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Characterization and Biological Effect of Silver Nanoparticles Synthesized by *Zingiber officinale* Aqueous Extract.

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ABSTRACT

Aqueous extract of ginger (AEG) has been used as green and eco-friendly technique in reduction silver nitrate (AgNO3) in order to synthesis Silver nanoparticles, it is believed that using biologically method contribute to avoid using chemicals so it will lead to protect environment from hazard pollution. Otherwise, green synthesized Silver nanoparticles (AgNPs) activity investigated as anti-biofilm formation of three different bacterial cultures (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) and studying ability of silver particles in curing plasmids of these bacteria. Ginger rhizomes were cleaned, dried and powdered, (10g) of the powder extracted with 100 ml of distilled water, then silver nanoparticles prepared by reduction of 1Mm of 45ml silver nitrate with 5ml of ginger extract. UV spectrophotometer and Atomic force microscope (AFM) have been used for characterization these particles; biological activity of green synthesized silver nanoparticles was investigated by detection anti-biofilm efficiency and its ability for curing plasmids of (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*). UV absorption was recorded 410 nm while AFM images showed that silver nanoparticles has average diameter about (83 nm) with no. of grain (685). Otherwise, silver nanoparticles inhibited biofilm formation ability of (*E. coli*, *K. pneumoniae* and *Staph. aureus*) as well as they have an activity in curing plasmid that may be carry pathogenic genes or antibiotic resistance genes. Investigating these important pathogenicity parameters of silver nanoparticles leading to an opinion in reduction the adhesion of bacteria with epithelial and mucosal tissue leading to decrease the chances of infection.

Keywords: *Zingiber Officinale*, AFM, silver nitrate, nanoparticles, plasmid, biofilm

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INTRODUCTION

Recently, there is much focusing on natural products obtained from plants and herbal resources, *Zingiber Officinale* (ginger) consider as an example of phyto-compounds richest resources. Rhizomes of ginger are highly recommended by clinical and food professionals for utilizing it in homemade remedies, spices and using for treatment of health disorders (Morakinyo *et al.,* 2008). Recently, nanotechnology field received highly attention and revolutionary growing in modern technology and sciences (B. edhaya naveena and S. Prakash, 2013; Priyaa and Satyan, 2014). Synthesized of nanoparticles carried out by using different metals such as: copper, gold, magnesium and silver, nanoparticles synthesis procedures including physically and chemically developed and improved by researches but the environmental pollution risks involved during using those procedures by accumulate toxic byproducts and chemical vapor so the researches focusing on biological or green process using plant extracts which are considered as non-toxic and eco-friendly materials (Reddy *et al.,* 2014; Preethi and Padma, 2016). The ability of bacteria of infects human body and pathogenicity is referred to formation biofilm and possesses plasmids (Srivastava *et al.,* 2014). Biological and pharmaceutical applications of silver nanoparticles represent effects against pathogenic bacteria due to wide surface of the particle which provide a great chance for adhere between bacteria and nanoparticle (Jones and Hoek, 2010). This study present biosynthesis of silver nanoparticles by ginger extract in addition to characterization. Biological activity, biofilm and plasmid curing tests of silver nanoparticles against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were performed.

MATERIALS AND METHODS

Preparation of *Zingiber officinale* aqueous extract

Ginger rhizomes were purchased from local market in Baghdad, cleaned with tap water then distilled water, cut in small pieces and left to dried after that grinded by pestle. (10 g) of powder shake with 100 ml of distilled water for 12 hr then infusion filtrated by Whatman filter paper no.1. Filtrates collected, drying by oven at (40^0C) and the final weight of the extract was 22 mg/ml, stored in air-tight bottled in 4^0C until used (Kumari, *et al.,* 2014).

Preparation of silver nanoparticles (AgNo3) solution by using Ginger extract

Biosynthesis of (AgNo3)was carried out by adding 5 ml of *Zingiber officinale* extract to 45 ml of 1 Mm aqueous silver nitrate solution (Sigma-Aldrich, Germany), reduction of silver nitrate by ginger extract performed at room temperature (26 ± 2) (Priyaa and Satyan, 2014).

AgNPs characterization

UV-Vis spectroscopy analysis

Spectrum data analysis investigated by using UV-Vis spectrophotometer as indicator for reduction of silver nitrate. The absorbance was measured between 190 and 1100 nm (Priyaa and Satyan, 2014).

