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Green Synthesis and Antimicrobial Activity of *Senecio glaucus* - Mediated Silver Nanoparticles.

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ABSTRACT

Silver nano particles (AgNPs) was synthesized using extract of *Senecio glaucus* extraxt. The effect of extract source on the shape of the Ag nanoparticles and antibacterial activity are investigated. The nature of AgNPs synthesized was analyzed by UV–vis spectroscopy and transmission electron microscopy (TEM). Silver nanoparticles were found to have an average size of 15-20 nm and mostly spherical. The antibacterial potential of modified extract containing synthesized AgNPs was compared with that of pure extract by cup plate method. Antibacterial activity of modified plant extract was reported and evaluated against drug resistant of bacterial isolates. Modified shoot extract gives higher response than that of modified root extract which facilitate them as a good alternative therapeutic approach in future.

Phytochemical analysis revealed that *Senecio glaucus* extract act as reducing and stabilizing agent for synthesized silver nanoparticles (AgNPs).

Keywords: Silver Nanoparticle; *Senecio glaucus*; Green synthesis

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INTRODUCTION

Wild plants are vital components of ecosystems. Plants supply animals with food, medicinal uses, recycle nutrients essential for agriculture and help to produce and maintain fertile soil [1]. Green synthesis of nanoparticles as an emerging intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis [2].

A great efforts introduced for green synthesis of metal nanoparticle using microorganisms and plants [3, 4]. Nanosilver used as an antimicrobial agent; it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances. Besides their antimicrobial features, silver nanoparticles exhibit strong optical features making the nanoparticles suitable for biological sensing and imaging [5]. Silver nanoparticles are also used as catalysts in several chemical reactions. Silver nanoparticles can be synthesized by reduction in solutions [6], thermal decomposition of silver compounds [7], microwave assisted synthesis [8], laser mediated synthesis [9] and biological reduction method ([10]. The latest is the most preferred way for the synthesis of nanoparticles as it offers one step, eco-friendly way of synthesis of nanoparticles.

Different authors explored leaf extract for different plants for the synthesis of silver and gold nanoparticles [11-13]. The main aim of the present study is to introduce an eco-friendly method for nano-particle synthesis and to study their antimicrobial effect under different conditions.

Senecio is the largest genus in the family Asteraceae and more than 1500 species have been reported and spread all over the world [14]. This genus is represented by 6 species in Egypt. This genus is rich in alkaloids [15, 16] and sesquiterpenes [17]. In folk medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have been reported [18, 19].

Senecio glaucus is an annual herbaceous species, erect, branched and grows in sandy soils of coastal plains. According to Greuter [20] *S. glaucus* is palearctic native to many regions of Northern Africa and Northern Asia. In Egypt, it is distributed in the Egyptian deserts and Mediterranean coastal strip [21]. Ecological studies in the Deltaic Mediterranean coastal land reveal that many species are of wide ecological amplitude [22-25]. Previous studies focused on phytochemical analyses of *S. glaucus* [26, 27], cytological study [28] and biological activity [29].

MATERIALS AND METHODS

Plant material and preparation of extract

In the flowering stage *Senecio glaucus* (family Astraceae) was collected from their different natural habitats in the Mediterranean coastal region of Nile Delta. The plant was taxonomically identified according to Boulos [21].

Plant sample divided into two parts: shoot system and root part, these parts of the *Senecio glaucus* were handly cleaned, dried at room temperature (20-23^{o}C) and ground into a powder using a blender. The dried plant powder (100 g) was extracted with 80% methanol (400 ml) by refluxing for 3 hours. The solution was filtered and evaporated to dryness. A stock solution of extract was prepared in dimethyl sulfoxide (DMSO) and kept at -20^{o}C for future use [30].

Phytochemical analysis

The amount of total phenol in the extracts was determined spectrophotometrically [31], the standard curve was prepared using different concentration of catechol. Flavonoid content was measured using aluminum chloride colorimetric assay developed by Zhishen, et al. [32], Quercetin was used as a standard. Harborne [33] method was used to determine alkaloid by using concentrated ammonium hydroxide. The amount of tannins was determined using the method of [31], while saponin content was estimated according to Obdoni and Ochuko [34].

Instrumental analysis

Synthesis of nanoparticles

The silver colloid was prepared by chemical reduction method, silver nitrate $AgNO_3$ (Sigma Aldrich, UK) of analytical grade purity was taken as the metal precursor, were used as starting materials without further purification. All solutions of reacting materials were prepared in dimethylesulfoxide (DMSO). In typical experiment 500 ml of 1×10^{-3} M $AgNO_3$ was prepared. Predetermined concentrations of silver nanoparticles in plant extract were added drop by drop and solution was mixed vigorously.

