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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles from *Macrocybe gigantea* and its Effect Against Food Borne Pathogens

S Nirmala[1], R Siva[2]*

1 Research Scholar, Department of Plant Biology and Plant Biotechnology, Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women, Chrompet, Chennai
2 Assistant Professor and Head, Department of Plant Biology and Plant Biotechnology, Shrimathi Devkunvar Nanalal Bhatt Vaishnav College for Women, Chrompet, Chennai

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* **Corresponding author**.

drsivagovind@gmail.com

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Abstract

Objectives: To evaluate the efficacy of edible mushroom, *Macrocybe gigantea* for the biosynthesis of silver nanoparticles, antioxidant property and antibacterial property. **Methods**: *M. gigantea* pure culture was isolated using tissue culture in a petri-plate comprising potato dextrose agar (PDA) medium, spawn was prepared using sorghum grains and mushrooms were grown indoors using paddy straw in an uniquely designed environment for mushroom growth. The green synthesis of silver nanoparticles with $AgNO_3$, antioxidant activity and antibacterial potential against a few food-borne bacteria were all investigated. The formation of AgNPs was further confirmed using a UV-Visible spectrophotometer. The silver nanoparticles were examined using Fourier-transform infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The disc diffusion method was used to test the bacteriostatic and bactericidal activity of synthesised silver nanoparticles against selected food-borne bacteria such as *Escherichia coli*, *Bacillus subtilis* and *Streptococcus mutans*. **Findings**: The existence of phytochemicals such as alkaloids, terpenoids, phenols, flavonoids, proteins, and carbohydrates was revealed. Furthermore, the AgNPs produced from *M.gigantea* demonstrated absorbance at 420nm in a UV-Visible spectrophotometer. According to the SEM pictures, AgNPs appear spherical with diameters ranging from 50 to 70nm. At 100g/mL, the DPPH antioxidant assay exhibited the maximum percentage of inhibition of 67.94%, with an IC50 value of 57.07g/mL. The produced AgNPs also inhibited bacterial growth significantly. Green synthesised AgNPs from *M.gigantea* were produced in an environmentally friendly, quick and simple technique that functions as an antibacterial agent against certain food-borne pathogens such as *E. coli*, *Bacillus subtilis*, and *Streptococcus mutans*. The antibacterial screening demonstrates the biocidal ability of AgNPs from *M.gigantea*, which could be employed in the food processing and packaging industries. **Novelty**: This study is regarded as the first attempt to synthesise AgNPs from *M.gigantea* for use against food-borne bacteria.

Keywords: Macrocybe gigantea; Agnps; MycoNanoparticle; Antibacterial Activity; DPPH And Antioxidant

1 Introduction

Silver nanoparticles are extensively used due to their promising antibacterial and anticancer properties[(1)]. Because the physical and chemical methods of synthesizing AgNPs are very expensive, time consuming and produce hazardous by products that harm the environment and human health. The biological method of silver nanoparticles synthesis was used for the current study, which is cost effective, non-toxic, stable, and harvests a high yield[(2)]. The biological sample also contains a variety of phytochemical and metabolic substances, which eliminates the use of harmful chemicals for reducing and capping throughout the reaction. Natural resources such as algae, fungi, bacteria, yeast plants, and plant products have been investigated for the manufacture of nanoparticles[(3)]. The bioactive substances found in living creatures, such as alkaloids, flavonoids, phenols, polyphenols, quinines, proteins, peptides, and enzymes, convert silver cations to AgNPs and stabilise them by encapsulating them with organic molecules[(4)].

Because of their high nutritious value and antioxidant properties, mushrooms have long been considered a special food and medicine. Mushrooms have a remarkable ability to generate novel secondary metabolites, which have important applications in a variety of sectors including medicine, biodegradation, biocatalysis, and bioleaching. Among the mushroom species used in the manufacture of silver nanoparticles are *Pleurotus sp*., *Volvariella volvacea*, *Ganoderma sp*., *Boletus edulis*, and *Lentinula edodes*[(5)]. Food borne diseases have been a major concern in recent years. The consumption of a lot of ready to eat food causes an outbreak of food-borne infections[(6)]. The multidrug resistant microbes are able to resist the drugs that are now accessible. At relatively low concentrations, biologically produced nanoparticles exhibit significant inhibitory activity against bacteria[(7)]. AgNPs have powerful antibacterial properties and are employed in a variety of medical devices, textiles, and materials. As a result, AgNPs can be employed in food-related tasks including food manufacture and packing to minimize the harm caused by pathogenic microorganisms in food[(8)].

