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# Bovin Serum Albumin Stabilized Silver Nanoparticles: Synthesis, Characterization and Antifungal Studies

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***Abstract :*** *The work outlines the fungicidal properties of silver nanoparticles synthesized through a simple and an effective reduction technique using silver acetate and dodecylamine in a single step, and the synthesized silver nanoparticles are encapsulated into the scaffolds of bovin serum albumin(BSA) conjugates, which offers enhanced stability and performance for both the host and and the guest. The average size, size distribution, morphology, and structure of particles were determined by UV/Visible spectroscopy, Scanning Electron Microscopy (SEM), and X-ray Diffraction analysis (XRD). We determined the fungal inhibition in order to evaluate the antifungal efficacy of silver nanoparticle encapsulated BSA against selected fungal strains using well diffusion method, which ensures its potential ability as an antimicrobial agent for therapeutic purposes as well as for antimicrobial coating materials.*

***Keywords-*** *antifungal, bovin serum albumin, encapsulation, silver nanoparticle.*

## I. Introduction

During the last two decades, metal nanoparticles with a high surface area and a high fraction of surface atoms, are witnessing extreme attention due to their application in various fields of science and technology [1]. The novel properties of these nanoparticles have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [2–5]. Recently, the confluence of nanotechnology and biology has brought to force metals in the form of nanoparticles as potential antimicrobial agents. Nanoparticles have unique and well defined physical and chemical properties which can be manipulated suitably for desired applications. Among the various metallic nanoparticles, silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in various areas such as catalysis, optics, medicine, antimicrobials, and biomaterial production and this is due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts [2,6].

The biological application of silver nanoparticles is a rapidly developing area of nanotechnology that raises new possibilities in the diagnosis and treatment of various diseases. Silver has a broad spectrum of biocide activity against 650 bacteria, fungi and viruses [7,8], that this excellent property makes them applicable in various fields of marketing as well as for medicinal applications[2,9]. The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high. So, the formulation of effective, resistance-free and low-cost antimicrobial agents is of great interest. Thus, the preparation, characterization, surface modification, and functionalization of nanosized silver particles opens the possibility of formulation of a new generation of antimicrobial materials[10].

In recent years, the synthesis of silver nanoparticles in microemulsion by chemical reduction that could prevent the nanoparticles from agglomeration was reported[11,12]. In spite of Ag nanoparticles with different shapes and sizes successfully obtained in various aqueous solution systems, most of the reported synthetic methods depend heavily on the use of organic solvents and toxic reducing agents. All these chemicals are highly reactive and can induce potential environmental and biological pollutions [11,13]. So a more efficient, simple and reproducible method could be used instead of the conventional methods, where simple reduction of metal acetates or chlorides with amines such as dodecyl amine can be applied [14]. The silver nanoparticle produced from silver salts can be further stabilized by encapsulation in polymeric scaffolds or by binding to suitably functionalized macromolecular or supramolecular systems. So we think about stabilized metal nanoparticles for antimicrobial applications by manipulating the host-guest protocols. Metal nanoparticle encapsulated macromolecular systems need special attention in this regard. So encapsulation of Silver nanoparticles in suitable macromolecular systems like bovin serum albumin (BSA), starch, cyclodextrins etc provide high

stability and excellent antimicrobial coating effect. They can effectively stabilize silver nanoparticles because of the presence of large number of easily accessible functional groups present in these systems and the unique scaffolds or pockets provided by them[15]. Besides, high solubility in different solvents and low viscosity of these modified materials provide added advantage.

## II. Experimental

### 2.1. Materials

All the reagents were of analytical grade and used without further purification. Silver acetate and dodecylamine were obtained from Loba, Mumbai, India. All the solvents were purchased from Merck, Worli, Mumbai, India. Bovine serum albumin was purchased from Sigma (A-7030). Double distilled water was used for all experiments. Three fungal strains such as Mucor ramosissimosus, Mucor circinelloides, and Conidiobolus coronatus were used for the antifungal studies.

