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Taxonomic and Phylogenetic Analysis of *Ganoderma gibbosum* from Kerala and Antibacterial Activity of Its Silver Nanoparticles

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ABSTRACT

***Ganoderma gibbosum* was collected from Thiruvananthapuram District, Kerala State, India and was characterized based on macroscopic, microscopic and phylogenetic data. The methanolic and distilled water extracts of *G. gibbosum* was used to synthesize silver nanoparticles and its antibacterial effect was studied against *Bacillus cereus, Bacillus subtilis, Escherichia coli,* and *Proteus mirabilis*. The maximum UV absorption observed at 420.25 nm corresponds to the specific surface plasmon resonance band of biosynthesized silver nanoparticles. *Proteus mirabilis* exhibited the highest susceptibility to the extract, as indicated by a substantial zone of inhibition measuring 20 mm. These silver nanoparticles demonstrated efficacy against pathogens by inhibiting their proliferation, thereby potentially mitigating environmental and public health risks.**

Keywords: AgNPs, Antimicrobial, nrITS, Polyporales, Taxonomy, White rot

INTRODUCTION

The genus *Ganoderma* was established by Karsten (1881) with *Polyporus lucidus* (Curtis) Fr. (=*Ganoderma lucidum* (Curtis) P. Karst.) as the type species (Moncalvo and Ryvarden, 1997). Many *Ganoderma* species are pathogenic in nature, causing various diseases to plants including various types of rot and wood decay (Ryvarden, 2004; Pilotti, 2005; Dai *et al.*, 2007; Cao and Yuan, 2013; Coetzee *et al.*, 2015). The genus has a worldwide distribution in the tropical and temperate regions, including Africa, America, Europe, and Asia (Pilotti, 2005; Cao *et al.*, 2012; Wong *et al.*, 2012; Cao and Yuan, 2013).

Ganoderma is characterized by distinctive laccate or non-laccate, sessile to stipitate basidiomata, double-walled basidiospores with interwall pillars (Karsten, 1881; Moncalvo and Ryvarden, 1997). There are 503 records in the Index Fungorum (http://www.indexfungorum.org/; accessed date: April 8, 2026).

Ganoderma has been used in traditional medicine in East Asia and is used to treat a wide range of pathological diseases (De Silva *et al.*, 2012; Hapuarachchi *et al.*, 2016). *Ganoderma* are also used as ingredients in cosmaceuticals (Hyde *et al.*, 2010) and neutraceuticals (De Silva *et al.*, 2013; Wachtel-Galor *et al.*, 2011).

During our ongoing studies on Macro fungi of Thiruvananthapuram District, Kerala, India, a *Ganoderma* species was collected. It was identified based on macro-micro morphology and molecular phylogenetic affiliation as *G. gibbosum*. This species is already reported from Kerala (unpublished data, GenBank), but no detailed taxonomic account or molecular study from Kerala State is available prior to the present study. Additionally, studies on the nanoparticle mediated antimicrobial effect of the extracts of this macrofungus has not been conducted previously and is being reported here for the first time. The present study investigated the capability of *G. gibbosum* to synthesize nanoparticles under mild conditions and at low costs. The methanolic and distilled water extracts of this macro fungus was used for silver nanoparticle synthesis and its antimicrobial effect was studied on *B. cereus, B. subtilis, E. coli* and *P. mirabilis.*

MATERIALS AND METHODS

Morphological studies

Gross morphological descriptions are based exclusively on fresh materials collected from Kerala State, India. Microscopic characters were studied on dried material using hand cut sections of basidiomata stained with 1% Congo Red and mounted in 3% solution of KOH and examined under an Olympus CX43 optical microscope (Olympus, Japan). For evaluation of the range of spore size, 20 basidiospores were measured for length and width. Spore quotient (Q = L/W) was also determined. Colour coding follows that of Kornerup & Wanscher (1978). Microphotographs were made with a Magcam DC10 digital camera attached to the same microscope (Olympus, Japan). The Specimen is deposited in the Mycological herbarium of Government College for Women, Thiruvananthapuram with accession number MHGCWT001.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh specimens following protocols in Izumitsu et al. (2012). The Internal Transcribed Spacer region (nrITS) was amplified and sequenced from the presented species. PCR reactions were performed with the primer pair ITS1, ITS4 (White *et al.,* 1990). The protocols for PCR amplification and sequencing followed Kumar *et al.* (2018). The newly generated sequences were deposited in GenBank. Sequences retrieved from GenBank and obtained in this study are listed (**Table 1**).

