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Eco-Friendly Green Synthesis of Silver Nanoparticles from Egyptian Honey: Evaluating its Antibacterial Activities

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THIS RESEARCH article describes a strong approach for the green synthesis of silver nanoparticles (AgNPs) that employs a local black seed honey (BSH). Honey was chosen as the eco-friendly reducing and stabilizing agent replacing most reported reducing agents which represent highly biological risk to the society and environment. Honey reduced silver ions into AgNPs after 20min in a stirred reaction mixture. Nanoparticles of different sizes were obtained, and the solution turned black over time. The antibacterial activity of AgNPs was investigated against eight pathogenic bacterial strains; five Gram-negative and three Gram-positive. AgNPs were potentially effective against the Gram-negative more than Gram-positive bacterial strains. *E. coli* and *P. aeruginosa* were the highly susceptible strains with 6.3µg/ml minimum inhibitory concentrations (MICs). The AgNPs were characterized by perform UV-visible spectroscopy, fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) imaging. The colloid obtained at a pH of 9 was found to be spherical in shape and was distributed with a certain amount of agglomeration. An intense surface plasmon resonance band at 400nm in the UV-visible spectrum clearly revealed the formation of AgNPs after 72hr. TEM showed spherically shaped AgNPs, and the sizes of the nanoparticles ranged from 25-70nm. AgNPs had broad spectrum antibacterial property thus they can be explored further for medical and therapeutic applications.

Keywords: Black seed honey (BSH), Green synthesis, Characterization of silver nanoparticles, Pathogenic strains, Antibacterial activity.

Introduction

One of the most promising and recent areas of research in modern science is nanotechnology (Albrecht et al., 2006). Nanocrystalline metal particles (in particular AgNPs) have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics (Schultz et al., 2000), antimicrobials and therapeutics (Elechiguerra et al., 2005; Ouf et al., 2015, 2017), catalysis (Crooks et al., 2001) and micro-electronics (Gittins et al., 2000). The use of chemical and physical methods in the synthesis of nanoparticles is very expensive and cumbersome and leads to the presence of some toxic compounds and may have adverse effects in their applications (Parashar et al., 2009). Therefore, the development of green synthesis of nanoparticles is an important aspect of nanoscience and nanotechnology by growing environmentally benign nanoparticles (Venu et al., 2011). Synthesis of nanoparticles by green approach is an emerging field because of its various advantages over the other processes like nontoxic, ecofriendly and low cost. Researchers have used biological extracts for the synthesis of nanoparticles by adopting simple protocols, involving the process of reduction of metal ions by using biological extracts as a source of reductants either extracellularly or intracellularly (Fayaz et al., 2009; Kamel et al., 2016; Sharma et al., 2009).

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Many bacterial and fungal species have been used for AgNP synthesis (Bhainsa & D'souza, 2006; Song & Kim, 2009). However, most of them have been reported to accumulate AgNP intracellularly. Microbe mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions and their maintenance. Therefore; the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures (Kalishwaralal et al., 2010). Honey has recently been identified as another exceptional candidate that has yet to be fully explored by researchers around the world. The use of honey to synthesize AgNPs in water was recently reported (Philip, 2010) for the development of cost-effective and environmentally benign synthesis (Philip, 2009; 2010). Eco-friendly honey contains ingredients such as glucose and fructose that are used as capping and reducing agents in the synthesis of nanoparticles. In addition, it has extraordinary healing properties that are attributed mainly to its acidic pH and the presence of hydrogen peroxide (French et al., 2005). It replaced synthetic reducing agents such as hydrazine and dimethyl formamide, which are not completely environmentally safe. Natural honey is a sweet viscous fluid produced by bees. Honey consists of 80-85% carbohydrates, 15-17% water, 0.3% proteins, 0.2% ashes and minor quantities of amino acids and vitamins as well as low concentrations of other components (Moniruzzaman et al., 2014). Honey mediated biological synthesis has advantages over other types of biological methods (Venu et al., 2011), including the avoidance of elaborate processes such as drying plant materials and maintaining cell cultures. In recent years, studies have focused on the composition of honeys and their biological properties such as their antioxidant, anti-inflammatory and antimicrobial activities. The components of honey that are reportedly responsible for its antioxidant effects are flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids and the products of Maillard reactionswere investigated by Chua et al. (2013). In addition, honey helps providing a protective barrier to prevent infections within wound environments as well as in the treatment of skin ulcers and gastrointestinal disorders (Lusby et al., 2005; Dahl et al., 2007).

