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Andrographis paniculate mediated biosynthesis of silver nanoparticles and its antibacterial activity against human pathogens

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Abstract

Development of reliable biological route to synthesis nanoparticles is essential for their potential applications in diverse fields, especially in antimicrobial activity against human pathogens. The present work is also evidencefor the plant mediated synthesis of silver nanoparticles showing a greater consequence against microbes.This study deals with the synthesis of silver nanoparticles by treating silver nitrate with aqueous extract of Andrographispaniculata at room temperature. The effect of the Andrographis paniculataaqueous extract on the formation of silver nanoparticles was characterized by UVvisible spectroscopy (UV-Vis), X-ray Diffraction Spectrum (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDX). The UV spectra results show a strong resonance centre and surface of silver nanoparticles (AgNPs) at 443 nm. XRD and SEM studies revealed that the synthesized AgNPs shows spherical shape.

Key words: 1. Silver nanoparticles 2.Green synthesis 3.Andrographispaniculata 4.Human pathogens.

Introduction

Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometres. In nanotechnology nanoparticles research is an important aspect due to its innumerable applications. Nanoparticles have expressed significant advances owing to wide range of applications in the field of bio medical,sensors, antimicrobials, catalysts, electronics, optical fibres, agriculture, bio-labelling and in other areas (1).

The nanoparticles are synthesized through physical, chemical and biological methods (2). The physical and chemical methods are extremely pricey (3). As well as the substances like hydrazine, sodium borohydride, hydrogen, heavy metals, and radiation chemicals which are used in chemical synthesis protocol causes great damage in the environment as well as side effects in human health (4-7).

An important area of research in nanotechnology deals with the biomimetic synthesis of nanoparticles by using biological sources like plants, microorganisms, and others. This offers numerous benefits of eco-friendliness and effective in various medicinal applications as they do not use any toxic chemicals in the synthesis.Also, it is rapid, non-pathogenic, economical protocol and provides a single step technique for the green synthesis process (8).

In this present study Andrographis paniculata plant has been used as a biological source for the synthesizing of silver nanoparticles. A.paniculata belongs to the family Acanthaceae and is commonly known as 'Kings of Bitters'. This plant has been used as medicinal herb for centuries in several traditional systems of medicine all over the world. This plant is used in treating skin eruptions, boils, scabies, and chronic undetermined fever (9).

Therefore, this study aims to explore the bio synthesis and its efficacy as a source of nanomedicine against various bacterial strains and to establish their therapeutic values in anti-bacterial potential of the plant.

Materials and Methods

Materials

The chemical silver nitrate (AgNO3) was purchased from SD Fine Chemical Pvt, Ltd., Mumbai, India.

Plant sample collection

The fresh and healthy leaves Andrographis paniculata were collected from Coimbatore district, Tamilnadu, India. The plant was identifiedAndrographis paniculata from the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Extract preparation

The A. paniculataplant leaf was washed with tap water followed by rinsed with distilled water thoroughly to remove dust and other any attached particles. The A. paniculata aqueous leaf extract was prepared by taking 20g of thoroughly washed and finely cut A. paniculata leaves in a 250 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 30 min at 60°C to obtain bioorganic compounds from A. paniculata leaves. Followed by this step, after heating treatment, the solution was then removed from the heat source and left at room temperature and obtained aqueous extract was then filtered through a normal filter paper followed by Whatman filter paper No. 1. The final filtrate of the A. paniculataleaf extract was used as a reducing agent to synthesis biomimetic silver nanoparticles.

Biosynthesis of silver nanoparticles

Aqueous extract (10 ml) of A. paniculata plant which was prepared was taken in a 150 ml conical flask and 90 ml of 1mM of AgNo3 was added & kept at room temperature for reduction process and change of colour was monitored. Entire process was carried out in darkness to avoid photoactivation of AgNo3 at room temperature.

Detection and Characterization of AgNPs

The bioreduction of silver ions in A. paniculata plant aqueous extract was monitored by various characterization process.

UV-Vis Spectroscopy

The pre-liminary bio-reduction of Ag+ in aqueous solution was detected by UV-Vis spectrophotometer (Perkin-Elmer lamda-25) at room temperature with the wavelengths of 200nm – 800nm at a resolution of 1nm to analyse the Surface Plasmon Resonance band.