Table 1. Species, their geographic location and GenBank accession numbers used for phylogenetic analyses in the present study

Species	Voucher/Strain	GenBank No.	Geographic Origin
Amauroderma calcitum	*FLOR:50931*	KR816528.1	Brazil
Ganoderma applanatum	*CLZhao170*	MH114668.1	China
G. applanatum	*KA17-0004*	MN294816.1	South Korea
G. applanatum	*hr4819*	OP803095.1	China
G. applanatum	*CLZhao*	MK268928.1	China
G. applanatum	*SWFU 0010576*	MK809458.1	China
G. applanatum	*Gap-1*	GU213472.1	China
G. applanatum	*Gap-2*	GU213473.1	China
G. applanatum	*D.K-1*	MZ618704.1	India
G. applanatum	*applanatum*	MF072395.1	India
G. australe	*GDGM25503*	JX195199.1	China
G. australe	*GDGM24918*	JX195197.1	China
G. australe	*GDGM25831*	JX195200.1	China
G. australe	*GIM5.288*	JX195196.1	China
G. eickeri	*IBSDM78*	OQ683303.1	India
G. gibbosum	*610723MF0082*	KY950512.1	China
G. gibbosum	*KUT0805*	AB733121.1	Japan
G. gibbosum	*JFRL66*	MN622779.1	China
G. gibbosum	*JFRL120*	MN622799.1	China
G. gibbosum	*SFC20140702-12*	KY364260.1	South Korea
G. gibbosum	*Pvc62*	MK280717.1	Taiwan
G. gibbosum	*MRC23*	PP582915.1	India
G. gibbosum	*CQ01*	OQ144181.1	China

Species	Voucher/Strain	GenBank No.	Geographic Origin
G. gibbosum	*GZ14070501*	MK345432.1	China
G. gibbosum	*JBRI-M22-078*	OP928177.1	South Korea
G. gibbosum	*AS5.624 type 3*	AY593856.1	China
G. gibbosum	*XSD-34*	EU273513.1	China
G. gibbosum	*ITS1-ITS4*	MW426270.1	China
G. gibbosum	*SFC20130404-21*	KY364259.1	South Korea
G. gibbosum	*SFC20150918-08*	KY364271.1	South Korea
G. gibbosum	*15388*	OM809727.1	South Korea
G. gibbosum	*KUMCC 17-0099*	MH035684.1	China
G. gibbosum	*L022*	KJ195663.1	China
G. gibbosum	*XSD-35*	EU273514.1	China
G. gibbosum	*EF423*	PP734699.1	China
G. gibbosum	*18004*	ON553388.1	South Korea
G. gibbosum	*XSD-B35*	EU273557.1	China
G. gibbosum	*19199*	OM809738.1	South Korea
G. gibbosum	*TH15062144*	MK345434.1	Thailand
G. gibbosum	*15301*	OM809723.1	South Korea
G. gibbosum	*XSD-62*	EU326218.1	China
G. gibbosum	*AS5.624 type 1*	AY593854.1	China
G. gibbosum	*AS5.624 type 2*	AY593855.1	China
G. gibbosum	*XZ160*	OQ457018.1	China
G. gibbosum	*JBRI-M22-025*	OP345214.1	South Korea
G. gibbosum	*JFRL67*	MN622780.1	China
G. gibbosum	*KUMCC 17-0005*	MH035682.1	China
G. gibbosum	*KUMCC 17-0003*	MH035681.1	China
G. gibbosum	*U21*	OM350473.1	Pakistan
G. gibbosum	*SFC20150612-11*	KY364263.1	South Korea
G. gibbosum	*HKAS123781*	ON994245.1	China
G. gibbosum	*KUMCC 17-0010*	MH035685.1	China
G. gibbosum	*CQ02*	OQ144171.1	China
G. gibbosum	*U1*	OM350446.1	Pakistan
G. gibbosum	*xsd08026*	EU918695.1	China
G. gibbosum	*AS5.624 type4*	AY593857.1	China
G. gibbosum	*CLZhao 11324*	MW742521.1	China
G. gibbosum	*FH039*	OQ984997.1	China
G. gibbosum	*DCY3111(HGASMF01-14014)*	MZ413055.1	China
G. gibbosum	*CBE1*	JN655531.1	India
G. gibbosum	*CH23_Kerala*	OQ586755.1	India
G. gibbosum	*KA6_Karnataka*	OQ586761.1	India