UV/Visible measurements

The reduction of pure Ag^+ ions into silver nanoparticles was monitored by recording the UV/Vis spectra of the reaction mixture after prolonged time to ensure complete reduction of silver ions. The UV/Visible spectra of the resulting silver nanoparticles were recorded in the range of 200-1000 nm using Jasco UV-Vis. spectrophotometer. The analysis was performed at room temperature using quartz cuvettes (1 cm optical path), and the blank was the corresponding *Senecio glaucus* aqueous solution.

Fourier transform infrared spectroscopy

FT-IR measurements were carried out for both the *Senecio glaucus* extract and the silver nanoparticles synthesized in the presence of the *Senecio glaucus* to identify the possible biomolecules in the *Senecio glaucus* extract that can participate in the reduction process of the Ag^+ ions and capping of the resulting silver nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on *Nicolet is10* FTIR spectrometer in the range of 4000–4000 cm^{-1} at room temperature.

Transmission electron microscope measurement

The size and morphology of the resulting silver nanoparticles was investigated by transmission electron microscopy, TEM (JEOL TEM-1230) attached to a CCD camera at an accelerating voltage of 120 kV. The samples were prepared by placing few drops of the nanoparticles suspension on carbon coated copper grids, followed by allowing the solvent to slowly evaporate under the sun light before recording the TEM images.

Microbial susceptibility testing

The antimicrobial activity of the plant extract was estimated by cup plate method. The sterile borer was used to prepare wells of 6 mm diameter containing the plant extract, in the medium of each Petri dish and other wells containing the methanol and DEMSO served as controls. The test samples diffuse from the cups through an agar layer. The plates were incubated at 37°C for bacteria. Diameters of inhibition zone (mm) were measured after 24 hours for bacteria [35-37].

Tested Bacteria

Bacillus subtilis, Candida albicans, Erwenia carotovora, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus biogenesis and *Staphylococcus aureus.*

RESULTS AND DISCUSSION

The biological function of plant secondary metabolites includes protection against allergies, inflammation, platelets aggregation microbes, ulcer, vineses, anticarcinogenic and antimutagenic activity and tumors [38, 39]. Flavonoids are the most common and widely distributed groups of plant phenolics. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anticancer activity [40, 41].

Phenolic compounds are present in almost all plant, fruits, vegetables and beverages are the major sources of these compounds in the human diet, and could be a major determinant of antioxidant potentials of

foods [42-44]. Saponins may be phytotoxic through their effects on membrane lipids or by effects on specific enzymes [45].Tannins are known to inhibit the activities of digestive enzymes and the nutritional effects of tannins are mainly related to their interaction with protein [46].

The phytochemical analysis of *Senecio glaucus* in the present study showed that it rich in saponins (26.53 and 23.52 mg/g dry weight in the root and stem, respectively), phenolic compounds (27.80 and 23.52 mg/g dry weight in the root and shoot, respectively), tannins (19.18 and 17.61 mg/g dry weight in the root and shoot, respectively), alkaloids (11.03 and 8.09 mg/g dry weight in root and shoot, respectively) and flavonoids (21.62 and 18.51mg/g dry weight in the root and shoot, respectively) (Table 1). These results are agrees with those investigated in *Senecio hypochionaeus* and *S. lorentii*, while lower than those reported in *S. pandurifolius* and *S. trapezuntinus* as mentioned by [47].

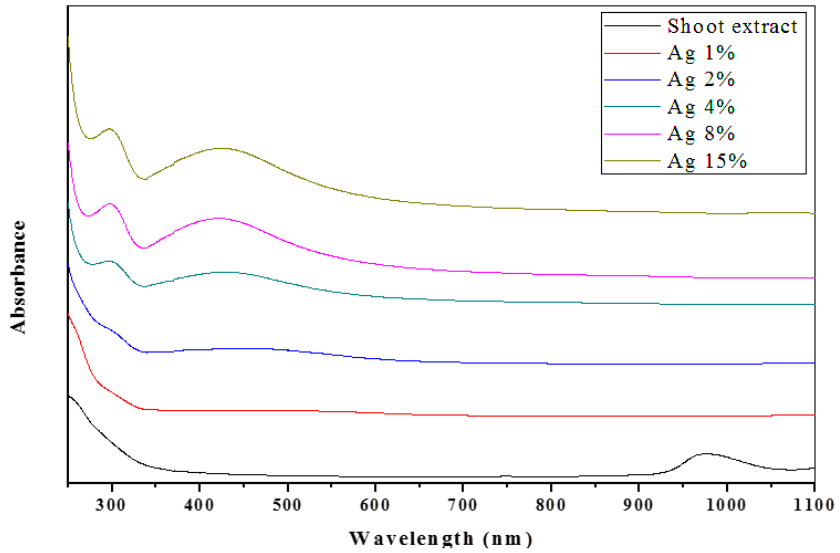
Djerdane, et al. [48] stated that the amount of phenols of *Anthemis arvensis* and *Artemisia campestris* (Asteraceae) were 32.32 and 20.38 mg/g dry weight, respectively. Also, it has been noted that the amount of saponins, flavonoids, alkaloids and tannins of *Conyza bonariensis* (Asteraceae) were 56.0 ± 0.03, 7.0 ± 0.06, 5.9 ± 0.07 and 21.13 ± 0.01[49]. According to these results, organic secondary compounds of *Senecio glaucus* which were tested are higher than these species belong to the same family.