Atomic force microscope (AFM) analysis

The prepared nanoparticles molecules were spreading on the slide then dried and subjected for characterization to detect morphological and structural properties by Atomic force microscope (AFM).

Biological activity

Biofilm assay

The activity of green synthesized silver nanoparticles against biofilm formation ability was investigated compared with activity of ginger extract, silver nitrate and ceftriaxone as (+ve control). 50 µL of bacterial culture (1.0 McFarland) were supplemented in 96-well culture plates along 50 µl of Müeller–Hinton broth media for each well. Silver nanoparticles, silver nitrate and ginger extract then added separately to wells and kept in incubator for 24 h at 37°C with 100 µl of 100µg/mL for each. Medium without treatment was used

as the control while growth control consist of bacteria and broth only (-ve control). Following incubation period, media along with un-adhered cells have been removed and rinsed with 200 µl of Phosphate Buffer Saline (PBS). Then the plate was left for dry then stained with 100 µl of 0.1% Crystal Violet (CV) (Sigma-Aldrich, Germany) after that incubated at 37±2 °C for 15 min then the plates have been rinsed 5 times by using sterile distilled water to descant remaining CV dye.

Presence/ reduction of biofilm biomass were assessed by monitoring the optical density (OD) at 630 nm for each well by ELIZA microplate reader (Nikolić *et al.,* 2014).

Plasmid Curing test

The fresh bacterial culture of (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) were incubated at 37^0C for 24 hr. with two different concentration (1% and 3%) of silver nanoparticles to investigate the ability of curing bacterial plasmid compared with ginger extract and silver nitrate.

Tested bacterial plasmid was obtained by a slight alteration of the Birnboim method (Birnboim and Doly, 1979). The bacteria, cultured for 18 hrs. in Luria Bertani broth medium which contain (Tryptone 1%, Yeast extract pH 7.9) then collected by centrifugation at 10 000g for 1 min after that re-suspended in 100 µl of the lytic solution consist of lysostaphin (Sigma-Aldrich, Germany) 20 mg/L added to 50 mM glucose with 25 mM Tris HC1 and 10 mM EDTA pH 8. Tubes kept in incubator at 37°C for 45 min. Lysis process carried out by adding 200 µl of sodium dodecyl sulphate (BDH, UK)(1%) in 0.2N NaOH(BDH, UK), which incubated at 0°C for 5 min after that added 150 µl of 3M sodium acetate (Sigma-Aldrich, Germany)(PH 4.8). The lysates were re-incubated at 0°C for 60 min then subjected to centrifuge at 10 000g for 7 min.

Cold ethanol (BDH, UK) (1 ml) was used by added to the supernatant , the tubes kept at - 70°C for 30 min. then centrifugation at 10000g for 8min, the precipitate which had the plasmid DNA suspended in 100 µ1 of 0.1sodium acetate, 0.05 M Tris HC1 (BDH, UK) (PH 8). Ethanol (BDH, UK) 200 µl added to the tubes preparation and held at - 70°C for 30 min. The final pellet obtained by centrifugation at 10 000g for 8 min, then suspended in 10 mM Tris HC1 , 1 mM EDTA (BDH, UK) (PH 8). Samples were electrophoresed in system contain agarose gel (Sigma-Aldrich, Germany) 0.75% in TPE (0.08M phosphate Tris (Sigma-Aldrich, Germany), 0.002M EDTA) in horizontal shape gel electrophoresis for 14 h at 20 V at room temperature (GELMI *et al.,* 1987)

RESULTS AND DISCUSSION

AgNPs synthesis by aqueous extract of ginger

Silver ion reduced by *Z. Officinale* can be visual observed in distinct color changed from pale yellow to brown which showed creation silver nanoparticles as shown in Figure (1). Initial changing in color intensity can be observed during first 40 min. of reaction time.