Species	Voucher/Strain	GenBank No.	Geographic Origin
G. gibbosum	*KA1*	OQ561191.1	India
G. gibbosum	*KA4*	OQ586451.1	India
G. gibbosum	*KA3*	OQ586450.1	India
G. gibbosum	*DQS37F*	MZ735417.1	Philippines
G. gibbosum	***MLHGCWT001***	**PP957441.1**	**India**
G. lingzhi	*Cui14342*	MG279179.1	China
G. pseudoapplanatum	*ZGCVN824*	MW541919.1	India
G. sp.	*PDD#101283*	KM229676.1	India
G. sp.	*BAB-4560*	KR154930.1	India
G. sp.	*PDD#101263*	KM229675.1	India

Sequence alignment and phylogenetic analysis

The nrITS sequences of *G. gibbosum* from present study along with species of *Ganoderma* retrieved from GenBank based on BLAST results in NCBI (above 99 % sequence identity, 98% query cover and 580 bp sequence length) were aligned using MAFFT web tool (www.ebi.ac.uk/Tools/msa/mafft/) with default settings. The final aligned data matrix of nrITS sequences from 50 taxa, including one species of *Amauroderma* as outgroup (**Table 1**), was then imported into BioEdit v7.2.6.1 (Hall, 1999) for manual adjustment. *Amauroderma calcitum* was selected as outgroup following Laungharn *et al.*, (2020). Maximum likelihood (ML) analysis was performed in the web platform http://iqtree.cibiv.univie.ac.at (Trifinopoulos *et al.*, 2016) with 1000 ultrafast bootstrap replicates. Kimura 2- parameter with Gamma variable (K2P+G4) was selected automatically as the best fit substitution model in the same web platform with the least BIC score (3622.2862). The phylogram inferred from ML analysis is displayed with MEGA 11.0.13 (Tamura *et al.*, 2021).

Nanoparticle assay

2g of powdered extract was dissolved in 20 mL of methanol and distilled water in small bottles. The mixture was kept at room temperature for 24 h and the extract was filtered through a Whatman No. 1 filter paper. 5 mL of the prepared fungal extract was combined with 45 mL of the silver nitrate (0.001M) solution, and the mixture was incubated for 24 h at room temperature to observe a colour change which will indicate the possible formation of silver nanoparticles.

Antibacterial assay

This study investigated the potential antibacterial activity of methanolic, and distilled water extracts derived from *G. gibbosum* against a panel of bacterial strains. The agar well diffusion method was employed on Mueller Hinton agar (MHA) to assess the inhibitory effects of the extracts. MHA plates were prepared and sterilized following standard protocols. After solidification, the sterile MHA surfaces were uniformly inoculated with 100 µL of actively growing cultures of *B. cereus* (ATCC 10876), *B. subtilis*, *E. coli*, and *P. mirabilis* [GC10 (Clinical strain)] using sterile cotton swabs. Sterile cork borers were used to create wells (5 mm diameter) in each MHA plate. Subsequently, 50 µL and 100 µl of methanolic and distilled water extracts were introduced into the wells. Chloramphenicol disc served as a positive control for antibacterial activity. The inoculated MHA plates were then incubated at 37°C for 24 h. Following incubation, the diameter of the inhibition zones surrounding each well was measured in millimeters. These zones represent the area where bacterial growth was inhibited by the respective extract or control antibiotic. The size of the inhibition zone was compared to the positive control to assess the relative antibacterial activity of each *G. gibbosum* extract. (Ramasamy *et al.*, 2022)

Statistical analysis

This study investigated Analysis of variance (ANOVA) was conducted using the Statistical

Package for Social Sciences (SPSS) version 13 to determine significance levels (at $p \leq 0.05$).

RESULTS

Molecular phylogeny

The phylogeny inferred from ML analysis of the data matrix of nrITS sequences is provided in **Figure 1**. Sequence of *G. gibbosum* from the present study was nested in a clade containing several sequences of *G. gibbosum* species from India with a strong ML bootstrap (98 %) confirming its identity as well.

