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Article

Ganoderma applanatum extract mediated synthesis of silver nanoparticles

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Nanotechnology has been used in the field of medicine and pharmacology for its greater efficacy of drug delivery than crude molecules of drugs. In the present study medicinal mushroom *Ganoderma applanatum* extract mediated silver nanoparticles (AgNPs) were synthesized, characterized by Ultraviolet-visible (UV-Vis.) spectroscopy, scanning electron microscopy (SEM), X-ray diffraction, Dynamic light scattering (DLS) and Furior transform-infrared (FTIR) spectroscopy. Maximum absorbance was recorded at 435nm by UV-Vis. The synthesized nanoparticles of 13.54nm-255nm in size with an average particle size of 58.77nm were analyzed by DLS. FTIR-Spectroscopy provided high transmission at 3606cm^{-1} corresponds for phenolic capping biochemical. Thus *G. applanatum* extract can be used for synthesis of silver nanoparticles and the synthesized nanoparticles may be used for development of future therapeutic agent for treatment of diseases.

Keywords: Nanoparticles. Particle size. Fungi. Mycochemical.

INTRODUCTION

In recent decades great attention has been paid for preparation of materials of small size and the technology has entered into nanoscalerange (Guisbiers, Mejia-Rosales, Deepak, 2012).Synthesis of nanoparticles using nanotechnology and its application in the various field of biological sciences and medicine has emerged a new field nanobiotechnology (Shah *et al*., 2015). Nanotechnology in the field of medicine and pharmacology concerns nanoparticles of 1 to 100 nanometres in size made up of carbon, metal oxides or organic matter (Iravani *et al*., 2014).

Day by day application of nanoparticles in drug and gene delivery, bio detection of pathogens, tissue engineering, fluorescent biological labels, tumour destruction via heating etc. (Hasan *et al*., 2018) is increasing due to their unique physicochemical properties such as ultra-small size, large surface to volume ratio, high reactivity and unique interactions with structural components and perform as carrier of therapeutic molecules and ligands for targeting location of biological systems, which improves the pharmacokinetics and therapeutic index of the drugs in contrast to the free drug counterparts (Dandapat, Kumar, Sinha, 2014a).

Medicinal application of silver and its compound have been experiencing for over 2000 years and found nontoxic but safe bactericidal agent (Husen, Siddiqi, 2014).Recently several physical and chemical methods of synthesis of silver nanoparticles have been developed but biological methods such as using plant and fungal material are quite easy, less expensive, safe and eco-friendly (Prabu, Johnson, 2015; Beyenea *et al*., 2017).

Macrofungi belong to genus *Ganoderma* has been traditionally used as medicine rather than fodder in China, Japan and India for therapy of various diseases (Deepalakshmi, Mirunalini, 2011;Wasser, 2011). The mushroom *Ganoderma* has been used high antioxidant capacity, *in vitro* antibacterial activity against pathogenic bacteria (Jogaiah *et al*., 2019).

In this work we reported the synthesis and characterization of silver nanoparticles mediated by aqueous extract of fruiting body of *G. applanatum* synthesis of silver nanoparticles using fungal extract specially *Ganoderma applanatum* is least explored.

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MATERIAL AND METHODS

Collection of macrofungi

Fresh fruiting body of *G. applanatum* (presented in Figure 1) was collected from Kaziranga National Park of Assam26°40′N 93°21′E and was match and identified on the basis of morphology with museum specimen in Department of Botany, Gauhati University, Guwahati, Assam and brought to Department of Zoology, Ranchi University, Ranchi for further studies.

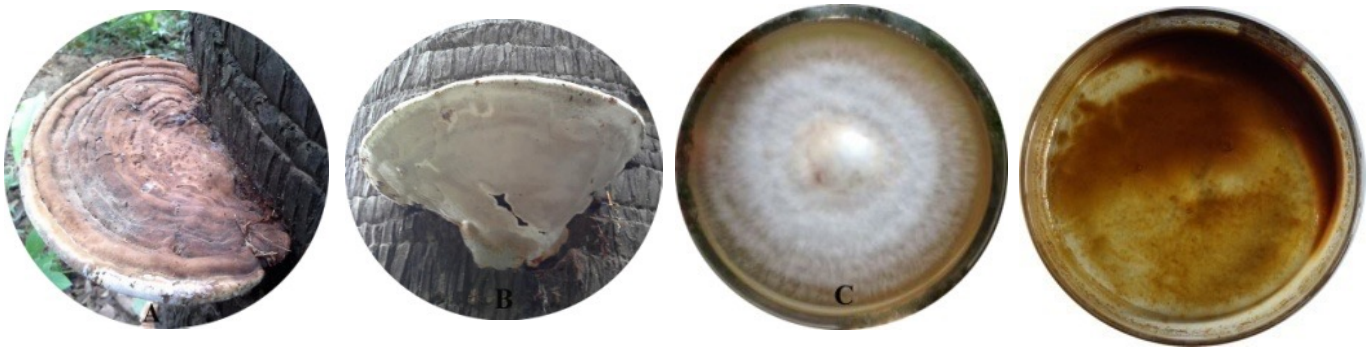


FIGURE 1 -Fruiting body of *G. applanatum* (A and B), cultured mycelia (C) and extract (D).

