCC 96), *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404), obtained from the culture stock from the department of Biotechnology, Manasagangotri, University of Mysore, Mysuru. Sterilised discs (16mm) loaded with 50ìg of silver nanoparticles and endophytic fungal extract were placed on Mueller-Hinton Agar (MHA) plates inoculated with bacterial cultures and PDA with fungal cultures. Sterile distilled water was used as negative control, while antibiotics tetracycline, vancomycin and Fluconazole (25ìg) were used as positive controls. Plates were incubated at 37 ± 2^{0}C for bacterial cultures and 25 ± 2^{0}C for fungi. The zone of inhibitions was measured after incubation period to assess antimicrobial potency.

Antioxidant activities of AgNPs

The radical scavenging property of the endophytic extract was evaluated by the DPPH assay using ascorbic acid as standard. In a 96-well plate, 50ìl of extract was mixed with 180ìl of DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was read at 514nm and activity was expressed as a percentage.

The FRPA assay measured the antioxidant potential of the endophytic extract (10mg/ml) by reducing Fe^{3+} to Fe^{2+}using ascorbic acid (40ìg/ml) as a standard. 0.10ml extract was mixed with 3ml FRAP reagent and incubated at 37^{o}C for 30 min and absorbance read at 593nm (Sheng *et al*. 2011; Suguna *et al*. 2023).

In vitro anti-inflammatory activities of AgNPs

Protein denaturation inhibition was assessed using bovine albumin in PBS (pH 6.4) with 0.02 ml extract. The mixture was incubated at 37^{0}C for 15 min, heated at 70^{0}C for 5 min and absorbance measured at 660nm. Inhibition percentage was calculated. The membrane stabilization assay involved incubating blood cell suspension with extract and phosphate buffer (pH 7.4) at 54^{0}C for 20min, followed by centrifugation at 2500rpm for 3 min. Absorption of the

supernatant was measured at 540nm. Aspirin served as the standard and haemolysis was calculated. Proteinase inhibition was assessed by incubating trypsin, Tris-HCl buffer and extract at 37^0C for 5 min later adding casein and incubation for 20 min. The reaction was stopped with perchloric and followed by centrifugation. The absorbance measured at 210 nm, inhibition percentage was calculated (Gunathilake *et al.* 2018; Suguna *et al.* 2023).

Cytotoxicity Potentials

Cytotoxicity of endophytic extract against HeLa cells was evaluated using MTT assay. Cells were cultured in MEME with FBS and antibiotics, treated with extracts or podophyllotoxin for 24 h and then incubated with MTT. Formazan crystals were dissolved in DMSO absorbance measured at 570 nm and cell viability calculated (Mahnashi *et al*. 2021).

RESULTS AND DISCUSSION

The biosynthesis of silver nanoparticles was visually affirmed by a colour change from light to dark brown, as shown in Fig 1. This color change indicated the reduction of silver ions (Ag^+) to elements silver nanoparticles (Ag^0) in the presence of aqueous extract of *Fusarium moniliforme.*

Characterisation of Silver nanoparticles

The UV–Visible spectroscopy analysis further supported nanoparticle formation, displaying a characteristic surface plasmon resonance (SPR) peak at 443 nm after 3 hours of reaction (Fig. 1B). These observations confirm the successful biosynthesis of silver nanoparticles using the endophytic fungus *F. moniliforme* isolated from the *C. pusillus.* These findings are aligned with earlier studies where fungal species such as *Fusarium oxysporum* and *Aspergillus niger* were reported to reduce silver ions into stable nanoparticles with potent bioactivities (Ahmad *et al*. 2003; Bhainsa and D Souza, 2006). These observations confirm the successful biosynthesis of silver nanoparticles using the endophytic fungus *F. moniliforme* isolated from *C. pusillus*. These findings are consistent with earlier reports where fungal species such as *Fusarium oxysporum* and *Aspergillus niger* were shown to reduce silver ions into stable nanoparticles that exhibited characteristic SPR peaks and potent bioactivities, including antimicrobial and anticancer effects (Gade *et al.* 2008; Raj *et al*. 2021). UV–Vis spectroscopy not only provides qualitative confirmation of nanoparticle formation but can also offer preliminary insights into nanoparticle stability and size trends, as shifts in SPR peak position and peak breadth have been linked to particle size distribution and degree of aggregation.