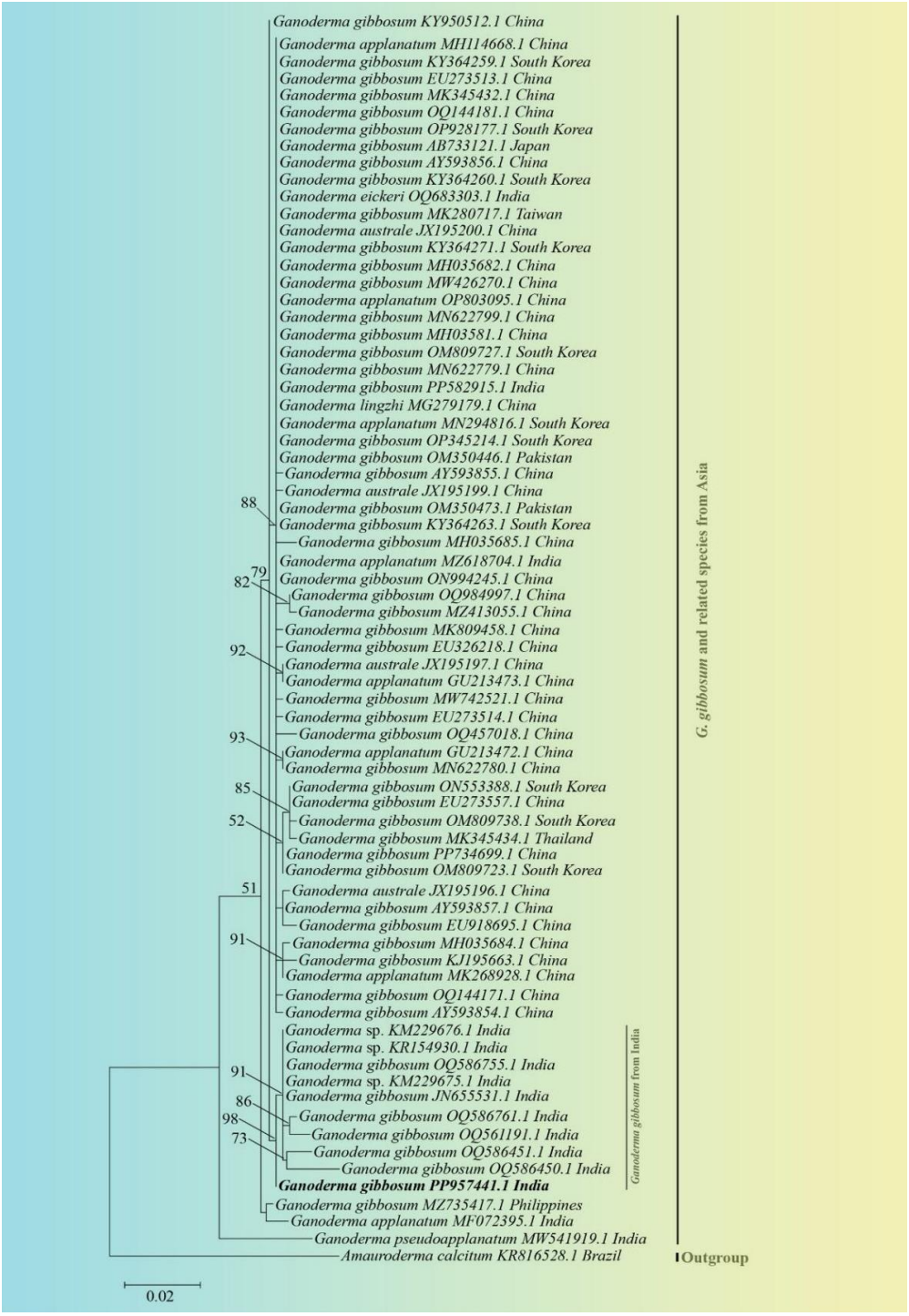


Figure 1: ML tree generated from nrITS sequences. Bootstrap values (ML>50%) are indicated above branches. *Ganoderma gibbosum* from present study is indicated in bold. GenBank accession numbers and geographical locations are given after the species names.