Scanning Electron Microscopy

The morphology of synthesized nanoparticles was examined by using Scanning Electron Microscopcic analysis. The reaction solution containing silver nanoparticles synthesized from A. paniculata leaf extract was made into powder by using Lyophilizer equipment. Thin flims of sample were prepared on carbon coated grids and SEM analysis was done. The images of biomimetic silver nanoparticles were obtained in SEM (Fb- Quanta 200 SEM machine) operated at 30 kV at different magnification level.

Energy-Dispersive X-ray (EDX) Analysis

The synthesized silver nanoparticles using A. paniculata aqueous extract subject to the Energy Dispersive Spectrum using SEM attached Fb-Quanta- 200 resolution to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particle.

XRD Analysis

The bio-reduced silver nanoparticles are dried in powder form by using lyophilizer equipment and they are coated on XRD grid and analysed for the formation of nanoparticles by using Philips PW-1830 XRay Diffractometer. X-Ray generator operated at a voltage of 40 kV and tube current of 30mA with Cu Kα1 radiation with λ of 1.5406. The scanning was done in the region of 2 from 300 to 800 at 0.02 min and the time constant was 2 sec. The average particle size was determined by using Scherr"s formula

$D = (0.9\lambda \text{ X } 1800) \dagger \beta \cos\theta$

Antimicrobial Assay

Collection of Microbial Strains

The clinically isolated bacterial cultures of gram positives Staphylococcus aureus, Bacillus subtilis and Gram-negative Helicobacter pylori, Aeromonas hydrophila were obtained from PSG medical college and hospital, Coimbatore, Tamil Nadu, India.

Preparation of Inoculums

A loopful of inoculums of each strain were suspended in 5ml nutrient broth & incubated overnight at 37 ^{0}C & those cultures were used for experiment.

Preparation of Media

The standard nutrient agar medium at standard concentration was prepared and its pH was adjusted to 7 & sterilized by autoclaving at 15 lbs pressure at 1210C for 15 minutes.

Determination of Minimal inhibitory concentrations

The minimal inhibitory concentrations of test antibacterial agents were determined by standard Broth micro dilution method using 96- well microtitre plates(CLSI) slightly modified according to the recommendations proposed for effective assessment of the anti-infective potential of natural products (10). Biological synthesized silver nanoparticles were dissolved in DMSO with the addition of tween 80% and diluted in muller hington broth to get initial reaction concentration of 1 mg/ml with maximum DMSO and tween contents of 1 and 0.5 % respectively. The solution was then two-fold diluted in muller hington broth (100 µL), inoculated with bacterial culture and then incubated at 37°C for 24 hours.

The bacterial growth was measured as turbidity with a cyberlab micro plate reader at 405 nm. The minimal inhibitory concentration was defined as the lowest concentration of the compound that inhibited the growth of the test bacteria by ≥ 50%. DMSO assayed as the negative control at the concentration of 1% did not inhibit any of the strains tested. All tests were assayed in triplicate in three independent experiments and median values were used for MICs calculation.

Results and Discussion

A wide range of secondary metabolites are presented in the plant extracts, nanoparticles produced by plants are more stable and the rate of synthesis is much faster in comparison to other biological sources. In the present study the aqueous silver nitrate solution was reduced during exposure to the A. paniculata plant leaf extract at 24- 48 hrs incubation at normal temperature.

Visual observation

The primary detection was done by visual observation. The formation of silver nanoparticles in the solution of 1mM AgNO3 & aqueous extract of A. paniculata plant sample was confirmed by change in colour of the mixture from light yellow to dark reddish brown, which indicates the formation of silver nanoparticles using A. paniculata biological sources act as reducing agent.

Control (without silver nitrate) shows no colour change, the colour change in the aqueous extract with silver nitrate solution which may be due the presence of bioactive compounds in aqueous extract like Lawsone & Gallic acid responsible for the reduction of silver nitrate to silver nanoparticles.The different type of antioxidants & various phyto-chemicals are responsible for the reduction of silver ions, similar type observations were reported by several authors.

