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Determination of Antifungal Activity of Silver Nanoparticles Produced from *Aspergillus niger*

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Abstract

AgNPs, over the past decades have attractive considerable interest because of their exclusive optical, electromagnetic, catalytic properties, and antifungal potency compared with other metal nanoparticles. This study was conducted to evaluate the antifungal effect of colloidal AgNPs gainst pathogenic Candida species such as *Candida albicans*, *Candida glabrata* and *Candida tropicalis* using disc diffusion method. Biosynthesis of AgNPs was confirmed by appearance of greyish black color of the fungal filtrate and UV visible spectrometry analysis reveals maximum absorption at 430 nm. The obtained resulted suggested that AgNPs was found to be effective against Candida species based on the diameter of the inhibition zone thus have potential implications to be used as antifungal agent.

Keywords: Antifungal activity; *Aspergillus niger;* Silver nanoparticles

Introduction

The term "nano" is originated from Greek language meaning extremely small [1]. The manufacturing of materials at Nano scale was described by Taniguchi in 1974 who coined the term "Nanotechnology" which is the manipulation, amalgamation and reduction of matter at nano scale to produce products that possess enhanced characteristics such as stronger, lighter, cleaner, definite and specific [2,3]. Even though nanotechnology is at its infancy, but is gaining momentum with the passage of time, opening opportunities for the scientists and researchers. Enhanced functionality and improved stability being converted into its nanosize particles [4-6].

Nano size objects have diverse applications in different disciplines such as biology, nanotechnology, physics, chemistry and material engineering etc. [7]. Besides many process being employed for the preparation of nanoparticles including aerosol technologies, laser ablation, lithography, photochemical reduction, ultrasonic fields, ultraviolet irradiation techniques etc. But these methods are expensive and involve the use of toxic chemicals that poses a serious threat to the environment.

Therefore microbial synthesis of nanoparticles is an eco-friendly approach utilizing microbial technology [8]. For example a number of bacterias have been reported to produce different types of metallic nanoparticles including photosynthetic bacteria of genus Serratia and *Rhodobacter sphaeroides* were reported to produce Copper and Cadmium sulfate nanoparticles respectively [9]. In addition to that, green amalgamation of platinum nanoparticles using diopyros kaki leaf extract [10], gold and silver nanoparticles from clove [11] and *Aloe vera* plant extract [12], gold nanoparticle using *Gnidia glauca* flower extract [13] have been reported.

Cadmium nanoparticles have also been synthesized by the fungi *Fusarium oxysporum*, *Candida glabrata* and *Schizosaccharomyce pombe* [14]. Among different metallic nanoparticles, silver nanoparticles are known to possess antifungal activity. In present research antifungal activity of colloidal silver nanoparticles produced from *Aspergillus niger* was evaluated.

Materials and Materials

Fungal cell culture

Aspergillus niger culture was inoculated by wire loop method in PD (potato dextrose) broth medium which consists of 20 gm sucrose, 200 gm potato dextrose dissolved in 1000 ml of distilled water. The culture media was autoclaved for 15 min at 121°C at 15 psi (pound/square inches). Inoculated culture was incubated at room temperature for five days on a shaker.

Purification of fungal filtrate (FF)

After five days of fungal growth mycelia was filtered from the medium by Whatman's filter paper no. 1. The obtained fungal filtrate was then passed through polyvinylidene fluoride (PVDF) membrane to obtain cell free fungal filtrate (Figure 1). The FF was then centrifuged at 10000 rpm for 20 minutes.