Taxonomy

Ganoderma gibbosum (Blume & T. Nees) Pat., Ann. Jard. Bot. Buitenzorg, suppl. 1: 114. 1897. **Figure 2**

Basidiomes annual or perennial, sessile, sub flabellate or sub dimidiate. *Pileus* 4–17 cm in length, 3–12 cm broad, and 0.5–3 cm thick. *Pileus* convex, imbricate, umbonate, uneven, flabelliform when seen from above, broadly attached when mature, thick at the base. *Pileus surface* non-laccate, smooth when young, somewhat spathulate to uneven, incised, compact, hard, and woody when older, covered with a crust, usually dull and faded when mature. *Pileus colour* brownish orange (5C5) at the base, and greyish orange (6B3), brownish orange (7C5), and light brown (6D4) at the disc of mature basidiomes, white (6A1) at the margin, becoming reddish brown (8E6) to dull red (10C3) when old. *Hymenophore* poroid. *Pores* 4–6 in number per mm. *Pore surface* white (11A1) to orange white (6A2) when fresh, turning brown on bruising. *Hyphal structure*: *hyphal system* trimitic, with clamp connections; generative hyphae 1.0–1.8 µm broad, hyaline, and unbranched; skeletal hyphae 3–5 µm broad, dark brown, thick walled; skeletoligative hyphae 3–4 µm broad, brown, thick walled, highly branched, arboriform. *Basidiospores* (6.5–)7–7.5(–8) × (4.3–)4.5–5(–5.2) µm, AvL=7.02 µm, AvW=4.71 µm, Q=1.42–1.6, Qm=1.49, ellipsoid. *Basidia* not observed.

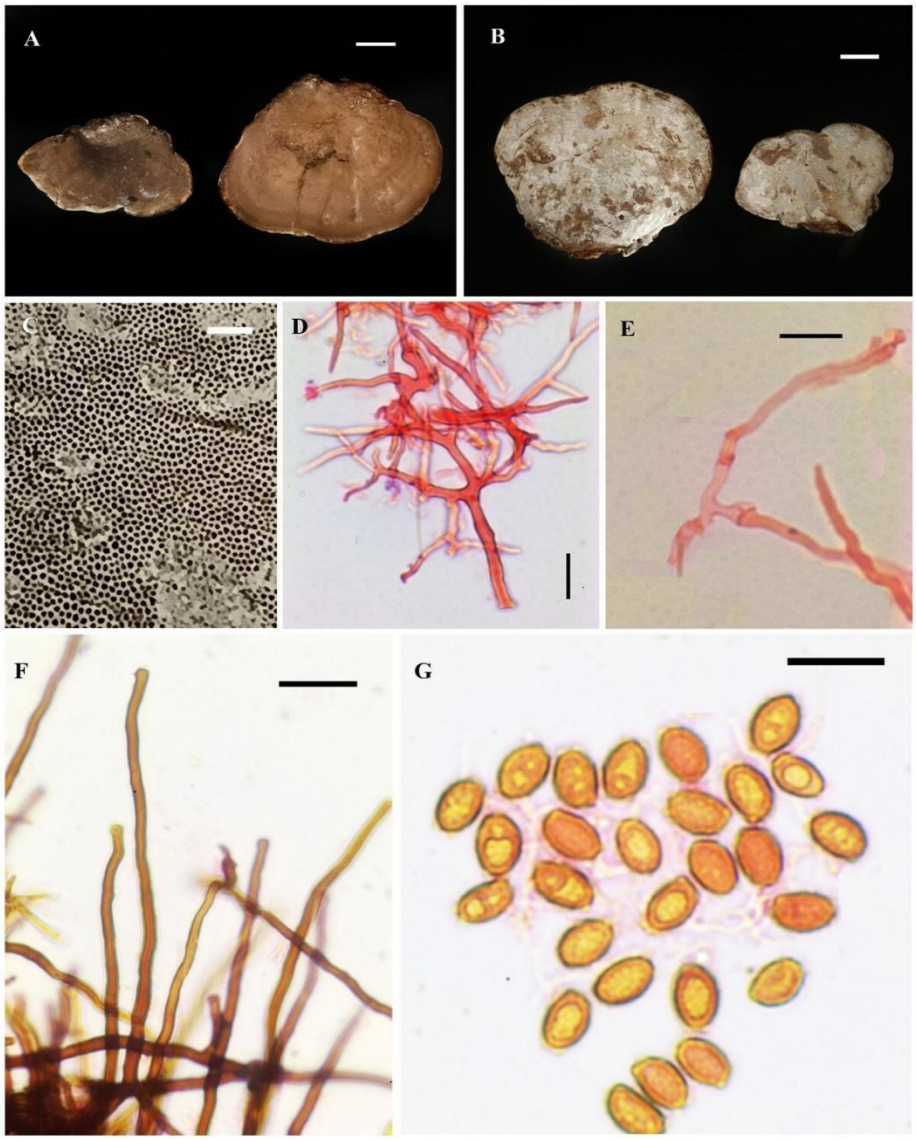


Figure 2: *Ganoderma gibbosum*. A-B, Habit; C, Pores; D, Skeletoligative hyphae; E, Generative hyphae with clamp; F, Skeletal hyphae; G, basidiospores. Scale bars A, B = 30 mm; c = 1 mm; D, E, G = 10 µm; F = 20 µm **.**

