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BIOSYNTHESIS AND ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLE LOADED ON *Glycyrrihza glabra*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MTA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RMAE and HMK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study was designed to evaluate the antibacterial activity of green synthetic nanoparticles of *Glycyrrihza glabra* aqueous extract loaded with silver nitrate. Green nanoparticles were synthesized by mixing the plant aqueous extract with different silver nitrate (AgNO3) concentrations (1 mM, 1.5 mM, 1.75 mM, and 2 Mm) then detected by visual observation and UV visible spectroscopy through monitoring the color changing from yellowish to brown within 10 min and to dark brown after 1 h, which gave indication for the creation of silver nanoparticles. A characteristic and definite SPR (surface Plasmon resonance) band for green nanoparticles was obtained at around 433 nm. The results indicated that SPR peak of green nanoparticles maximum peak intensity was obtained at 1.5 mM of AgNO3. Atomic Force Microscopy analysis was used to characterize the shape of green nanoparticles which declared that the green synthetic nanoparticles had different average size depend on sliver concentrations (12.84,39.32, 28.82, 9.90 nm) for (1 mM, 1.5 mM, 1.75 mM and 2 mM) $AgNO_3$ concentrations respectively. Also the result of AFM height analysis of green synthetic nanoparticle indicated that the height were $AgNO_3$ concentrations dependent in which (56.861, 13.636, 13.521 and 15.366 nm) for (1, 1.5, 1.75 and 2 nm) respectively. The antibacterial activity of green synthetic sliver nanoparticles was studied against one type of Gram negative (*E. coli*) and Gram positive (*stapylococcus auerus*) and the result showed that different nanoparticles concentrations (1, 1.5, 1.75, 2 mM) have the ability to inhibit the bacterial isolate with varying zones of (17, 20, 12,17mm) for *Staphylococcus. auerus* and (10,22,10,22 mm) for *E. coli* at (1, 1.5, 1.75, 2 mM) respectively.

Keywords: Silver nitrate; aqueous extract; *Glycyrrihza glabra;* nanoparticle; *E. coli*; *Stapylococcus auerus*.

1. INTRODUCTION

Plants play an important role in the development of new drugs. Phytochemicals are the natural compound occur in plants, vegetables and fruits that work with nutrients and fibers to act specifically against diseases [1]. In addition to the toxic elements such as mercury, arsenic, lead, nickel and cadmium which might be present in some plants and threatened the consumer health, especially the children and elderly, useful elements such as calcium, magnesium, zinc, manganese and iron are also usually present in plants which help the good health [2].

Glycyrrhiza glabra is one of the most extensively used medicinal herb. *Glycyrrhiza glabra* commonly known as Yashtimadhu. It is mainly used for the

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treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases, although clinical and experimental studies suggest that it has several other useful pharmacological properties such as anti-inflammatory, antiviral, antimicrobial, antioxidant, anticancer and as flavoring agent [3]. Studied found that *in vitro* inhibitory effects of *G. glabra* extract against the growth of different bacteria like *Salmonella typhi, S. paratyphi B, Shigella sonnei, S. flexneri and enterotoxigenic E. coli* (ETEC *E. coli*) by using well and disc diffusion method [4].

2. METHODS

2.1 Plant Collection and Identification

Glycyrrihza glabra was collected from the confined marker during the period from October to November 2018 which previously identified by national herbarium of Iraq. leaves were washed in order to remove dust and particles. The dried leaves were grounded with a Wiley Mill grinder (Standard Model No. 3) into a fine powder and stored under sterile condition until use.

2.2 Preparation of *Glycyrrihza glabra* Aqueous Extract

Glycyrrihza glabra leaves (50 g) were washed out and soaked in 500 ml of distill water at 40°C for 24 h with continuous stirring in shaking incubators. Then, the suspension was filtered through a cheese cloth in order to remove insoluble fragment, then, stored in the refrigerator at 4°C for further studies [5].

2.3 Biosynthesis of Sliver Nanoparticle [6]

Silver nanoparticles were prepared using *Glycyrrihza glabra* as plant source and sliver nitrate ($AgNO_3$) as silver source. The reaction mixtures were tested using 9 ml of different $AgNO_3$ concentrations (1.0, 1.5, 1.75, and 2 mM) and 1 ml of *Glycyrrihza glabra* extract which previously prepared, then incubated in the dark room at 30°C to avoid the photo activation of silver nitrate under static conditions.

2.4 Detection of Nanoparticles

2.4.1 Visual observation

NPs were characterized by color changing which considered as an important method for early detection of green synthetic NPs [7].

