Production of Ag nanoparticles Using *Aloe vera* Extract and its Antimicrobial Activity

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Abstract

The study was carried out to explain the role of *Aloe vera* extract as a reducing agent for the production of Ag nanoparticles. The UV-VIS spectrophotometer showed shift peak at 400nm and scanning electron microscope (SEM) showed the rectangular morphology of as prepared Ag nanoparticles with a size of (500) nm. These nanoparticles gave significant effect on Gram negative bacteria *E.coil* and Gram positive bacteria S.*aureus* at concentration 3.5 mg\ml, but it did not have any antifungal effect on *Candida albican, Pencillium spp* and *Aspergillas niger.*

Keywords: Nanoparticles, Biosynthesis of Ag NPs, *Aloe vera,* Antimicrobial activity.

Introduction

The field of nanotechnology is one of the most active areas of research in modern material sciences. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology [1]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanoparticles of noble metals, such as gold, silver, platinum, and zinc oxide are widely applied in products that directly come in contact with the human body, such as detergent, cosmetic products, and toothpaste, besides medical and pharmaceutical applications. Nanoparticle formation has been reported using chemical and physical methods. There are various methods for NPs formation such as sol-gel process, chemical precipitation, hydrothermal method, microwave, chemical vapour deposition [2,3], The above methods involve the usage of hazardous reagents for synthesis of nanoparticles. In view of an environmental sustenance, there is an urgent need to develop an ecofriendly method of synthesis of nanomaterials Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods [4, 5]. Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The use of materials like plant extract [6] bacteria, fungi [7] and enzymes [8] for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria Silver nanoparticles have unique catalytic, optical, electrical and antimicrobial properties [9], which inhibiting 650 types of microbe's growth as well as because of its reputation of limited toxicity to humans [9]. Various methods are available for synthesis of silver nanoparticles, which sign thereto above. various plant extracts such as Cinnamon camphora, Cinnamon zeylanicum, Geranium, Neem leaf broth, Lemongrass extract, Tamarind leaf extract and Acalyphaindica .In this study we report a simple, effective, low cost and environmental safe synthesis of silver and other metal nanoparticles using phyllostachy ssp leaves extract and measurement its therapy effect of it, were

characterized by UV-VIS spectroscopy and scanning electron microscopy (SEM).

Materials and Methods

Preparation of Plant Extract (Aqueous Extract)

Aqueous extracts of *A. vera* were prepared according to (10). Thirty gram portion of thoroughly washed *A. vera* leaves were finely cut and boiled in 100 mL of sterile distilled water. The resulting extracts were used for further experiments.

Biosynthesis of Sliver nanoparticles [10]

The synthesis of silver nanoparticles, 2.5 mL of 30% ammonia solution was added to 5 mL of 10^{-2} M $AgNO_3$ solution followed by addition of 5 mL of the *A. vera* extract. The concentration of $AgNO_3$ was adjusted to 10^{-3} M by making up the final volume to 50 mL with water. The observation of faint yellow color after 24 h of reaction indicated the formation of silver nanoparticles, which was further characterized by UV\VIS absorbance and SEM measurements.

Characterization of nanoparticles

UV–visible Spectroscopy (11)

Excellent technique for measuring nanoparticles concentration in pure nanoparticles suspensions with relatively low detection limits (g/L range). contains information on size, aggregation and surface chemistry since the peak shifts in response to change in these parameters. We used this technique to measurement the peak shifts of sliver and zinc oxide nanoparticles, the measured begin with 300 t0 750 nm.

Atomic Absorption Spectroscopy

The selection of the atomic absorption spectroscopy model depends on the concentration of silver and ZnO in the sample. For extremely low concentrations, the graphite furnace is used. We used this technique to measure the concentration of sliver and zinc nanoparticles measured with (ppm) unit.

Scanning Electron Microscopy (SEM) (11)

Scanning Electron Microscopy (SEM) offer nanometer resolution for measuring nanoparticle size. We used this technique used to measure the size of sliver and zinc oxide nanoparticles. The plant extract biomass after reaction spontaneously precipitated at the bottom of the tubes. After the precipitation, the suspension above the precipitate was sampled for SEM observation. SEM samples of the aqueous suspension of silver nanoparticles were fabricated by dropping the suspension onto clean electric Stubs and allowing water to completely evaporate.