***Habitat*:** On a dead unidentified angiosperm log in tropical evergreen forest.

***Specimen examined*:** INDIA. Kerala State, Thiruvananthapuram district, Palode, 23 August 2023, MHGCWT001.

Silver nanoparticle synthesis

The formation of silver nanoparticles (AgNPs) was confirmed through UV-Vis spectroscopy within the 200-800 nm range. The graphs obtained **(Figure 3 and Figure 4)** illustrates respectively a distinct absorption band for methanolic and distilled water extract biosynthesized AgNPs at 420.25 nm. This absorption peak at 420.25 nm signifies the stability of the synthesized AgNPs. The band, however, is asymmetrical, indicating the presence of aggregated particles (Constantin *et al.,* 2023).

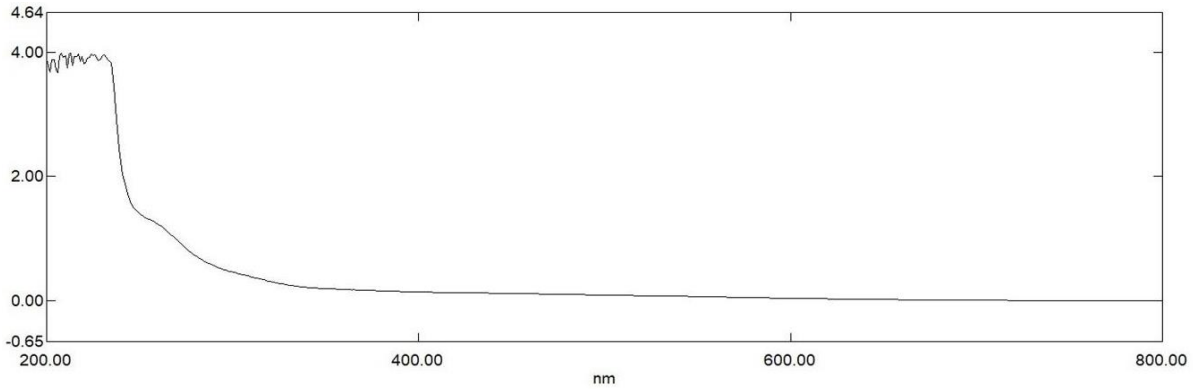


Figure 3: The UV spectra of methanolic extract biosynthesized AgNPs.

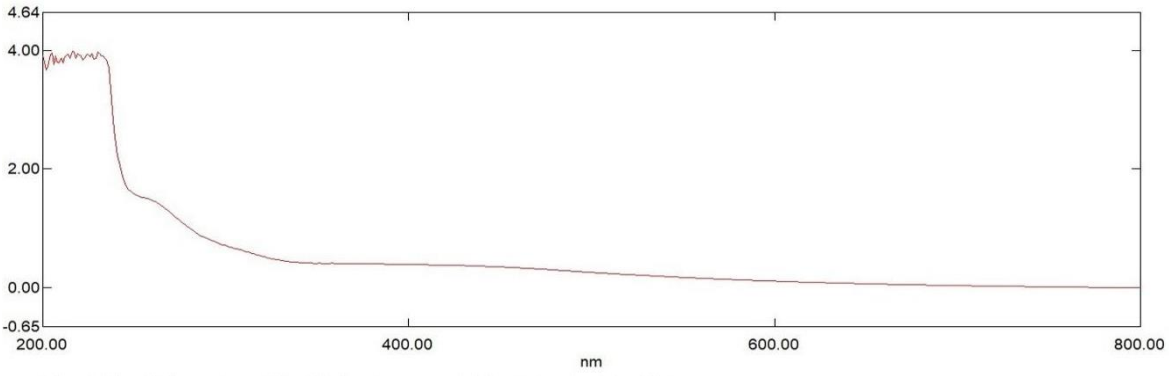


Figure 4: The UV spectra of distilled water extract biosynthesised AgNPs

Antibacterial activity

The antibacterial activity of both the methanolic and distilled water extracts of *G. gibbosum* was assessed, demonstrating varying degrees of efficacy against both Gram-positive and Gram-negative bacteria known to cause diseases in plants and humans. The results, summarized **(Figure 5, Table 2)**, indicate that among the four bacterial strains tested, *P. mirabilis* exhibited the highest sensitivity to the aqueous extract, with a zone of inhibition measuring 20 mm at a concentration of 50 µl and 100 µl.