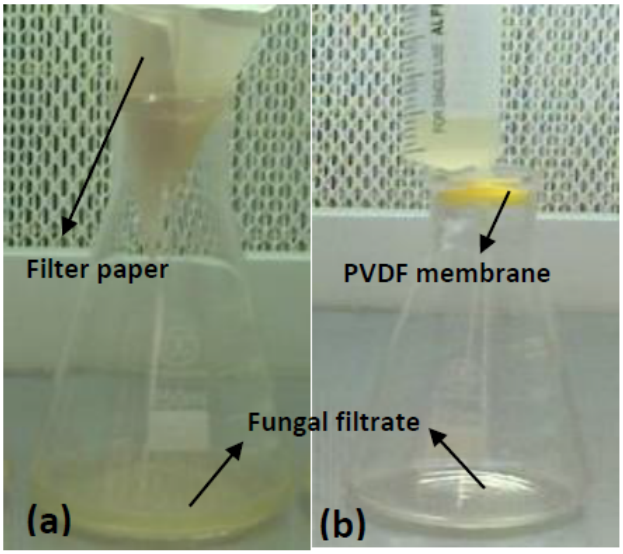

Figure 1: Purification of fungal filtrate (FF) using (a) Whatman's filter paper no. 1 and (b) PVDF membrane.

$AgNO_3$ synthesis

20 ml of FF incubated with 80 ml of 1 mM of silver nitrate solution in an incubator for 48 hours in dark to avoid any photochemical reactions. Change in color will indicate silver nanoparticles formation. Control containing freshly prepared PD broth incubated with silver nitrate was run as a control.

UV-visible spectrometer analysis

Using UV-Visible Spectrometer (JENWAY 6305), absorption analysis was carried out from 360 nm to 460 nm to obtain λ_{max}. Double distilled water was used as a reference blank (Figure 2).

Figure 2: UV-visible spectrometer analysis.

Antifungal activity

Antifungal activity of $AgNO_3$ was determined by disc diffusion method.

Preparation of YM agar plates

Yeast malt extract agar plates were prepared by dissolving 10 gm glucose, 3 gm yeast extract, 3 gm malt extract, 20 gm agar and 5 gm peptone in 1000 ml distilled water. The culture medium was autoclaved for 15 min at 121°C at 15 psi (pound/square inches). Before pouring 0.5 ml antibiotic ampicillin was added to the medium to inhibit bacterial contamination and 1 ml of 10% sterilized tartaric acid solution to adjust pH of media (4.2-4.5). The agar surface was impregnated with the test pathogens such as *C. glabrata*, *C. albicans* and *C. tropicalis* (Figure 3).

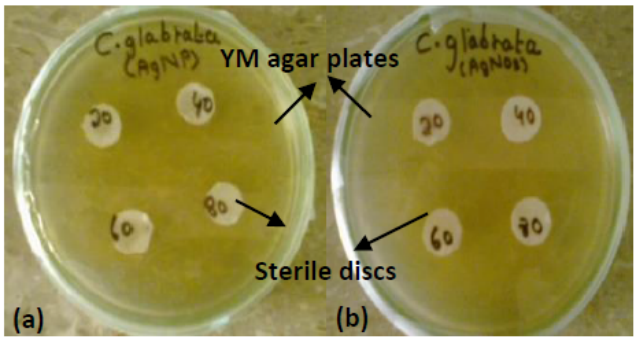

Figure 3: Disc diffusion method (a) Experiment (b) Control.

Preparation of sterile discs

Spherical shaped discs of size one centimeter was prepared from Whatman's filter paper no. 1and autoclaved at 121°C at 15 psi (pound/square inches) for 15 minutes. The prepared discs were than impregnated with different concentration of colloidal AgNPs i.e., 20 µL, 40 µL, 60 µL and 80 µL. Silver nanoparticle impregnated discs were then place on the surface of YM agar impregnated with test pathogens and incubated for 48 hours at 37°C. Width of the inhibition zone was measured in centimeters using a ruler scale. Silver nitrate impregnated discs were used as a control.

Results and Discussion

Appearance of greyish black color in the experimental flask containing the fungal filtrate incubated with $AgNO_3$ solution depicted formation of colloidal silver nanoparticles formation (Figure 4).