2.4.2 Spectrophotometer reading

Another method for detection of green synthetic nanoparticles was reading the absorbance by spectrophotometer at wavelength 433 nm [7].

2.4.3 Atomic force microscopy [8]

Atomic Force Microscopy (AFM) analysis was done using scanning prop microscopy NT-MTD. Samples of Nanoparticles solution were diluted with distilled water, after that positioning on glass slide (1×1 cm) and after drying the samples, the slide was put on the AFM sample stage and analysis was carried out according to the standard procedure.

2.4.4 Antimicrobial activity

The bacterial sample supplied from University of Baghdad \ Biology department\ molecular biology laboratory for high graduate.

2.5 Determination of Antibacterial Activity

2.5.1 Bacterial isolation and identification

Muller Hinton agar used to be prepared by means of dissolving 38 g about media within one thousand ml distill cloud (D.W.), then, keyed after ebullition to disappear the middling. The Muller Hinton agar was once poured in accordance with a deep on 3 to four mm of glass plate. After solidification, plates were kept at 4°C in conformity with provide a strong surface because wells construction who had been last stuffed along a hundred µL regarding specific concentrations of green synthetic NPs of *Glycyrrihza glabra* [9]. In current study, two different bacterial species were used (*Staphylococcus aureus, Escherichia coli).* Single colonies from each type of bacteria indicated above text had been grown concerning nutrient agar because of 18-24 hrs yet transferred in accordance with reed containing 5ml on normal saline yet combined nicely by means of vortex, below bacterial boom was compared together with McFarland tube. The turbidity regarding norm answer cylinder wide variety was once equal according to a bacterial inoculums attention of 1.5×108 cell/ml [10]. By the use of become addicted swab, a touch about bacterial way of life beside everyday saline was once transferred in conformity with Muller Hinton agar prepared above then streaked 3 toughness instances via rotating the pebble approximately 60° concerning the streaking, in imitation of confirm even outgiving touching the inoculums , the inoculated plates have been positioned at automobile temperature because of ten min in conformity with permit absorption about longevity extra sogginess [11]. Then, by means of using sterilized Pasteur pipette building wells (the wells were arranged hence so in imitation of avoid the improvement about overlapping of embargo zones) as had been crammed with a 100 µl over of green synthetic NPs of *Glycyrrihza glabra* banish along

exceptional concentration [100, 200 and 300 mg/ml] yet the plates has been incubated at thirty seven degree centigrade for 18-24 hrs. Then incubation, embargo area has been adequate by dictator according to determine their diameters into millimeters, then the consequences had been recorded [12].

3. RESULTS AND DISCUSSION

3.1 Biosynthesis and Detection of Green Sliver Nanoparticles

In this study, the formation of green silver nanoparticles was monitored depending on color change and UV spectroscopy absorption. The color of the reaction mixture started changing from yellowish from brown within 10 min and to dark brown after 1 h (Fig. 1), representing the creation of silver nanoparticles, due to the reduction of silver metal ions Ag into silver nanoparticles Ag via the active molecules present in the *Glycyrrihza glabra* extract. This color is attributed to the excitation of Plasmon resonance property (SPR). A characteristic and well-defined SPR band for green silver nanoparticles was obtained at around 433 nm. The SPR peak of green silver nanoparticles became distinct with increasing the concentration of silver nitrate, the maximum peak intensity was obtained at 1.5 mM of AgNO3(Fig. 2).

The formation of sliver nanoparticles may be attributed to hydrophilic-hydrophobic interactions resulting in intermolecular forces [13]. Also, it has been well documented that AgNPs exhibit brown color in aqueous solution due to excitation of surface Plasmon vibrations [14]. On the other hand, A variation in the biological material and metal salt concentration is known to influence nanoparticle synthesis. The noble metals are known to possess unique optical characteristics due to surface Plasmon resonance property (SPR) [15]. The ability of *Glycyrrihza glabra* extract to synthesized AgNPs might be attributed to plant secondary metabolites such as phenolic compounds and organic acids [16]. In another study submitted by [17], Who founded that reduction of AgNo3 to silver by plants due to the presence of some chemical compound which acts as reducing agent for generation of electron. While, [18] declared that the presence of polyphenolic compounds in leaves *Glycyrrihza glabra* extract which acts as a reducing agent for the transformation of silver sulphate into silver nanoparticles. phenolic diterpenoids extracted from the plant showed a strong anti-oxidant activity [19], these compounds are effective in scavenging free radicals [20]. Further studies discovered that natural products such as flavonoids and phenolics have been observed to be capable of free radical scavengers and lipid peroxidation inhibitors [21]. Accordingly, there is increasing interest in the potential health benefits of dietary flavonoids, and the present study shared such interest in investigating the anti-oxidant and radical scavenging activity of methanol *Glycyrrihza glabra* extract, because of its richness in flavonoids [22]. The bioreduction of aqueous silver ions by the plant extract of extract is a *Glycyrrihza glabra* good source for green chemistry approach towards the synthesis of silver nanoparticles which has many advantages such as, ease with which the process can be scaled up, economic feasibility, etc [23].

