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Antifungal Activity of Biogenic Selenium Nanoparticles Synthesized from Electronic Waste

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Abstract: Fungal infections can occur in different parts of the body and can sometimes be difficult to treat due to their antibiotic resistance. While selenium nanoparticles (SeNPs) has been used for a wide range of applications including antibacterial, antioxidant and anticancer applications, the effects of SeNPs on fungal strains remain for the most part unknown to date. This is a pioneering attempt to evaluate the antifungal activity of SeNPs synthesized from Electronic waste (E-waste). The antifungal effectiveness of SeNPs was tested against two important clinical fungal genera, *Candida* and *Aspergillus*. The antifungal efficacy was determined by disc diffusion method and sensitivity in terms of zone of inhibition formed around the disc. Results of this study provided the first evidence of SeNPs was effective against the fungal strains tested. Therefore, the present study reveals that SeNPs synthesized from E-waste is not only a potential alternative path of managing electronic wastes but also a mode of converting macro-electronic components into nano - antifungal agent.
Keywords: Selenium Nanoparticles; E-waste; Candida sp; Aspergillus sp; Antifungal activity.

Introduction

Nanotechnology plays a key role in the fabrication of different nanoparticles that can exhibit novel antimicrobial properties[1]. Selenium nanoparticles (SeNPs) have become the focus of intensive research owing to their wide range of applications in areas such as antioxidants[1-4], antibacterial activity[5] and anticancer[5,6] applications. However, so far not much has been reported on the evaluation of antifungal activity of SeNPs with an exception of a recent study[1]. Studies of Shahverdi et al (2010)[1] have shown that antifungal formulations in the form of nanoparticles could be used as potential fungicidal material.

Selenium sulfide is an effective antifungal agent[1] and it has been used in anti-dandruff hair shampoos[7] for treatment of scalp fungal infections[1]. Nevertheless, while compared with micronparticles, nanoparticles have increased surface areas and therefore have increased interactions with microbial cells. In addition, nanoparticles were easily entered into the cells than micronparticles[5]. Due to this ability, nanoparticles were synthesized from different sources and they may be used as an antimicrobial agent. In our previous study, we have used *Bacillus thuringiensis* (Bt) as a bionanofactory for the recovery of selenium from photocopier selenium drum (E-waste). Hence, the aim of the present study was to evaluate the antifungal activity of selenium nanoparticles synthesized from E-waste against the important clinical fungal strains of *Candida albicans* and *Aspergillus niger*. In doing so, this study revealed a new dimension for the convention of materials recovered from the electronic waste as a potential antifungal agent.

Experimental Methods

Synthesis of selenium nanoparticles

In our previous study, SeNPs was synthesized from E-waste using *Bacillus thuringiensis*. Briefly, the surface sterilized layer was scrapped from photocopier selenium drum (E-waste) and then ground to fine powder (E-scrap). 80 mg of lyophilized E-scrap was added into Enrichment medium and then 100µl of cell suspension of Bt was inoculated into the medium. The reaction solution was incubated at 37°C for 24- 48h. The culture solution was centrifuged at 10,000 rpm at 4°C for 10min and the pellet was washed twice with deionized distilled water by repeating the two centrifugation steps[6]. The harvested samples were freeze dried on Lyo Guard Chamber to carry out further characterization studies[8]. The size and morphology of the SeNPs was investigated by using Transmission Electron Microscope (TEM) and Field Emission Scanning Electron Microscope (FESEM). The composition of SeNPs was confirmed by Energy Dispersive Spectroscopy (EDS).

Antifungal Activity

The antifungal activity of the biogenic SeNPs synthesized from E-waste was tested against the following clinical strains: *Candida albicans* and *Aspergillus niger* and these strains were obtained from Bose Laboratory, Madurai, TamilNadu, India. The antifungal activity of SeNPs against these two strains was evaluated by agar well diffusion method[9]. Using a sterile cotton swab lawn cultures of the test organisms were made on the agar plates. Selenium loaded disc along with standard and control (water) disc were kept into the Petri dish. The plates were then incubated at 30°C for 36h. Fluconazole was used as a standard antifungal drug. The antifungal activity was evaluated by measuring the zone of inhibition around the disc.