The green synthesis of AgNPs involves three primary steps, that must be evaluated in accordance with green chemistry perspectives (Dahl et al., 2007) selection of the solvent medium (Hutchison & Rodriguez, 2008) selection of an environmentally benign reducing agent, and (Anastas & Warner, 2000; Raveendran et al., 2003) selection of non-toxic substances for AgNP stability. Based on this approach, we have reviewed green chemistry type AgNP synthesis processes.

Advancements in nanotechnology have prompted microbiologists to apply metal nanoparticles as an effective method to control certain pathogenic microbes that are involved in infectious diseases (Nanda & Saravanan, 2009; Allahverdiyev et al., 2011; Li et al., 2011; Rosarin & Mirunalini, 2011; Singh et al., 2011; Ahmed et al., 2015; Prabu & Johnson, 2015). It is expected that the high specific surface area and high fraction of surface atoms on AgNPs will lead to high antimicrobial activity when compared with that of bulk silver metal (Cho et al., 2005). These nanoparticles are now primarily used as catalysts or antibacterial/antifungal agents (Anuj & Ishnava, 2013). One approach for countering bacterial drug resistance is the application of metal composites, especially at the nanoscale, to control bacterial infections. Scientists are developing new ways to control resistant pathogens (Nambiar et al., 2014).

In vitro evidence shows that AgNPs exert a unique antibacterial action on cells. There is an electrostatic attraction between nAg^+ and the negative charge on bacterial cell membranes and nAg^+ binds to the modified phospholipid bilayer and induces massive proton leakage (Dibrov et al., 2002). When nAg^+ anchors to the bacterial cell wall and causes structural change by forming irregular-shaped "pits" on the bacterial outer membrane, the permeability of the membrane changes and it becomes porous (Sondi & Salopek-Sondi, 2004; Mirzajani et al., 2011). The cell will then progressively release lipopolysaccharides and membrane protein, which will ultimately cause the cell to die (Li et al., 2010). Pal et al. demonstrated that the surface area to volume ratio of AgNPs and the crystallographic surface structures are important factors that determine the antibacterial activity of AgNPs (Pal et al., 2007).

The production of AgNPs is relatively inexpensive, and the addition of these particles into goods such as plastics, clothing, creams and soaps increases their market value. New classes of

compounds that include nanoparticle–antibiotic conjugates are undergoing clinical evaluations (Ping et al., 2005; Rai et al., 2009; Fayaz et al., 2010; Sekhon, 2010; Allahverdiyev et al., 2011; Kollef et al., 2011). The collaboration between nanotechnology and nanomedicine resulted in the emergence of new trend in both therapeutic and pharmaceutical fields in order to reveal novel therapeutic and diagnostic tools for human use (Asharani et al., 2009; Blanco et al., 2015).

Materials and Methods

Bacterial strains

Eight pathogenic bacterial strains comprising both Gram-positive and Gram-negative species were used. These bacteria were kindly provided by members of the culture collection of Genetic Engineering and Biotechnology Research Institute in Scientific Research and Technology Applications City, Egypt. Five strains of Gram-negative *(Escherichia coli, Proteus mirabilis, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Shigella* spp.) and three strains of Gram-positive *(Staphylococcus aureus, Streptococcus mutans* and *Bacills subtilis*) bacteria. All species were subcultured on nutrient agar and incubated aerobically at 37°C. The organisms were maintained in the laboratory on nutrient agar slopes at 4°C.

Honey samples

The natural local honey (black seed; BSH) used in this study was purchased from Al-Dakhakhny market of Alexandria, Egypt.

Honey-mediated biosynthesis of AgNPs

Silver nitrate was purchased from Sigma-Aldrich (St. Louis, MO). An aqueous solution (0.1M) of silver nitrate ($AgNO_3$) was prepared and used to synthesize AgNPs. Five millilitres of honey was added to 95ml of 0.1M aqueous $AgNO_3$ and stirred well for 1min to induce reduction into Ag+ ions. The intensity of the colour was then investigated.

Characterization of the biologically synthesized AgNPs

UV- visible spectrum analysis

UV-visible spectra were measured with a UV-visible spectrophotometer (T70 Split-Beam UV/VIS Spectrophotometer, England). The reduction of pure Ag^+ ions was monitored by measuring the UV-visible spectrum of the reaction medium over a range from 300 to 900nm. One millilitre of the sample was pipetted into a test tube after incubation for 20min and subsequently analysed at room temperature (Philip & Unni, 2011). The two parameters that were optimized were the pH and the reaction incubation time, which were identified as the factors that affected AgNP yields.

pH

The above mentioned procedure was performed to optimize the pH, the reaction pH values were maintained at 5, 6, 7, 8 and 9. The pH values were adjusted using 0.1N HCl and 0.1N NaOH. The absorbance of the resulting solutions was measured spectrophotometrically after 24hr.