Table 1: The concentration of the active constituents in mg/g dry weight for the selected plant species.

Active constituents	*Senecio glaucus* L.	
	Shoot	Root
Tannins	17.61±0.23	19.18±0.11
Saponins	22.42±0.17	26.53±0.25
Flavonoids	18.51±0.27	21.62±0.43
Alkaloids	8.09±0.24	11.03±0.35
Total phenol	23.52±0.15	27.80±0.14

UV/Visible measurements

Figure (1 a, b) shows the UV/Vis. Optical absorption spectra of pure shoot and root extract and samples that doped with different concentrations of silver nano-particles. Pure extract shows a strong charge transfer UV band at 230 nm which attributed to trace iron impurities even in a ppm level in the shoot extract with shoulder at about 260 nm with no visible bands.



(a)

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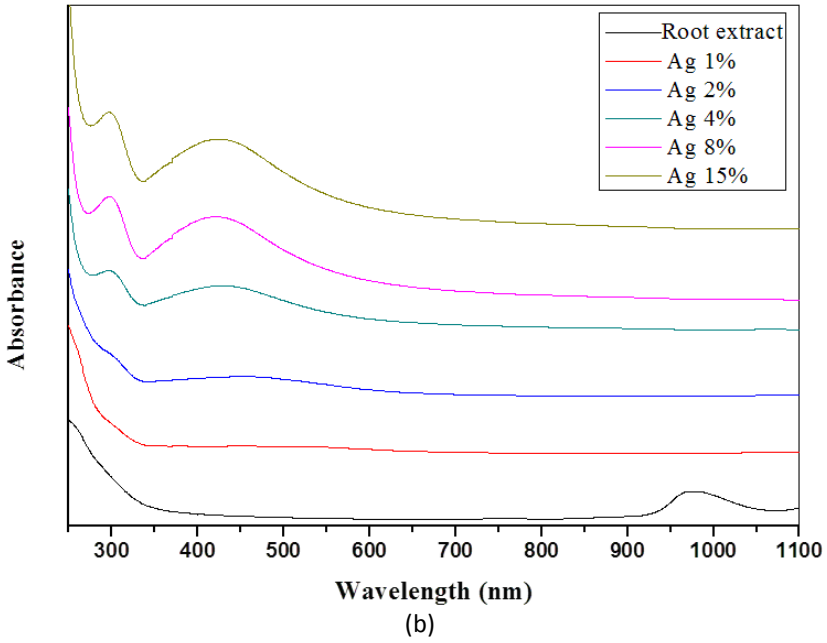

Figure 1: UV/Vis Absorption spectra of (a) shoot (b) root extract with different concentrations of silver

All samples that contain silver in the filling level (1, 2, 4, 8 and 15wt%) shows additional visible band at about 430 nm attributed to the surface plasmon resonance of silver nano-particles with increasing intensity with increasing silver content within the shoot and root extract.

Figure (2) shows the variation of optical density in visible region for both shoot and root according to the change in silver content.

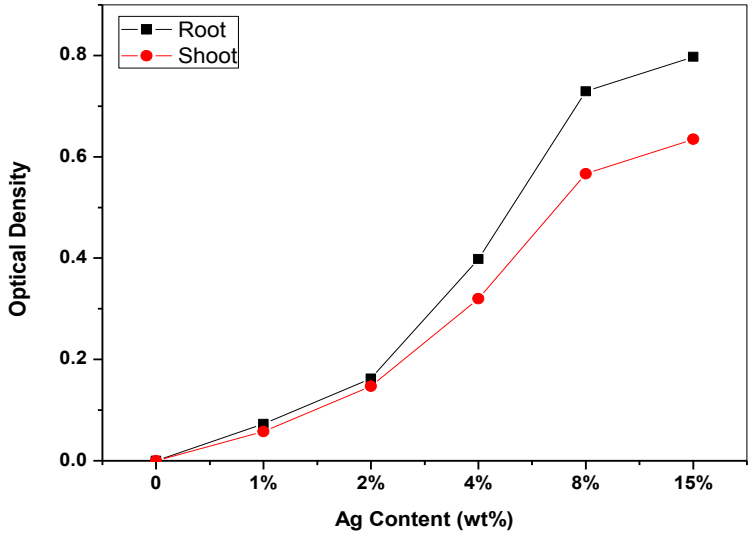

Figure 2: Variation of optical density of both shoot and root with silver content