Preparation of extracts

The fresh fruiting body of *G. applanatum* was washed by distilled water and then by absolute ethyl alcohol (99.8%) to avoid microbial contamination. The mushrooms were dried in shade under room temperature for six to seven days, powdered and sieved. 50g of the fine powder was subjected to extraction chamber of Soxhlet and 300mL distilled water was taken in boiling flask as extraction solvent for aqueous extraction. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at 45ºC for proper dehydration and the dried extracts were stored in air tight containers at room temperature for further studies (Dandapat, Sinha, 2015).

Qualitative analysis of mycochemicals

Freshly prepared extract was used for mycochemical analyses. Presence of various biochemicals in the aqueous extract of *G. applanatum* was analyzed followed protocols described by Arya,Thakur, Kashyap (2012).

Test for carbohydrates

Presence of carbohydrate was determined by addition of few drops of Molisch's reagent to the test solutions (1mg/mL extract), this was then followed by addition of 1 mL concentrated H_2SO_4 (98%) by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted by adding 5 mL of distilled water. The mixture was observed for appearance of purple violet ring.

Test for glycoside

Glycoside was determined by addition of 1mg/mL of extract to 3mL of anthrone reagent and was mixed properly. The mixture was observed for appearance of green colour complex.

Test for proteins

Protein was estimated by addition of 0.5 mg/mL of the extract and 2mL of Bradford's reagent were left for few minutes. The mixture was observed for appearance of blue colour.

Test for alkaloid

Presence of alkaloid was determined by stirring of 1mg/mL extract with 5 mL of 1% HCl on hot water bath and then filtered. 1 mL of the filtrate was taken individually into 2 test tubes and few drops of Dragendorff's reagent were added into the test tube. The mixture was observed for appearance of dark brown colour.

Test for steroid

Presence of steroid was determined by addition of 2mL concentrated H_2SO_4 (98%) with 2mg/mL of extracts was mixed vigorously. The mixture was observed for initially formation of red colour followed by blue and finally development of green colour.

Test for triterpene

Triterpene was estimated by addition of 1mg/mL extract with one drop chloroform and concentrated H_2SO_4 (98%). The mixture was observed for formation of red colour.

Test for phenol

Presence of phenol was estimated by phenolic -catechol method. Dilute aqueous extract (0.5 mL of 1:10 g/L) was pipette out in series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteau reagent (0.5mL) was added to each tube and incubated 3 minutes at room temperature and then sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 minute in boiling water bath. The mixture was observed for the emergence of a blue-green colour.

Test for flavonoid

Flavonoid was estimated by dissolved 1mg/mL extracts in water and later addition of 2 mL of the 10% aqueous sodium hydroxide and then addition of dilute hydrochloric acid as an indicator. The mixture was observed for formation and disappearance of yellow colour.

Test for tannin

Tannin was estimated by stirring0.5 mg/mL of the extracts with 10 mL of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 mL of the filtrate. The mixture was observed for formation of yellow precipitate.

Test for saponin

Saponin was determined by heating1mg/mL extracts with alcoholic KOH and boiled for 1 min and cooled, and then the mixture was acidified with 1mL of concentrate HCl. Later few drops of 5% NaOH added drop wise and observed for froth formation.

Test for lipid

2 mL extract was taken and iodine solution was added drop wise. Disappeared of original colour of iodine indicate the presence of lipid. The mixture was observed for disappearance of original colour of iodine.

Biosynthesis of nanoparticles

The synthesis of silver nanoparticles was done slight modification of previous method of Dandapat,Kumar, Sinha (2014b) and Kumar, Sinha (2017). Synthesis of nanoparticles were done by mixed 3mL (41mg/mL) of *G. applanatum* fruiting body aqueous extract and 197 mL of 0.1M silver nitrate (169.87g/mol) solution (i.e., 3.35g $AgNO_3$/197 mL of distilled water) and incubated by using hot plate at 80ºC and continuous stirring using magnetic stirrer bar, until the light yellow colour of the solution was changed to dark brown. Then the solution was cooled to room temperature and centrifuged at 15000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed with distilled water and was dried in the incubator at room temperature for characterization.

Characterization of nanoparticles

UV-Visible spectra analysis

Nanoparticles sample for UV-Visible spectra analysis was prepared by dilute 1mL of pure nanoparticles solution in 4 mL of deionised water and 1mL of diluted sample was taken in standard quartz cuvette and placed in sample compartment. UV-Visible spectra analysis was done by using Parkin Elmer Lambda-25 UV-Visible spectrophotometer (PerkinElmer Inc., USA). The UV-Visible spectrophotometer was operated at 240V, 20±2°C, 60-70% humidity and light test specification at 200-800 nm wave length (Kumar *et al.*, 2018).

Scanning electron microscopy (SEM)

Scanning electron microscopy was done using JEOL JSM-6390 LV (Japan) machine provided with Vega TC software. Thin layer of nanoparticles powder sample (1mg) was prepared on glass slide and then press on a carbon taped copper grid for SEM. Excess powder on surface of carbon taped copper grid was blown away with compressed air and the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min and was coated with platinum using ion sputter (Bini *et al.*, 2018).