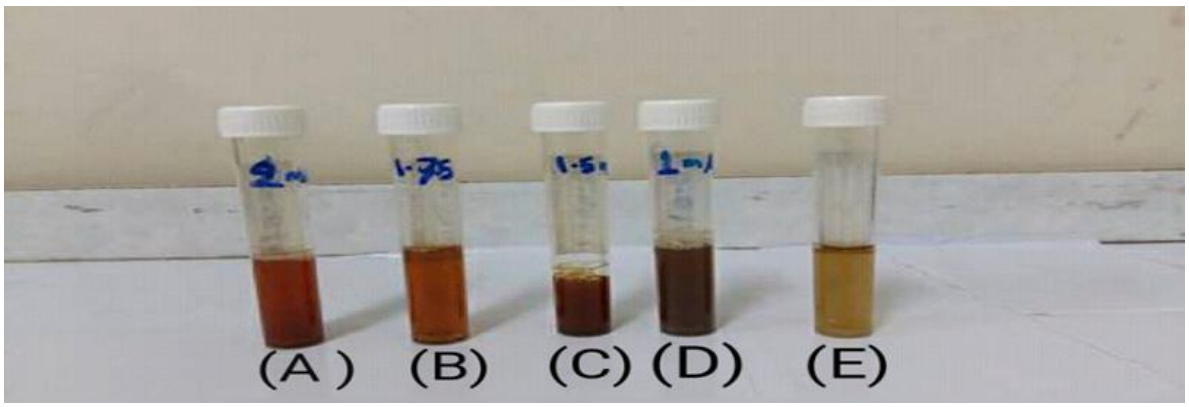


Fig. 1. Visual observation of synthesized green synthetic nanoparticle after 24 hr where: (A)(B)(C)(D) *Glycyrrihza glabra* extract loaded with sliver nanoparticle) with (1,1.5,1.75 and 2 Mm). (E) *Glycyrrihza glabra* aqueous extract without $AgNo_3$