Determination of Minimum Inhibitory Concentration (MIC)

The synthesized nanoparticles can be tested for minimum inhibitory concentration by microtitre broth dilution method[9]. Sabouraud broth was used as diluents for fungal species. About 10^6CFU/ml cells can be inoculated, and the final volume in each microtitre plate well will be 0.1 ml. Nanoparticle concentration ranges from 0.19µg/ml to 100µg/ml. After incubation for 30°C for fungal strains, the microtitre plates are read at 450 nm using microtitre plate reader prior to and after incubation to determine the minimum inhibitory concentration (MIC) values. The MIC is defined as the lowest concentration of compound, which inhibited 90% of the growth of tested organisms.

Results and Discussion

Synthesis of SeNPs from E-waste and its Characterization

The cell suspension of Bt strain when challenged with lyophilized E-scrap and incubating for 24 - 48h in the EM medium, show evidence of a time dependent change in color of the medium from light yellow to red. The characteristic red color of the solution was due to excitation of the surface plasmon vibrations of the SeNPs[6].

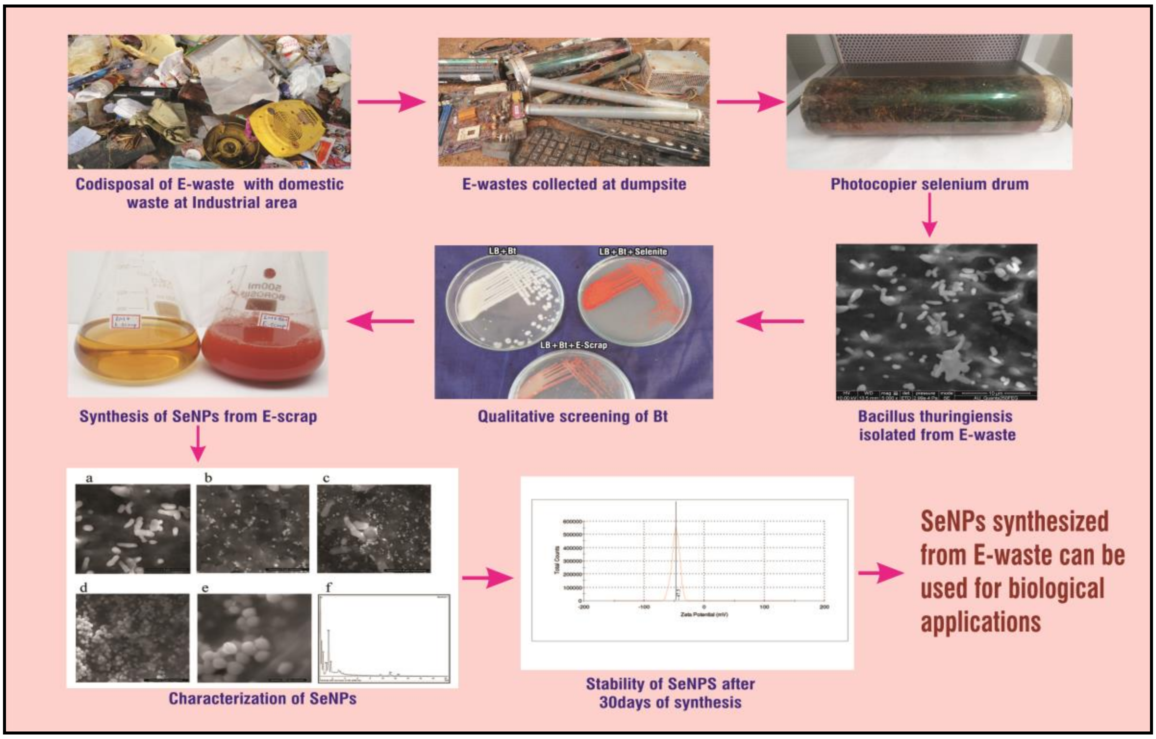
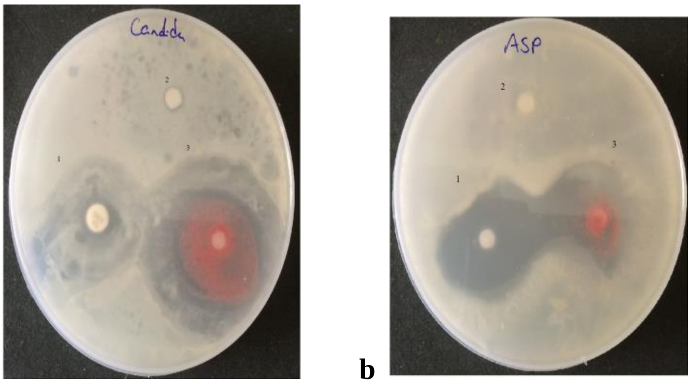