X-Ray diffraction analysis

XRD analysis of the prepared sample of nanoparticles was done using a Rigaku-smartlab powered diffraction XRD machine with 40kV operating voltage and 15mA current, Cu-Kα X-rays of wavelength (λ)=1.54056 Å and data was taken for the 2θ range of 10° to 90° with a step of 0.02°. The particle size was calculated by considering the peak at degrees and by using Debye-Scherrer formula (Akbari,Tavandashti, Zandrahimi, 2011).

$$D = \frac{0.9\lambda}{\beta cos\theta},$$

Where, 'λ' is wave length of X-Ray (0.1541 nm), 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter size.

Dynamic light scattering analysis

The sample was diluted, filtered and 0.1mg/mL concentration nanopaticle colloidal solution was ultrasonicated at 20 % sonication amplitude with continuous mode during 882 second to avoid agglomeration and for proper dispersion of nanoparticles in the solution. The dynamic light scattering for particle size and zetapotential analysis of nanoparticles was carried out using Malvern Nano ZS green badge) ZEN3500 (U.K.) zetasizer provided with zetasizer nano software (ZNUM, 2013).

FTIR spectra analysis

Fourier transform infrared (FTIR) spectra analysis was carried out IPRresting-21 (Shimadzu Corp., Kyoto, Japan) in the diffuse reflectance mode operated at resolution of 4 cm^{-1} in the range of 400 cm^{-1} to 4 000 cm^{-1} wave number and KBr as standard to identify the potential biomolecules present in fruiting body of *G. applanatum* extract which are responsible for reducing and capping the bioreduced silver nanoparticles. The FTIR machine was operated at 25±5°C, 60-70%humidity and 240V AC (IMUSG, 2002).

RESULTS

The collected fruiting body of *G. applanatum*, cultured mycelia and extract obtained is presented in Figure1.The collected basediocarp was semi circular in shape, 13cm in diameter. Outer surface of carp having wrinkled zones of brownish to grayish-brown colour and the lower surface is white.

Mycochemical screening

Result of mycochemical screening is presented in Table I. In the present study different mycochemicals such as carbohydrate, protein, alkaloid, flavonoid, saponins, steroid, phenolics etc. were found in the aqueous extract of fruiting body of *G. applanatum*.

TABLE I - Screening of proximate mycochemicals present in aqueous fruiting body extract of *G. applanatum*

Mycochemicals	Present(+) or Absent (+)
Carbohydrate	+
Glycosides	+
Protein	+
Alkaloid	+
Steroid	+
Triterpene	+
Flavonoid	+
Tannin	+
Lipid	+
Saponin	+

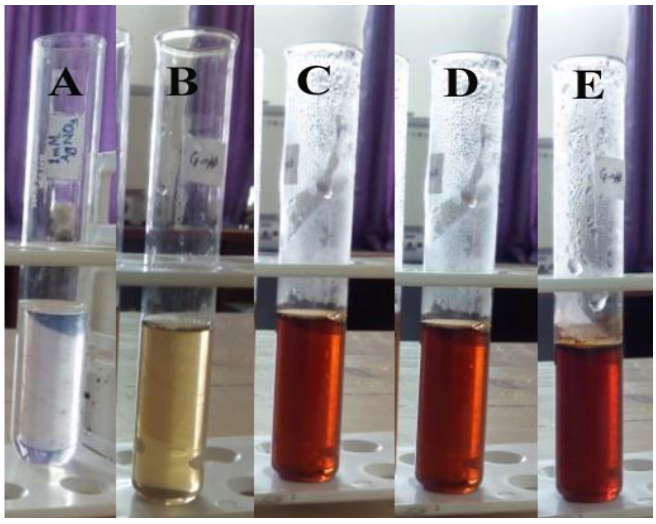


FIGURE 2 - Change in colour of mixed solution. (A) $AgNO_3$ solution; (B) $AgNO_3$ solution and extract at room temperature; C, D and E-mixed solution after heat stirring of 30 min, 1h and 2hrespectively.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles mediated by aqueous fruiting body extract solution is presented in Figure2. Result showed the change of pale yellow colour of mixed solution of extract and $AgNO_3$ solution turns into dark brown as the temperature and incubation time period increase, which indicates the formation of nanoparticles.

Characterization of synthesized nanoparticles

UV-Visible spectroscopy analysis

The absorption spectrum of nanoparticles obtained from UV-visible absorption spectroscopy is presented in Figure3, which shows peak at 400nmcorresponds to the surface plasmon resonance.