UV-Visible Spectral Analysis

Formation of silver nanoparticles (AgNPs) by reduction with silver nitrate (AgNO3) by aqueous extract of A. paniculataleaf after 24 hrs incubation samples were characterized by UV-Visible Spectroscopy and the results obtained from them confirmed, the biological AgNPs formation in reaction mixture. In UV Visible spectrum, a strong, broad peak located between 420nm – 471nm was obsereved (Fig 1). This reveals that the formation of AgNPs occurs rapidly within 24 hrs, and it is stable even after 24 hrs of completion of the reaction. Similar observations were reported in Geranium leaf extract, aqueous extract of Areca nut, pomegranate peel extract (11). In this present study, the synthesized AgNP's were shown characteristic peak at 443 nm in visible light regions.

SEM Analysis

SEM analysis shows high-density AgNPs synthesized by A. paniculata leaf extract was shown that relatively spherical(Fig 2). The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating

stabilization of the nanoparticles by a capping agent. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

EDX Analysis

EDX spectra recorded from the silver nanoparticles were shown in (Fig 3). From EDX spectra, silver nanoparticles reduced by A. paniculata shown maximum peaks around 3.18 keV correspond to binding energies of silver ions. Throughout the scanning range of binding energies, some additional peaks belonging to bioorganic compound present in the reaction mixture. The EDX analysis revealed strong signals in the silver region and confirms the formation of silver nanoparticles by using biological source. There were other EDX spectrum peaks for Cl, k, O and Ca suggesting that they are mixed precipitates present in the plant extract (12).

XRD Analysis

The XRD patterns obtained for the Ag-NPs synthesised using A. paniculata extract is shown in (Fig 4). The Braggs reflections were observed in the XRD pattern at 2θ =32.78, 38.99, 46.22 and 77.98. These Braggs reflections clearly indicated the presence of (202), (111), (200), (220), and (311) sets of lattice planes and further on the basis that they can be indexed as Face- Centred-Cubic (FCC) structure of silver. (13) Reported that the XRD pattern green synthesized silver nanoparticles showed number of Bragg‟s reflections that may be indexed based on the face centred cubic structure of silver. Since, the present study clearly indicated the X-ray diffraction pattern of biological synthesized silver nanoparticles formed crystalline in nature.

Anti - bacterial Activity

In the present study achieved biosynthesized silver nanoparticles showed very good antibacterial activity against both gram positive and gram-negative pathogens. The antibacterial activity of silver nanoparticles against gram positive bacteria Staphylococcus aureus, Bacillus subtilis and found that the nanoparticles achieved maximum activity against Staphylococcus aureus (Fig 5 and Fig 6). Whereas the antibacterial activity of the nanoparticles against gram negative bacterium such as Helicobacter pylori, Aeromonas hydrophila and found that the maximum activity was against Helicobacter pylori.

The maximal inhibitory concentrations of silver nanoparticles against gram positive bacterium such as staphylococcus aureus, Bacillus subtilis were found to be 796.742 µg/ml (table 1)and 988.782 µg/ml (table 2)whereas minimal inhibitory concentrations of silver nanoparticles against gram negative bacterium such as Helicobacter pylori, Aeromonas hydrophila were found to be 647.109 µg/ml (table 3) and 815.827 µg/ml (table 4 and table 5) respectively. Biological route synthesis of Andrographis paniculatasilver nanoparticles showed dose depended on activity against test organisms. The activity of the compound against Helicobacter pylori was very significant compared to other organisms. When compared to gram positive bacterium the green synthesised Andrographis paniculatasilver nanoparticles showed maximum activity against gram negative bacterium. The minimal inhibitory concentration of Andrographis paniculatasilver nanoparticles was between 640-990µg/ml. This result suggests that the test compound is a potential antimicrobial agent.

In conclusion, a simple & environmental free green route was used to synthesize the AgNPs from silver nitrate using the aqueous extract of Andrographis paniculata plant. Theaqueous extract showed dark reddish-brown colour. From UV Vis spectrum synthesized AgNPs were shown characteristic peak at 443 in visible light regions. From XRD & SEM studies revealed that the

synthesized AgNPs shows spherical in shape with EDX Potential of 3.18keV.The Anti-bacterial activity of synthesized AgNPs showed much promising positive results.