Incubation time

The optimization of reaction mixture incubation times was required to complete the reaction. The above mentioned procedure was repeated after different incubation periods of 20 min, 24, 48 and 72 hr at a 9 pH value. The absorbance of the resulting solutions was measured spectrophotometrically.

FTIR spectroscopy

FTIR spectroscopy is useful for probing the chemical composition of the AgNP surface and the capping agents on the nanoparticles. A drop of sample is directly placed on the ZnSe crystal with a medicine dropper. A 9d spectrometer then records the IR spectrum of the sample. AgNPs were evaluated for their fluorescence characteristics with a Perkin-Elmer LS 50B luminescence spectrophotometer (Spectrum one, Perkin Elmer, Germany). Spectra over the wavelength range of 4000-650cm-^{1} were recorded (Honary et al., 2013).

TEM

The size and size distribution of AgNPs in an aqueous solution were measured using TEM. A drop of synthesized AgNPs was placed on a carbon coated copper grid and loaded onto a specimen holder. TEM micrographs of the sample were taken using the JEOL JSM 100cx. The transmission electron microscope was operated at an accelerating voltage of 80.0kV. TEM provided further insight into the morphology and particle size distribution profile of the AgNPs and revealed a pattern similar to that described of pervious reports of synthesized AgNPs.

SEM

A scanning electron microscope (JOEL,

Japan, Model-6360) was employed to image the sample surface via scanning with a high energy electron beam. This scanning was undertaken to identify the sizes and shapes of AgNPs that were biosynthesized using honey. The colloidal solution containing AgNPs was centrifuged at 6000rpm for 10min and the pellet was mixed and suspended in a small amount of sterilized double distilled water. A small amount of the suspension was sprayed on a glass slide, creating a thin film that was placed carefully on a glass cover slip followed by air-drying. The sample was then coated with gold using a coater. The sizes of the AgNPs are indicated in the images.

Assessing the antibacterial activity of synthesized AgNPs

Agar well diffusion method

The biosynthesized AgNPs were tested for their antibacterial activity against eight pathogenic strains of Gram-negative and Gram-positive bacteria by the well diffusion method (Ibrahim, 2015). Mueller-Hinton agar plates were swabbed with 100µl of each tested strain using a sterile cotton swab. Wells approximately 5mm in diameter were produced with the help of a sterile gel puncture. Using a micropipette, synthesized AgNPs at 5mg/ml were loaded at concentrations of 25, 50, 75, 100 and 125µl/well. A control sample (BSH) was used to detect the antimicrobial activity of the natural honey. The plates were incubated at 37°C for 24hr. The antibacterial activity was expressed as the diameter of the inhibition zone, as measured in millimetres. The assays were implemented in triplicate. The mean values for the inhibition zones were detected. The inhibition zones were measured and individually compared with those of the BSH, $AgNO_3$ solution and AgNPs.

Determination of minimum inhibitory concentrations (MICs)

The MIC of the synthesized nanoparticles was tested using a standard microdilution method (Sarker et al., 2007). MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism after overnight incubation (Qi et al., 2004). MICs were determined by monitoring the growth of the bacteria in a microplate reader at 600nm. Serial two-fold dilutions of AgNP solutions were prepared in sterile 96-well plates. One hundred microlitres (100µl) of the nutrient broth was added to each well with the exception of row one, which was the positive control. Then, 100µl of the nanoparticles were added to each well of the first row (row 1), starting with the positive control, followed by the negative control. Fifty microlitres of freshly prepared bacterial suspension were mixed with the prepared extract dilutions, and they were incubated at 37°C for 24hr. All experiments were performed in triplicate. Bacterial growth was measured as the increase in absorbance at 600nm using a spectrophotometer (Williams et al., 2006).

Statistical analysis

All assays were performed in triplicate, and the experiments were repeated at least three times. The results are presented as the means±SD. All experimental data were compared using Student's t-test. A p value of less than 0.05 was considered statistically significant (D Steel & Torrie, 1986).

Results and Discussion

Honey mediated biosynthesis of AgNPs

An aqueous solution (0.1M) of $AgNO_3$ was prepared and used to synthesize AgNPs. Five millilitres of honey were added to 95ml of 0.1M aqueous $AgNO_3$ and stirred well for 1min to initiate the reduction into Ag+ ions. The intensity of the colour increased with increasing time as showed in Fig. 1. The colour of the resulting samples changed rapidly from amber to dark brown after 20min indicating the formation of AgNPs due to the reduction of silver metal ions from Ag+ into AgNPs (Philip, 2010).