Antimicrobial activity

Testing for antibacterial activity

The cup-plate agar diffusion method was used [12] to assess the antibacterial activity of the prepared *A. vera* extracts. aliquot of 0.6 ml of standardized bacterial stock suspensions of 10^5-10^6 colony- forming units per ml was thoroughly mixed with 60 ml of sterile nutrient agar. Then 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates, 1 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 0.05ml of each extract and nanoparticles green synthetic of Ag nanoparticles with concentration 3.5 mg\ml and 500 nm in size) using micropipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the growth inhibition zones were measured.

Testing anti-fungal activity

The same method as for bacteria was followed. Instead of nutrient agar media, Potato extract agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for *Aspergillus niger* and *Pencillium spp.*

Aloe vera extract

Water extract

Results showed that the weight of the residue obtained after evaporation of water was 1.5 g, the appearance of the extract was brawn color.

Biosynthesis of sliver Nanoparticles

Results showed that the Silver nanoparticles were synthesized using the *A. vera* extract from silver nitrate. The reaction proceeded only in the Presence of ammonia, which facilitates the formation of soluble silver complex [Ag $(NH_3)_2$)] which then facilitates the reduction. The reaction mixture turned pale yellow after 24h of reaction and exhibited an absorbance peak at 400nm (Fig. (3-2)) which is a characteristic of silver nanoparticles due to its surface Plasmon absorbance. SEM analysis revealed that the silver nanoparticles were predominantly spherical (Fig. (1A, B)), and the average size of the spherical silver nanoparticles was estimated to be 500nm. These results are in agreement with [10] in other study Ag nanoparticles without adding ammonia [13]. A plenty of Ag^+ were added into the extract of *Aloe vera* which contains many active small and large molecules. On the other hand, the biological molecules might act as reducer to reduce Ag^+ to Ag NPs; On the other hand, they were also responsible for the stabilization of resulting nanoparticles [13].

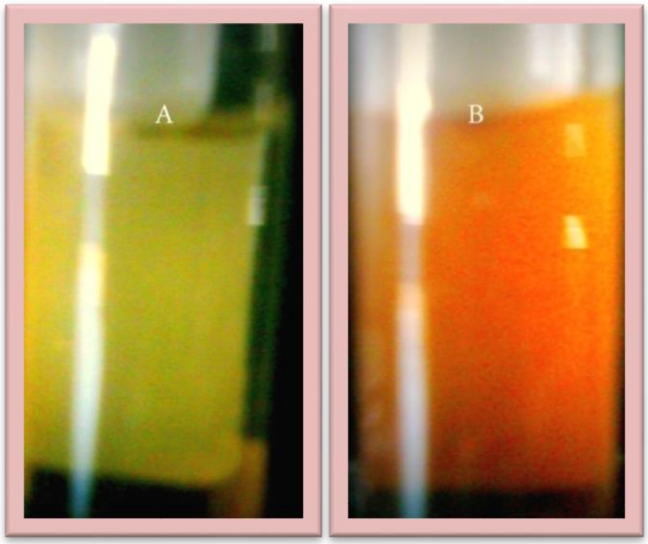

Fig. (1) A: A. vera extract before add $AgNo_3$and ammonia.
B: A. vera extract with $AgNO_3$& NH_4 after 24hrs.

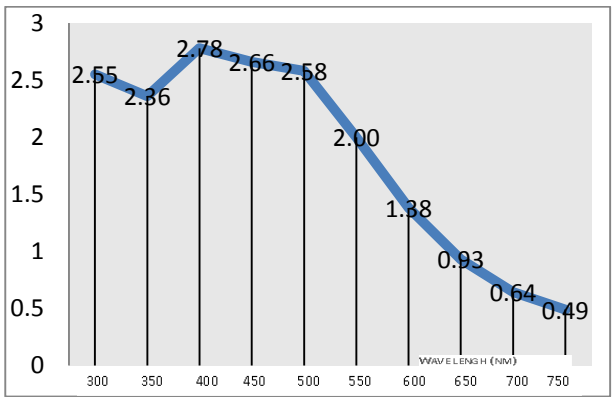

Fig. (2) VIS absorption spectra of Ag nanoparticles in A. vera solution, after 24hs at 25 C^0.