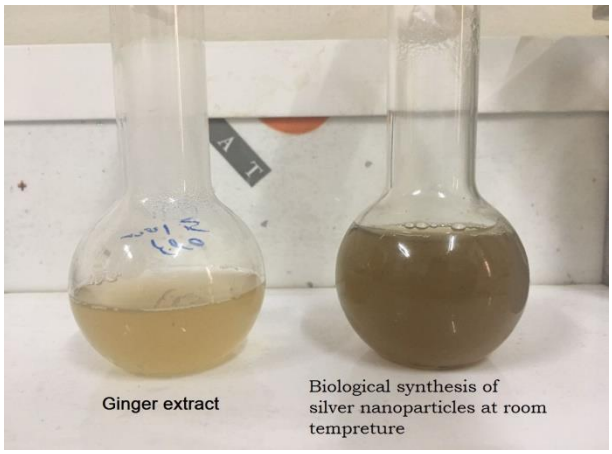

Fig. 1: formation of silver nanoparticles by ginger extract

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AgNPs characterization

UV-Vis spectroscopy analysis

Silver metal was reduction by incubated it with 20% of *Zingiber Officinale* extract, AgNPs seems to be brown color as a signed of reduction silver nitrate (Subha V *et al.,* 2015), the absorption maximum peak of UV-VIS spectrum was around 410 nm , results showed formation of silver nanoparticles by reduction of silver ion to Ag^0 which carried out by using ginger root aqueous extract (Fig 1).

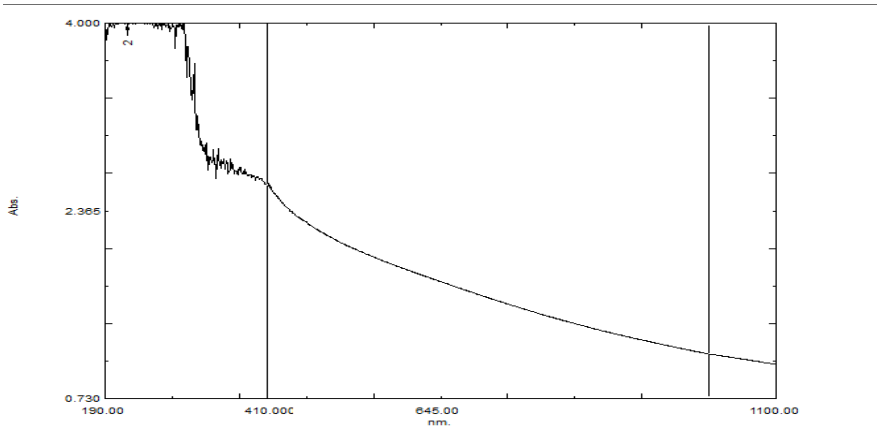
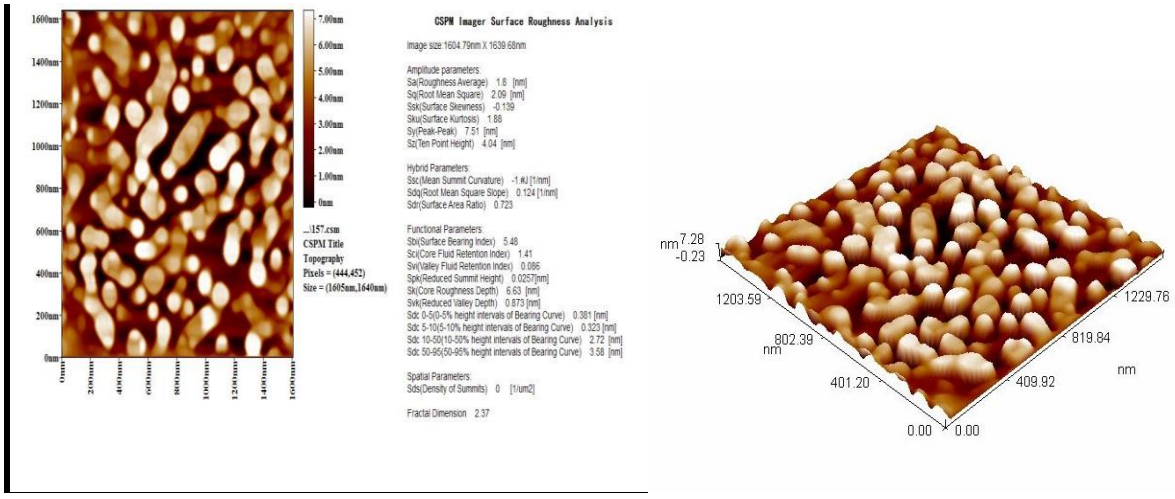


Fig. 2: UV-visible absorption spectrum of silver Nanoparticles (AgNPs) synthesized by reduced silver ion with 10% ginger extract and incubated at room temperature.