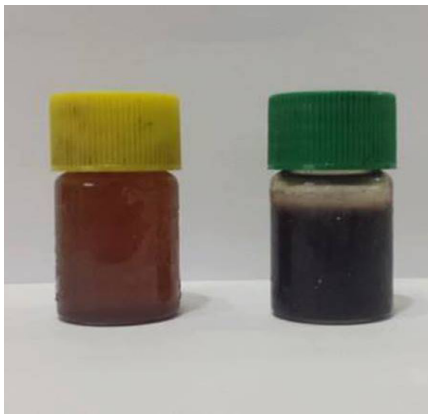


Fig. 1. Colour intensity, Left: Natural BSH; right: Synthesized AgNPs after 20 min.

Characterization of biologically synthesized AgNPs

UV-visible spectroscopic analysis

Metallic nanoparticles display characteristic

optical absorption spectra known as surface plasmon resonance (SPR) in the UV-visible region. This effect becomes influential when the wavelength of the valence electrons is equal to or less than the size of the particle,(Ahmad & Sharma, 2012).

The two parameters that were optimized were the pH and the reaction incubation time, which were identified as the factors that affected AgNP yields. The first factor under consideration was the pH of the reaction mixture. Large nanoparticles formed at a lower pH (pH 5) that suppresses the formation of AgNPs (Fig. 2 a); this phenomenon may be attributable to the instability of the nanoparticles at an acidic pH (Sadowski et al., 2008). The UV-visible spectra showed a distinct maximum absorbance at 400nm at pH 9, whereas small and highly dispersed nanoparticles were detected. A similar result was observed in previous study, which showed that highly monodispersed nanoparticles were obtained at a pH of 10 (Ndikau et al., 2017). We next investigated the reaction incubation time, and the UV-visible spectrum of AgNPs was recorded (Fig. 2 b). The rate of silver ion reduction was slow during the first 20min, and then the intensity of the SPR peak showed a gradual increase until reaching its maximum absorption with a sharper peak at 400nm after 72hr. The time required for a complete reduction in metal ions during the biosynthesis of metal nanoparticles using bacteria and fungi ranges from 24 to 124hr (Korbekandi et al., 2009). The sharpness of the absorbance peak depends on the size of the synthesized nanoparticle (Philip, 2010). The broadening of the peak at that wavelength is attributed to the formation of poly dispersed AgNPs, (Ponarulselvam et al., 2012).

FTIR analysis

To identify the compounds responsible for reducing silver ions, the functional groups present in AgNPs were investigated by FTIR analysis. The FTIR analysis results for this study showed different stretches of bonds over a wave length range from 4000-650cm^{-1} at different peaks; 3318cm^{-1} and 1636cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, respectively. The band at 2119 cm^{-1} was characteristic of carbonyl group C=O - stretching, and the band at 1364cm^{-1} corresponds to C-N stretching (aliphatic amines) vibrations (Fig. 3). The results obtained by FTIR spectroscopy suggest that honey has the ability to reduce and stabilize the AgNPs (Sheela et al., 2010 and Philip, 2009, 2010). Natural biological components are known to interact with metal salts via these functional groups and to mediate their reduction to nanoparticles (Sheela et al., 2010).

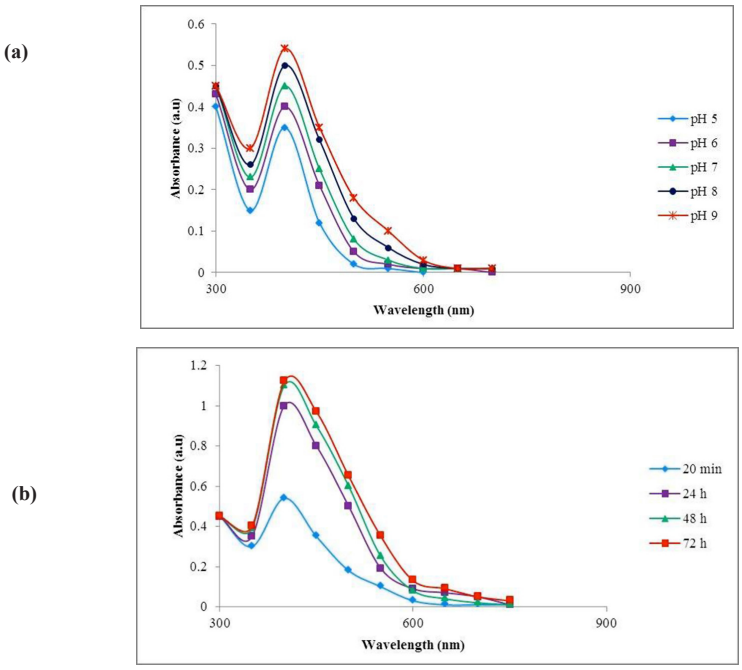


Fig. 2. UV-visible absorption spectra of synthesized AgNPs at different pH values (a) and at different incubation periods for the reaction mixture (b).

