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POLYSACCHARIDE INDUCED PRODUCTION OF SILVER NANOPARTICLES (AG-NPS) AND THEIR ANTIBACTERIAL EFFICACY AGAINST SELECTED BACTERIAL PATHOGENS

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ABSTRACT

Synthesis of silver nanoparticles (Ag -NPs) using sustainable thermo-chemical method have drawn considerable interest in the field of nanotechnology. Polysaccharides have been used as stabilizing and reducing agents for the synthesis of silver nanoparticles. In the present investigation two different sources of starch (synthetic starch, rice starch) have been used to reduce precursor of Ag ions for the rate of nucleation and size distribution of silver nanoparticles. The synthesized silver nanoparticles were adopted to characterize by UV-visible spectroscopy, field emission scanning electron microscope (FESEM) analysis and antibacterial assay. The surface plasmon resonance (SPR) absorption of nanoparticles was monitored by using UV-visible spectrophotometer and maximum peaked at 440nm and 420nm respectively. The FESEM micrograph of synthetic starch mediated silver nanoparticles shows that an irregular shaped with size range of 60-100nm and natural starch mediated silver nanoparticles demonstrate that spherical shaped nanoparticles having the size range of 60-80nm. Natural starch mediated silver nanoparticles showed highest antibacterial activity (25 mm) against *Shigella* sp. Among the bacterial pathogens tested Gram negative bactria are more susceptible than the Gram positive bacteria by the nanoparticle synthesized using natural starch as a reducing and stabilizing agent. Thermo-chemical mediated natural starch induced silver nanomaterials can be considered as reliable, eco-friendly and cost effective approach and evident by the properties of the nanoparticles.

KEYWORDS: silver nanoparticles, polysaccharide, starch, FESEM analysis, antibacterial

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INTRODUCTION

Recent decade nanomateriales are the most important field of nano-biotechnology and nanomedicine. However lacks of sufficient informations are available on the adverse effects of nanoparticles on human health and environmental hazards. Nanoparticles obtained from different metals and process techniques possess certain antibacterial properties which can be used against certain drug resistant bacteria and other biomedical applications. Antibacterial resistance has increased in recent years due to uncontrolled and over dose of antibiotics for the conventional chemotherapy. The researchers are keep on searching and approaching alternative methods using novel resources to develop new and unique chemotherapeutic drugs[1]. Nanotechnology is the ideal unique and highly demanding fields in the current scenario particularly to synthesize of metallic nanoparticles. Now-a-days metal nanoparticles (NPs) have attracted much more attentions because of their good conductivity, chemical stability, use as catalysts[2] and their applications in various industries including the field of medical sciences such as antimicrobial incredients or wound dressings[3-6]. The properties of the silver nanoparticles are strongly influenced by the experimental conditions, the interaction of silver ions with reducing agents and stabilizing agent with metal nanoparticles[7]. Metallic nanoparticles have drawn much attention due to their unusual chemical and physical properties. Various nanoparticles have been synthesized among the diverse researchers community across the globe, among these metal nanoparticles is one of the most promising substances. This is due to their broad spectrum of antibacterial, antiviral and anticancer properties. Changing their shape, size or surface of the constituent can be changed by physical and chemical properties of the nanoparticles[8,9]. Different types of production preparation path ways have been reported for the synthesis of silver nanoparticles. Silver nanoparticles can be synthesized using different techniques methods such as electrochemical, γ-radiation, photochemical, thermal decomposition of silver compounds, sono-chemical, micro wave assisted process and recently *via* chemical route *etc*. The biological synthesis and physical methods are numerous but many of these methods are highly expensive and time consuming process[10]. Using chemical reduction methods of different morphologies with varying size of nanoparticles can be synthesis over other biosynthetic processes. Though, green synthesis approach of Ag-NPs has advantages over physical and chemical reduction process. Chemical mediated reduction technique is commonly used to synthesize Ag-NPs because of its availability to generate Ag-NPs under suitable and gentle conditions. Moreover it has clinical and other intense biomedical applications[11]. The present study has been focused on the development of thermo-chemical polysaccharide mediated (TCPM) synthesis of Ag-NPs using synthetic starch and natural starch as a reducing and capping agent. The main objectives of the present investigation are to synthesize the silver nanoparticles using starch under moderate temperature at different stirring time and their characterization. The synthesized nanoparticle was subjected to perform antibacterial activity against selected pathogens by the standard method and compared with selected commercial antibiotic discs.