Atomic force microscope (AFM)

The AFM of 2D and 3D result images that shown in Fig (3) revealed the morphological and structural characterization of green synthesized (AgNPs) with average diameter (83 nm) , no. of grain (685) and about 90% of granularity accumulation distribution centered at75 nm as shown in Fig (4):-



a- AFM images 2D of $AgNO_3$ particles **b- AFM images 3D of $AgNO_3$ particles**

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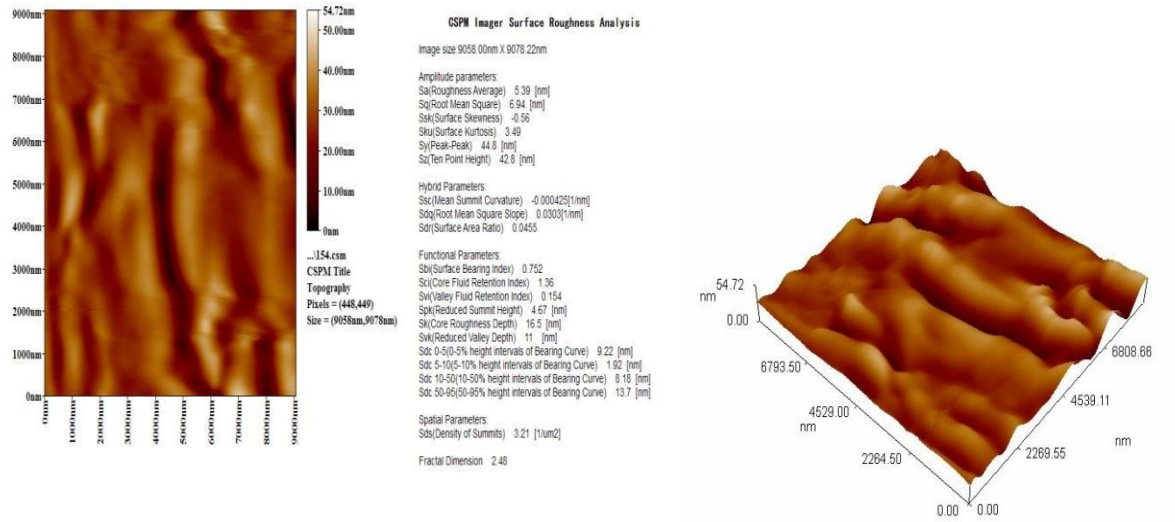

a- AFM images 2D of $AgNO_3$ particles **b- AFM images 3D of $AgNO_3$ particles**

Fig. 3: AFM images of Silver nanoparticles synthesized by Ginger extract /a- 2 D; b- 3D of $AgNO_3$ particles