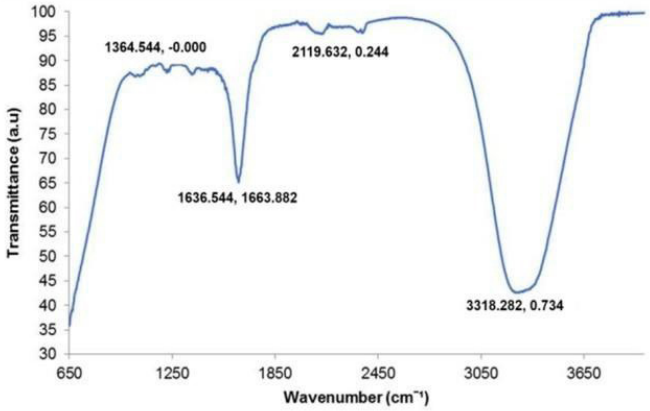


Fig. 3. FTIR spectra of the biosynthesized AgNPs over the wavelength range from 4000-650cm^{-1}.

Morphological characterization via TEM

TEM is one of the best-adapted techniques for studying the sizes, shapes, and distribution of nanoparticles (Bar et al., 2009). TEM micrographs revealed that the AgNPs were well dispersed and predominantly spherical in shape, while some were irregularly shaped. The AgNPs were widely separated over a range from 5-22nm (Fig. 4 a), and agglomerated AgNPs were also observed in some places, thereby indicating their possible sedimentation at a later time over a range of 18-70nm (Fig. 4 b). Similar results have been reported for spherical-shaped AgNPs with an average diameter ranging between 20 and 34nm (Jyoti et al., 2016; Kamel et al., 2016; Ndikau et al., 2017). Interestingly, our data suggest that using honey produces smaller AgNPs, which exhibit better antimicrobial and anticancer activities.

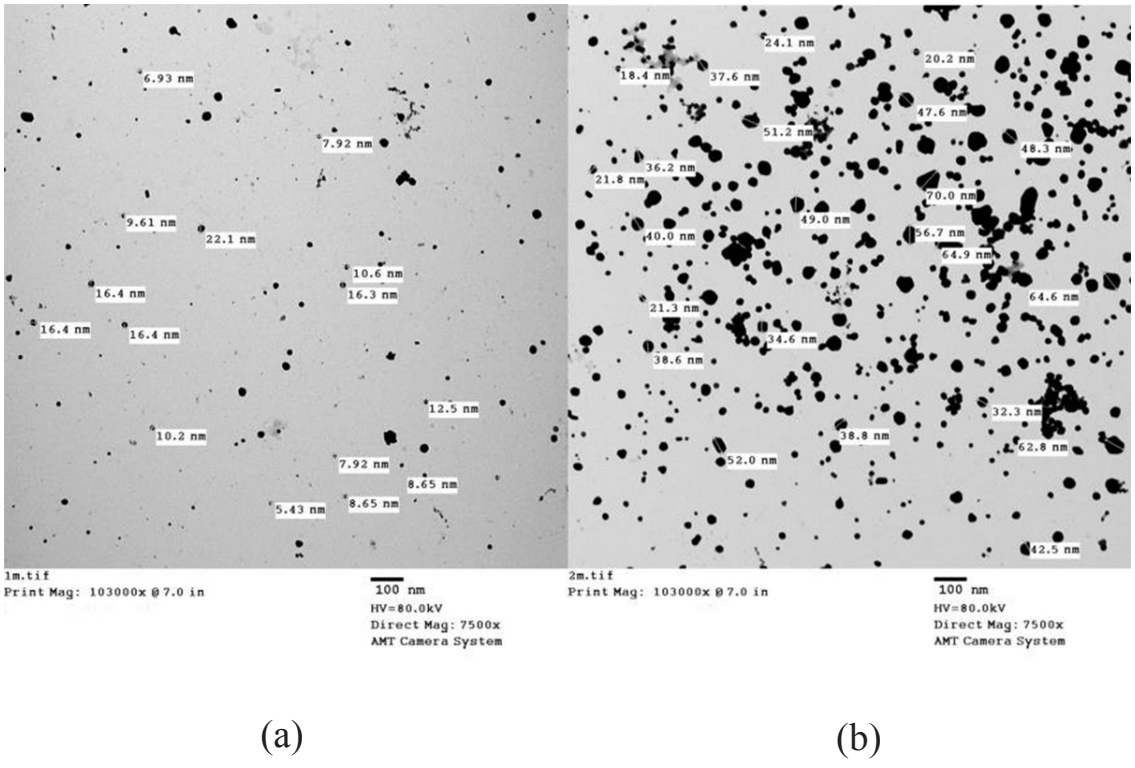


Fig.4. TEM micrographs of AgNPs showing spherical shapes over range 5-22nm (a) and agglomerated structures at 18-70nm (b).