### 2.2. Synthesis and Encapsulation of Silver Nanoparticles

Silver nanoparticles were synthesized by a versatile and a reproducible method by reducing silver acetate using dodecylamine in a single step, following the procedure reported by Hiroki and Osterloh[14], with slight modification. Silver acetate (50 mg) was dissolved in dodecylamine (2ml) and refluxed with toluene (200ml) at $110^0$C for 8 hrs. It was concentrated to 10 ml, and methanol was added to precipitate the product and centrifuged. The ultimate powder was then dried in vacuum at room temperature for 2 hrs. The powder showed deep grey colour. It was dissolved in hexane and then added to an aqueous saturated solution of BSA. The mixture was vigorously stirred at room temperature for 5 hours, which caused the the phase transfer of BSA from the aqueous layer to the organic layer of hexane and the subsequent formation of silver nanoparticle encapsulated BSA. The encapsulated product separated from the solution was dried in vacuum.

### 2.3. Characterization

The UV-visible spectra were measured on a Systronics 2203 UV-Visible spectrophotometer operating in the range 200-1100nm. The surface analysis and the internal structure were probed with a JEOL JSM-6390 model scanning electron microscope (SEM) operated at 20KV. X-ray diffraction measurements were carried out using an X'Pert Pro x-ray diffractometer (PAN analytical BV) with CuKα radiation in a θ-2θ configuration. All measurements were performed at room temperature.

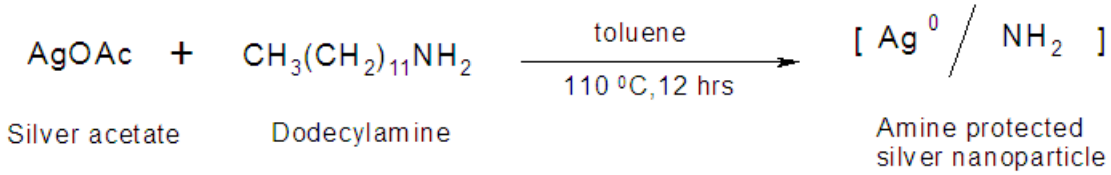
### 2.4. Antimicrobial Analysis

For in vitro screening, three fungal strains, M. ramosissimosus, M. circinelloides, and C. coronatus were selected. The strains were collected, sub cultured and stocked on the semi solid nutrient Agar slants. The micrograms were transferred to the Nutrient Broth prior to inoculation. Antifungal susceptibility test was performed by the agar well diffusion method[16, 17]. The fungal spore suspension was thoroughly mixed with melted potato dextrose agar (PDA) and poured into sterilised petri plates. Wells were performed with 6 mm in diameter in the culture medium. These holes were filled with the testing sample and the control. The Petri plates were incubated. All culture plates were examined after 24–96 hrs. The antifungal activity was evaluated by measuring the zone of inhibition in millimetres. Experiments were repeated six times and the average diameter was calculated.

## III. Results And Discussion

### 3.1. Synthesis and Encapsulation of Silver Nanoparticles

For the effective synthesis of silver nanoparticles, we adopted an effective and a reproducible reduction technique instead of the conventional methods, where simple reduction of silver acetates with a long chain aliphatic amine such as dodecylamine was applied. Here the dodecylamine acts as a reducing agent as well as a capping agent. The schematic representation [18] of the synthesis is shown in scheme 1.




Scheme1. Synthesis of silver nanoparticles

The long hydrocarbon chain of the aliphatic amine prevents the nanoparticles from aggregation and is responsible for the hydrophobicity of the nanoparticles. As the reaction proceeded, the colour of the reaction mixture slightly changed from pale yellow to reddish brown, then to dark brown, indicating the formation of silver nanoparticles. In order to overcome the stability problem of the synthesised silver nanoparticles, we encapsulated them into macromolecular matrices which offer enhanced stability and performance for both the host and guest. For the encapsulation process, bovin serum albumin was used as the trapping agent. The BSA matrix can encapsulate the nanoparticles and the resultant host–guest complex is stabilized by the polar interaction of the peripheral hydroxyl groups of BSA and amine-protected silver nanoparticles. The macromolecular matrix of BSA provided a scaffold for immobilizing NPs and prevented aggregation and served as a capping agent for limiting NP growth.

### 3.2. UV/Visible Spectroscopic Studies

The UV–Visible spectra of the reaction mixture were recorded on a time-dependant manner. The spectrum recorded after 30 min shows a shallow absorption band (Figure 1. (a)). As the reaction proceeded, this band became well defined and more and more intense. The spectra recorded after 1 h and 8 hrs are shown in Figure 1. (b) and (c), respectively. The spectrum recorded after 8 hrs shows a strong and well-defined peak at 408.8 nm.