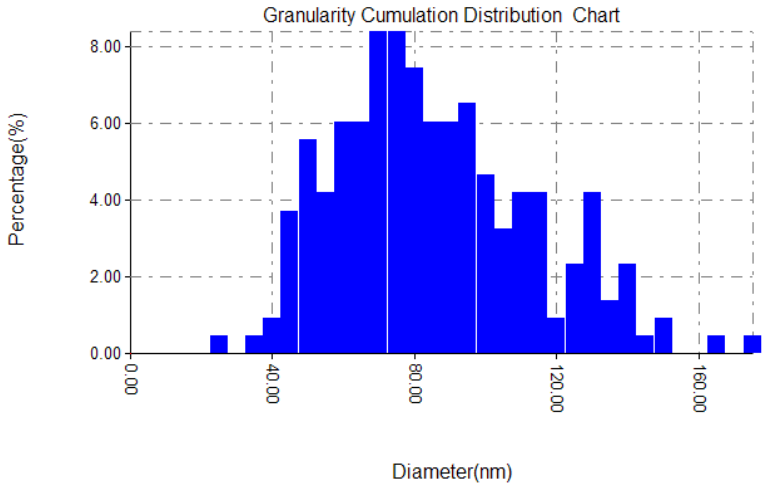

Fig. 4: Percentage of Granularity Distribution of AgNPs diameter

Biological activity

Biofilm assay

Antibiofilm effect of silver nanoparticles was estimated by using crystal Violet stain and reading absorption by ELIZA reader against three bacterial cultures which were (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*), biofilm inhibition activity also estimated for ginger aqueous extract and silver nitrate. Fig(5) revealed the ability of silver nanoparticles in preventing biofilm formation against all bacterial strains used in the current study with nearly identical activity compared to control while ginger extract caused inhibition in *Staph. aureus* only and increasing the biofilm formation of *E. coli* and *K. pneumonia,* studies mentioned that some plant extracts especially which contain phenolic compounds had ability to stimulate formation of biofilm by various bacteria while other reports explained that this phenomena as self-protecting behavior against risky agents in the environment when it presence in a dosage less than lethal dose or may be due to presence some compounds which were enhanced adhesion of the bacteria (Varposhti *et al.,* 2013; Selim *et al.,* 2014) . Silver nitrate treated cells presented no-activity against *E. coli* compared with control but it affected K*. pneumonia* and *Staph. aureus*.

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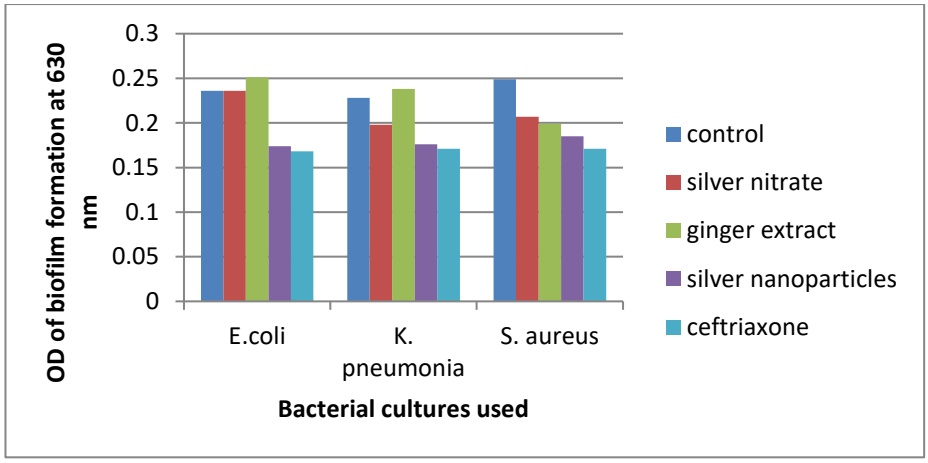

Fig. 5: Anti-biofilm activity of (Silver nanoparticles, $AgNo_3$, and ginger extract) against three different bacterial isolates (*Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) recorded by ELIZA microplate reader at 630 nm.

However, there is no-sample was capable to prevent cell adhesion totally, as well as the positive control, ceftriaxone. The ability of silver nanoparticles in preventing cell attachment as present in current study is a suitable for decreasing bacterial colonization on surfaces and epithelial mucosa which consequently caused infections (Selim *et al.,* 2014).

Plasmid Curing analysis

The results of the current study revealed the ability of bio-synthesized silver nanoparticles to curing plasmids of different bacterial isolates which were *E.coli*, *K. pneumoniae* and *Staph. aureus* in the both concentrations used (1% and 3%), plasmid profile of tested isolates indicated the occurrence of plasmid/s in these microorganisms. Curing analysis of *Zingiber Officinale* extract also revealed efficiency against tested bacteria with both concentrations.

E.coli and *Staph. aureus* showed curing efficiency by Silver nitrate $AgNo_3$ solution at concentration 3% but resistance activity at 1% of $AgNo_3$. Otherwise *K. pneumoniae* plasmid was cured by silver nitrate solution with both concentrations.

There is assumed that microbes have -ve charge while nanoparticles have +ve charge. This formed an "electromagnetic" force between microbe and particle surface. If the communicate is happened, the bacteria start to suffering from oxidation effect and dead rapidly. Furthermore, introducing of metal ions could be interact with nucleic acid and insert between DNA strands. Otherwise, presence of Silver ions inside the microorganism structures can interrupt biochemical processes. The complex mechanism of action may affect different microbial structures and offering benefits compared to drugs with high particular mechanism of action. In general the Goals of nanoparticles are outer and inner microbial cells including cell wall, plasma membrane, proteins and DNA (El Behiry, 2014). Moreover bacterial isolates resistance takes a place by various mechanisms such as efflux pump, release enzymes which inhibit the effect of the medicine and modification the coal of drug. The resistance action can be transferred between microbes of the similar species by give-and-take of genetic materials (Soman *et al.,* 2015). However, based on researches the bacterial isolates became susceptible to antibiotics and drugs due to losing plasmid that's encoding for resistance property (Lara *et al.,* 2010).