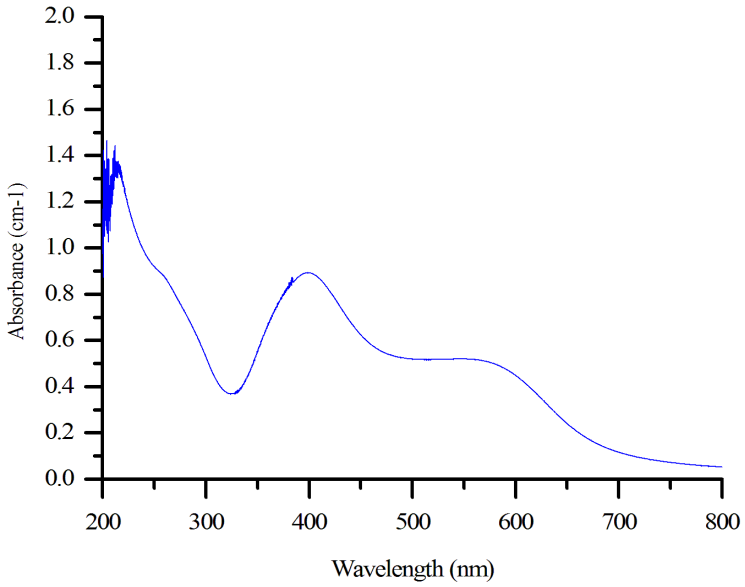
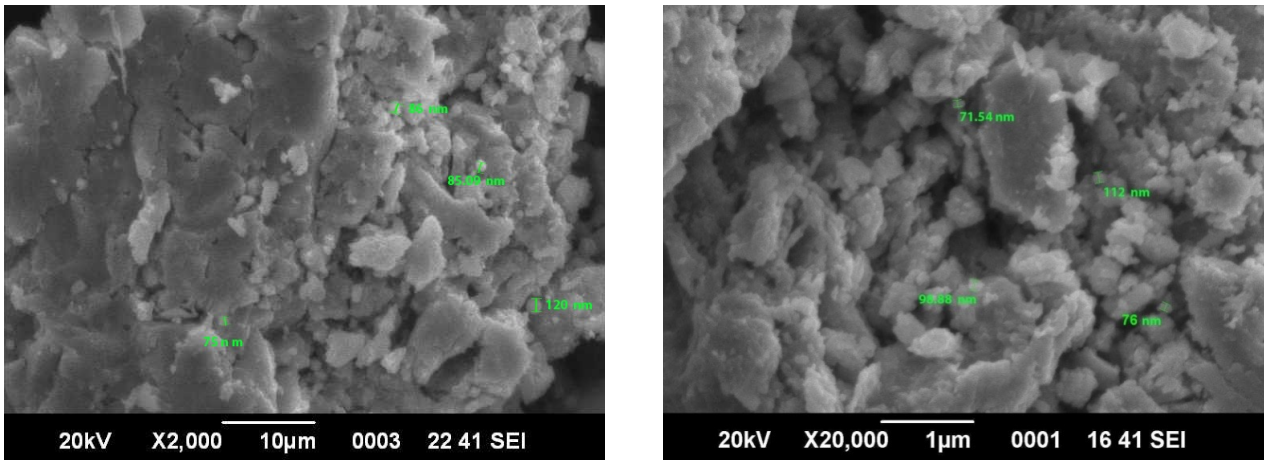


FIGURE 3 -UV-Visible spectrum of synthesized silver nanoparticles mediated by aqueous fruiting body extract of *G. applanatum*.

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy provided the confirmation about the morphology of synthesized green nanoparticles. Result of SEM analysis is presented in figure4 which showed the synthesized nanoparticles are spherical shaped and size ranges from 70nm to 120nm in diameter.



A B

FIGURE 4 (A and B) - Scanning electron microscopy photograph of silver nanoparticles mediated by aqueous fruiting body extract of *G. applanatum*.

X-ray diffraction analysis

The information pertaining to phase formation, translational symmetry present and size and shape of the unit cell are obtained from peak positions in the diffraction pattern of a sample. The X-ray diffraction pattern of the *G. applanatum* extract mediated synthesized silver nanoparticles is shown in Table II and Figure5. The result showed particles of 60.60nm to 121.19 nm were formed with average particle size 102.08 nm. The diffraction pattern has been analyzed and refined using open source full proof analysis software. It consists of the major peaks of SNPs with fcc type lattice and some additional unassigned peaks, which may be attributed to the formation of bio-organic phase acting as surfactant for the silver nanoparticles.

TABLEII - Average size estimation of *G. applanatum* extract mediated nanoparticles using X-ray diffraction analysis of and using Scherrer formula

Copper K radiation: Wavelength λ (nm) = 0.154								
2θ of the major peaks (deg.)	**θ of the peak s (deg.)**	**d-spacing (Å)**	**Intensity (cps)**	**FWHM of major peaks (β: deg.)**	**FWHM of the major peaks (β: rad.)**	**Size (Å)**	**Size (nm)**	**Avg. Size (nm)**
35.51	17.75	2.52581	9732.26	0.0796	0.0013	1093.7	109.37	102.08
29.66	14.83	3.00943	1690.34	0.0732	0.0012	1171.7	117.17	
21.73	10.86	4.08588	1652.76	0.1393	0.0024	606.0	60.60	
75.09	37.54	1.26401	1411.44	0.0863	0.0015	1211.9	121.19	

