KOMBUCHA FUNGUS MEDIATED SILVER NANOPARTICLES AND THEIR BIOLOGICAL ACTIVITIES

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Abstract

The biosynthesis of Silver nanoparticles (AgNPs) was carried out using the extract of tea fungus (Kombucha consortium). "Kombucha" is commonly known as tea fungus that consists of bacterial and fungal strains. The Kombucha biomass was used for the synthesis of AgNPs. The synthesized AgNPs were characterized by UV-Visible spectroscopy, Dynamic Light Scattering Microscopy (DLSM), FTIR, Atomic Force Microscopy (AFM) and Scanning Electron Microscope (SEM). Biosynthesized AgNPs exhibited antibacterial activity against gram positive and gram negative bacteria (*S. aureus* and *E.coli*). The anti-cancer activity was also conducted against human breast cancer cells (MCF-7). The synthesized AgNPs are simple, less expensive and environmental friendly. The AgNPs have wide applications in various fields namely medicine, catalysis and chemistry.

Keywords: Kombucha fungus, Silver nanoparticles, SEM, Antibacterial activity, Anticancer activity.

I. INTRODUCTION

In the present nanotechnological research, nanoparticles have been playing a prominent role due to their tiny size and higher efficiency. The metallic nanoparticles are widely used in the different field, as they possess unique optical, electrical and chemical properties. Applications of AgNPs include drug delivery, biosensors, cosmetics, catalysts in biological reactions, etc. AgNPs are synthesized by chemical [1], photochemical [2], electrochemical [3], and biological methods [4, 5, 8]. In chemical method, the chemicals used for the synthesis of nanoparticles are harmful to the environment. The biosynthesis of AgNPs eliminates this hazardous chemicals and also eco-friendly and biocompatible. In microbial synthesis, prokaryotic bacteria *Bacillus sp*. has been extensively used [6]. Reports showed that AgNPs exhibited anti-microbial activity against the *Staphylococcus aureus* and *Escherichia coli* [9, 19]. Antibacterial and antifungal activities of AgNPs have been reported against *E.Coli* and *Yeast* strains [10, 7,11].

Kombucha consortia are commonly known as "tea fungus" which can be obtained from the fermented tea. Tea fungus constitutes "Symbiotic Culture of Bacteria and Yeasts." The fungal strains such as *Zygosaccharomyces, Pichia, Brettanomyces, Schizosaccharomyces, Saccharomycoides, Saccharomyces, Torulaspora, Candida* etc., are present in this consortium [13, 14]. *Acetobacter xylinum* [33], the strains of *Glucanobacter* and *Lactobacillus* [13] were also present in this consortium. Kombucha is being marketed as a healthy fermented beverage which is embodied with several healthy nutrients and also been utilized as medicine for various ailments. It is a specialized drink with detoxifying and energizing properties. Kombucha has been found to cure Atherosclerosis and cardiovascular diseases [12]. The major compounds consist in the fermented liquid are acetic acid, gluconic acid and lactic acid, glucuronic acid and some antimicrobial compounds [21, 13, 15]. This report has been focused mainly on the synthesis of AgNPs using the Kombucha consortia to identify their biological activities such as antibacterial and anticancer. Concerning the Kombucha fungus, this is the first report for the synthesis of AgNPs.

II. MATERIALS AND METHODS

A. *Microorganisms*

The Kombucha consortium was used from the Biological Materials Laboratory, CSIR-Central Leather

Research Institute, Chennai. The microbes *Staphylococcus aureus* MTCC3160 and *E. coli* MTCC40 were procured from the Institute of Microbial Type Culture Collection (MTCC), Chandigarh, India.

B. Preparation of Biomass:

The biomass extract of the tea fungus was grown aerobically in 250 mL Erlenmeyer flask containing 100 mL of liquid growth medium (black tea and sucrose). The culture was agitated in the orbital shaker at 150 rpm at 35°C for 7days. The fungal biomass was isolated after 7days by filtration using a suction pump and Whattman filter paper. The biomass was repeatedly washed with MilliQ water to remove medium components. 10 g of biomass was suspended in 100 mL of MilliQ water in 250 mL Erlenmeyer flask. The flask was kept in the orbital shaker for agitation at 150 rpm for 3days at 35°C. The entire process was carried out in complete darkness.

C. Synthesis of AgNPs:

5 mM AgNO3 solution was prepared and stored in the amber color bottle. The biomass extract was added to 5 mM AgNO3 solution. The color change of the solution from pale yellow to the dark brown color indicated the formation AgNPs by Kombucha fungus.