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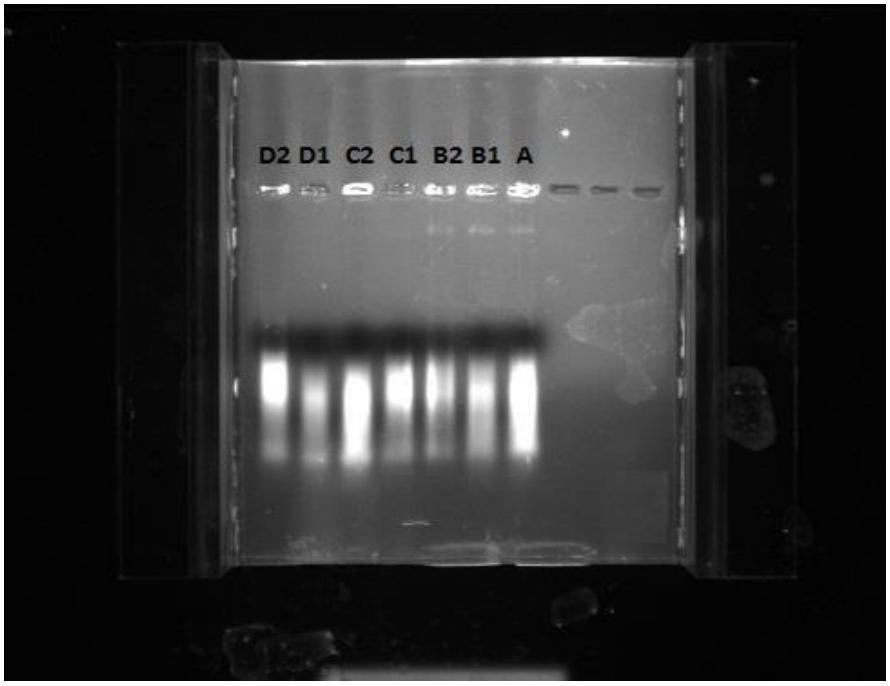

Fig. 6: Gel electrophoresis of *Escherichia coli* plasmid profile as (a) control: *E. coli* without treatment (B1), *E. coli* treated with 1% silver nitrate, (B2) *E. coli* treated with 3% silver nitrate, (C1) *E. coli* treated with 1% ginger extract, (C2) *E. coli* treated with 3% ginger extract, (D1) *E. coli* treated with 1% silver nanoparticles, (D2) *E. coli* treated with 3% silver nanoparticles.