The FTIR spectrum of silver nanoparticles (AgNPs) synthesized using the endophytic fungus *F. moniliforme* from *C. pusillus* shows the presence of several functional groups involved in the reduction and stabilization of the nanoparticles (Fig.2). The FTIR shows a broad-spectrum peak at 3242 cm^{-1} indicating O-H stretching from hydroxyl groups, likely involved in the silver ion reduction. Similar observations have been reported in biologically synthesized AgNPs, where hydroxyl-containing biomolecules act as effective reducing agents. The absorption peak at 2920, 2850 and 2360 cm^{-1} represent C-H stretching from aliphatic chains. The sharp peak at 1694 cm^{-1} and bends at 1632, 1534 and 1384 cm^{-1} indicate the presence of carboxyl and amide groups, suggesting protein involvement in nanoparticles stabilization. These proteinaceous biomolecules likely act as capping agents, preventing aggregation and contributing to nanoparticle stability. Furthermore, the characteristic peak at 779, 605 and 471 cm^{-1} in the fingerprint region correspond to Ag-O vibrations, confirming the interaction between silver ions and functional groups in the fungal extract. Thus, FTIR analysis confirms that multiple functional groups, indicating hydroxyl, carbonyl and amide groups are actively involved in the biosynthesis and stabilization of silver nanoparticles (Fig.2).These biomolecules from *F. moniliforme* not only facilitate the reduction of silver ions but also cap the nanoparticles enhancing their stability and potential bioactivity. FTIR analysis confirmed the role of protein and phenolic groups in reduction and capping aligning with findings by Narayanan and Sakthivel (2010), who highlighted the involvement of amide and hydroxyl groups in nanoparticles stabilization.

Table 1 : Antimicrobial activity of mycosynthesized AgNPs and endophytic *Fusarium moniliforme extract*

	Zone of inhibition (in mm)			
	Candida albicans	*Aspergillus niger*	*Staphylococcus aureus*	*Escherichia coli*
Endophytic F. *moniliforme* extract (25µl)	18± 0.54	19± 0.28	18± 0.44	20± 0.35
Ag NPs 50 µg	21± 0.98	20± 0.86	21± 0.55	21± 0.98
Fluconazole 25µg	22± 0.73	20± 0.49	-	-
Vancomycin 25 µg	-	-	21± 0.75	20± 0.75
Tetracycline 25 µg	-	-	21± 0.95	21± 0.65
Negative control DW	NI	NI	NI	NI

Table 2 : Antioxidant activities of mycosynthesized AgNPs and endophytic *F. Moniliforme* extract

	AA (%)*	EC_{50} (µg/mL)	FRAP (µmol TE/g)
Endophytic *F. moniliforme* extract (25µl)	93.58[a]	984[a]	205[a]
AgNPs	91.58[a]	975[b]	201[b]
Quercetin	98	9	NT
Ascorbic acid	NT	NT	161.8

* Assays were carried out with the endophytic fungal extracts at the concentration of 10mg/ml. Quercetin and Ascorbic acids were tested at 40 µg/ml. NT-Not tested. Results were expressed as the means of experiments in triplicate. Means that do not share a letter are significantly different ($p<0.05$) according to the Z-test.

Table 3. *In vitro* anti-inflammatory activities of mycosynthesized AgNPs and endophytic *F. moniliforme* extract

	Albumin denaturation	Membrane stabilization	*Proteinase Inhibition*
Endophytic *F. Moniliforme* extract (25µl)	73± 0.05[b]	86± 0.08[a]	95± 0.09[a]
AgNPs	73.58[a]	82± 0.18[a]	93± 0.69[a]
Aspirin (200 µg/ml)	78±0.08	86±0.19[a]	96±0.46[a]

Experiments repeated three times for each replicate, according to Duncan's Multiple Range Test (DMRT), values followed by different subscripts are significantly different at $P < 0.05$, SE-standard error of the mean.

Table 4 : Cytotoxicity Potentials of mycosynthesized AgNPs and endophytic *F. moniliforme* extract against HeLa Cells

	IC_{50} (µg/mL)
Endophytic *F. moniliforme* extract	55.36
AgNPs	56.58
Positive control (Podophyllotoxin)	10.96

The Atomic Force Microscopy (AFM) analysis of the mycosynthesized silver nanoparticles provided clear evidence for their nanoscale morphology and uniform distribution. The 2D topographic image (Fig. 3A) reveals a relatively smooth surface with well dispersed nanoparticles, while the 3D image (Fig. 3B) illustrates the surface texture and particle height variations in greater detail. The observed particles heights range from 0 to approximately 5.5 nm, indicating the successful formation of ultra-small silver nanoparticles. The size distribution histogram (3C) shows that the majority of nanoparticles fall within the range of 2.8 to 4.5