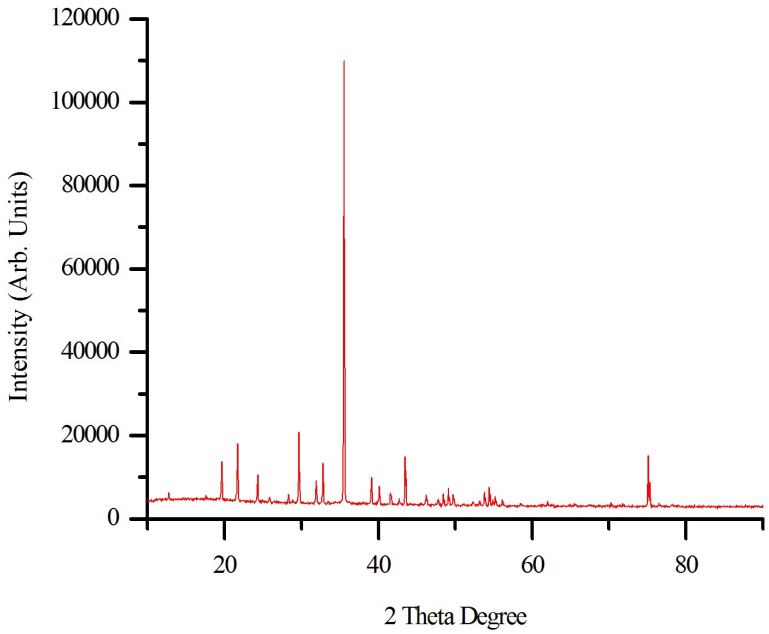


FIGURE 5 -X-Ray diffraction peaks of *G. applanatum* extract mediate nanoparticles powder.

Dynamic light scattering (DLS) analysis

Size and distribution of nanoparticles play a fundamental role in quality control of nanoparticles synthesis. It also basically associated diffusivity and passage of nanoparticles through cell membranes in the field of nanobiotechnology. In the present study size distributions of number, intensity, volume and zeta potential of synthesized *G. applanatum* extract mediated nanoparticles were analysed by DLS method. The results of DLS analysis are presented in Figures6, 7, 8, 9 and10 (hypothetical figure).Cumulants mean (Z-average) obtained by DLS analysis for the synthesized silver nanoparticle is 58.78d.nm corresponds to average size in diameter. Figure6 (particle size distribution by intensity) represents high peak for nanoparticles of 77.25nm diameter with 99.6% intensity and small peak for 5177nm diameter nanoparticles with 3.4% intensity. Figure7 (particle size distribution by volume) represents high peak and low peak for nanoparticles of 88.4% and 11.3% size distribution by volume of 38.21nm and 5282 nm diameter nanoparticles respectively. Figure8 (particle size distribution by number) represents single peak for nanoparticles of diameter of 23.64nm with 100% size distribution by number. Zeta potential is the electrostatic charge distribution, develops in liquid layer or capping materials on surface presented in Figure9. In present study the zeta potential analysis of nanopaticles provides peak at -13.8mV potential with100% area distribution, presented in Figure10.

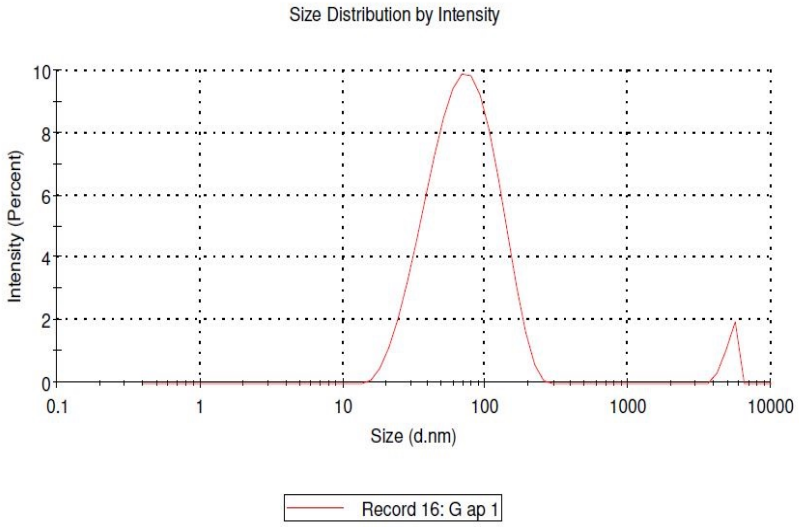


FIGURE 6 -DLS Size distribution by intensityof G. applanatum extract mediate silver nanoparticles.

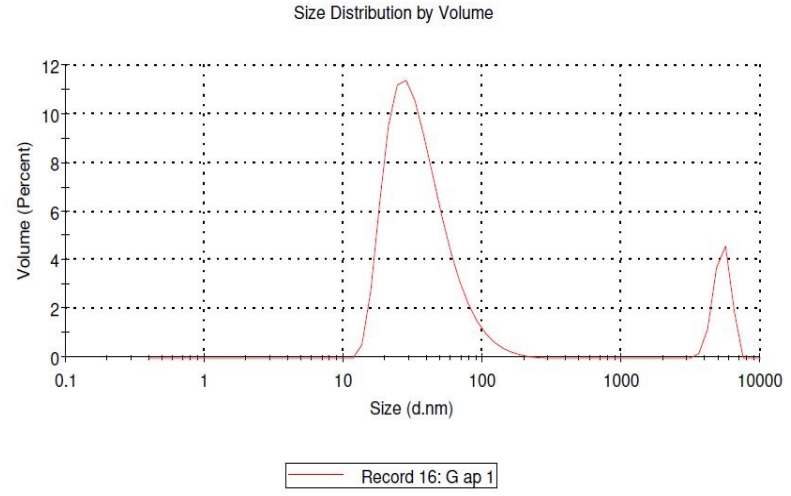


FIGURE 7 -DLS Size distribution by volume of *G. applanatum* extract mediate silver nanoparticles.