MATERIALS AND METHODS

Test organisms

Silver nanoparticles produced by TCPM method were subjected to perform to antibacterial assay against selected gram negative human pathogens *viz., Salmonella paratyphi* A*, Shigella* sp*., Enterococcus* sp*.,* and gram positive pathogen *Staphylococcus aureus.* Bacterial pathogens were preserved and maintained as auxenic culture in our microbiology laboratory was used for the present investigation.

Chemicals and media components

All chemicals and media components were obtained form Hi media Laboratory Private Limited (Mumbai, India) for the current nanoparticle synthesis and their characterization studies.

TCPM synthesis of silver nanoparticles

Two different polysaccharides of synthetic starch and natural starch as a reducing and stabilizing agent have been adopted for the reductions of silver salt in order to obtain silver nanoparticles with potential biomedical application and their characterization.

Natural starch (NS) mediated synthesis of the silver nanoparticles

Natural starch as a reducing agent and silver nitrate ($AgNO_3$) as starting material were used for the synthesis of nanoparticle under constant heating. Natural starch was prepared (1% v/v) from boiled rice water for the synthesis of nanoparticles. 5mM of $AgNO_3$ solution was prepared in 250ml of Erlenmeyer flask by adding 85 mg of silver nitrate in Milli-Q water. Natural starch was added drop by drop and the reaction mixture was stirred continuously for 30 minutes until a clear colourless solution was obtained. The reaction mixture was heated at 50°C and stirred at regular intervals for 30-45 minutes until a colour change was evident from colourless to yellowish brown. The resultant colloidal solution thus obtained was adapted to centrifuge at 10000 rpm for 15 to 20 minutes. The clear supernatant was discarded and slurry precipitate was transferred into watch glass for drying at room temperature for 48-72 hrs to achieve nanoparticles. The dried nanoparticles were stored in dark container for further studies.

Synthetic starch (SS) mediated synthesis of the silver nanoparticles

Synthetic starch of analytical grade (AG) as a reducing agent and silver nitrate ($AgNO_3$) used as a starting material were used for the production of silver nanoparticle under constant heating. 5mM of $AgNO_3$ solution was prepared in 250ml of Erlenmeyer flask by adding 85 mg of silver nitrate in Milli-Q water. Starch solution (1% w/v) was added drop by drop and the reaction mixture was stirred continuously for 30 minutes until a clear colourless solution was obtained. The reaction mixture was heated at 50°C and stirred at regular intervals for 30-45 minutes until the colour change was evident from colourless to yellowish brown.

The resultant colloidal solution was centrifuged at 10000 rpm for 15 minutes. The upper clear supernatant was discarded and lower slurry precipitate was transferred into watch glass for drying at room temperature for 48-72 hrs to obtain nanoparticles. The dried nanoparticles were stored in dark container for further studies. The synthesized silver nanoparticles by both the techniques were characterized by using UV-visible spectroscopy, FESEM analysis and antibacterial assay.

UV-Visible spectroscopy analysis

The development of silver nanoparticles was confirmed by UV-visible spectroscopy analysis by using CARY Conc 100 – EL06023680 model UV-Vis spectrophotometer. Thermo-chemical mediated reduction of silver ions was monitored by diluting small aliquots of silver nanoparticles into double distilled water after 2 hours for measuring the UV-Vis spectral analysis.

Field emission scanning electron microscopy (FESEM) characterization

FESEM study was carried out using SUPRA-55, ZEISS (German) microscopy for the morphological characterization and surface topography of silver nanoparticles. FESEM provide structural characterization, size and morphology of the nanoparticles under specific consideration. This confers the information about the structure of a specimen at micrometer and even sub-micrometer range. Thin film of dried silver nanoparticles was primed on a carbon incorporated copper grid by dropping a trace amount of the sample on the grid. The thin film on the SEM coated grid was allowed to dry provided under a mercury fluorescent lamp for 5-10 minutes.