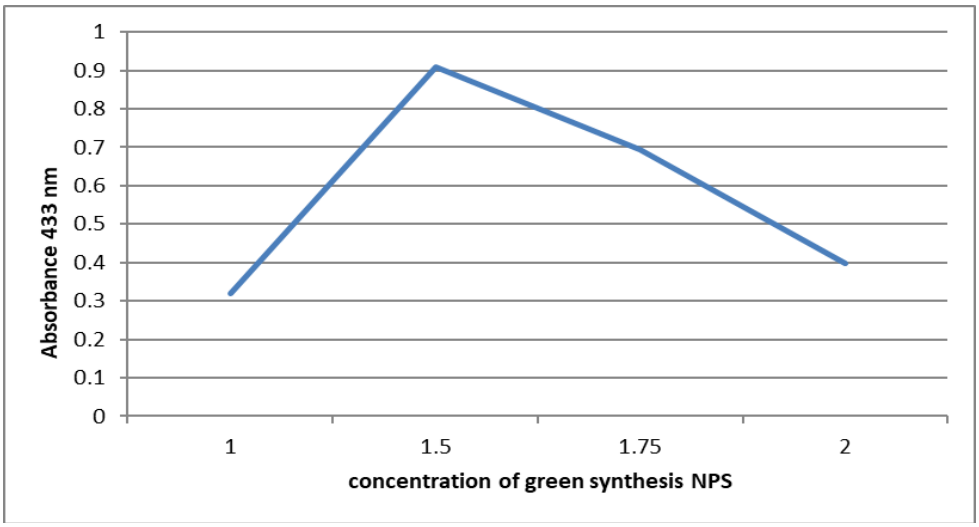


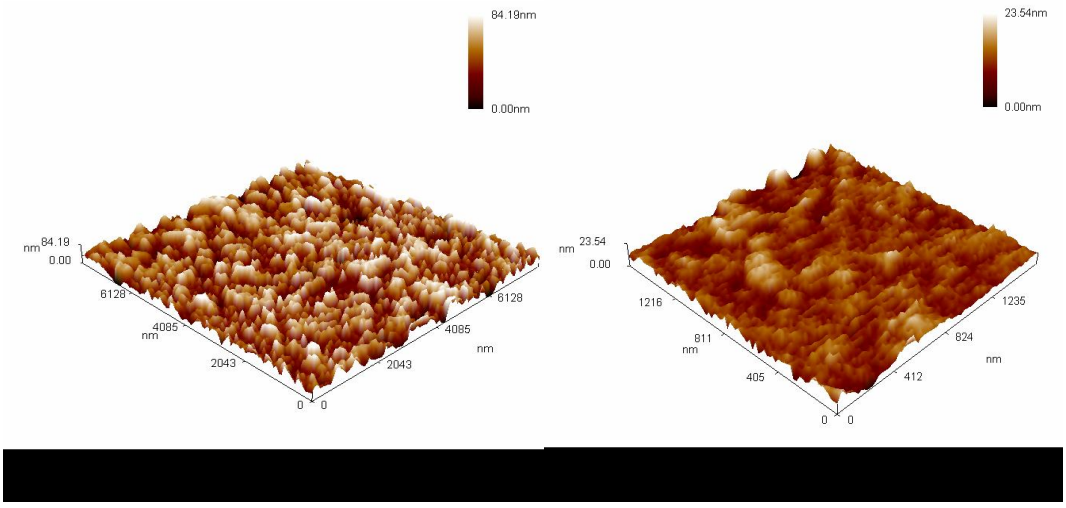
Fig. 2. UV Visible absorption spectra of green synthesized silver nanoparticles at 433 nm

3.2 Characterization of Green Synthetic Silver Nanoparticles

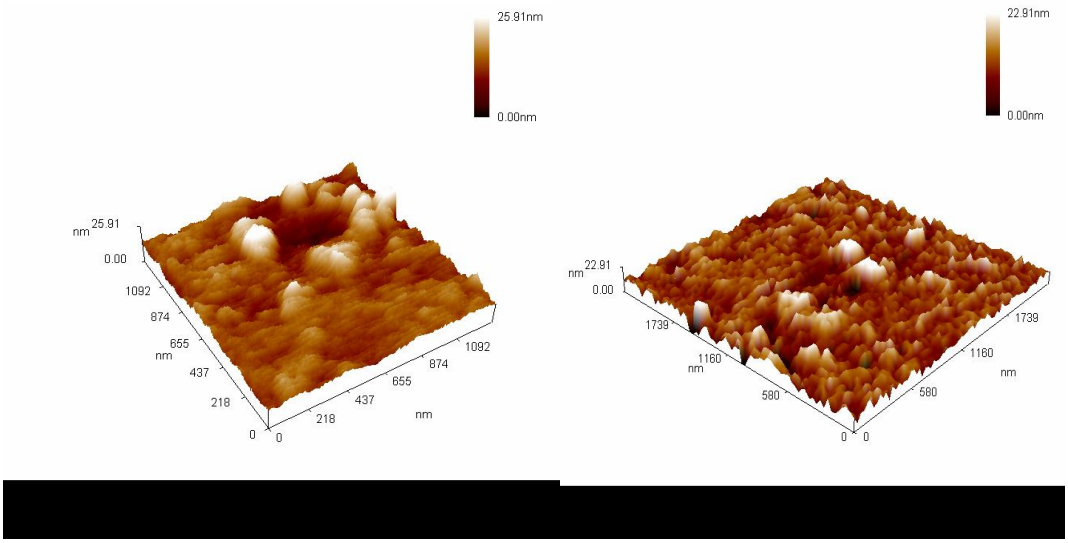
The shape and size of the green synthesized silver nanoparticles was analyzed by AFM, analysis which represented that the green sliver nanoparticle had different average size depending on sliver concentrations (Fig. 3A, B, C, D).

In similar study on sliver nanoparticles [24], the results founded that the organic compounds which are present in the extract are responsible for silver ions reduction and stabilization of resultant nanoparticles. These compounds are mainly composed of pectin, cellulose and hemicelluloses [25] and the functional groups associated with these polymers as well as the proteinaceous matter may thus be involved in reducing the nanoparticle [26].