Fourier transform infrared spectra

Figure (3) shows Fourier transform infrared (FTIR) absorption spectra of pure shoot and root extracts. It is obvious that the FTIR spectra of both shoot and root contains similar peak position that represent the individual structural groups present in the corresponding extract due to a similar composition of both extract but with different intensities which are related to the percent of structural units present in the measured samples.

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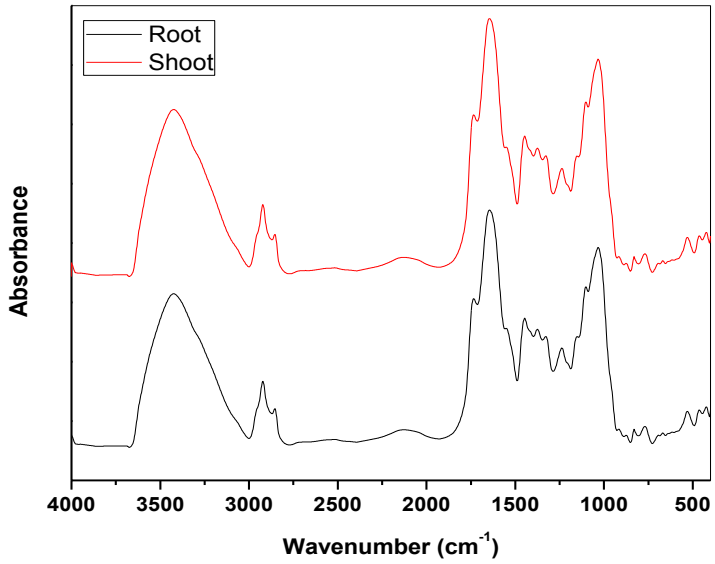

Figure 3: FTIR spectra of root and shoot extract before doping with silver nanoparticles