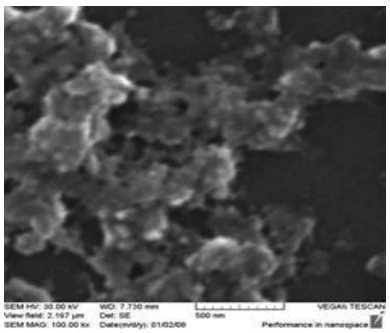

Fig. (3-3-A)

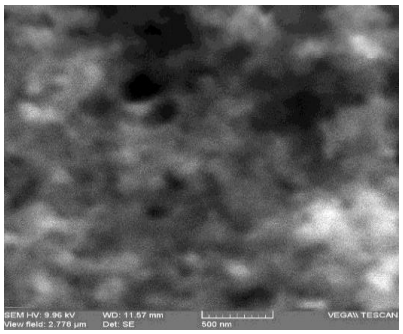

Fig. (3-3-B)
Fig. (3A, B) SEM sections of the green synthetic of Ag Nps with size(500 nm).after 24hs at 25C^0.

In plants the probability of reduction of $AgNO_3$ to silver may be illustrated due to the mechanism known as glycolysis. Plants fix CO2 in presence of sunlight. Carbohydrates are the first cellular constituent formed by the photosynthesizing organism on absorption of light. This carbohydrate is utilized by the cell as glucose by Glycolysis. This is the metabolic pathway that converts glucose $C_6H_{12}O_6$ into pyruvate and hydrogen ion.

Glycolysis is a definite sequence of ten reactions involving ten intermediate

compounds. Large amount of H^+ ions are produced along with ATP. Nicotinamide adenine dinucleotide, abbreviated NAD+, is a coenzyme found in all living cells. NAD is a strong reducing agent. NAD+ is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is therefore found in two forms in cells. NAD+ is an oxidizing agent—it accepts electrons from other molecules and becomes reduced. This reaction forms NADH, which can donate electrons. These electron transfer reactions are the main function of NAD:

$AgNO_3 \rightarrow Ag^+ + NO_3$,
$NAD^+ + e \rightarrow NAD$,
$NAD + H^+ \rightarrow NADH + e^-$,
$e^- + Ag^+ \rightarrow Ag^0$(3)

NAD+ keeps on getting reoxidised and gets constantly regenerated due to redox reactions. This might have led to transformations of Ag ions to Ag^0. Another mechanism for the reduction of Ag ions to silver could be due to the presence of water-soluble substances like (Aloeemodin, Chrysophonal, and Helminthospor). These substances are contained in fresh *Aloe Vera* extract., may be made as reducing agent and can reduce, and there by neutralized, reactive oxygen species leading to the formation of electron. This free electron reduces the Ag+ ion to Ag^0 [13, 14].

Antimicrobial activity of water extract of *Aloe vera* extract and Ag nanoparticles

Antibacterial activity

Results showed that the water extract of *A. vera* and nanoparticles had different antibacterial effect on Gram negative bacteria (*E.coli*) and Gram positive bacteria (*S.aureus*) as shown in table (1). In this table showed that there is no effect of water extract of *A. vera*. While green synthetic Ag nanoparticles (3.5mg\ml) had effect on (*E.coli & S.aureus*) with Inhibition (20-30) mm successively. These results showed in Fig.(4).

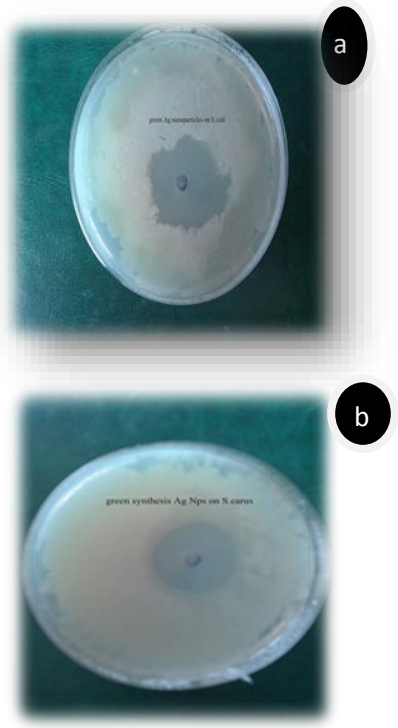

Fig. (4) The effect of green synthesis mixture Nps , with concentration (3.5)mg\ml after incubation at 37c^0 for 24 hrs.
(a) The effect of Green synthesis of Ag Nps on S.aureus.
(b) The effect of Green synthesis of Ag Nps on E.coli.