SEM

SEM was employed to analyse the structure of the nanoparticles that formed. According to scanning electron micrographs, the morphology of synthesized AgNPs consisted of spherical nanoparticles with some nanoclusters. The larger silver particles may be attributable to the aggregation of smaller particles, indicating stabilization of the nanoparticles by a capping agent (Priya et al., 2011). The particle sizes obtained here range from 70nm to 163nm (Fig. 5 a) and between 26nm and 91nm (Fig. 5 b). These results suggested that AgNPs are synthesized due to the actions of the honey, which acts as a good capping and reducing agent for nanoparticles biosynthesis.

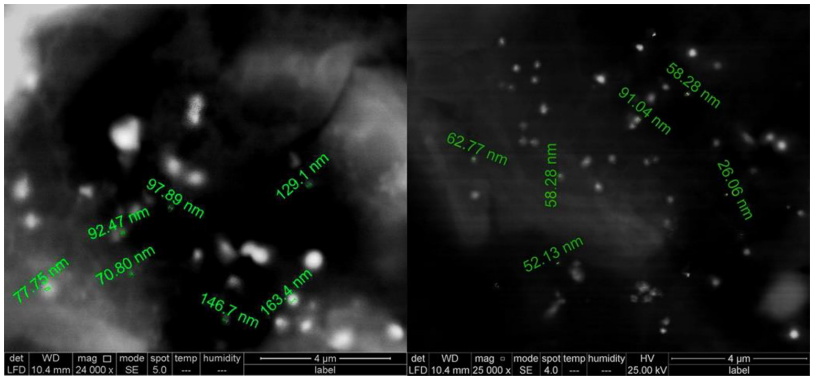
Antibacterial activity of the synthesized AgNPs

Agar well diffusion method

The results revealed that AgNPs were potentially effective with variable potency in suppressing microbial growth of the pathogenic bacterial strains (Figs. 6, 7). *E. coli* and *P. aeruginosa* were the most susceptible strains to AgNPs with larger inhibition zones of 29.33 and 25.00mm at 125µl, respectively, followed by *K. pneumonia*e with 23.33mm zone of inhibition at 100µl (Fig. 6). The most susceptible Gram-positive bacteria was *Staphylococcus aureus* with inhibition zone 20.00mm followed by 19.00 and 18.20mm for *Streptococcus mutans* and *Bacillus subtilis,* respectively. Natural BSH (as the control) showed moderate antibacterial activity compared with the nanoparticles. The combination of BSH with $AgNO_3$ to form AgNPs enhances its antimicrobial activity (Fig. 6). The results revealed larger inhibition zones for Gram-negative than for the other tested Gram-positive bacteria of both natural honey and nanoparticles. This result could be attributed to differences in bacterial cell walls, Gram-negative have thinner cell walls in comparison to Gram-positive bacteria (Shrivastava et al., 2007; Rai et al., 2009). Several studies have explained the bactericidal effects of AgNPs; they may attach to the negatively charged cell surface, alter the physical and chemical properties of cell membranes and the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration (Sondi & Salopek-Sondi, 2004; Kvitek et al., 2008; Nel et al., 2009; Su et al., 2009; Marambio-Jones & Hoek, 2010). AgNPs generate free radicals, which damage the bacterial cell membrane (Sondi & Salopek-Sondi, 2004; Mirzajani et al., 2011). It is also possible that AgNPs not only interact with the membrane surface but also penetrate bacteria (Morones et al., 2005).

Determination of minimum inhibitory concentrations (MICs) of the most susceptible strains

The MICs of the AgNPs against the most susceptible pathogenic bacteria (*E. coli, P.aeruginosa* and *K. pneumoniae)* were shown in Fig. 8. The lowest MIC was detected at 6.3µg/ml for *E. coli* and *P. aeruginosa*, respectively, while *K. pneumonia* was less sensitive and its MIC reached to 12.5µg/ml. The results of MICs suggested that AgNPs can be used to control the pathogenic bacterial growth. These results are in accordance with those of (Erjaee et al., 2017) who proved that 7.8µg/ml was the lowest MIC for *E. coli*. Our results are in contrast with some studies reporting negligible inhibitory effect of silver nanoparticles on *E. coli* up to 1mg/ml (Sondi & Salopek-Sondi, 2004; PanáčeK et al., 2006). Additionally, (Ping et al., 2005) reported the MIC of silver nanoparticles appeared to be 40µg/ml.