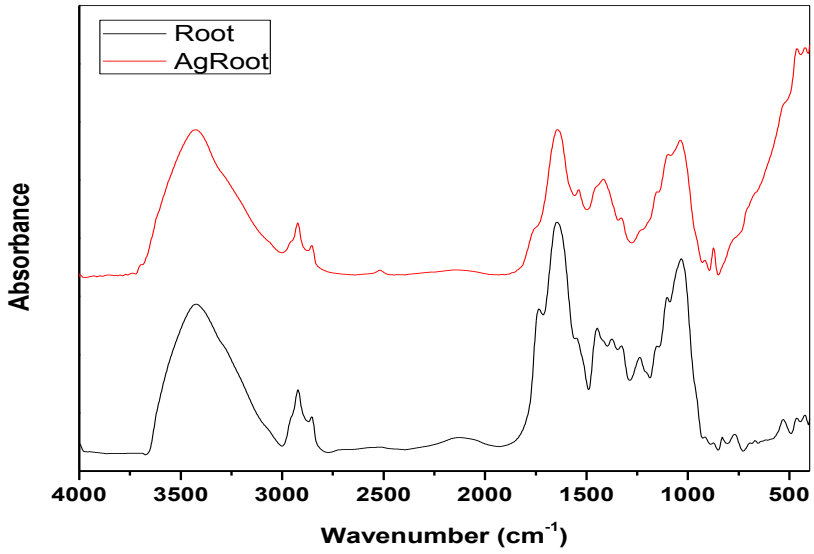

Figure 4a: FTIR spectra of pure and silver doped root extract

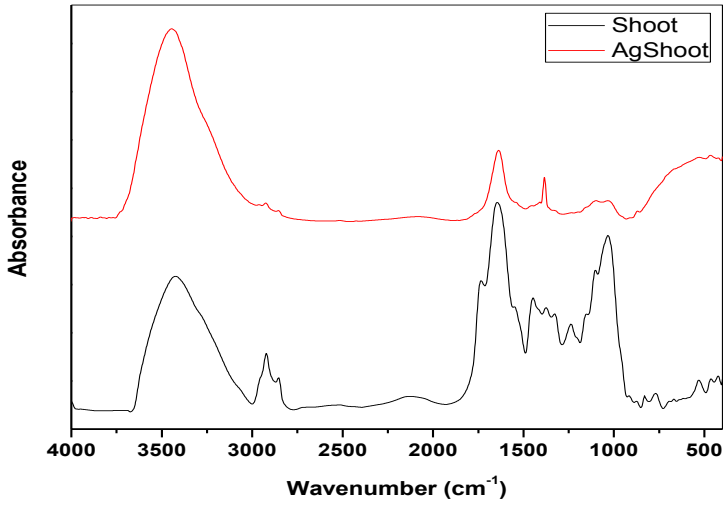

Figure 4b: FTIR spectra of pure and silver doped shoot extract

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Figure (4 a,b) shows Fourier transform infrared (FTIR) absorption spectra of pure root and shoot extracts compared to samples that contain a higher ratio filling with silver nano-particles. It is noticed that not only some of the main peaks was preserved indicating the presence of structural groups in the root and shoot extract but it is clear also, that some peak intensities was changed and some other are disappearing indicating a strong interaction between the plant extract and silver nitrate added and hence a presence of reducing agent in the plant extract (antioxidant).

Transmission electron microscope

Figure (5, 6) shows TEM image with diffraction pattern for shoot and root systems filled with silver nano particles. TEM analysis confirmed that the metal particles are in nano-range, they are nearly spherical in shape and the average diameter of silver nanoparticles was estimated to be ranged from 20 to 40 nm after 30 min. From the spectroscopic and imaging data obtained, it is obvious that 30 min is sufficient for the reduction of all silver ions present in solution. Most of the silver nanoparticles in TEM images are not in physical contact but they are separated by a fairly uniform interparticle distance. From TEM images it is observed that a second material coats the silver nanoparticles.

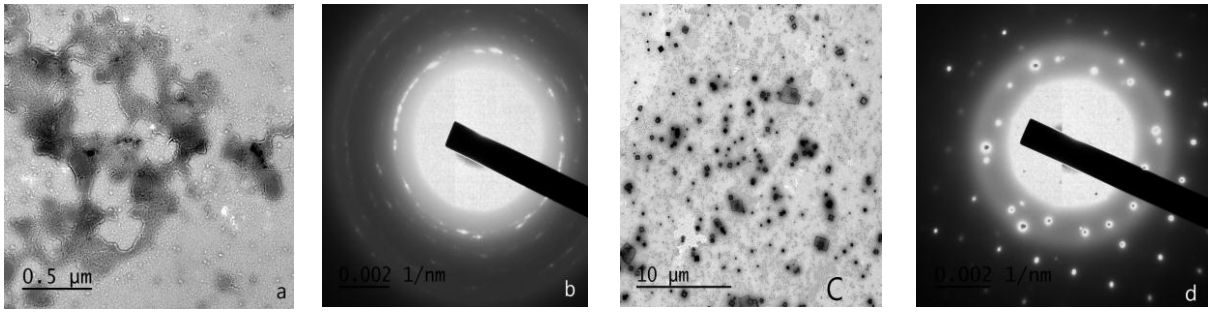

Figure 5: TEM image of shoot extract (a) before, (c) after adding silver nanoparticles and their

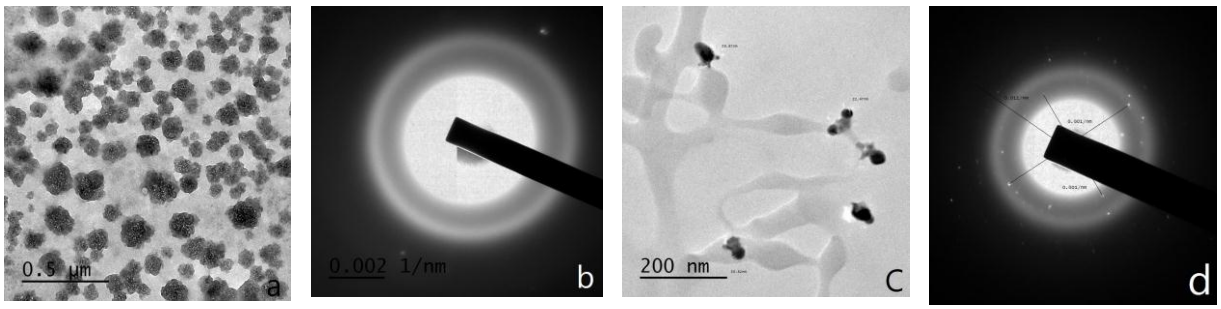

Figure 6: TEM image of root extract (a) before, (c) after adding silver nanoparticles and their corresponding diffraction pattern (b, d).