The change in color indicates that silver metal ions have been reduced to form the corresponding silver nanoparticles. The exact mechanism behind silver nanocrystals formation is not known however it is given in the literature that trapping and reduction of the silver ions take place with the help of nitrate reductase and protein complexes that act as electron shuttle [15,16]. These enzymes and protein complexes were detected in the fungal filtrate and possess redox properties [17]. As it is evident that microorganism releases electron shuttles or other reducing agents such as hydroquinones which enables metal ions reduction to their respective nanoparticles. However absence of nitrate reductase in the control flasks does not result in silver ion reduction therefore no color change was observed. UV visible spectrum obtained clearly indicates the formation of silver nanoparticles as a single band was obtained with maximum absorption at wavelength of 430 nm which is specific for silver nanoparticles [18-20] (Figure 5).

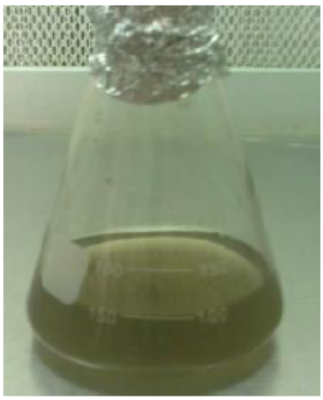

Figure 4: AgNPs synthesis by appearance of greyish black color of the incubated fungal filtrate.

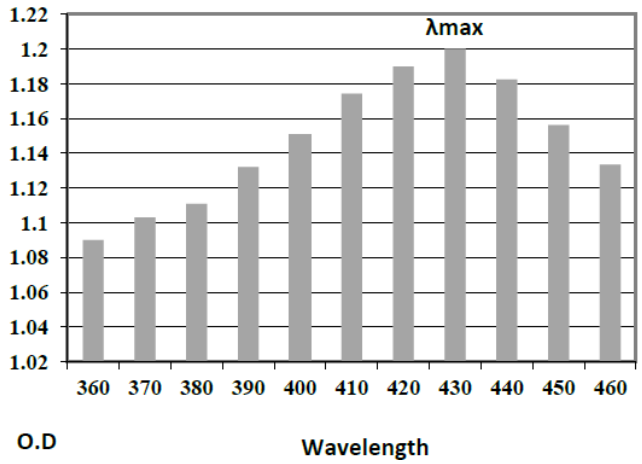

Figure 5: UV visible spectrum.

The increase in intensity of the color occurred due to gradual increase in the amount of silver nanoparticles present in the aqueous medium. The mycosynthesized silver nanoparticles were found to be well distributed with not much accretion [21]. Obtained results suggested that AgNPs formed by *Aspergillus niger* showed antifungal property against pathogenic Candida species as indicated by the clear zone around the silver nanoparticle impregnated discs (Figure 6) and the diameter of the inhibition zone increases with increased concentration of colloidal silver nanoparticles. Similar results were reported while using silver nanoparticles produced at different physioculture conditions [22-24]. However no zone of inhibition was obtained in case of the control. Width of the inhibition zone around the sterile discs impregnated with different concentration of silver nanoparticles indicates silver nanoparticles broad antifungal spectrum (Table 1).

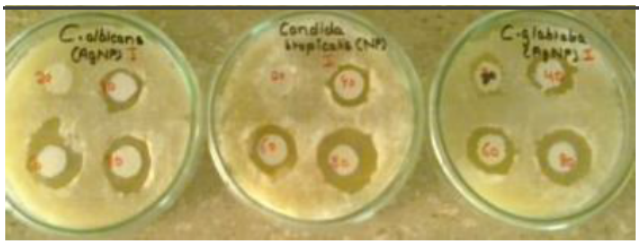

Figure 6: Antifungal activity determination of silver nanoaprticles by disc diffusion method.

S.no	Pathogens	Zone of inhibition (cm)			
		Silver nanoparticle (AgNPs)			
		20 µL	40 µL	60 µL	80 µL
1	*Candida glabrata*	0.1	0.5	0.6	0.9
2	*Candida albicans*	-	0.5	0.9	1
3	*Candida tropicalis*	-	0.4	0.4	0.9

Table 1: Inhibition zone.

Conclusion

Colloidal silver nanoparticles produced extracellularly from the fungus *Aspergillus niger* possess broad antifungal spectrum thus could be used in clinical medicine.

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