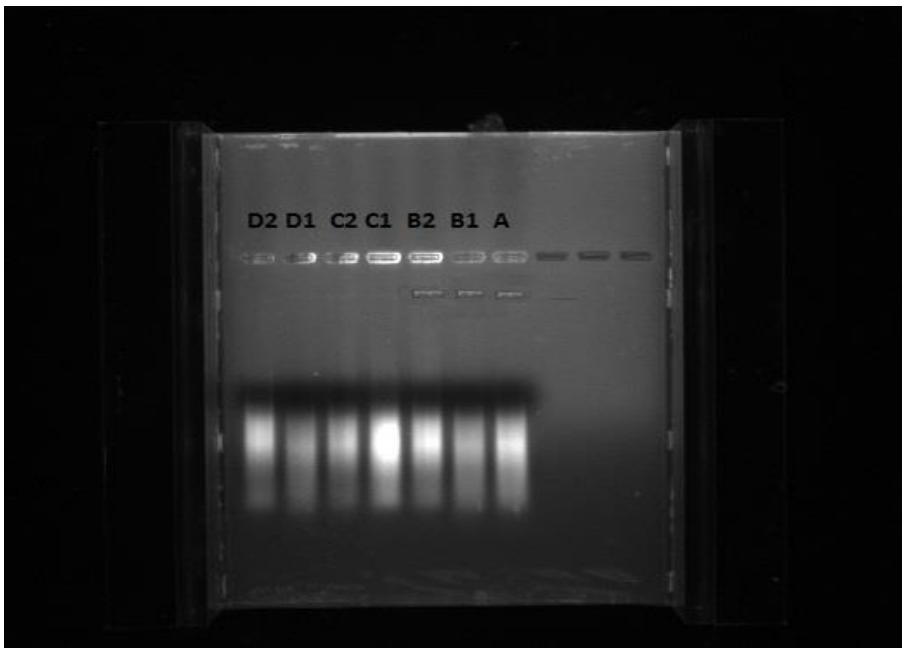

Fig.7: Gel electrophoresis of *Staphylococcus aureus* plasmid profile as (a) control: *Staph. aureus* without treatment (B1), *Staph. aureus* treated with 1% silver nitrate, (B2) *Staph. aureus* treated with 3% silver nitrate, (C1) *Staph. aureus* treated with 1% ginger extract, (C2) *Staph. aureus* treated with 3% ginger extract, (D1) *Staph. aureus* treated with 1% silver nanoparticles, (D2) *Staph. aureus* treated with 3% silver nanoparticles.

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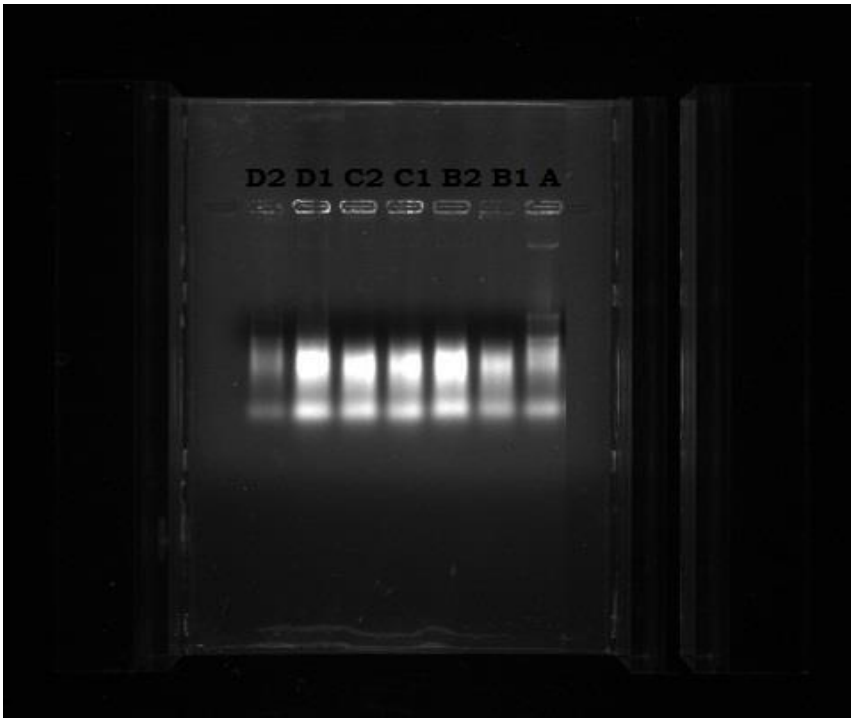

Fig.8: Gel electrophoresis of *Klebsiella pneumoniae* plasmid profile as (a) control: *K. pneumoniae* without treatment (B1), *K. pneumoniae* treated with 1% silver nitrate, (B2) *K. pneumoniae* treated with 3% silver nitrate, (C1) *K. pneumoniae* treated with 1% ginger extract, (C2) *K. pneumoniae* treated with 3% ginger extract, (D1) *K. pneumoniae* treated with 1% silver nanoparticles, (D2) *K. pneumoniae* treated with 3% silver nanoparticles.

However, in spite of microbial cultures presented susceptibility to ginger aqueous extract but it is likely to use biosynthesis silver nanoparticles in antimicrobial studies due to recording anti-biofilm data in current study in addition to bactericidal activity of AgNPs against both Gram +ve and -ve bacteria and even drug resistance bacteria while ginger extract effective against Gram +ve more than Gram –ve bacteria due to presence Lipopolysaccharide molecules in the cell wall (Lara *et al .,* 2010 *;* Kaushik and Goyal, 2011).

CONCLUSION

Depending on the presented results, it is concluded that *Zingiber officinale* aqueous extract was effective in bio-reduction of silver nitrate in order to green synthesis of silver nanoparticles. UV spectroscopy and AFM analysis were useful tools in characterization the morphological and structural properties of these particles. In addition, green synthesized silver nanoparticles revealed potential biological effect such as anti-biofilm and plasmid curing noted *in vitro* against three bacterial isolates (*E. coli, Staph. aureus and K. pneumonia*), further studies could be performed to detect molecular mechanism of the nanoparticles.

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