The inhibition effect of *Senecio glaucus* shoot and root extract against bacterial strains after adding silver nanoparticles are shown in Table (2) and Figure (7 & 8). At the concentration 1% of $AgNO_3$ showed the inhibition effect against *Staphylococcus aureus* and *Bacillus subtilis* for shoot extract and against *Staphylococcus aureus* and *klebsiella pneumonia* for root extract. At the concentration 2%, the inhibition effect against *klebsiella pneumonia* and *Salmonella typhi* for shoot extract and against *Erwenia carotovora, Escherichia coli* and *Pseudomonas aeruginosa* for root extract. At the concentration 4%, the inhibition effect against *Escherichia coli* and *Pseudomonas aeruginosa* for shoot extract and against *Candida albicans* for root extract. At the concentration 8 and 15% of $AgNO_3$ showed the inhibition effect against all tested bacterial strains for shoot and root extract. It is of interest to denote that, the concentration 1% of $AgNO_3$ showed the inhibition effect against Gram-negative bacterial strains for shoot and root extract. At concentration 4% of $AgNO_3$ showed the inhibition effect against all tested bacterial strains for shoot extract, while at concentration 8% of $AgNO_3$ exhibited the inhibition effect against all tested bacterial strains for root extract. Also, the antimicrobial spectrum of *Sencio glaucus* methanol extract and in combination with different concentrations of $AgNO_3$ increase from plant extract to 15% of $AgNO_3$ against tested pathogenic bacteria (Figure 9).

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Table 2: The inhibitory activity of the plant extract against the tested bacteria as demonstrated by diameters of the inhibition zone (mm)*.

Tested pathogenic bacteria strains	Zone of Inhibition											
	Sencio glaucus L.											
	Shoot extract	$AgNO_3$ Concentration					Root extract	$AgNO_3$ Concentration				
		1%	2%	4%	8%	15%		1%	2%	4%	8%	15%
Gram-positive												
Bacillus subtilis	-	17	18	20	22	22	16	18	18	23	24	24
Staphylococcus aureus	-	18	24	24	27	30	-	19	17	20	20	22
Gram-negative												
Erwenia carotovora	12	14	16	17	18	21	-	-	15	17	18	19
Escherichia coli	-	-	-	16	18	19	-	-	16	18	18	20
Candida albicans	11	15	15	17	19	19	-	-	-	17	22	22
klebsiella pneumonia	-	-	14	15	18	21	-	15	15	19	20	35
Pseudomonas aeruginosa	-	-	-	16	17	19	-	-	15	18	19.5	28
Salmonella typhi	-	-	15	18	19	20	-	-	-	-	15	18
Staphylococcus biogensis	15	16	19	21	21	23	15	14	16	14	28	31

-Zone of inhibition, including the diameter of the cup plate method (6.0 mm)
-The recorded value is mean value of 3 replicates.

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Figure 7: Percentage of the inhibitory activity of the methanolic extract of *Sencio glaucus* shoot and in combination with different concentrations of $AgNO_3$ from which nanoparticles synthesized against tested pathogenic bacteria