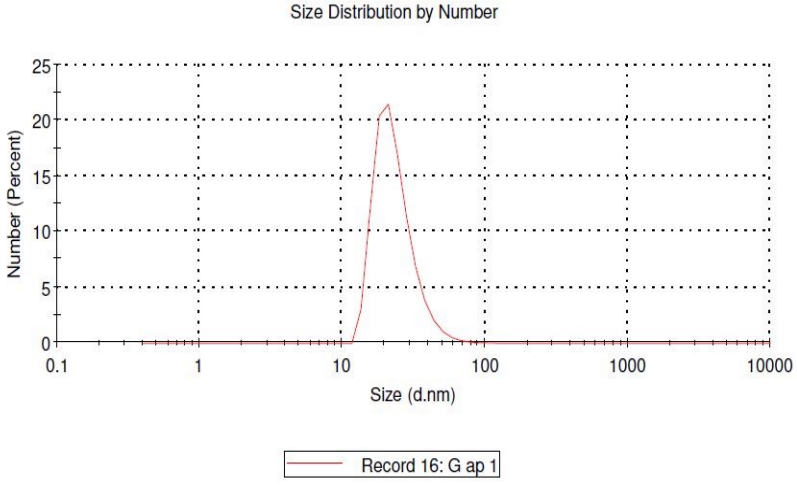


FIGURE 8 -DLS Size distribution by number of *G. applanatum* extract mediate silver nanoparticles.

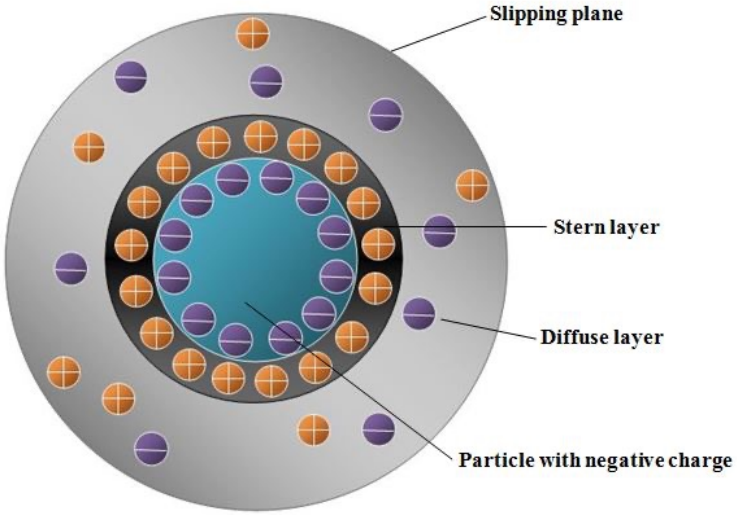


FIGURE 9 -Dielectric potential exists at the boundary of a nanoparticle (ZNUM, 2013; Haider, Kang, 2015).

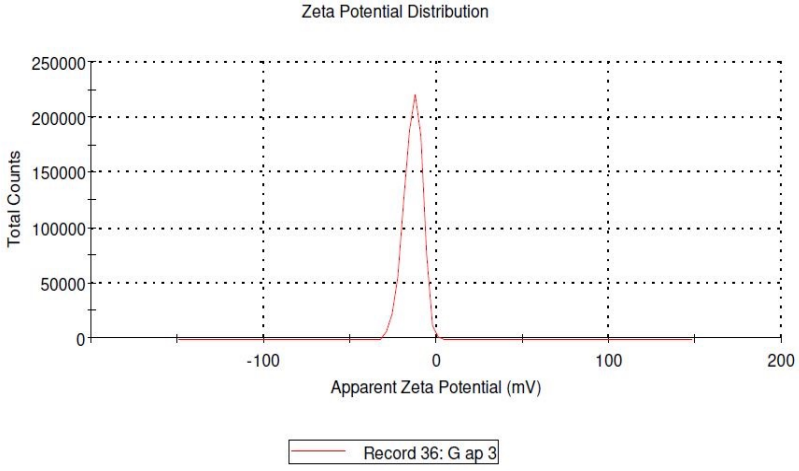


FIGURE 10 -DLS zetapotential analysis of *G. applanatum* extract mediate silver nanoparticles.