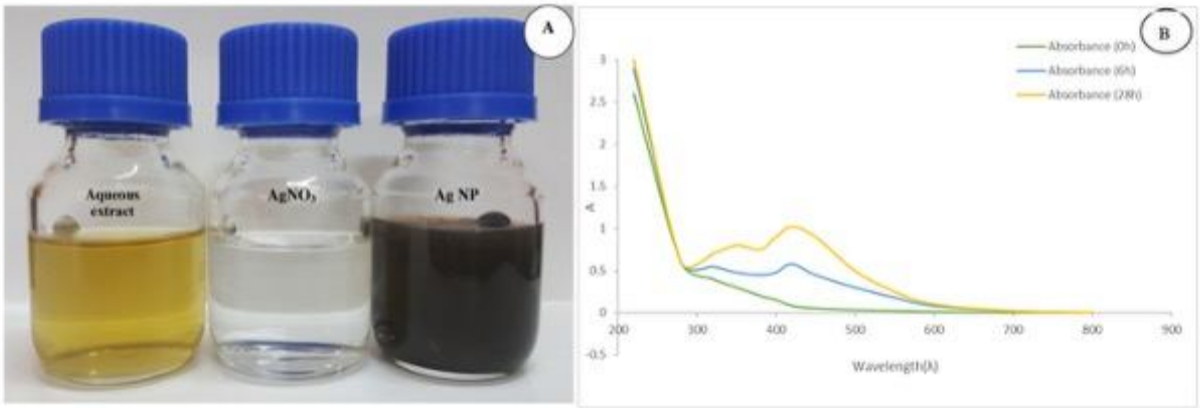

Fig 1: Mycosynthesis of AgNPs using endophytic *F. moniliforme* isolated from C. *pussilus.* A) Change in the color intensity of *F. moniliforme* aqueous extract before and after the reduction of $AgNO_3$ (1 mM); B) UV–Vis spectrum of silver nanoparticles after the 3 hours of the reaction.

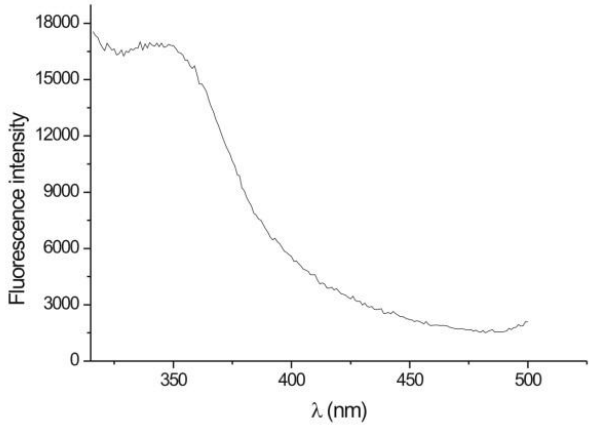

Fig 2 : FTIR spectrum of AgNPs synthesized using endophytic *F. moniliforme* isolated from *C. pusillus.*

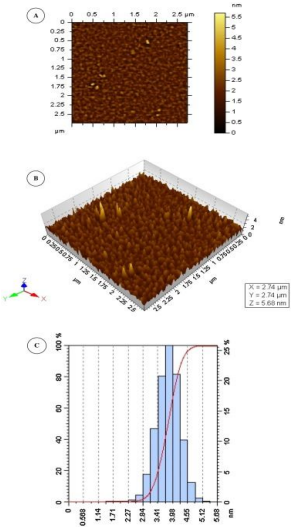

Fig 3: AFM micrograph of AgNPs synthesized using endophytic *F. moniliforme* isolated from *C. pusillus.* (A) 2.5 ìm resolution studies of 1–5 nm size, spherical shaped, polydispersed particles, (B) 3D image of silver nanoparticles analyzed by NOVA-TX software, (C) Histogram showing the particle size distribution.

nm, with a peak around 3.4 nm, suggesting a narrow and uniform size distribution, these results confirm that the nanoparticles synthesized using the endophytic fungus *F. moniliforme* are monodispersed, stable and consistent in size, highlighting the efficiency of the green synthesis method and supporting their potential use in biomedical and antimicrobial applications.

The X-ray diffraction (XRD) pattern of silver nanoparticles (AgNPs) synthesized using endophytic fungus *F. moniliforme* isolated from *C. pusillus* exhibits distinct diffraction peaks at 20 values corresponding to 38.1°, 44. 3°, 64.5° and 77.3° which are indexed to the (111), (200), (220) and (311) planes, respectively. (Fig. 4).