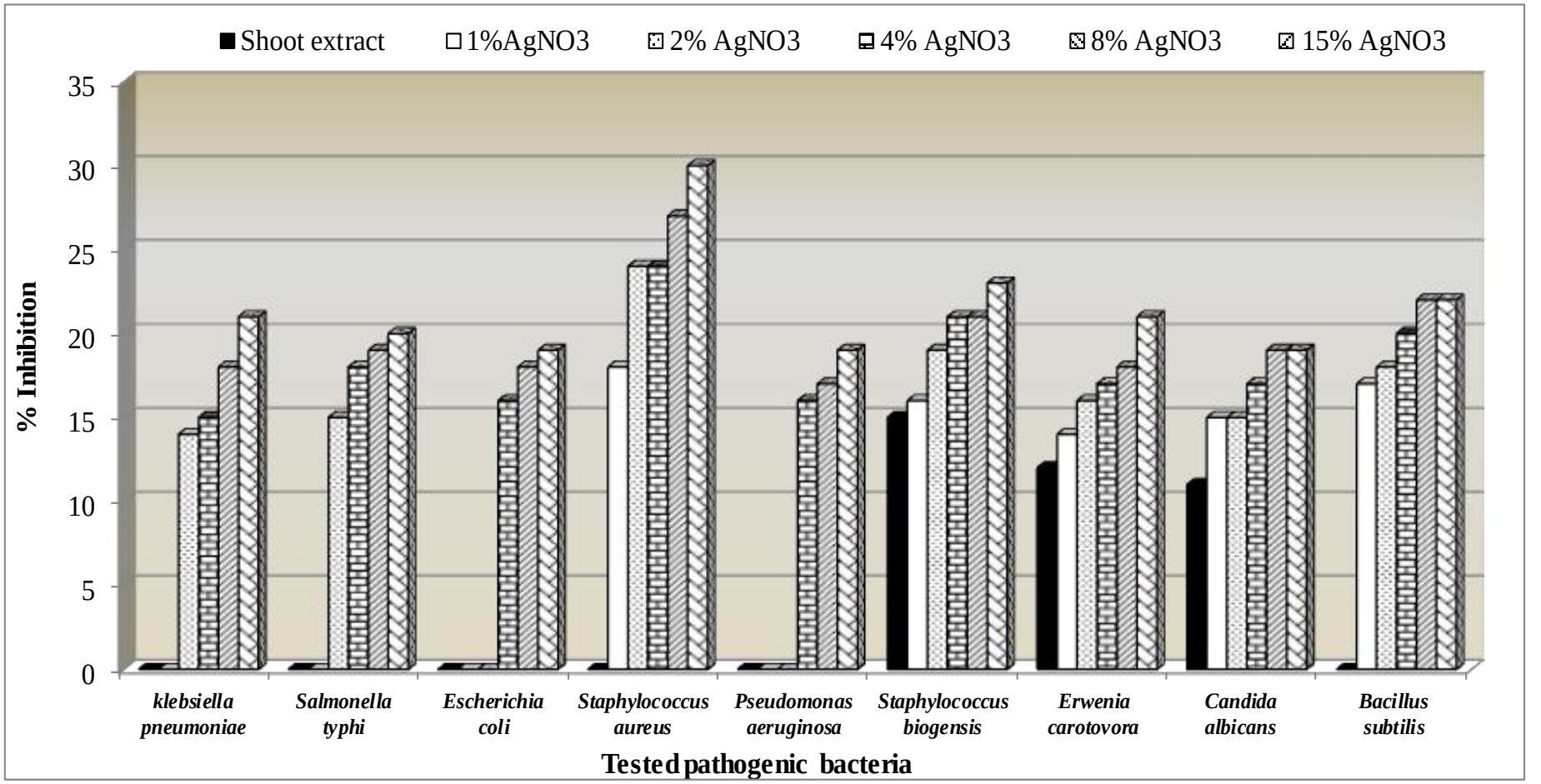

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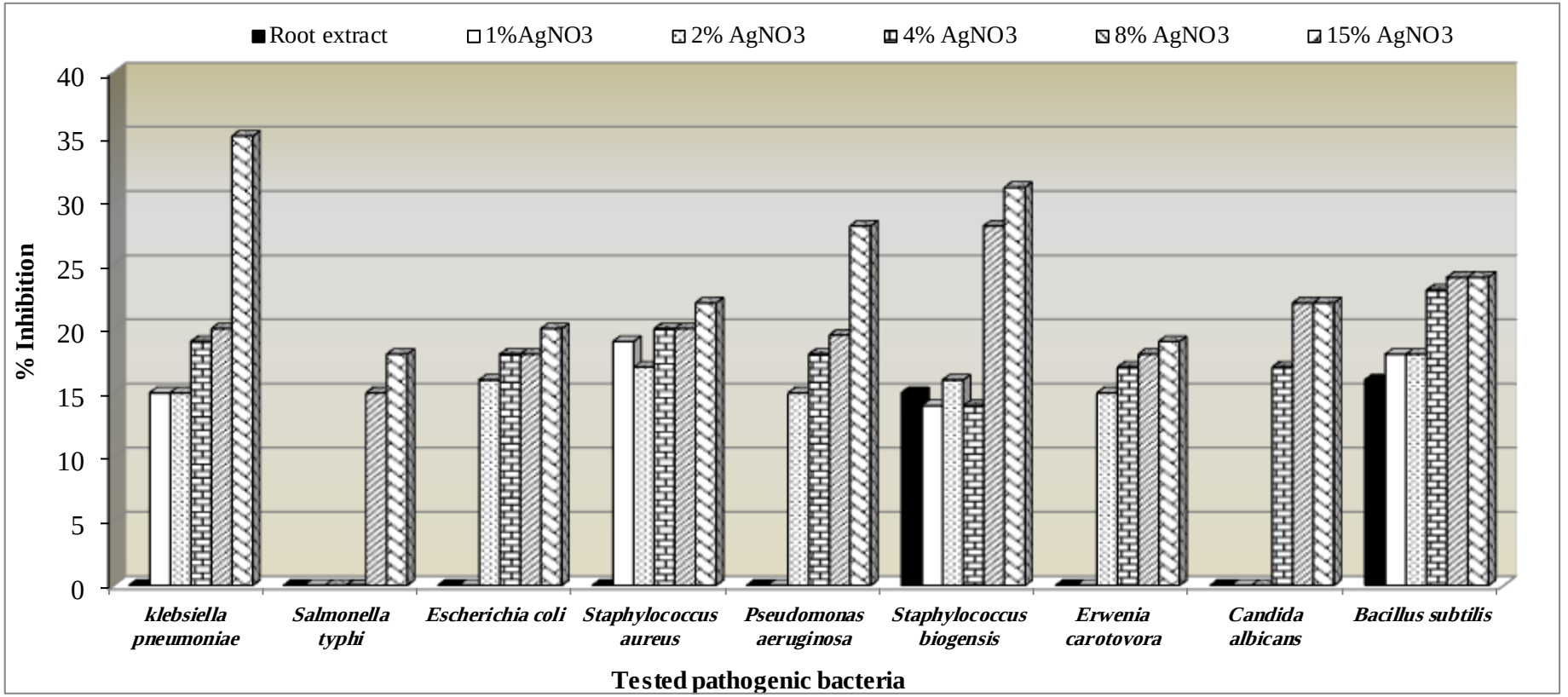
Figure 8: Percentage of the inhibitory activity of the methanolic extract of *Sencio glaucus* root and in combination with different concentrations of $AgNO_3$ from which nanoparticles synthesized against tested pathogenic bacteria.