Table 2. Antibacterial activity of *G. gibbosum* methanolic and distilled water extracts. GM50 and GM100 represent 50 µL and 100 µL of methanolic extracts while GD50 and GD100 represent 50 µL and 100 µL of distilled water extracts from *G. gibbosum*. Results represented as Means ± Standard Error (n=5)

Bacterial strains	**Zone of inhibition (mm)**			
	GM50	GM100	GD50	GD100
Bacillus cereus	17 ± 0.03	14 ± 0.03	15 ± 0.03	9 ± 0.03
Bacillus subtilis	15 ± 0.03	18 ± 0.03	14 ± 0.03	16 ± 0.03
Proteus mirabilis	18 ± 0.03	19 ± 0.03	20 ± 0.03	20 ± 0.03
Escherichia coli	11 ± 0.03	16 ± 0.03	12 ± 0.03	18 ± 0.03
Antibiotic disc	33 ± 0.03			

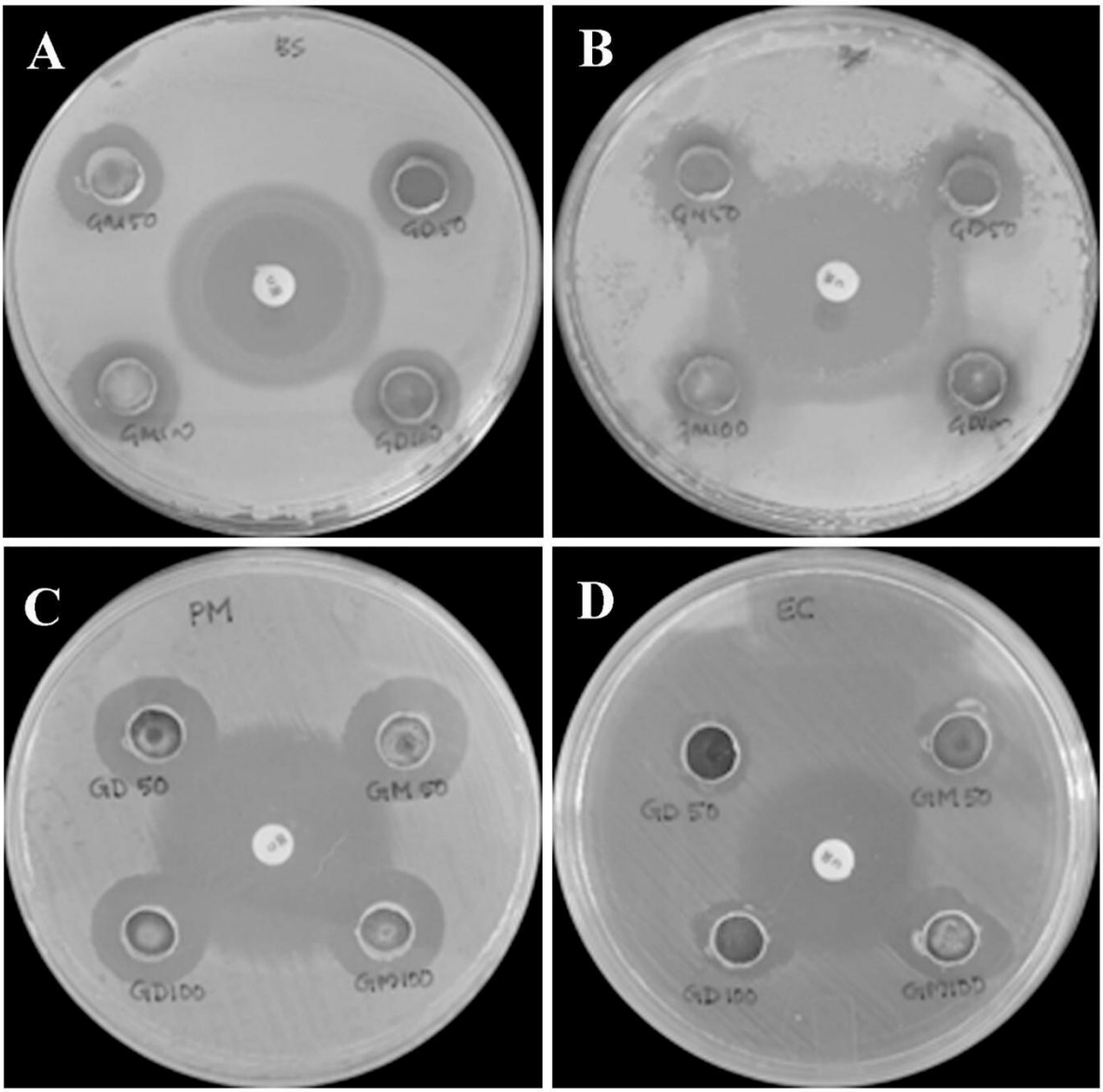


Figure 5: Antibacterial activity of methanolic extracts of *G. gibbosum* against a, *Bacillus subtilis*; B, *Bacillus cereus*; C, *Proteus mirabilis*; D, *Escherichia coli*

DISCUSSION

Ganoderma gibbosum is a widely distributed polypore with presence known in tropical and temperate areas (Cao *et al.,* 2012). *Ganoderma gibbosum* has been reported to cause white rot and several other diseases in hard woods (Ryvarden, 2004). From Asia, It has been reported from China (Zhao *et al.,* 1983), India (Kaliyaperumal and Kalaichelvan, 2007), Indonesia (Parmasto, 1986), Japan (Jargalmaa *et al.,* 2017), Korea (Jargalmaa *et al.,* 2017), Laos (Hapuarachchi *et al.,* 2019), Myanmar (Hapuarachchi *et al.,* 2019), Pakistan (Umar *et al.,* 2022), Papua new Guinea (Pilotti *et al.,* 2004), Philippines (Dulay *et al.,* 2020), Taiwan (Wei *et al.,* 2021), Thailand (Hapuarachchi *et al.,* 2019; Laungharn *et al.,* 2020, 2021) and Vietnam (Parmasto, 1986). This species is distinctive in having non-laccate basidiomes, and ellipsoid with double-walled basidiospores (Zhao *et al.,* 1983). Our collection of *G. gibbosum* is similar to *G. gibbosum* described by Hapuarachchi et al. (2019) from China especially in the basidiospore size (6.9–7.6 × 4.6–5.6 µm). In the NCBI BLAST results, our material shows 99.83% sequence similarity with a *Ganoderma* sp. from India (KM229676.1) as the top hit. However, the detailed description of this material is unavailable for macro-micromorphological comparison.

The UV-Vis spectroscopy analysis, conducted within the 200-800 nm range, confirms the formation of silver nanoparticles (AgNPs). The appearance of a distinct absorption band at 420.25 nm (Fig. 3, Fig. 4)