Furior transform infrared spectra (FTIR) analysis

FTIR spectroscopy spectra of *Ganoderma applanatum* extract is presented in Figure11. FTIR spectroscopy analysis of *G. applanatum* extract showed major transmittance peaks at 3248. 13cm^{-1} for phenol O-H stretch, 2939.52cm^{-1} for alkyl C-H stretch, 2086.98cm^{-1} for terminal alkyne C≡C stretch, 1651.07cm^{-1} for amide C=O stretch, 1597.06cm^{-1} for primary amine N-H stretch, 1392.61cm^{-1} for fluro alkane C-F stretch, 783.10cm^{-1} for aromatic (metadisub bengene) C-H stretch, 617.22cm^{-1} for chloro akane C-Cl stretch and 520.78cm^{-1} for bromo alkane C-Br stretch.In the present study FTIR analysis of silver nanoparticles synthesized from *G. applanatum* extract is presented in Figure12. The result represents absorption peaks at 3606cm^{-1} corresponds to O-H stretch for alcohol and phenol, 2430cm^{-1} corresponds to N-H stretch for primary and secondary amines, 2156cm^{-1} corresponds to C≡ stretch for alkynes, 1681cm^{-1} corresponds to C=N for amines or C=O stretch for unsaturated aromatic carboxylic acid, 1234cm^{-1} corresponds to C-O stretch for aromatic compound, 1091cm^{-1} corresponds to C-F stretch for fluroalkanes, 925cm^{-1} corresponds to C=C stretch foralkanes and also stretch for O-H and 613cm^{-1} corresponds to C-Cl or C-Br stretch for chloro and bromo alkanes.

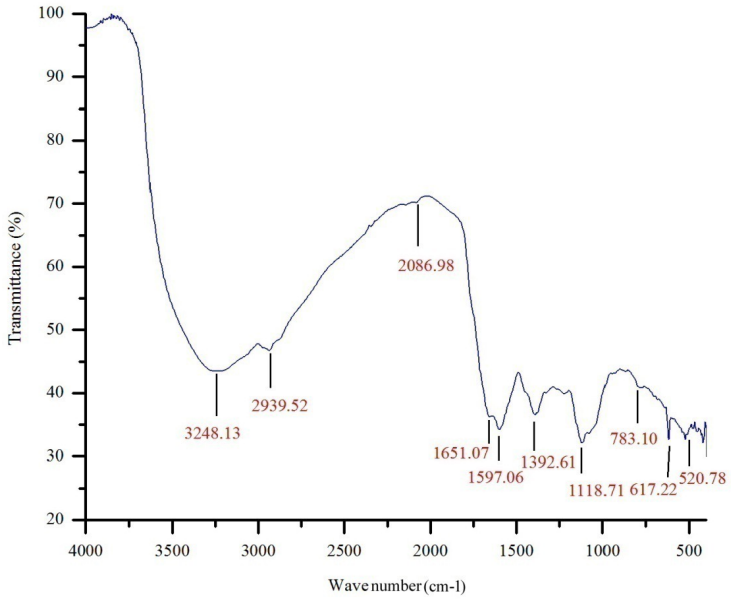


FIGURE11 - FTIR spectra of *G. applanatum* extract.

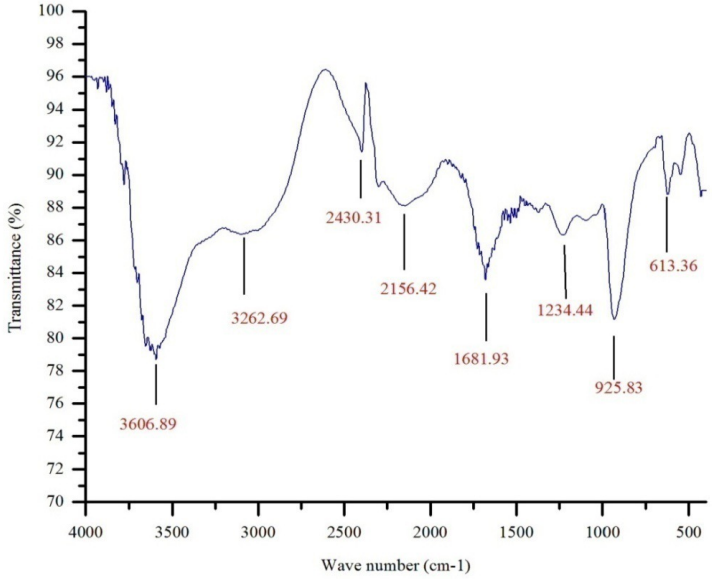


FIGURE 12 -FTIR spectrum of *G. applanatum* extract mediated silver nanoparticles.

DISCUSSION

Ganoderma applanatum is a polypore macrofungi with hard, woody, less fan-shaped, semicircular, fruiting bodies with a dull, unvarnished outer surface having wrinkled zones of brownish to grayish-brown colour on carp surface and white colour pore surface (Niemela, Miettinen, 2008; Mushroom Expert Com, 2018). In the present study collected basidiocarp of *G. applanatum* (Figure1) showed similar morphology. Medicinal mushrooms belong to genus *Ganoderma* have high antioxidant activity and therapeutic value because of presence of compounds such as phenolics, organic acids, alkaloids, carbohydrates etc. and these mushrooms can be used as a fodder (Menaga *et al.*, 2012; Dandapat *et al.*, 2015; Dandapat *et al.*, 2019b). It has been reported species of *Ganoderma* contain different mycochemical such