Antibacterial assay

The antibacterial profile of TCPM silver nanoparticles was evaluated by standard Kirby-Bauer disc diffusion assay method against selected bacterial pathogens. Different volume of 5 µl, 10 µl, 15 µl, 20 µl and 25 µl per disc of silver nanoparticles synthesised by two different sources of starch was saturated with commercially available sterile disc (Hi-media, Mumbai) with the size of 6mm diameter. Actively growing broth cultures of test pathogens (10^8 cells) were swabbed on sterile Muller Hinton agar (MHA) plates separately. Silver nanoparticle impregnated disc was placed at equal distance from center to center of the Petri plate under aseptic condition. Three replicates were retaining for each test pathogens corresponding to different concentrations of nanoparticles to obtain mean value of zone of inhibition. Negative control was maintained by the disc impregnated with 25µl/disc of natural starch (1% v/v) and synthetic starch (1% w/v) to compare the antibacterial efficacy of silver nanoparticles. The different intensity of zone of inhibition in the region around of the discs were measured and recorded in mm diameter after 18 – 24 hrs of incubation at 37^{o}C for each test pathogen.

Statistical analysis

The antibacterial assay of silver nanoparticle synthesized by two different sources of starch and commercial antibiotic discs were measured. The mean diameter of zone of inhibition was calculated and expressed in mm ± standard deviation (mean ± SD).

RESULTS

UV visible spectroscopy

The reduction of silver ions into silver nanoparticles by the mixing of starch following heating at 50^{o} C could be understood by changing of colour of the reaction mixture. Starch effectively acted as electron donors and reduced silver ions to silver. Structural characterization and the formation of Plasmon resonance absorption of silver nanoparticles was determined by UV-visible spectroscopy technique. The formation and initiation of nanoparticles was indicated that by changing of yellowish brown colour of the solutions. UV-visible absorption spectrum of yellowish brown silver nanoparticles was prepared using synthetic starch as reducing and stabilizing agent is portrayed in fig.1. The reaction mixture showed band of Plasmon resonance surface absorption with a maximum peak of 440 nm demonstrating the presence of nanoparticles. Silver nanoparticles were prepared using natural starch as a reducing and stabilizing agent conferred by UV-visible absorption spectrum is displayed in fig. 2. The resultant solution exhibit typical Plasmon resonance absorption band peaked at 420 nm confirming that the occurrence of nanoparticles.

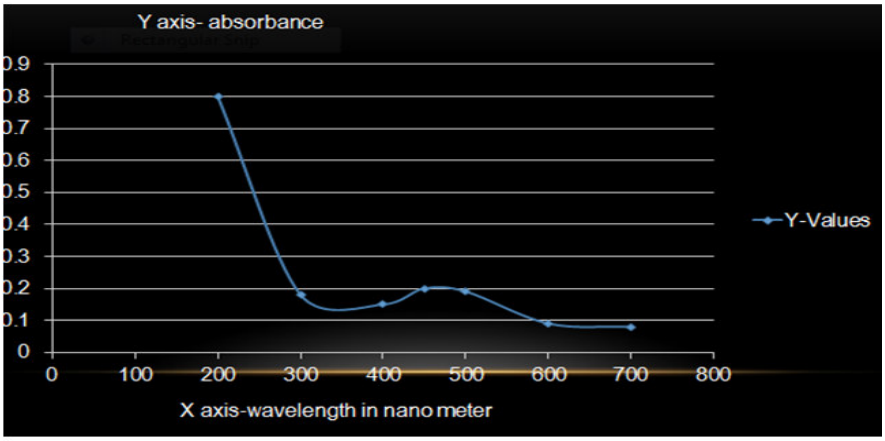

Figure 1
UV- Visible spectrograph of silver Ag-NPs synthesized by synthetic starch