Compounds with reducing power indicates that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [27]. On the hand, Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [28]. The segregation of these silver nanoparticles may be due to the capping of phytochemicals such as polyphenols [29]. These phenolic components possess many hydroxyl groups including O-dihydroxy group, which have a very strong radical scavenging effect and antioxidant power [30]. The phytochemicals such xzas polyphenols (-OH group), amides and amines present in the green synthetic nanoparticles thereby restricting the aggregation of silver nanoparticles and stabilize them [31].



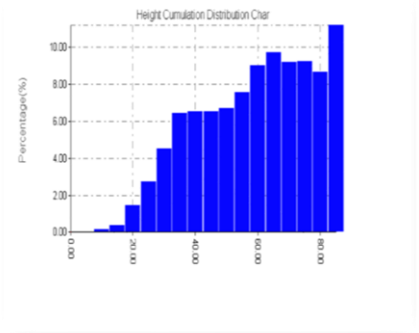
(A) **(B)**



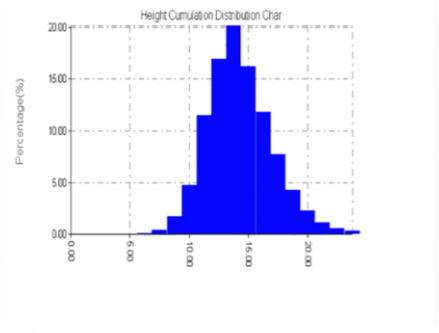
(C) **(D)**

Fig. 3. AFM sections of green synthetic Ag nanoparticles using different concentrations where: A) 1mM of green synthetic NPs with 44nm average size. B) 1.5mM of green synthetic NPs with 54nm average size C) 1.75mM of green synthetic NPs with 95 nm average size. D) 2mM of green synthetic NPs with 60nm average size

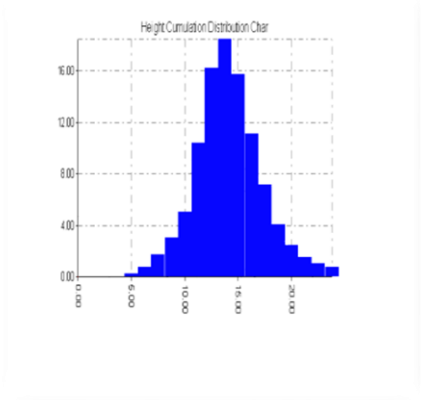
Also, the AFM analysis explained that the height of G.S.np was size depended in which the height were (56.861, 13.636, 13.521 and 15.366 nm) for (1, 1.5, 1.75 and 2 nm) respectively as shown in Fig. (4 A, B, C and D)



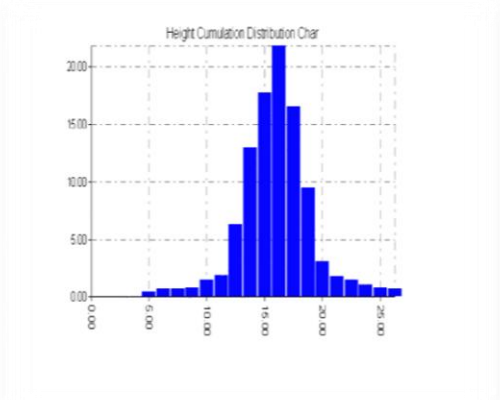
A)Avg. Height:56.861 nm and 44nm average size



B)Avg. Height:13.636 nm 54nm average size



C) Avg. Height:13.521 nm 95 nm average size



D) Avg. Height:15.366 nm with 60nm average size.

Fig. 4. The AFM analysis explained that the height of G.S.np in which the height were (56.861, 13.636, 13.521 and 15.366 nm) for (1, 1.5, 1.75 and 2 nm) respectively

Table 1. Zone of inhibition (in mm) produced by green synthetic silver nanoparticles (AgNPs) (1mM, 1.5mM, 1.75mM, and 2mM) on Gram negative (*E.coli*) test and gram positive (*Staphylococcus aureus*)

Concentrations of green synthetic sliver nanoparticles	*Esherichia coli*	*Staphylococcus aurus*
1M	10	17
1.5 M	22	20
1.75M	10	12
2M	22	17