is indicative of the successful biosynthesis and stability of the AgNPs. The position of this peak is consistent with the characteristic surface plasmon resonance of silver nanoparticles. However, the observed asymmetry of the plasmon band suggests the presence of aggregated particles within the sample. This asymmetry could imply variations in particle size and distribution, possibly due to the synthesis conditions or the nature of the biological reducing agents used. Such aggregation might affect the optical properties and potential applications of the AgNPs, warranting further investigation into optimizing synthesis parameters to achieve more uniform nanoparticles.

In this study, the antibacterial activity of the methanolic and distilled water extract of G. gibbosum was evaluated against a range of Gram-positive and Gram-negative bacteria known for their pathogenicity in both plants and humans. The findings, detailed (Table 2), reveal a spectrum of antibacterial efficacy, suggesting that the extract possesses broad-spectrum antimicrobial properties. Notably, P. mirabilis demonstrated the highest susceptibility to the extract, as evidenced by a significant zone of inhibition measuring 20 mm. This pronounced sensitivity of P. mirabilis highlights the potential of G. gibbosum extract as a promising candidate for developing novel antibacterial agents, particularly against Gram-negative bacterial infections. Further studies are warranted to isolate and characterize the specific bioactive compounds responsible for this activity, and to evaluate their mechanisms of action and potential therapeutic applications.

CONCLUSIONS

In conclusion, the study demonstrates that *G. gibbosum* is a widespread species with its presence in Kerala State too and can be effectively utilized in the biosynthesis of silver nanoparticles, which exhibit potent antibacterial properties. *Ganoderma gibbosum* derived silver nanoparticles could be valuable in developing eco-friendly antibacterial strategies with applications in public health and environmental management.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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