Table 1: Antibacterial activity of AgNPs against S. aureus

Concentration (µg/ml)	OD at 405nm	% of inhibition	MIC (µg/ml)
Control	1.31	0	796.742
100	1.24	5.34	
200	1.18	9.92	
300	1.08	17.55	
400	1.00	23.66	
500	0.93	28.24	
600	0.87	33.58	
700	0.79	39.69	
800	0.68	48.09	
900	0.57	56.48	
1000	0.42	67.93	

Table 2:Antibacterial activity of AgNPs against B. subtilis

Concentration (µg/ml)	OD at 405nm	% of inhibition	MIC (µg/ml)
Control	1.74	0	988.728
100	1.62	6.89	
200	1.56	10.34	
300	1.49	14.36	
400	1.40	19.54	
500	1.31	24.71	
600	1.20	31.03	

700	1.12	35.63	
800	1.03	40.80	
900	0.94	45.97	
1000	0.86	50.57	

Table 3: Antibacterial activity of AgNPs against B. subtilis

Concentration (µg/ml)	OD at 405nm	% of inhibition	MIC(µg/ml)
Control	1.74	0	988.728
100	1.62	6.89	
200	1.56	10.34	
300	1.49	14.36	
400	1.40	19.54	
500	1.31	24.71	
600	1.20	31.03	
700	1.12	35.63	
800	1.03	40.80	
900	0.94	45.97	
1000	0.86	50.57	

Table 4: Antibacterial activity of AgNPs against H. Pylori

Concentration (µg/ml)	OD at 405nm	% of inhibition	MIC (µg/ml)
Control	1.10	0	647.109
100	1.01	8.18	
200	0.96	12.72	
300	0.87	20.90	
400	0.75	31.81	
500	0.68	38.18	

600	0.59	46.36	
700	0.40	63.36	
800	0.31	71.81	
900	0.26	76.36	
1000	0.19	82.72	

Table 5:Antibacterial activity of AgNPs against Aeromonas hydrophila

Concentration (µg/ml)	OD at 405nm	% of inhibition	MIC (µg/ml)
Control	1.04	0	
100	0.96	7.69	
200	0.90	13.46	
300	0.84	19.23	
400	0.78	25.00	
500	0.71	31.73	815.827
600	0.68	34.61	
700	0.60	42.30	
800	0.53	49.03	
900	0.46	55.76	
1000	0.38	63.46	