D. Characterization of AgNPs:

The formation of AgNPs were confirmed by UV-visible, single beam spectrophotometer (Shimadzu UV 2450). Infrared (IR) spectrum of extracts was obtained using the KBr pellet technique. The spectrum was recorded in the range of 4000-400 cm-1 (Bruker Tensor). The size distribution and zeta potential of the nanoparticles in the solution were determined using particle size and zeta analyzer (Malvern Zetasizer). For scanning electron microscope (SEM) analysis, the synthesized AgNPs were diluted and had been spread on Aluminium foils and allowed to dry. The SEM micrograph was obtained using Hitachi-SU 6600.

E. Antibacterial activity:

The synthesized AgNPs were tested for antibacterial activity against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) by using agar well diffusion method. Muller Hinton agar was used for the bacterial growth. The bacterial strains were spread equally on the surface of the medium and were kept for incubation at 37 ^{0}C for 24 hrs. Wells were loaded with different concentrations of AgNPs along with control (Kombucha extract alone). The zone of inhibition was observed.

F. MTT assay:

The synthesized AgNPs were tested for cytotoxic activity against MCF-7 cell line at different concentrations (12.5 to 200 µg/mL).Stock solutions of AgNPs have been prepared and added to the cells and kept for incubation at 37^{0} C for 3 days with control. At the end of the exponential phase, the cells were harvested and counted using hemocytometer. Using 96- well culture plate, the cell suspension was assigned in triplicates in the concentration of 1×10^{5} cells/well. MTT (3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide) colorimetric assay was performed. This colorimetric assay determines the viability of cells. The readings were taken using spectrophotometer at 520 nm and using microplate absorbance was taken. The cells were observed under an inverted microscope to find their morphological changes. The percentage cell viability was then calculated on control as follows:

Cell viability (%) = Mean OD/ control OD × 100

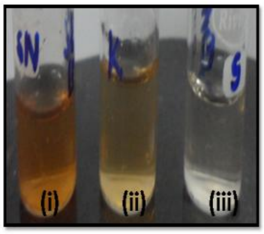

Figure 1 (a)

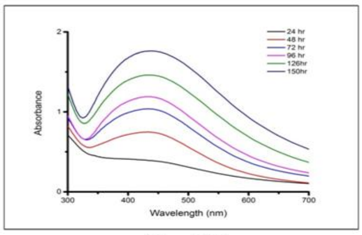

Figure 1 (b)

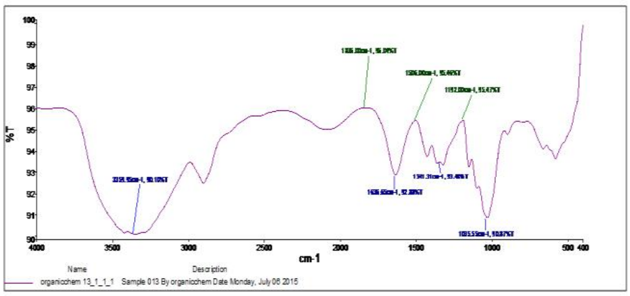

Figure 1 (c)

1. Fig. 1-a (i) : AgNPs
   Fig. 1-a (ii) : Kombucha biomass extract
   Fig. 1-a (iii) : $AgNO_3$ solution
   Fig. 1-b: UV-Vis Spectroscopy of AgNPs
   Fig. 1-c: FT-IR Spectroscopy analysis of AgNPs

III. RESULTS AND DISCUSSION

A. Microbial synthesis of silver nanoparticles:

The microbial synthesis of AgNPs through the extract of Kombucha fungus was identified by the color change of the solution. After 24 hours, the color of the solution had been changed from pale yellow to dark brown color as shown in Figure 1(a) [30, 31, 32]. The color change was due to the surface Plasmon resonance of the synthesized AgNPs [18, 26, 28]. Further confirmation done by UV-Vis spectroscopy and the peak was observed at 420 nm (Figure 1(b)) [24].