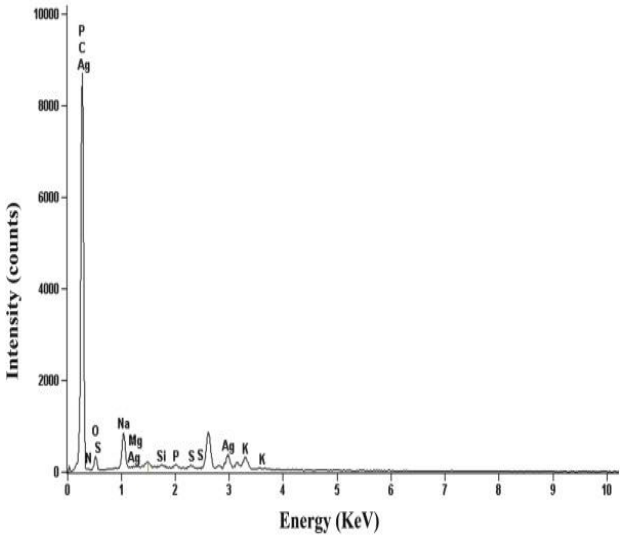

Fig 4: XRD pattern of AgNPs synthesized using the endophytic *F. moniliforme* isolated from *C. pusillus*.

These peaks are in good agreements with the standard face-centred cubic (FCC) structure of metallic silver, confirming the crystalline nature of the synthesized nanoparticles. The intense peak at 38.1° corresponding to the (111) plane indicates it as the dominant crystalline facet, which is typical for biosynthesized silver nanoparticles. The absence of additional impurity peaks further suggests high purity and successful synthesis. This XRD analysis clearly confirms the formation of crystalline silver nanoparticles through fungal –mediated green synthesis. Comparable XRD profiles have been reported for AgNPs synthesized using fungal species such as *Fusarium* and *Aspergillus*, further validating the effectiveness of fungal systems in producing crystalline and phase-pure nanoparticles (Bhainsa and D'Souza, 2006; Ahmad *et al*., 2003).

Overall characterization of silver nanoparticles synthesized using *F. moniliforme* confirms their successful formation, stability, and purity. UV–Visible spectroscopy revealed a characteristic surface plasmon resonance peak at 443 nm, confirming nanoparticle formation; FTIR analysis identified functional groups involved in reduction and stabilization; AFM confirmed nanoscale dimensions and monodispersity; and XRD demonstrated a crystalline FCC structure with a dominant (111) facet. Collectively, these results indicate the efficient synthesis of high-quality silver nanoparticles through a green, fungal-mediated approach, supporting their potential application in biomedical and antimicrobial fields.

Antimicrobial activity of AgNPs

The antimicrobial activity assay revealed that AgNPs synthesized using endophytic *F. Moniliforme* from *C. pusillus* showed remarkable inhibition against all tested pathogens (both fungi and bacteria) (Table 1). At 50ìg concentration. AgNPs exhibited inhibition zones (20-21mm) surpassing the activity of the crude fungal extract (25ìl), which recorded smaller inhibition zones (18-20), compared to standard antibiotics, AgNPs exhibited activity close to fluconazole against fungi and comparable to vancomycin / tetracycline against bacteria. The negative control (distilled water) exhibited no inhibition, confirming that the antimicrobial extracts were due to the test samples. Antimicrobial evaluation revealed that AgNPs exhibited broad-spectrum activity against both Gram-positive bacteria as well as pathogenic fungi, with inhibition zones comparable to standard antibiotics. This aligns with earlier reports highlighting the enhanced antimicrobial efficiency of AgNPs due to their nanoscale size, high surface area and ability to disrupt microbial membrane and generate reactive oxygen species (Rai *et al*. 2009; Ahmad *et al*. 2003). Similar results were reported by Bhainsa and D'Souza (2006), where AgNPs synthesised using *Aspergillus fumigatus* showed strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. The enhanced antimicrobial properties are attributed to the small size of the nanoparticles, which increases surface area and facilitate interaction with microbial cell membranes, leading to disruption and cell death (Rai *et al*. 2009). In another study AgNPs synthesized using *Fusarium oxysporum* exhibited potent antimicrobial activity and were found to penetrate bacterial membranes, generating reactive oxygen species (Ahmad *et al*. 2003). The present study also demonstrated superior antimicrobial effects of AgNPs compared to the fungal extract alone, confirming the enhanced potency through nanoparticles formation. These findings reinforce that fungal-mediated AgNPs serve as effective antimicrobial agents and offer a green alternative to combat antibiotic-resistant infections.