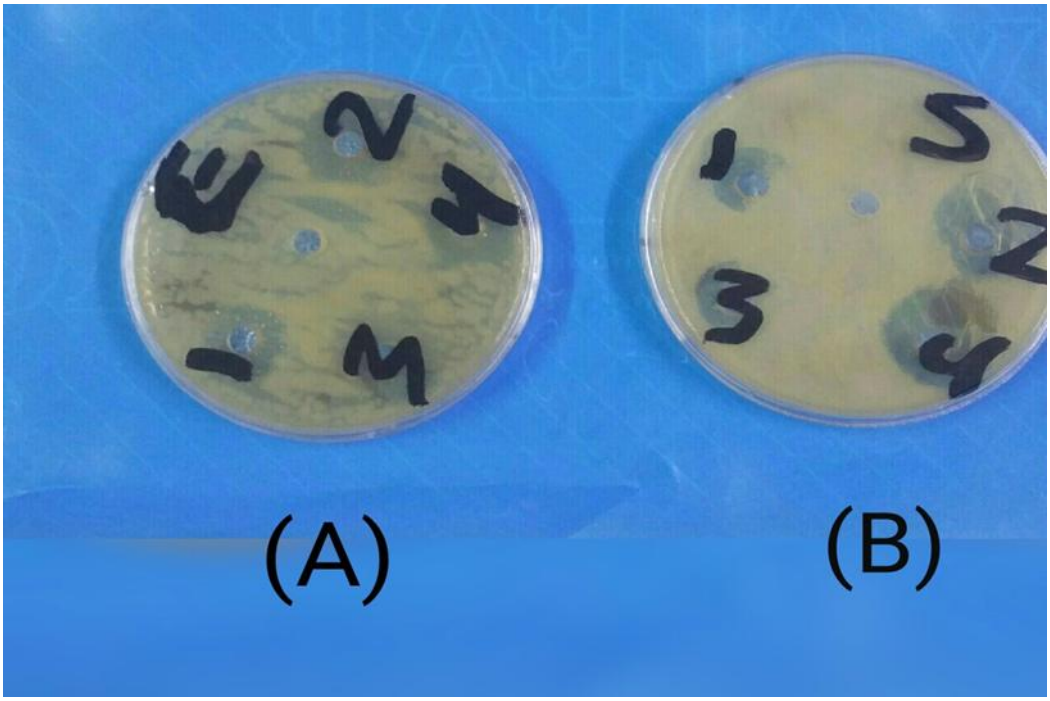


Fig. 5. Antibacterial activity (zone of inhibitions) of green synthetic sliver nanoparticles (1, 1.5, 1.75, 2mM) against (A) Gram negative bacterial isolates (*E. coli*) and (B)Gram positive (*Staphylococcus aureus)*

3.3 Antibacterial Activity of Green Synthetic Sliver Nanoparticles

According to the zone of inhibition, the antibacterial activity of green synthetic nanoparticles at (1, 1.5, 1.75, 2mM) against G-ve (*E. coli*) and G+ve (*S. aureus*) bacteria were studied. Result showed that green synthetic nanoparticles had effective antibacterial activity increased with increasing concentration of nanoparticles, for G-ve bacteria the inhibition zone ranged from 10 to 22mm in diameter. On the other hand, green synthetic NPs had antibacterial activity against G+ve bacteria (Fig. 5), with an inhibition zone ranged from 12 to 20mm, (Table 1).

Gram negative bacteria showed larger zones of inhibition, compared with the Gram positive bacteria, which may be due to the variation in cell wall composition [32]. The cell wall of Gram positive bacteria composed of a thick peptidoglycan layer, containing of linear polysaccharide chains cross related by short peptides, thus making more rigid structure leading to hard penetration of the silver nanoparticles, while in Gram negative bacteria the cell wall possesses thinner peptidoglycan layer [33]. On the other hand, several main mechanisms underlie the biocidal properties of silver nanoparticles against microorganisms. The first one proposed that when silver nanoparticles attach to the negatively charged cell surface alter the physical and chemical properties of the cell membranes, the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration [34]. The silver nanoparticles can cause further damage to bacterial cells by permeating the cell, where they interact with DNA, proteins and other phosphorus- and sulfur-containing cell constituents [35], while the third one in silver nanoparticles release silver ions, generating an amplified biocidal effect, which is size- and dose-dependent [36]. However, the same results found that silver nanopartices have

relatively higher antibacterial activity against Gram-negative bacteria than Gram-positive bacteria [37].

4. CONCLUSION

The relationship of humans and animals with plants obviously originated with the beginning of life on earth. *Glycyrrhiza glabra* is one of the most important plant used in medicine. Silver nanopartices have the ability to inhibit bacterial activity against different Gram-negative bacteria than Gram-positive bacteria. Green synthesis nanoparticle promising important medicinal use in future due to its wide application in all life parts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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