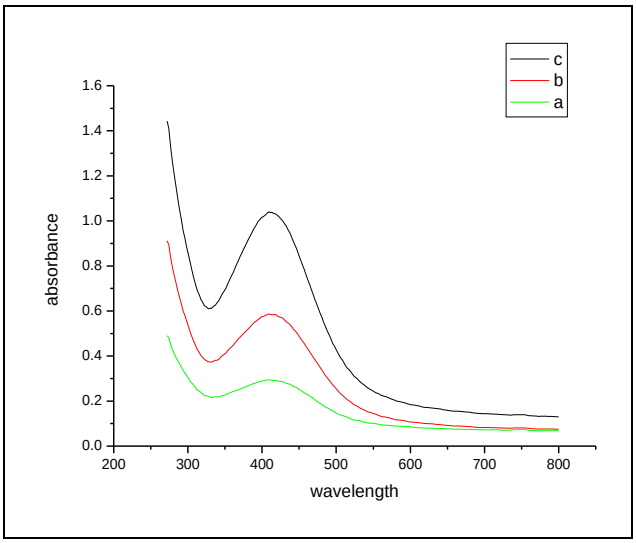
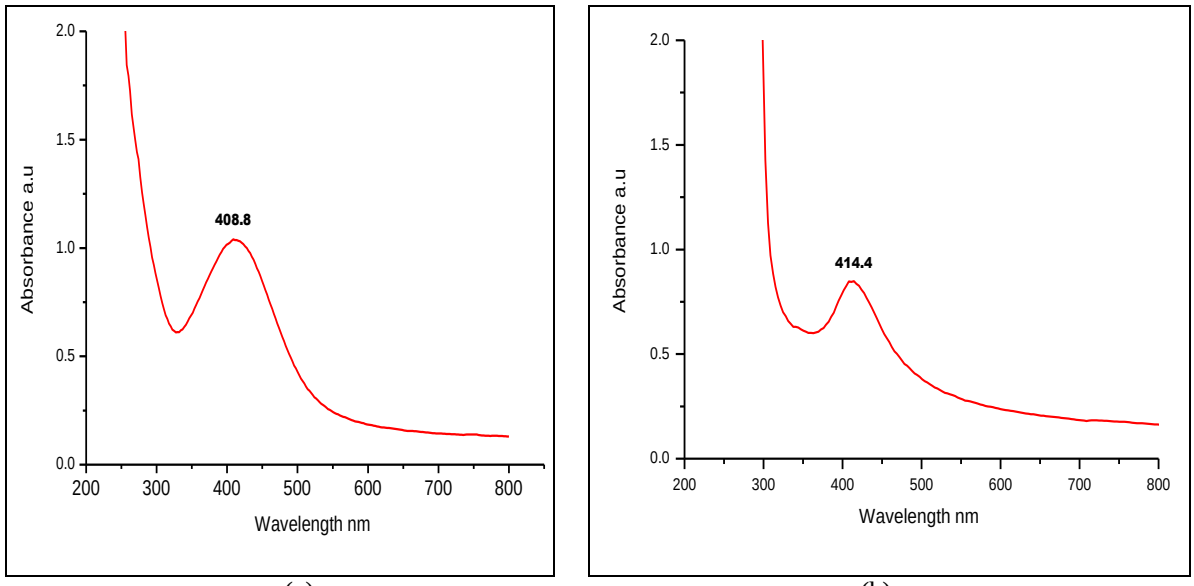



**Figure 1.** UV–visible spectra of the reaction mixture during different stages of nanoparticle formation: (a) after 30min, (b) 1 hr and (c) 8hrs.