Based on morphological, ecological, and ribosomal DNA sequences, Macrocybe is a new genus in the tricholomataceae family, replacing Calocybe or Megatricholoma[(9)]. While there have been several reports on nutritional contents, green synthesis of AgNPs, and antibacterial properties of various mushooms, there are very few or no reports on *M.gigantea* AgNPs and their antibacterial efficacy against a few food borne diseases.

2 Methodology

2.1 Culture, Spawn Preparation and Cultivation of *M. gigantea*

M. gigantea fruit body was taken from the soil at Shrimathi Devkunvar Nanalal Bhatt Vaishnav college campus in Chennai, Tamil Nadu, India and identified using the publication "The pantropical genus *Macrocybe* gen. Nov." by Pegler, Lodge, and Nakasone. Tissue culture was used to acquire a pure culture of *M. gigantea.* Employing forceps, a small piece of tissue was extracted from the pileus and stalk junction and deposited in petri plates over potato dextrose agar media. After a week of incubation at 25±2°C, the tissue produces mycelium and spreads around the entire petri plate[(10)]. Spawn was created utilising Sorghum grains, paddy straw as a culture substrate, and soil vermicompost and sand (1:1) casing[(11)].

2.2 Preparation of ethanolic extract

M. gigantea fruit body was cleansed and dried in a hot air oven at 35°C for three days. The dehydrated mushroom was pulverised in a blender and refrigerated at 4°C for later investigation. 20g of dried mushroom powder was steeped in 100ml of distilled water overnight before being filtered through a Whatman No.1 filter paper. The residue was then diluted twice more with distilled water. The mushroom aqueous extract was then mixed and pulverised using a rotary evaporator set to 40°C. The dry powder was resolubilized in distilled water for further research [12].

2.3 Phytochemical screening [13]

Chemical tests were carried out with the ethanolic extract of *M. gigantea* for the determination of phytoconstituents qualitatively.

2.4 Test for alkaloids (Dragendorff test)

One drop of Dragendorff's reagent was added to 5 mg of sample, and the orange-red precipitate that resulted indicates the presence of alkaloids.

2.5 Test for glycosides

10 ml of the sample and 1 ml of concentrated H_2SO_4 were combined with 4.0 ml of glacial acetic acid and 1 drop of 2.0% $FeCl_3$. Between the layers, a brown ring that represents the substance of cardiac steroidal glycosides is visible.

2.6 Test for saponins (Foam test)

In a test tube containing sample, about 20 ml of distilled water was added, and the mixture was then cooked for two to three minutes. After cooling, the solution is shaken. Saponins can be detected by the 10 – 15minutes formation of foam.

2.7 Test for flavonoids (Alkaline Reagent Test)

Sample was combined with 2 ml of a 2.0% NaOH solution; the intense yellow colour indicates the presence of flavonoids.

2.8 Test for terpenoids

The 5 ml sample was mixed with 2 ml of chloroform, evaporated on a water bath and was then heated with 3 ml of concentrated H_2SO_4. Terpenoids cause a grey colour to appear, indicating their presence.

2.9 Test for proteins (Biuret´s test)

Biuret's reagent was added in a few drops to the 5 mg of sample. The resultant mixture was well mixed and warmed for 1 - 5 minutes. Proteins are present when red or violet colour is present.

2.10 Test for amino acids (Ninhydrin test)

The violet colour that occurred after boiling 5 mg of sample in 2 ml of 0.2% Ninhydrin solution for 2 minutes in a water bath confirms the presence of amino acids.

2.11 Test for carbohydrates (Fehling´s test)

The presence of carbohydrates is shown by the reddish-brown precipitate that forms after boiling 5 mg of sample with a few drops of Benedict's reagent.

2.12 Test for phenol

The powdered mushroom sample was boiled and filtered in 20ml of distilled water in a test tube. Three to four drops of 0.1% v/v ferric chloride was added to the filtrate. The presence of phenol is indicated when the colour changes from brownish green to blue.