The plant extract showed no effect on tested bacteria and these result agreed with [15]. While the green synthesis of Ag nanoparticles and other nanoparticles showed significant effect on the tested bacteria, This effect may due to the interaction with the cell wall of bacteria lead to the formation of pores in these walls. Accumulation of the Ag nanoparticles in the pits caused the permeability of the cell membrane suggestion is agreed with [16,13]. Other reason caused the death of the bacterial cells may be is the effect of nanoparticles on the proteins in the cytoplasm of the cells which lead to reregulation in the functional cells, also the nanoparticles such as (Ag Nps) can effect on the DNA replication which will disrupt the replication mechanism ,this suggestion is agreed with [17].

Table (1)
Effect of Aloe vera and nanoparticles on E.coli and S.earus s with concentration (3.5)mg\ml after incubation at 37C^0 for 24hrs.

Sample	*Concentration (mg\ml)*	*Ecoli Inhibition Zone (mm)*	*S.aureus Inhibition Zone (mm)*
Green synthesis Ag Nps extract	**3.5**	**30**	**20**

Antifungal activity of *Aloe vera* water extract and nanoparticles

The results showed that the water extract of *Aloe vera* and nanoparticles had different effect on two fungi Genus *(pencillium spp, Aspergillus niger*) and one yeast *(Candida albican*) as in Table (2). there were no effect of (extract with concentration (3.5*mg*\ml), Ag nanoparticles with concentration (3.5) *mg*/ml and green synthesis extract of Ag Nps with concentration (3.5mg\ml) on the three fungi species as in Fig.(3-5). The no activity results of water extract of *Aloe Vera* on tested fungi are agreed with [15]. Results of other studies revealed green synthesis of Ag nanoparticles and, in other studies showed that some Ag nanoparticles which was green synthesis also have no effect on the tested fungi [18].size and shape of nanoparticles are the most important factors that affect, the effect of Ag nanoparticles on fungi [19].

Table (2)
Effect of Aloe vera and nanoparticles on Candida albican and Aspergillus niger and Pencillium spp. with concentration (3.5mg\ml) after incubation at 28 C0 for 3 days.

Sample	*Concentration mg\ml*	*Candida albican*	*Aspergillus niger Inhibition Zone (mm)*	*Pencillium spp Inhibition Zone (mm)*
Green synthesis of Ag Nps	**3.5**	**-**	**-**	**-**

Conclusions

The biosynthesis method represent an easily, eco-friendly and low-cost process to prepare NPs by reduction of silver nitrate solution with *Aloe vera* extract at room temperature. Using UV-VIS spectrophotometer and scanning electron microscope (SEM) for the identification of biosynthetic Ag nanoparticles and and using atomic absorption spectrophotometer (ASS) to measure concentractions of nanoparticles.

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الخلاصة

الدؤاسة نفذت لغرض توضيح دور مستخلص نبات الصبار Aloe vera كعامل مختزل لأنتاج دقائق الفضة النانوية بأستخدام مطياف الاشعة الضوئي/ فوق البنفسجية لوحظ التغير في (٤٠٠) نانومتر، وبأستخدام جهاز المجهر الألكتروني الماسح SEM تم قياس حجم دقائق الفضة النانوية وكانت بحجم (٥٠٠) نانومتر. وقد تمت دراسة الفعالية الميكروبية لهذه الدقائق وقد تبين أن لقائق النانوية فعالية ضد بكترية ضد كل من بكترية السلبة لصبغة غرام E.coli والبكتريا الموجبة لصبغة غرام S.aureus بتركيز ٣,٥ ملغم/ مل. ولكن لم يكن لها أي تأثير على الفطريات المستخدمة في الدراسة Aspergillas niger, Candida albican and Pencillium spp.