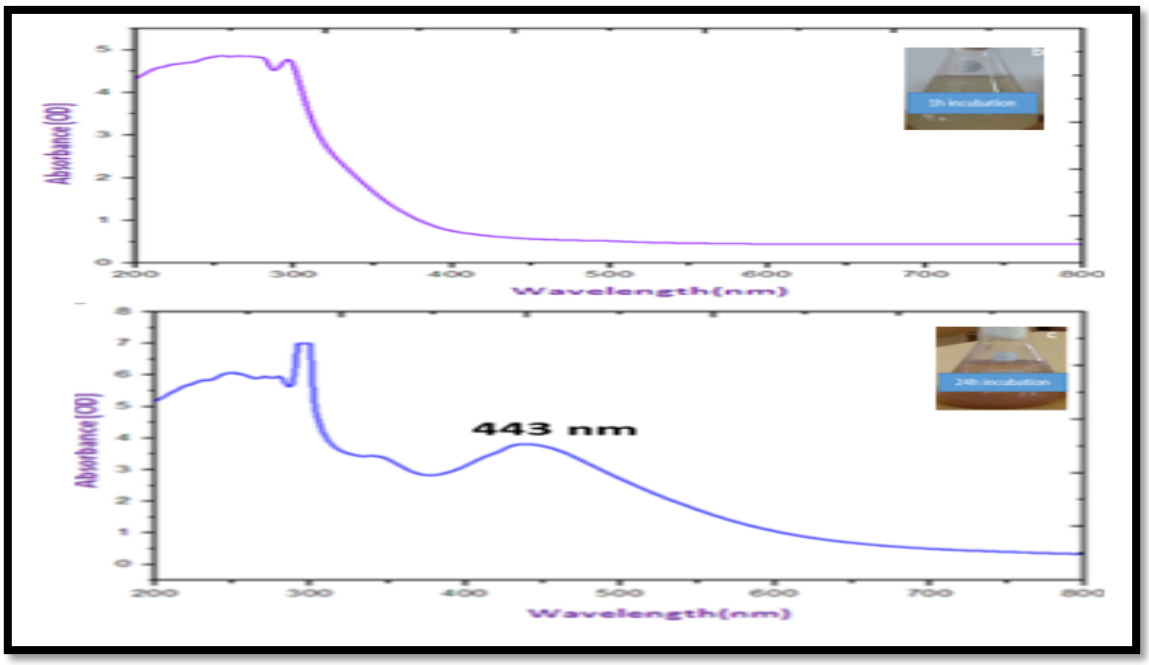

Fig 1: Absorbance spectrum of biological synthesized silver nanoparticles

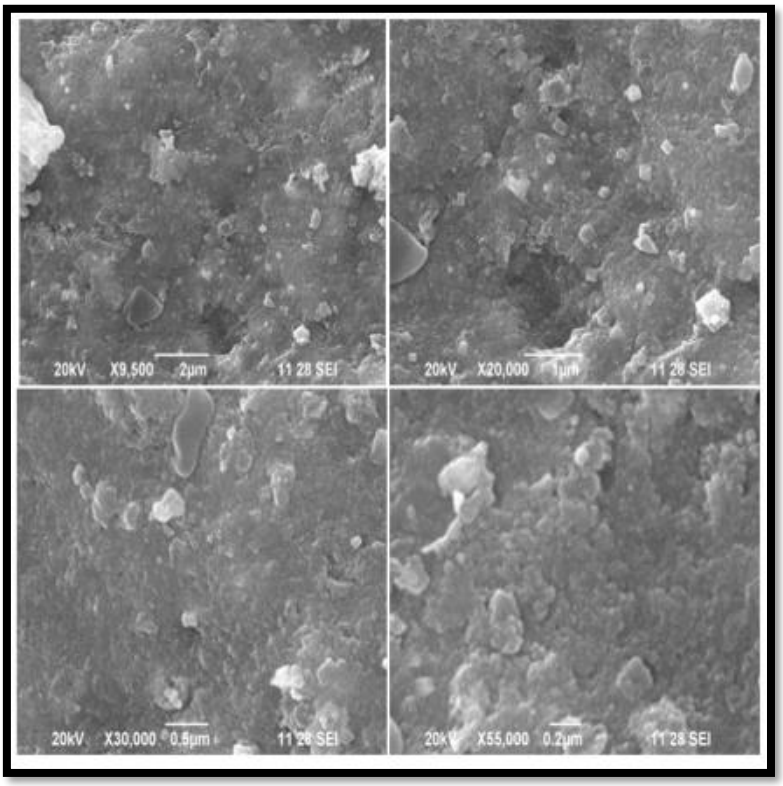

Fig 2: SEM image of green synthesized silver nanoparticles

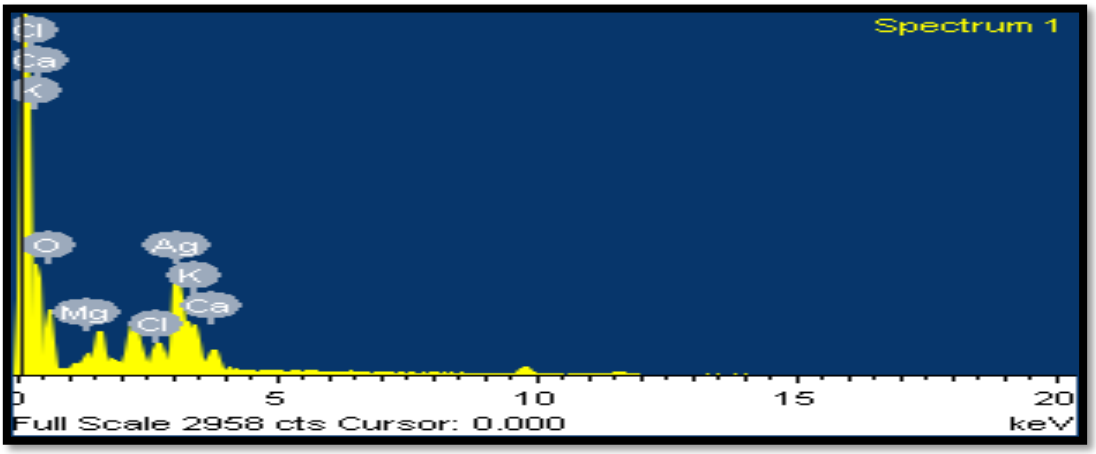

Fig 3: EDX image of green synthesized silver nanoparticles