2.13 Antioxidant activity (DPPH assay)

About 0.5mL of sample, 1mL of methanol, and 1mL of DPPH radical solution in 0.5mM in methanol make up the reaction mixture. DPPH is reduced when it interacts with an antioxidant substance that contributes hydrogen. When maintained in the dark condition for 100 minutes, the colour shifts from deep violet to pale yellow. The absorbance was measured using a UV-VIS spectrophotometer at 517nm [14].

Percentage of inhibition= Control O.D - Sample O.D x100 / Control O.D

2.14 Preparation of Mushroom Extract

For the synthesis of NPs, the *M. gigantea* basidiocarps were oven dried at 40°C for 2 hours after being rinsed with double-distilled water. 50mL of hot, boiled, distilled water was added to 6g of powder and allowed to cool at room temperature before being filtered using Whatman-4 filter paper and employed as mushroom extract [15].

2.15 Green Synthesis of silver nanoparticles (AgNPs)

Aqueous extract (10 mL) was combined with 90 mL of freshly produced, 1 mM silver nitrate solution, and the mixture was left at room temperature for 24 hours to facilitate the reduction process. *M. gigantea* extract was used as a positive control at the same time as AgNO3 solution was used as a negative control. While no colour change was seen in either the solution held without silver nitrate or mushroom extract, the colour changes from pale yellow to reddish brown [16].

2.16 Characterization of Silver NPs

Using a UV-Visible absorption double beam spectrophotometer with a wavelength range of 300 to 700 nm, the synthesised nanoparticles were examined. The functional groups present in the biomolecules were identified via FTIR analysis. The colour-changed suspension was centrifuged three times at 3000 rpm for 15 minutes to get rid of the undesired contaminants for this examination. The resulting pellets were cleaned with distilled water before being allowed to air dry at room temperature. The sample was ground up and subjected to FTIR examination between 450 and 4500 cm^{-1}. The surface and morphology of the produced AgNPs were determined by scanning electron microscopy (SEM) examination. The synthetic AgNPs made with *M. gigantea* were allowed to completely dry before being ground to a high-quality powder for examination.

2.17 Antibacterial activity

Lyophilized AgNPs from *M. gigantea* were diluted in water (5 mg/5 mL) to test their efficacy against *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus mutans* using the disc diffusion method. Sterilized water served as the negative control while streptomycin served as the positive control. Using a sterile cotton swab, the bacteria that had been cultured overnight in Luria broth media were swabbed onto the Muller Hinton Agar plates. AgNPs were produced at various concentrations such as 25μl, 50μl and 100μl, which then finally loaded onto the sterilised disc and incubated at 35°C for 24 hours [17].

3 Results and Discussion

3.1 Taxonomy

Macrocybe gigantea (Massee) Pegler Lodge comb.nov. *Tricholoma giganteum* Massee in Bull. Misc. Inf. Kew 1912: 254. 1912. Pileus 25-37 cm diameter, convex when young and broadly convex when mature, surface white paler at the margin, glabrous and smooth but cracking on drying; margin incurved. Lamellae sinuate, pale yellow, moderately crowded with lamellulae of different lengths. Stipe 12-17 × 4-7cm, cylindrical, surface white with fibrillose-striate. Context up to 3 cm thick at disc with thin-walled hyphae, 3-5μm diameter, inflated to 16μm diameter, with clamp connections. Spore print white. Spores 5.2-6.9 × 4.2-5.1 (6.10 ± 0.82 × 4.10 ± 0.33) μm, Q = 1.46, ovoid to ellipsoid, hyaline, in amyloid, thin walled. Basidia 22-34 × 5-6 μm, clavate with basal clamp connection with four sterigmata. Lamellar edge fertile; cystidia absent. Hymenophoral trama regular with septate thin-walled hyphae and clamp connections. Pileus surface loosely interwoven with thick walled and thin-walled hyphae. All the hyhae have clamp connections. The characteristic features of the above specimen are the presence of abundant clamp connections and lacks siderophilous granules in the basidia made the specimen to be placed in the genus Macrocybe. Basidocarp solitary to few and up to 37cm with smooth cracking white surface, cylindrical smooth stipe concolorous with the pileus, pale yellow lamellae resembles Macrocybe gigantea [18]