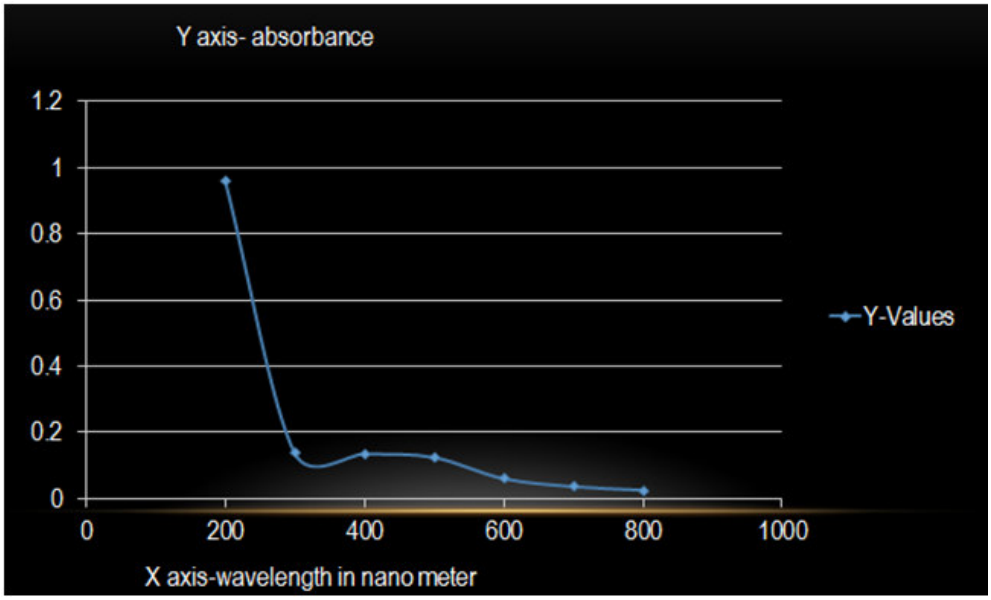

Figure 2
UV- Visible spectrograph of Ag-NPs synthesized by natural starch

Field emission scanning electron microscopy (FESEM) of Ag-NPs

The FESEM of silver nanoparticles mediated by synthetic starch and natural starch is depicted in figures 3 & 4 respectively. Synthetic starch mediate synthesized Ag-NPs have strong signals of silver ions at 5keV with magnetic resonance of 100.12Kx. Whereas natural starch mediated Ag-NPs have strong signals of silver ions at 20keV with magnetic resonance of 50.14Kx. The micrograph of synthetic starch exhibits that the particles have an irregular shaped and the size range of 60-100nm. Small crystals of nanoparticles aggregates over a period of time to produced inflorescence like silver nanoparticles mediated by synthetic starch were confirmed by SEM imaging. SEM micrograph of natural starch mediated silver nanoparticles demonstrate that spherical shaped nanoparticles was randomly agglomerates having the size range of 60-80nm. The variation in particle size is due to the fact for the formation of nanoparticles is being formed by the reducing starch used over the period of time.