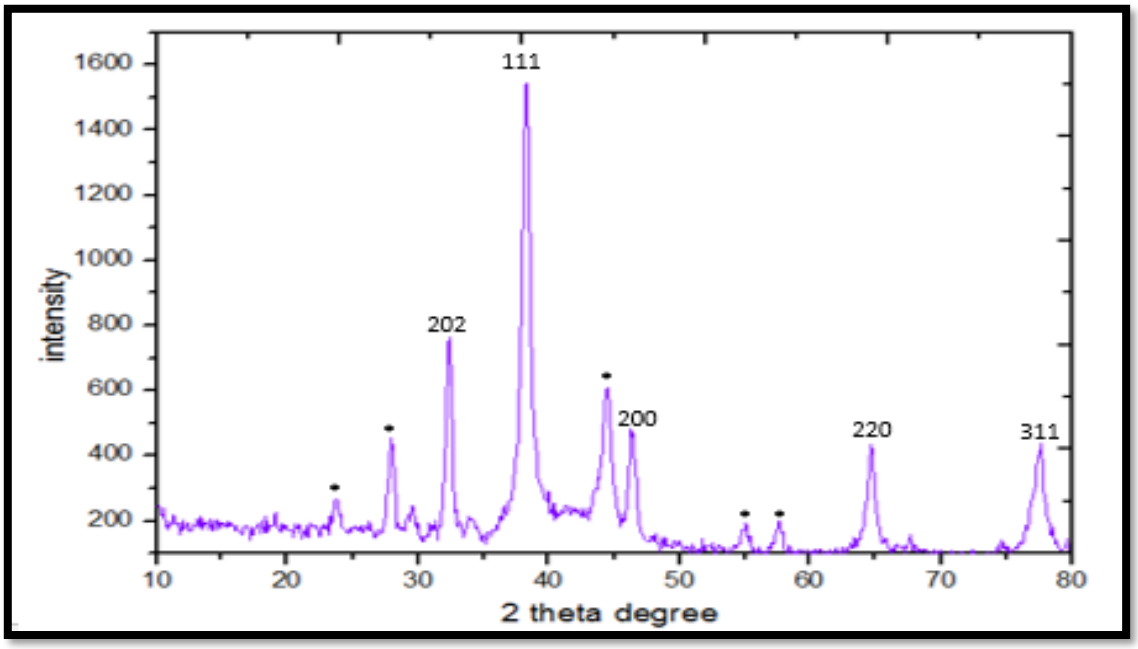

Fig 4: XRD analysis of silver nanopartilces

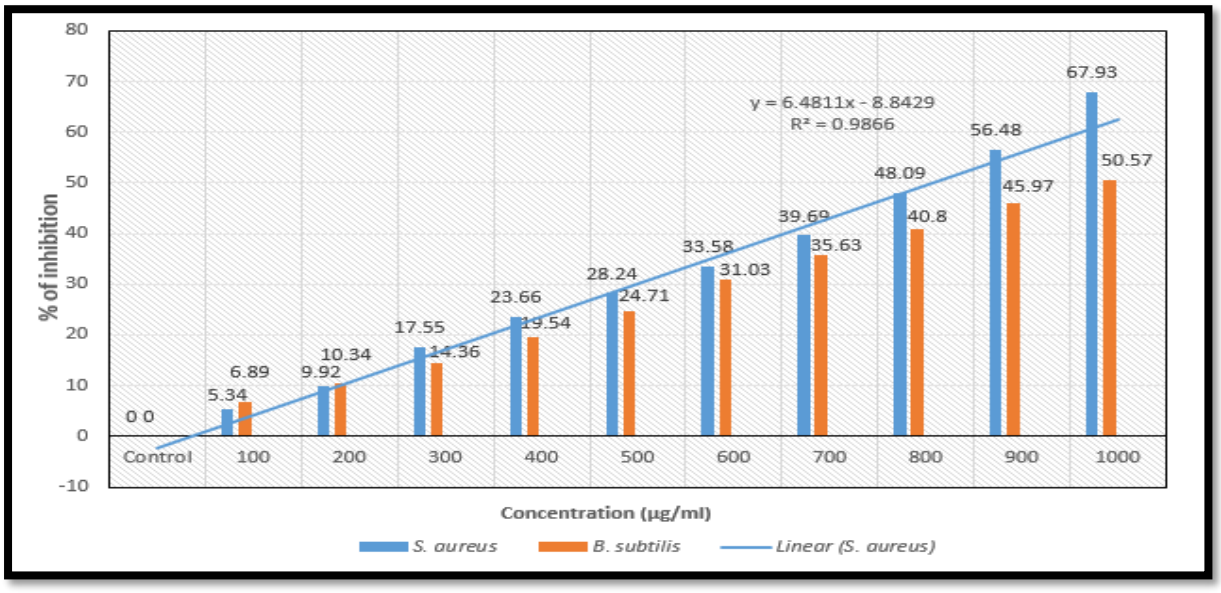

Fig 5: Antibacterial activity of silver nanoparticles against Stapylococcus aureus and Bacillus subtilis

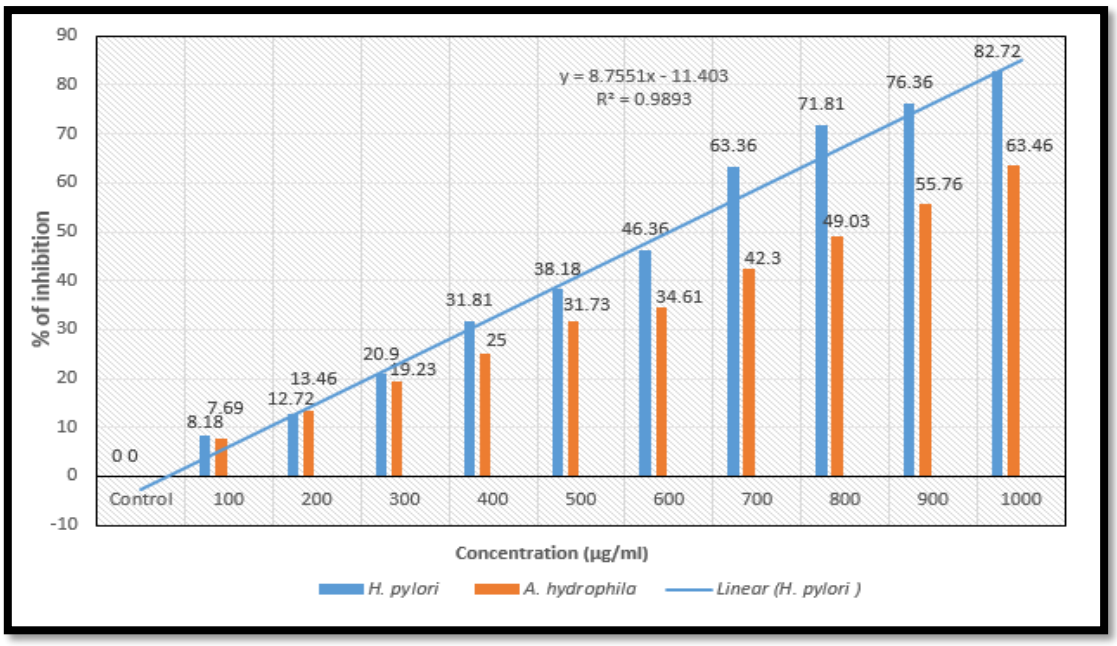

Fig 6: Antibacterial activity of silver nanoparticles against Helicobacter pylori and Aeromonas hydrophila

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Conflict of Interest:
The authors declare no conflict of interest.
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