Metal nanoparticles exhibit absorption bands or broad regions of absorption in the UV-visible range. These are known to be due to the excitation of plasma resonances or interband transitions, characteristic properties of the metallic nature of the particles [19]. The low concentration of silver in organic solvents such as toluene, hexane, etc give a bright yellow color due to the intense bands around the excitation of the surface plasmon resonance. Figure 2 shows UV-visible spectra of (a) silver nanoparticles and (b) silver nanoparticles encapsulated BSA. The silver nanoparticles display an optical absorption band peak at 408.8 nm, which is typical of the absorption of metallic silver nanoparticles due to the surface plasmon resonance. It was reported that the surface plasmon resonance (SPR) spectrum depends upon the refractive index of the surrounding medium, particle size, shape of particle and absorption substance of their surface [20]. This is the reason that when incorporated into BSA, the absorption peak was red shifted to 414 nm. Even a small chemical change in its surroundings causes a monitorable shift in the occurrence of SPR. When the macromolecule BSA had interacted with the surface of silver nanoparticle, its surface charge density was deformed by the electrostatic attraction resulting in a red shift.

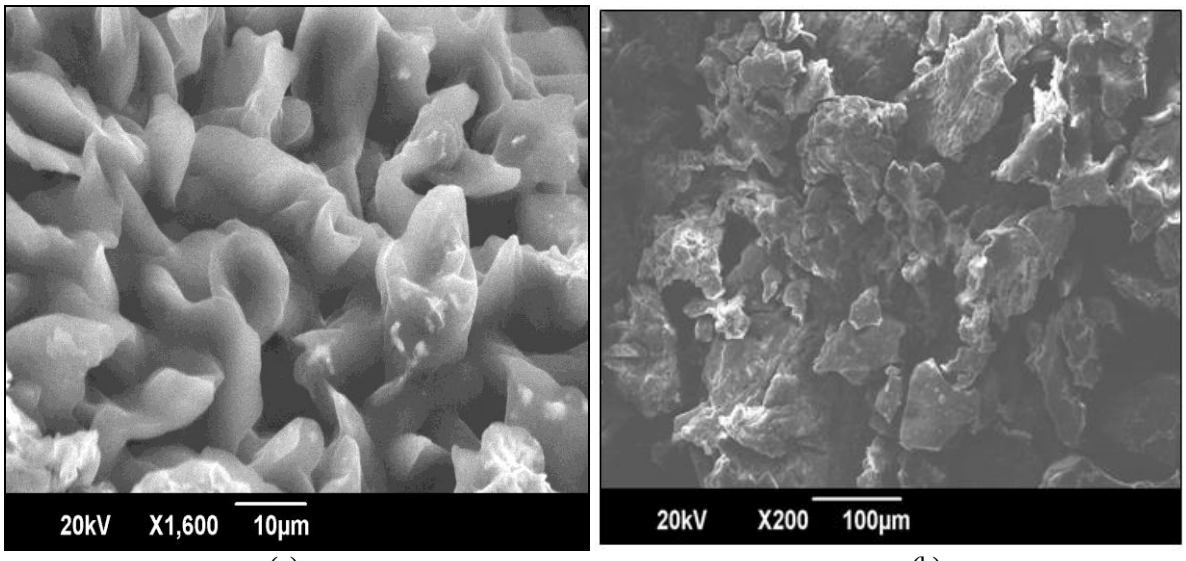



(a) (b)

**Figure2.** UV/visible absorption spectra of (a) silver nanoparticles and (b) silver nanoparticles encapsulated in BSA

### 3.3. Scanning Electron Microscopic Analysis

Surface analysis of the silver nanoparticles was carried out by scanning electron microscopy (SEM) in order to investigate the morphology of the particle. The samples were coated with a thin film of platinum to make their surface conducting. The scanning electron microscopy (SEM) image of silver nanoparticle is represented in figure 3.(a) and indicates more or less uniformly dispersed particles which are almost flowery shaped. The SEM image of the encapsulated product is shown in figure3.(b). It demonstrates a fairly sharp distribution BSA capped silver nanoparticles. Surface roughness has increased and this may due to the well dispersion of silver nanoparticles into the albumin matrix and was stabilized by the amino groups and hydroxyl groups of BSA. The stability of the silver nanoparticles has greatly increased on encapsulating them into the aggregates of BSA.