Antioxidant activities of AgNPs

The antioxidant evaluation showed that both the endophytic *F. moniliforme* extract and its synthesized AgNPs had high free radical scavenging activity (AA), with values of 93.58% and 91.58% respectively at 10 mg/ml, comparable to the standard quercetin (98% at 40ìg/ml) (Table 2). The EC50 values indicated strong activity, with AgNPs (975ìg/ml) slightly more effective than the fungal extract (984ìg/ml). In the ferric reducing antioxidant power (FRAP) assay, the fungal extract (205ìmol TE/g) exhibited little higher reducing power than AgNPs (201ìmol TE/g), while ascorbic acid recorded 161.8ìmol TE/g). Statistical analysis showed minor but significant differences between the extract and AgNPs. Over all, both fungal extract and its AgNPs exhibited

potent antioxidant activities close to natural antioxidant standards. The antioxidant potential assessed *via* DPPH and FRAP assays exhibited that both the fungal extract and AgNPs possessed strong radical scavenging and reducing abilities, comparable to natural standards like quercetin and ascorbic acid. This could be attributed to phenolic and proteinaceous compounds from the fungal extract that also play a role in nanoparticle stabilization (Narayanan and Sakthivel, 2010).

In vitro anti-inflammatory activities

The *in vitro* anti-inflammatory assays demonstrated that both the endophytic *F. moniliforme* extracts and its synthesized AgNPs exhibited strong activities comparable to standard drug aspirin (Table 3). The fungal extract showed 73% albumin denaturation inhibition, 86% membrane stabilization and 95% proteinase inhibition, while AgNPs recorded 73.58%, 82% and 93% respectively. Statistical analysis indicated minor but significant differences between the extract and AgNPs in some parameters. Results revealed the inti-inflammatory potential close to that of the standard drug. *In vitro* anti-inflammatory assays including albumin denaturation, membrane stabilization and proteinase inhibition, revealed activities close to aspirin, suggesting potential in mitigating inflammation-mediated disorders (Gunathilak *et al.* 2018). The observed activities may result from bioactive metabolites in the fungal extract capping the nanoparticles enhancing stability and biological efficacy (Narayanan and Sakthivel, 2010).

Cytotoxicity Potentials of AgNPs

The cytotoxicity assay against HeLa cells showed that both endophytic *F. moniliforme* extract and its synthesized AgNPs had similar inhibitory effect with IC 50 values of 55. 36ìg/ml and 56.58ìg/ml respectively (Table 4). The standard anticancer agent podophyllotoxin exhibited much strong activity with an IC50 of 10.96ìg/ml. This indicated moderate cytotoxicity potential for both the extract and AgNPs compared to the potent standard drug. Furthermore, cytotoxicity testing against HeLa cells indicated moderate anticancer potential, comparable to other green synthesized nanoparticles **(**Mahnashi *et al*. 2021**).** The activity aligns with earlier reports where myco synthesized AgNPs induced cancer cells apoptosis though ROS generation, mitochondrial dysfunction and DNA fragmentation (Gurunathan *et al*. 2013; Sulaiman *et al*. 2013). The moderate potential suggests possible selectivity towards cancer cells while minimizing toxicity to normal cells, making these AgNPs attractive for further targeted delivery and combinational therapy research. These findings reinforce the therapeutic promise of *F. moniliforme* derived AgNPs especially as eco-friendly antimicrobial, antioxidant, anti-inflammatory and anticancer agents.

CONCLUSION

Silver nanoparticles synthesized using the endophytic fungus *Fusarium moniliforme* isolated from *Catharanthus pusillus* were successfully produced and thoroughly characterized, confirming their stable, crystalline, and nanoscale nature. The biosynthesized AgNPs exhibited pronounced antimicrobial activity against both bacterial and fungal pathogens, indicating their potential application as broad-spectrum antimicrobial agents. In addition, the nanoparticles demonstrated significant antioxidant and anti-inflammatory activities, suggesting their usefulness in mitigating oxidative stress– and inflammation-related conditions. Moderate cytotoxic effects observed against HeLa cells further highlight their prospective role in anticancer applications. Notably, the biological performance of these AgNPs was comparable to that of standard reference drugs in several assays, underscoring their multifunctional bioactivity.

The eco-friendly mycosynthesis approach employed in this study represents a sustainable and cost-effective alternative to conventional chemical synthesis methods, eliminating the need for toxic reagents and high energy input. Owing to their diverse bioactivities, the endophyte-derived AgNPs show promising potential for applications in biomedical fields, including the development of antimicrobial coatings, antioxidant therapeutics, anti-inflammatory formulations, and adjunct anticancer strategies. Furthermore, this green synthesis strategy contributes to the advancement of environmentally benign

nanotechnology solutions, aligning with current demands for sustainable approaches in nanomaterial production.

DECLARATION

Conflict of Interest . Authors declare no conflict of interest.

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