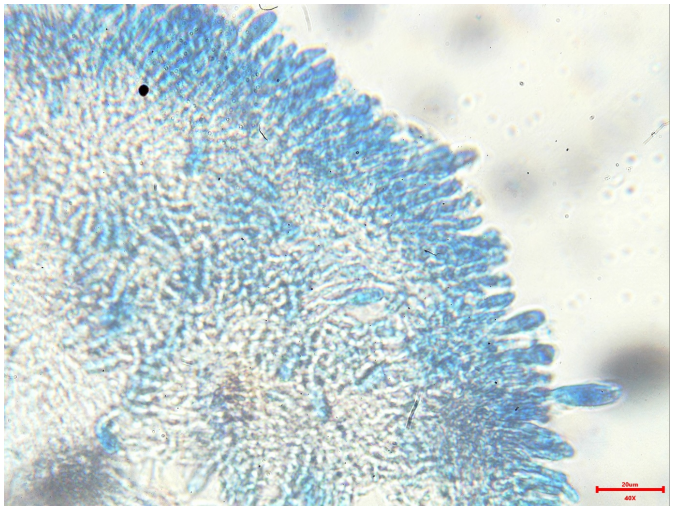

Fig 1. T.S Section of *Macrocybe gigantea*

3.2 Culture and Cultivation

M. gigantea was grown, kept alive, and incubated at 27°C using PDA medium. The highest development was visible in the spawn made from sorghum grains between days 14 and 16. In the paddy straw substrate, the spawn run rate and pin head formation peaked at 18 to 22 days. Sorghum, Maize, and Wheat grains were reported earlier, but the maximum yield was obtained in Sorghum grain prepared spawn and Paddy straw prepared substrate for cultivation. Agricultural wastes such as Paddy straw, Wheat straw, and maize cobs were used for cultivation and for the preparation of spawn. For spawning and cultivation, a maximum of 55 days was observed, although this isolate only needed 38 days for pinhead development. In an earlier assessment, the biological efficiency was 55%, up from 42 to 81% [(18)].

3.3 Screening of Chemical Constituents

The ethanolic extract of *M. gigantea* showed the presence of alkaloids, proteins, terpenoids, flavonoids, carbohydrates and phenol whereas saponins and amino acids are absent (Table 1). The phytochemical profile of Pleurotus and Calocybe already showed the presence of alkaloids, steroids, flavonoids, saponins, tannins and phenolic components. Quinone and triterpenoids were absent [(19)].

Table 1. Qualitative Analysis of Ethanolic Extract of *Macrocybe gigantea*

Phytoconstituents	Ethanolic Extract
Alkaloid	+
Saponin	-
Glycosides	-
Carbohydrates	+
Protein	+
Amino acid	-
Phenol	-
Terpenoids	+
Flavonoids	+

3.4 Scavenging Activity of DPPH Radicals

By accepting an electron or hydrogen to become stable, DPPH is a free radical scavenging activity used to assess the antioxidant capacity of the mushroom. Examining quercetin as a positive control, the free radical scavenging abilities of *M. gigantea* were compared. The scavenging activity of mushroom extract at various concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90, and

100μg/mL) is 6.75, 22.26%, 27.18%, 30.65%, 35.21%, 55.29%, 57.48%, 59.85%, and 67.94%, respectively (Figure 2). The synthetic antioxidant quercetin, on the other hand, had a 92.78% radical-scavenging activity, making *M. gigantea* a superior scavenger. The lyophilized methanolic extract of *M. gigantea* was previously discovered to possess a significant antioxidant activity of 75.73% at 200μg/mL[20], but the current investigation demonstrated the maximum percentage of inhibition of 67.94% at 100μg/ml and lowest of 6.75% at 10μg/ml concentration.