(a). (b)

**Figure 3.** SEM images of (a) silver nanoparticle and (b) silver nanoparticle encapsulated in BSA

### 3.4. X-ray diffraction (XRD) analysis

The crystalline nature of silver nanoparticles has been confirmed by X-ray diffraction studies. The XRD pattern of Ag nanoparticles is shown in figure 4 which shows that they held a cubic crystal structure. The major strong characteristic peaks of Ag particles are at 2θ around 38.252$^o$, 44.313$^o$, and 64.649$^o$ which were corresponding to crystal faces of (111), (200), and (220) of Ag. All the reflection peaks could be indexed to

face-centered cubic (FCC) silver nanoparticles. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula, $D = 0.9\lambda/\beta\cos\theta$, where D is the mean diameter of the nanoparticles, $\lambda$ is wavelength of X-ray radiation source, $\beta$ is the angular FWHM of the XRD peak at the diffraction angle $\theta$ [21]. The size of nanoparticles estimated by XRD is 9-20 nm range.

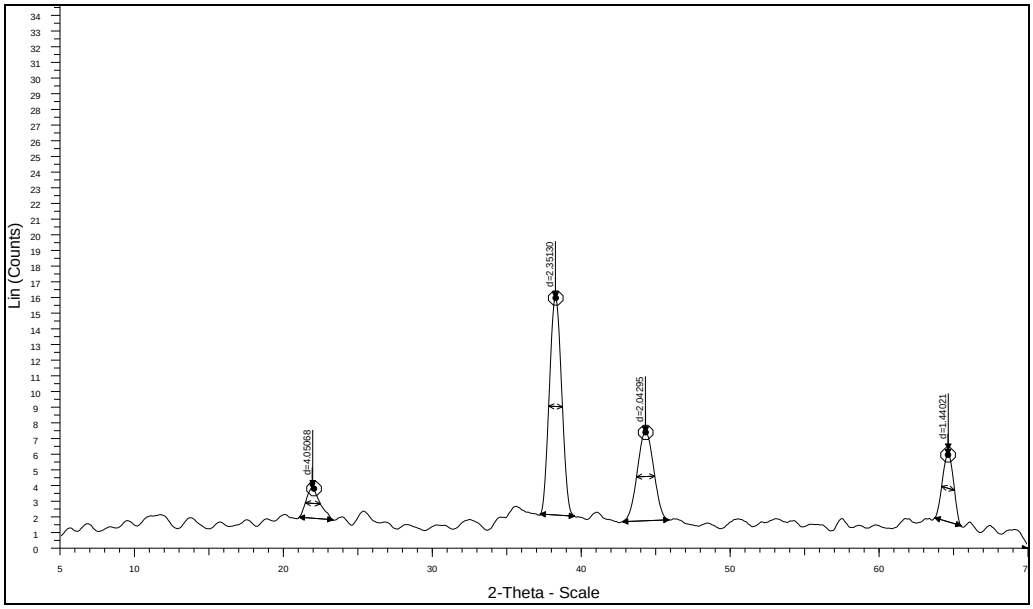
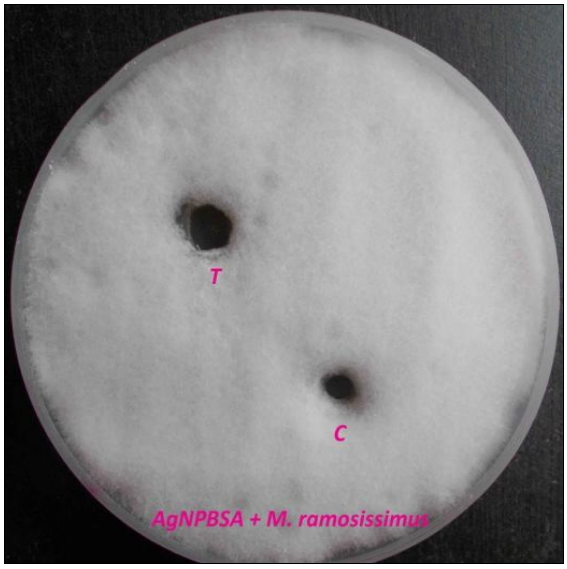