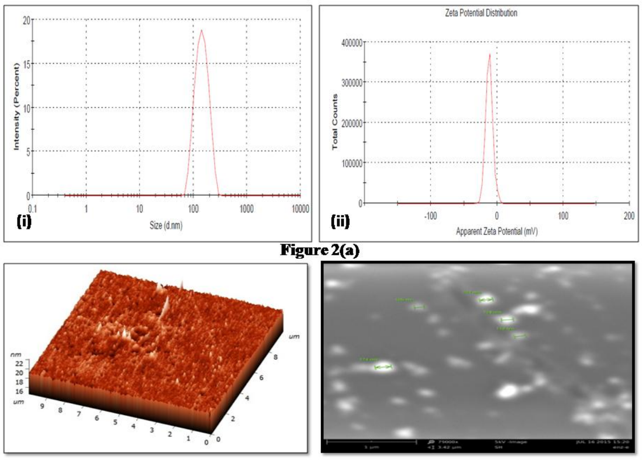

Figure 2(a)

Figure 2 (b) Figure 2 (c)

Fig. 2-a (i): Average particle size determination of AgNPs
Fig. 2-a (ii): Apparent zeta potential
Fig. 2-b: AFM (Atomic Force Microscopy)
Fig. 2-c: SEM image of AgNPs

B. Characterization of silver AgNPs:

The Fourier transform infrared spectroscopy (FT-IR) spectroscopic analysis was carried on the lyophilized powder of AgNPs. The measurements were taken using Nicolet Impact 400 FT-IR spectrophotometer with KBr pellets in the wave number region of 4000 to 400 cm-1. The synthesized AgNPs showed peaks at 1035.55, 1341.31, 1636.65 and 3359.95 cm-1(Figure 1(c)). This indicates the presence of various functional groups which were responsible for the formation and stabilization of AgNps [23, 25]. The particle size distribution was determined using Dynamic Light Scattering measurements. The average particle size of the synthesized AgNps was found to be 155 nm (Figure 2(a)). Zeta potential analysis of the synthesized AgNPs has also been confirmed the result. A similar report was observed by Umoren et al., in AgNPs synthesized by red apple extract at 150nm [20]. For scanning electron microscope (SEM) and atomic force microscopy (AFM) analysis image recorded the drop coated films of AgNPs were in spherical shape as shown in Figure 2(c) [22, 27, 29]. The surface properties and morphology of biosynthesized AgNps, characterized by AFM were reported in Figure 2(b) and also revealed that the AgNPS are irregular in shape.

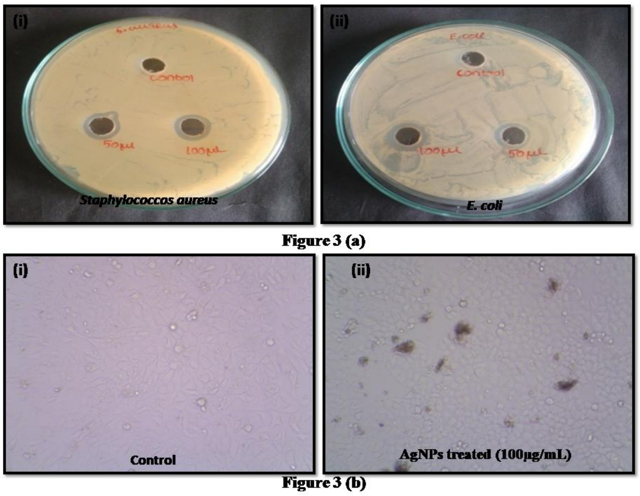

Figure 3 (a)

Figure 3 (b)

Fig. 3-a (i): *S. aureus* culture showing inhibitory zone
Fig. 3-a (ii): *E .coli* culture showing inhibitory zone
Fig. 3-b (i): Control (MCF-7)
Fig. 3-b (ii): AgNPs treated (100 µg /mL)

C. Screening of antibacterial and anticancer activity of the synthesized AgNPs:

The biologically synthesized AgNPs were tested for antibacterial activity against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli* using agar well diffusion method. The biomass extract of Kombucha fungus has been found to be effective against all tested bacteria especially Gram-positive bacteria *S. aureus* which showed 14.0 mm inhibitory zone. On the other hand, Gram-negative bacteria *E. coli* showed 9.0 mm inhibitory zone (Figure 3(a)). Similar result was observed in Gram-negative bacteria *E.coli* [19] and in both bacteria [28].The Kombucha synthesized AgNPs exhibited cytotoxic activity against human breast cancer (MCF-7) cell line. The cell growth was inhibited (IC50 = 172.83 µg/ml) (Figure 3(b)) in MCF-7 by AgNPs . This result is similar to the AgNPs synthesized by the seaweed *Ulva Lactuca* and the fungal extracts of *Hypocrea lixii* against MCF-7 cell line [16,17].

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