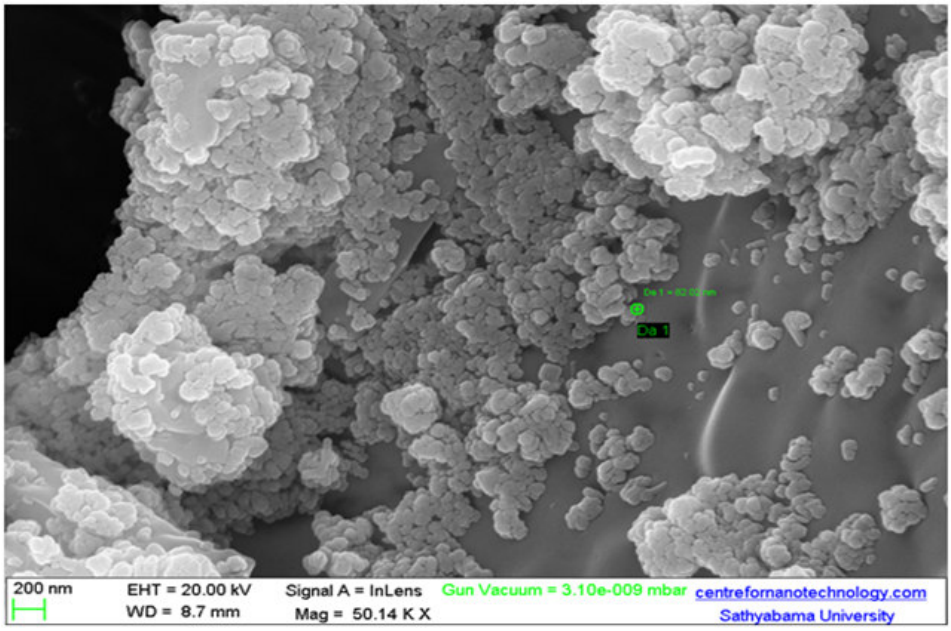

Figure3
FESEM image of silver nanoparticle synthesized using synthetic starch

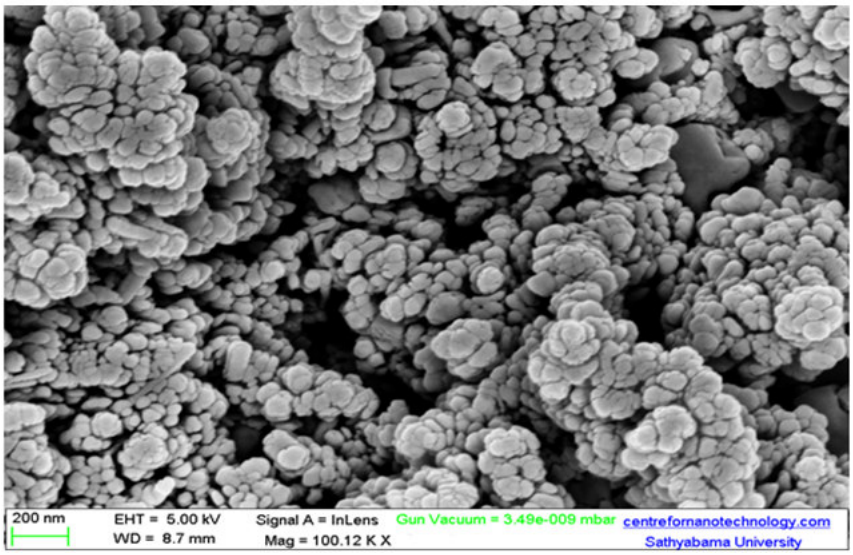

Figure 4
FESEM image of silver nanoparticles synthesized using natural starch

Antibacterial activity

Antibacterial pattern of silver nanoparticles mediated by synthetic starch and natural starch were assayed and the results were displayed in table 1 & 2 respectively. The antibacterial action of silver nanoparticles manufactured using two different sources of starch by thermo-chemical method was studied against selected bacterial pathogens. Different concentrations of of silver nanoparticles have been used to optimize the nanoparticle to compare with commercial antibiotic discs. The different levels of antibacterial efficacy were measured and recorded in terms of zone of inhibition in mm in diameter. Thermo-chemical mediated synthesis of silver nanoprticles using natural starch exhibited highest inhibition zone of 25mm against *Shigella* sp. followed by *Salmonella paratyphi* A of 22mm with the concentration of 25µl/disc. Among the bacterial pathogens tested Gram negative bactria are more susceptible than the Gram positive bacteria by the nanoparticle synthesized using natural starch as a reducing and capping agent. Moreover from the results revealed that 20µl/disc is the optimum concentration of nanoparticle is enough to inhibit the growth of tested bacterial pathogens.

Table 1
Antibacterial efficacy of Ag-NPs synthesized using synthetic starch

S.No	Selected pathogens	Inhibition zone in mm in diameter				
		5µl /disc	10µl/disc	15µl/disc	20µl/disc	25µl/disc
1	*Shigella* sp.	8	10	11	14	17
2	*Salmonella paratyphi* A	5	7	9	10	12
3	*Enterococci* sp.	7	11	13	17	13
4	*Staphylococcus aureus*	12	15	16	18	20

Values are the average of triplicates

Table 2
Antibacterial activity of silver nanoparticle synthesized using natural starch

S.No	Selected pathogens	Inhibition zone in mm in diameter				
		5µl /disc	10µl/disc	15µl/disc	20µl/disc	25µl/disc
1	*Shigella* sp.	15	18	20	22	25
2	*Salmonella paratyphi* A	10	13	15	19	22
3	*Enterococci* sp.	10	11	14	16	19
4	*Staphylococcus aureus*	9	11	14	15	21