(a) (b)

Fig. 5. SEM image (micrograph) of the AgNP structures over the ranges of 70-163nm (a) and 26-91nm (b).

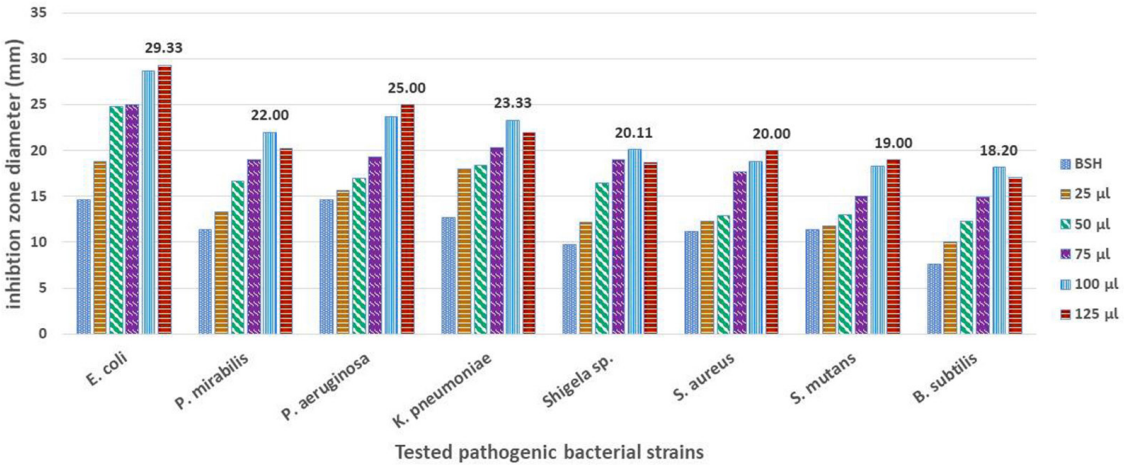
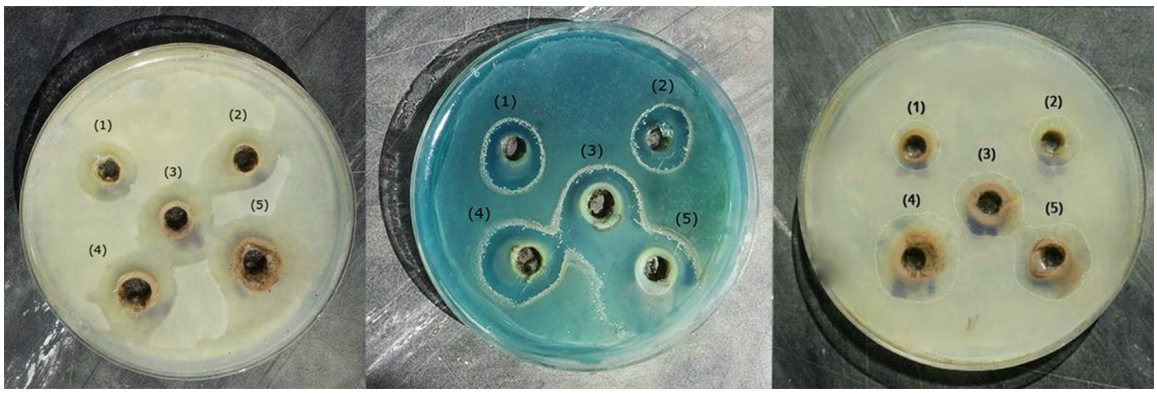


Fig. 6. Antibacterial activity of the synthesized AgNPs against various pathogenic bacterial strains by well diffusion method.



(a) (b) (c)

Fig. 7. Inhibition zones of synthesized AgNPs against (a) *E. coli,* **(b)** *P. aeruginosa* **and (c)** ***K. pneumoniae*** **at concentrations of (1) 25, (2) 50, (3) 75, (4) 100 and (5) 125µl/ml.**

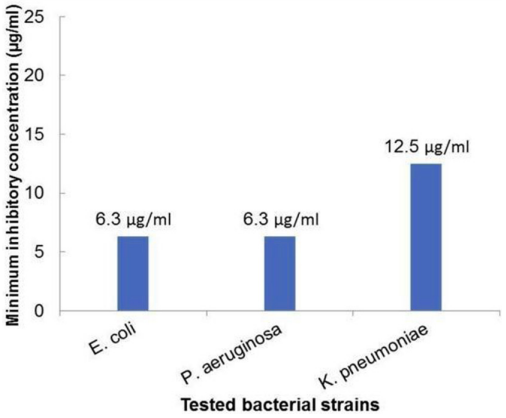


Fig. 8. MICs of the synthesized AgNPs against the most susceptible organisms.