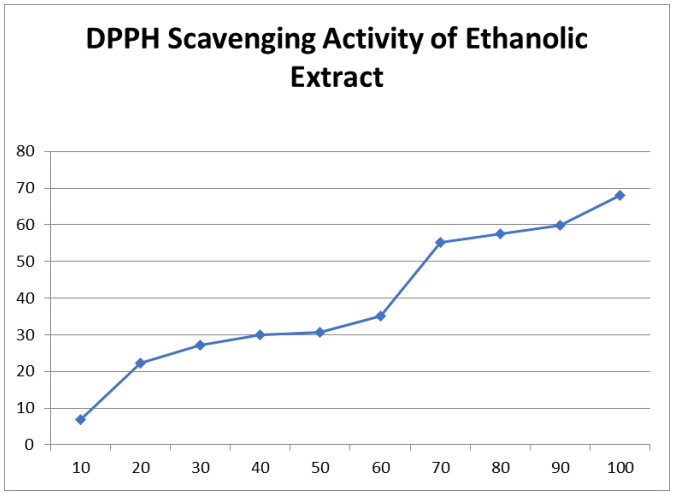

Fig 2. Free Radical Scavenging Effect of *Macrocybe gigantea* against DPPH

3.5 Synthesis of Silver Nanoparticles

Numerous bioactive compounds with anti-inflammatory, antioxidant, antibacterial, antiviral, anti-fungal, anticancer, cardiovascular, hypotensive and hepatoprotective activity are produced by mushrooms, which are well recognised[21]. Therefore, employing edible mushroom extracts to synthesise AgNPs is both cost-effective and biocompatible, with essentially no adverse reactions. The emergence of brown colour in the reaction solution during the biosynthesis of AgNPs strongly implies the production of AgNPs. Only the flask containing the mushroom extract and AgNO3 solution exhibits the brown colour (Figure 3). Within 24 hours of incubation, the colour changed, and the metal nanoparticles' surface plasmon vibrations, which are responsible for the brown colour, were excited[22]. The intensity of the colour increased with time due to surface plasmon resonance (SPR) and reduction of $AgNO_3$.

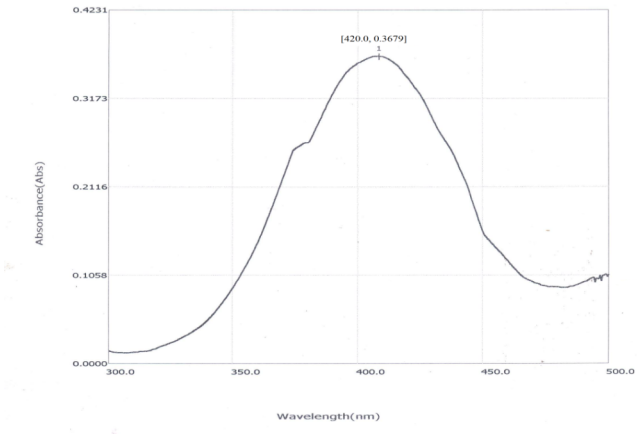

Fig 3. UV Spectra of AgNPs

3.6 Characterization of AgNPs

3.6.1 Spectral Analysis

The synthetic AgNPs from *M. gigantea* produced a distinctive peak at 420 nm, compared to the studies that are published on the absorbance peaks produced by AgNPs, which range from 430 to 450 nm [23].

The biomolecules capable of encapsulating and effectively stabilising the metal nanoparticles created using mushroom extract were visible in the FTIR absorbance peaks. Peaks at 3677.7, 2976, 2871, 2088, 1363, 1011,853,620, and 515 cm^{-1} were noted. The presence of alcohol and phenol groups in the *M. gigantea* mushroom is shown by the appearance of a sharp peak at 3677 cm^{-1}, which suggests O-H stretching. The presence of an amide group or proteins is shown by the peaks at 2976 cm^{-1}, 1363 cm^{-1}, and 1011 cm^{-1}. The FTIR data demonstrate that the functional groups O-H, -C-O, and N-H were responsible for the reduction of silver ions. to metallic silver. The protein in the extract clearly serves as a capping and stabilizing agent for artificial AgNPs.

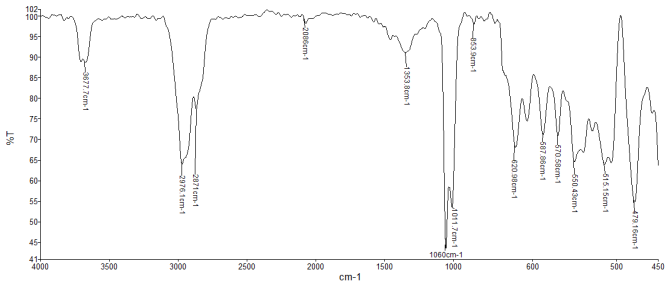

Fig 4. FTIR Analysis of AgNPs

3.7 SEM analysis

According to SEM images of nanoparticles made from an aqueous extract of *M. gigantea,* AgNPs are spherical in shape and range in size from 50 to 70 nm on average (Figure 5). While the size of the produced AgNPs in *Helvellaleucopus* ranges from 80 to 100nm [16].