as polysaccharides, proteins, amino acids, fatty acids, terpenoids, steroids, alkaloids, phenolic compounds, etc. (Shikongo, 2012;Singh *et al.*, 2014; Dandapat *et al.*, 2019a). In present study crude aqueous extract obtained from *G. applanatum* basidiocarp also possess good number of biochemicals (Table I). Transformation of pale yellow to dark brown colour of mixed silver nitrate solution and extract revealed the reduction of silver nitrate into siver nanoparticles (Vilchis-Nestor *et al.*, 2008; Firdhouse,Lalitha, Sripathi, 2012). Similar changes in colour of mixed solution of silver nitrate and extract were observed as the temperature and duration incubation time increased (Figure2).In nanotechnology Ultraviolet - visible spectroscopy is used to monitor the formation and stability of nanoparticles (Englebienne,Hoonacker, Verhas, 2012). It has been reported formation and stability of silver nanoparticles mediated by biological extracts showssurface plasmon resonance (SPR) within 400-500 nm (Khan *et al.*, 2013; Gujral, 2015).Sujatha *et al.*, (2013) reported the SPR for synthesized silver nanoparticles mediated by *Ganoderma lucidum* and *Agaricus bisporus*at 420nm. Scanning electron microscopy analysis provides the surface morphology. Previous study reported SEM analysis of *Ganoderma lucidum* extract mediated silver nanoparticles were spherical shaped and 5nm to 30 nm diameter. Gurunathan *et al.*, (2014b) also reported the SEM analysis of AgNPs synthesized using*Boswelliaovali foliolata* extract were spherical shaped, 30-40 nm diameter. In fact X-Ray diffraction pattern of a powder sample is supposed to be the fingerprint of that sample (Hansford, Turner, Degryse , Shortland, 2017). Mohanta *et al.*, (2018) reported average 45.26nm SNPs, after analyzed X-ray diffrection pattern of SNPs sample mediated from *Ganoderma sessiliforme* extract. Previously it has also been reported nanoparticles synthesized using *Ganoderma lucidum* mycelia *extract* of average size 6nm were analyzed by XRD (Kumar *et al.*, 2017). In this study the average size of the synthesized nanoparticles is 102.08 nm.

Dynamic light scattering (DLS) is also known as photon correlation spectroscopy (PCS) and has been widely used for analysis of nanoparticles size in liquid phase, particle shape, colloidal stability, and surface coating (Phenrat *et al.*, 2009; Lim *et al.*, 2013).The result of DLS analysis of nanoparticles distribution by intensity in the colloidal solution depends upon the rate of fluctuation of intensity of the laser beam by the particles of different size bean (Nanocomposix, 2015). Intensity distribution of nanoperticles is the fundamental size distribution generated by DLS (ZNUM, 2013). The DLS size distribution by volume analysis of nanoparticles represents the total volume of particles of different size bins (Nanocomposix, 2015; Kumar, Sinha, 2017). The DLS size distribution by number analysis of nanoparticles represents the total number of particles of different size bins (Nanocomposix, 2015; Kumar, Sinha, 2017). Zeta potential is the electrostatic charge distribution, develops in liquid layer or capping materials on surface (stern layer) of the nanoparticles presented in hypothetical Figure9 (ZNUM, 2013; Haider, Kang, 2015). It has been reported nanoparticles dispersion with ± 10 to 20mV are moderately stable (Tucker*et al.*, 2015). Zeta potential of nanoparticles within -25mV provides efficiency of the capping material to stabilize thenanoparticles in colloid solution and their evenly distribution (Almeida,Larentis, Ferraz, 2015; Bhattacharjee, 2016). In our study the average zeta potential of synthesized nanoparticles is -13.8mV (Figure10) which showed the nanoparticles were stable. FTIR analysis provides confirmation of presence of biomolecules by analysis of functional groups and provides the confirmation of capping tendency of biomolecules of biological extracts present on the surface of synthesized nanoparticles (Khan *et al.*, 2013; Kumar *et al.*, 2014). Gurunathan *et al.*, (2014a)synthesized gold nanoparticles (AuNPs) using extract of *G. lucidum* and reported strong bands of FTIR spectra at 602, 1096, 1201, 1388, and 1636 cm^{-1} correspond to the amide polypeptides or proteins which served as capping agents in AuNPs. Zhu, Tan (2015) also reported FTIR spectra analysis of crude extract of *G. lucidum* and reported the presence of biochemicals such as terpenoids and polysaccharide showed peaks at 1150 to 1000 cm^{-1} and 1760 to 1600 cm^{-1}corresponds to terpenoids, polysaccharide and carbonyl compounds. In our results the above absorption peaks represents the presence bioactive mycochemicals such as tannins, saponins, flavonoids, phenols, alkaloids, proteins etc. as capping agent on the surface of synthesized nanoparticles. In our study FTIR analysis of spectra of

extract (Figure11) and synthesized silver nanoparticles (Figure12) mediated by *G. applanatum* extract. Hence, present study concluded that *G. applanatum* extract can be used for the synthesis of nanoparticles with minimum cost and eco-friendly. Further the synthesized silver nanoparticles may be used for different purposes for pharmacological and medicinal aspects.

CONCLUSION

Ganoderma applanatum extract can be used for the synthesis of silver nanoparticles within nanoscale range with moderate stability. Yet the bioactivity of the synthesized nanoparticles were not studied but the SNPs may be used for various pharmacological and medicinal applications.

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