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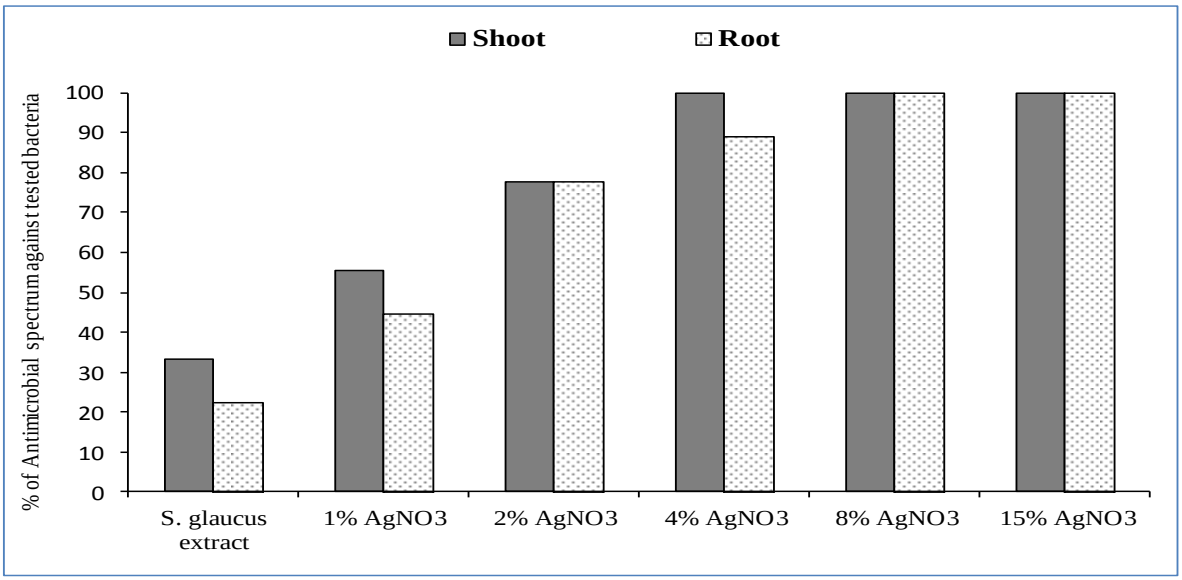

Figure 9: Percentage of the antimicrobial spectrum of *Sencio glaucus* methanol extract and in combination with different concentrations of $AgNO_3$ from which nanoparticles synthesized against tested pathogenic bacteria.

CONCLUSION

Senecio glaucus extract were classified according to their origin (root extract and shoot extract). *Senecio glaucus* is wide ecological amplitude in Egypt rich of secondary chemical constituents (phenols, tannins, alkaloids, saponins and flavonoids) as reveled by the phytochemical analysis. These phytochemicals act as reducing and stabilizing agent for synthesized silver nanoparticles (AgNPs).

The effect of extract source on the reaction rate, concentration and shape of the synthesized Ag nanoparticles are investigated. The silver nanoparticles were found to have an average size of 15-20 nm and mostly spherical. The antibacterial potential of synthesized AgNPs was compared with that pure extract by cup plate method. AgNPs showed broad spectrum antibacterial activity at different low concentration while shoot extract gives higer response than root extract which facilitate them as a good alternative therapeutic approach in future.

Therefore, the conservation of natural habitats of this species, which will be threatened by agriculture, urban expansions and exposed to serious erosion, are of vital importance.

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