Figure 1: Schematic Representation of Biogenesis of SeNPs from E-waste and its Characterization

The results of the FESEM and TEM studies were depicted that the SeNPs synthesized from E-waste was spherical in shape and in size range from 50 to 500nm, with an average size between 50 to 200nm. In the analysis of EDAX, the SeNPs produced peaks at 1.37keV (peak SeLα), 11.22keV (peak SeKα), 12.49keV (peak SeKβ), which was specific absorption peak of SeNPs[4,10]. Figure- 1 shows the schematic representation of biogenesis of SeNPs from E-waste using *Bacillus thuringiensis* isolated from the same E-waste and its characterization.

Antifungal Activity of SeNPs

The antifungal activity of SeNPs was analyzed against *Candida albicans* and *Aspergillus niger* by disk diffusion method[9] (Figure 2a& 2b). The common antifungal agents are enormously irritant and lethal and it is necessary to formulate new types of safe and cost-effective fungicidal material. Shahverdi et al (2010)[1] have reported that nanoparticles synthesized by eco-friendly manner could be used as a potential fungicidal material.



a **b**

Figure 2: Antifungal activity of SeNPS, a- *Candida albicans*; b- *Aspergillus niger*
1- Standard disc, 2- Control disc and 3- SeNPs.

Accordingly, the results of the present study illustrate good antifungal activity against *Candida albicans* and *Aspergillus niger*. While compared with *Aspergillus niger*, *SeNPs* showed better activity against *Candida albicans.* In addition, it was proved that the redness SeNPs has high biological activities and low toxicity[11-13]. Fluconazole, a potent antifungal drug used as a standard, showed strong antifungal activity against test strains and no zone of inhibition in control disc. To the best of our knowledge this is the first report on the evaluation of antifungal activity of SeNPs synthesized from E-waste with positive results.

MIC Value of SeNPs

SeNPs synthesized from E-waste showed a good antifungal activity against test fungal strains, with ranging from 0.19µg/ml to 100µg/ml for two strains. *Candida albicans* was the most sensitive to SeNPs when compared with *Aspergillus niger*, with MICs of 6.5 µg/ml for *Candida albicans* and 12.5 µg/ml for *Aspergillus niger*. Nano-selenium and selenium have similar biological activity[14] in terms of antioxidant[15], antibacterial and anticancer[16-18]. Although, due to the high surface- to- volume ratios and their nano scale sizes provide better activity against the biological materials. In addition, Nano-Se has significantly lower toxicity than other inorganic and organic forms of supplemental selenium[19-20].

Conclusion

The present study highlights the importance of SeNPs recovered from photocopier selenium drum as antifungal agent. Elemental selenium, when supplied in the form of nanoparticles, undoubtedly can give out as an effective ingredient for the preparation of new antifungal formulations. Hence, the present investigation is not only a potential alternative route of managing electronic wastes but also a mode of converting macro-electronic components into nano- antifungal agent. The results of the antifungal activity of SeNPs recovered from E-waste holding true to the phrase “Treasure from Trash”.

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