Values are the average of triplicates

DISCUSSION

Metallic nanoparticles are chemical moiety exhibiting higher chemical and physic-chemical activity when compared to their organic complement of the nanoparticles. Use of polysaccharides to stabilize metal nanoparticle is an interesting and inexpensive process because the hydroxyl rich group can be exploited as support medium as well as reducing agent. Thermo-chemical method of silver nanoparticles was prepared using high molecular weight polysaccharide of natural starch and synthetic starch as reducing and stabilizing agent. This technique is based on chemo-thermal decomposition of metallic compound in starch. It has been successfully applied for synthesize of nano sized particles with high crystallinity and high thermal stability. The size and shape of the nanoparticles depended on the tendency of the organic substrate used to reduce the silver ions. The size, shape and distribution pattern of the nanoparticles was highly depended on the tendency of the starch substrate used to reduce the silver ions in

the present investigation. The reaction condition and the concentration of precursors influence the shape, size and controllability of nanoparticles. Ultraviolet-visible spectroscopy of colloidal solution of thermo-chemical mediated nanoparticles was measured as it is an effective technique for the analysis of nanoparticles[12]. The small size of the nanoparticles decreases consequently the degree of the interacting atoms highly increases towards the target bacterial cell and neoplastic cells. Moreover smaller sized nanoparticles are high affinity to interact with the bacteria and cause membrane damage to penetrate inside of the cell. The ability of silver nanoparticles to penetrate the cell membrane is sensible to believe that of small nanoparticles are more capable of piercing the cell membrane than larger sized nanoparticles[13]. In the present study correlated with the previous research by which natural starch mediated silver nanoparticles are smaller in size (60-80nm) has more susceptible than the synthetic starch mediated silver nanoparticles (60-100nm) against tested bacteria. It is confirmed that the small sized nanoparticle could enter the bacterial cell membrane and affect the structural and osmotic stability of the bacterial cell. Oberdorster *et al*[14] reported that NPs are small enough to penetrate into biological species including microorganisms as deposition of metal ions. The exact mechanism of antibacterial efficacy and other cytotoxic activity of silver nanoparticles are not completely understood and still in infant stages. However the antibacterial property is related to the morphological as well as structural changes in the cell wall of bacteria has been studied by the researchers[15, 16]. Franci *et al*[17] was reported that the antibacterial effect of Ag-NPs emerges out to be granted by their ultra-smaller size and increased their surface area. The nanoparticls can crosses through the body of the bacteria and there by destroy the membrane permeability and create intracellular damage. Ag-NPs have considerably least effect on the multiplication of Gram positive bacteria because of the differences in chemical composition of the cell wall. The antibiogram activity of Ag-NPs towards Gram negative bacteria is believed that the negative charge on the lipopolysaccharides (LPS) is involved to the positive charge of Ag-NPs. According to previous literature reports shows that positively charged silver nanoparticles and electrostatic desirability between negatively charged bacterial cells is play a vital role for the antibacterial action of nanoparticles as biocidal materials[18, 19]. This is also confirmed by the present thermo chemical mediated silver nanoparticle synthesised by using synthetic and natural starch as reducing and stabilizing agent. It is suggested that the organism losses the osmotic stability by inflex and outflex of ions and molecules there by disturb its function by releasing silver ions leads to death. Ashour[20] reported that Ag-NPs have broad spectrum of antibacterial activities towards both Gram negative and Gram positive bacteria. But in the present study shows that Gram negative bacteria are more susceptible than the Gram positive bacteria. However, Krishnakumar *et al*[21] reported that starch mediated synthesis of silver nanoparticles with DMSO exhibited maximum inhibition activity against *Candida albicans* than the bacterial pathogens tested. Further detailed studies are required for the absolute understanding of actual cellular interactions between microbes and molecular mechanism of Ag-NPs is essential to treat infectious diseases caused by bacteria. The antibacterial efficacy of silver nanoparticles against tested Gram negative bacteria is high due to their cell wall composition bacteria and it is believed that silver nanoparticles attached to the surface of the bacterial cell membrane, consequently might be penetrate the bacteria and disturb its cellular metabolic function by releasing silver in the form of silver ions.

CONCLUSION

The research mainly focused on thermo chemical mediated production of silver nanoparticles using starch by the reduction of silver salts. Natural starch mediated silver nanoparticles exhibited mean particle size of 60nm showed highest antibacterial activity against Gram negative pathogens. The results of the present studies concluded that the polysaccharide mediated silver nanoparticle can be used as a replacement for the ingredient for topical application of conventional antibiotic ointment. However the stability of the nanoparticle and the toxicity testing and biocompatibility of the nanoparticles should be tested using cell lines in future before practical application. These nontoxic silver nanoparticles could be produced by simple and cost-efficient technique which might be suitable for the formulation of novel types of therapeutic drugs and leads components to treat infectious and contagious diseases caused by Gram negative bacteria.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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