**Figure 4.** XRD pattern of synthesised silver nanoparticles

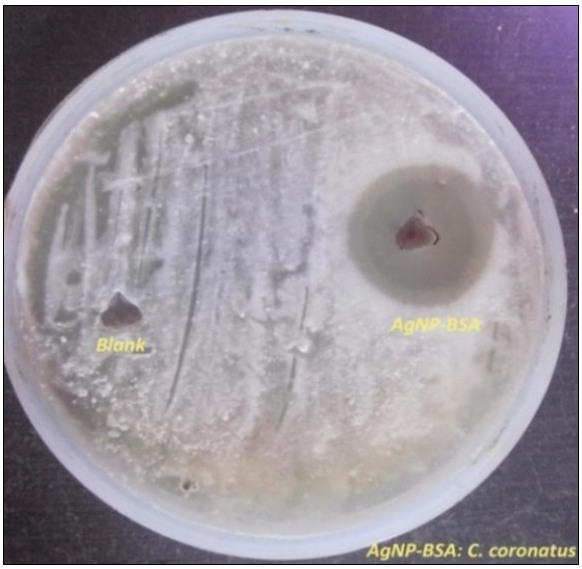
### 3.5.Antifungal activity of silver nanoparticles encapsulated in bovin serum albumin

Antifungal susceptibility test was performed by the agar well diffusion method. All the cultured plates were examined after 24–96 hrs. Six tests were conducted. The average diameter of inhibition zones for Mucor ramosissimosus, Mucor circinelloides, and Conidiobolus coronatus, were 21mm, 23mm and 37mm respectively. Among the tested strains, Conidiobolus coronatus showed maximum zone of inhibition. The inhibitory zone indicates the disruption of the fungal cell wall membrane by the action of silver nanoparticles. This result shows the efficiency of silver nanoparticle encapsulated macromolecular system to kill the pathogenic fungal strains. Figure 5 shows the antifungal effect against various tested strains.




(a)


(b)




(c)

**Figure 4.** Antifungal effect of silver nanoparticle encapsulated bovin serum albumin against (a) Mucor ramosissimosus, (b)Mucor circinelloides, and (c)Conidiobolus coronatus

The binding of the particles to the fungal cell wall depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more fungicidal effect than the larger particles. The antimicrobial activity of silver nanoparticles has been previously reported [8,11,12]. When encapsulated into the scaffolds provided by the BSA aggregates, the AgNPs preserve their antimicrobial efficiency. Here the silver nanoparticle encapsulated BSA solution was able to saturate and cohere to fungal hyphe and to deactivate the pathogenic fungi. Reports on the mechanism of inhibitory action of silver ions on microorganisms have shown that upon treatment with $Ag^+$, DNA loses its ability to replicate [22], resulting in inactivated expression of ribosomal subunit proteins, as well as certain other cellular proteins and enzymes essential to ATP production [23]. It has also been hypothesized that $Ag^+$ primarily affects the function of membrane-bound enzymes, such as those in the respiratory chain [24].Thus silver nanoparticle encapsulated bovin serum albumin had a wide spectrum of antifungal activity against most of the tested species although for a very low concentration of Ag inclusion complex.

## IV. Conclusion

Compounds containing silver ions which are known to have long lasting biocidal properties have received much attention because they are stable and exhibit a low toxicity to human cells. Efficient antimicrobial agents have been made by incorporating Ag nanoparticles into polymeric scaffolds or by binding to suitably functionalized macromolecular or supramolecular systems. The present work describes the preparation, characterization and surface modification of nanosized silver particles, which opens the possibility of formulation of a new generation of antimicrobial materials. Ag nanoparticles were successfully synthesized using a simple, versatile and reproducible reduction technique with low polydispersity and the synthesized silver nanoparticles were encapsulated into the scaffolds of bovin serum albumin conjugates, which offers enhanced stability and performance for both the host and the guest and provide wide utility in antimicrobial applications. Findings from the current investigation demonstrated that AgNPs with low toxicity and a broad spectrum of antimicrobial activity were very effective against various pathogenic fungi. However, the current study is based on in vitro petri dish evaluation; therefore, extrapolation of these findings to more general cases is limited. Still, data from this study provide valuable preliminary efficacy data on silver compounds for use in control of pathogens. In summary, AgNP encapsulated BSA exerted potent antifungal effects on fungi tested in vitro, probably through destruction of membrane integrity; therefore, it was concluded that they have considerable antifungal activity. The pronounced inhibition exhibited by silver nanoparticle encapsulated BSA could be tested on more fungal strains under various conditions and it can provide a more generalized picture of the antifungal activity of these systems.

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