The highest antibacterial activity was investigated by testing AgNPs against bacterial strains and comparing the results for silver salt and natural BSH as illustrated in Fig. 9. The combination of natural BSH with silver nitrate to prepare AgNPs enhances the bactericidal effects. Antibacterial activity against *E. coli, Staphylococcus aureus*, *Streptococcus mutans, Proteus mirabilis, Pseudomonas aeruginosa,* and *K. pneumonia* increased by 2.0-, 1.8-, 1.7-, 1.8-, 1.8- and 1.7-fold, respectively. The highest antibacterial activity of AgNPs was attributed to their extremely large surface area, which provides better contact with microorganisms (Ibrahim, 2015). Moreover, AgNPs have the ability to generate more reactive ionic silver with small

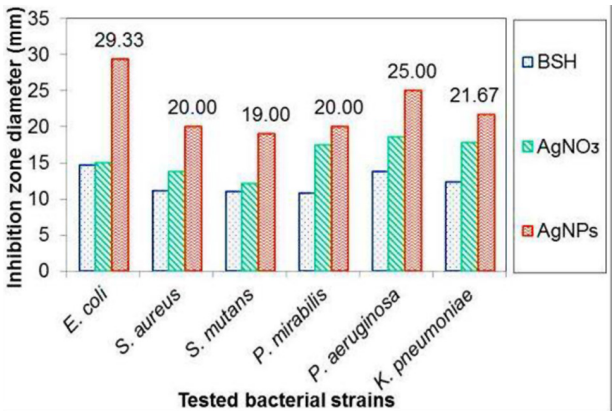


Fig. 9. Comparative antibacterial activity of BSH, $AgNO_3$ and AgNPs against the tested pathogenic bacterial strains.

surfaces (Wijnhoven et al., 2009).

Conclusion

The present study showed a strong, simple and pollutant free eco-friendly approach for synthesizing AgNPs. A local, natural, low cost biological reducing agent, BSH, was applied in an aqueous medium containing 0.1M $AgNO_3$, employing a green nano-chemistry methodology. The UV-visible spectra confirmed the reduction of the silver ions at 400nm after incubation for 72hr at 9 pH value . The sizes and morphologies of the particles were characterized using SEM and TEM images. The synthesized AgNPs showed more efficient antibacterial activities against the tested Gram-negative than that of Gram-positive bacterial strains. These nanoparticles are effective growth inhibitors against various microorganisms, and thus, they are applicable to diverse medical devices and antimicrobial control systems.

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التصنيع الحيوى للجسيمات النانوية الفضية الصديقة للبيئة من عسل النحل المصري: تقييم أنشطتها المضادة للبكتيريا

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يهدف هذا البحث إلى التصنيع الحيوى لجزئيات الفضة النانوية (AgNPs) الصديقة للبيئة من عسل النحل كمنتج طبيعيا مستخرج محليا من الحبة السوداء. ولقد أشارت النتائج الحصول على جسيمات نانوية ذات أحجام مختلفة بعد 20 دقيقة من إضافة محلول نترات الفضة بتركيز M0.1، وتحول المحلول إلى اللون البنى الغامق تدريجيا مع مرور الوقت دليل على إختزال ايونات نترات الفضة. وتبين من خلال الدراسة أن هذه الجسيمات لها فاعلية عالية كنشاط مضاد للبكتيريا تجاه ستة سلالات بكتيرية مسببة للأمراض أربع منهم سالبة الجرام واثنين موجبة الجرام حيث اظهرت جسيمات الفضة النانوية المصنعة حيويا تأثيرا فعالاً ضد السلالات الجرام السالبة أكثر من إيجابية الجرام. ولقد اهتم البحث بدراسة بعض الخصائص النانوية لتحديد تقنية الجسيمات الفضية النانوية بإجراء التحاليل الطيفية للأشعة فوق البنفسجية، والتحليل الطيفي بالأشعة تحت الحمراء (FTIR)، والمجهر الإلكتروني النافذ (TEM)، والمجهر الإلكتروني الماسح (SEM). وبإستخدام المجهرالاكترونى النافذ TEM تم الحصول على اشكال كروية من جزيئات الفضية النانوية حيث تراوحت أحجامها من 70-25 نانومتر. وتشير النتائج إلى استعمال الجسيمات النانوية الفضية المخلقة حيويا من عسل النحل هو الحل الأمثل لإيقاف انتقال الأمراض المعدية وذلك يعود إلى الصفات الضد ميكروبية لهذه الأجسام بيحث يمكن استخدامها فى العديد من المجلات الطبية.