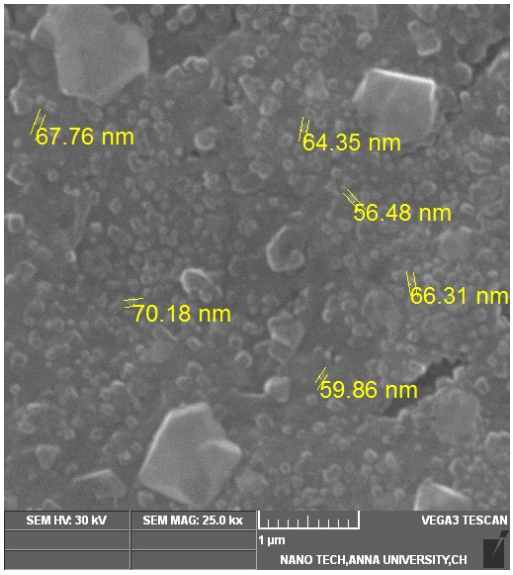

Fig 5. SEM Image Silver Nanoparticles Synthesized using *Macrocybe gigantea*

3.8 Antibacterial potential of biologically synthesized AgNPs

The synthesised AgNPs of *M. gigantea* demonstrated promising antibacterial activity against the following foodborne bacteria: *Escherichia coli, Streptococcus mutans* and *Bacillus subtilis* at three different concentrations: 25μL, 50μL and 100μL. The results were compared to standard antibiotics such as streptomycin and distilled water. At 25μL, the highest inhibition zone against *Escherichia coli* (6mm) was recorded, followed by *Streptococcus mutans* (5mm) and *Bacillus subtilis* (5mm) (3mm). The highest inhibitory zone was detected at 50 L in both *E. coli* (7mm) and *Streptococcus mutans* (7mm), followed by *Bacillus subtilis* (6mm). *Streptococcus mutans* (9mm) seemed to have the maximum inhibitory zone at 100L, followed by *Escherichia coli* (8mm) and *Bacillus subtilis* (7mm). The bactericidal properties of AgNPs are also influenced by their size, shape, surface charge, dosage and particle dispersion state. Furthermore, the destructive effect of AgNPs on bacteria rises with decreasing particle size [19].

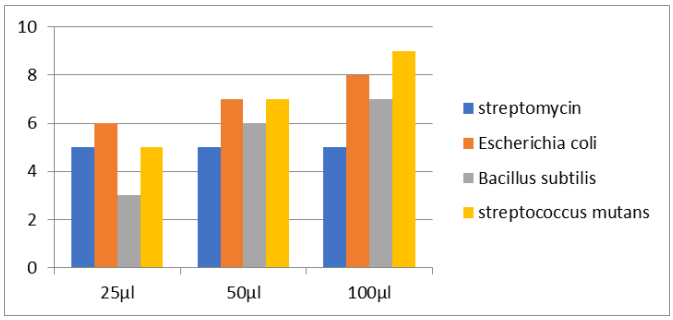

Fig 6. Antimicrobial activity of AgNPs by Disc Diffusion Method

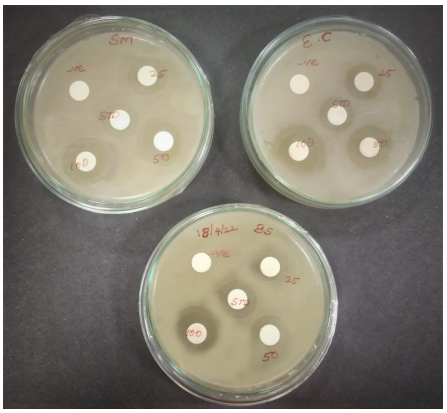

Fig 7. Zone of inhibition by different concentration of AgNPs *Streptococcus mutans*, *E.coli* and *Bacillus subtilis*

4 Conclusion

The study revealed that the green synthesis of silver nanoparticles using *M. gigantea* aqueous extract plays an important role in the food processing and food packing industries. The silver cations produced by AgNPs, which function as reservoirs for the Ag+ bactericidal agent, are responsible for the potent bactericidal activity against a restricted range of food-borne pathogens. To the best of the authors' knowledge, this is the first study on the biosynthesis of silver nanoparticles utilizing *M. gigantea* against a few food-borne pathogens.

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