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The effect of pH, Temperature on the green synthesis and biochemical activities of silver nanoparticles from *Lawsonia inermis* extract

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Abstract

Nanotechnology is an innovative technique which includes the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale. It covers the size range of 1 nm to 100 nm. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution. In this study, green synthesis of silver nanoparticles (AgNPs) from silver nitrate (AgNO3) conducted using lawsonia inermis leaves extract as reducing agent in pH and temperature different. The biosynthesized nanoparticles were characterized by UV-Vis at range 300 -800, the results shown an increase in the rate of formation of silver nanoparticles with increasing temperature, and there was monodispersive silver nanoparticles were obtained at pH equal to 9.Antioxidant activity of silver nanoparticles was tested by (DPPH) and was compared with standard ascorbic acid. The antioxidant test of the DPPH showed that these AgNPs could scavenge free radicals at different levels and a high-test inhibition percentage better than that of ascorbic acid , where found to be 72.57% at pH=9, 65.34% at pH=7 for AgNPs, methanolic extract showed antioxidant activity 59.67% as compared to standard ascorbic acid 62.67% , at a concentration of 100 µg/mL.

Key words: *Lawsonia inermis*, Silver nanoparticles, Antioxidant activity, Antibacterial activity.

1. INTRODUCTION

Nanotechnology is an innovative technique which includes the design, characterization, production, and application of structures, devices, and systems by controlling shape and size at the nanometer scale. It covers the size range of 1 nm to 100 nm [1]. Nanotechnology is nowadays an active domain for the synthesis of nanoparticles by using natural sources such as plant leaf, stem, bark and root for novel metals such as platinum, gold, and silver and is widely applied in biomedical applications such as diagnostic, imaging, drug delivery and therapeutics using metal nanoparticles [2]. “Nano” is derived from the Greek word “nanos”, meaning “dwarf, tiny, or very small” [3]. Nanoparticles are the materials with the overall size of 100 nm. Nanoparticles can be classified as polymeric (natural and synthetic), lipoidal (biodegradable), and metal nanoparticles (iron oxide, gold nanoparticles, and silver nanoparticles) [4]. Nanoparticles of noble metals have attracted immense interest incurring applications in catalysis, electronics, optics, environmental and biomedical applications due to their quantum confinement effects, antimicrobial activity and their large reactive surfaces[5]. Silver nanoparticles exhibit new or improved properties depending upon their size, morphology, and distribution. Various approaches using plant extract have been used for the synthesis of metal nanoparticles [6].

Nanoparticle synthesis methods can be classified as bottom-up and top-down. Chemical methods involve the reduction of chemicals [7], electrochemical procedures [8], and reduction of photochemicals. Plant-based synthesis of nanoparticles is in contrast faster, safer and lighter; works at low temperatures; and requires only modest and environmentally safe components [9]. Green synthesis of nanoparticles using plant extracts has several advantages over other environmentally green synthesis methods, because plants are broadly distributed, readily scalable, easily available, safe to handle and less expensive [10]. In this research, the methanolic extract of *lawsonia inermis* was used for rapid, simple and biosynthetic synthesis of silver nanoparticles. Lawsonia inermis (Henna) is a dwarf shrub belongs to Lythraceae family. *L. inermis* is generally used as traditional medicine worldwide to treat various diseases such as edema, bronchitis, rheumatism, small pox, spermatorrhoea, menstrual disorders and hemorrhoids [11]. *L. inermis* leaves consist of a diverse range of biomolecules, which formulate it as a rich source of different types of medicine [12]. Improvements in chemical composition, size, shape, and dispersity of nanoparticles would permit the use of nanobiotechnology in a variety of other applications [13]. The hydroxyl and carbonyl groups present in carbohydrates, flavonoids, terpenoids, and phenolic compounds are powerful reducing agents that may be responsible for the bio reduction of Ag+ ions necessary for AgNP synthesis [14].

Several characterization techniques for analysis, certain tabular data representing source, shape and size and information regarding various applications. Ag nanoparticles were characterized by UV-Vis Spectra and SEM techniques. Nowadays, the most important applications of silver nanoparticles in biotechnology science correspond to their antibacterial and antioxidant activities [15]. The antimicrobial activity of AgNPs is widely recognized, though their activity can change with physical characteristics of the nanoparticle, such as its shape, mass, size, and composition, and conditions of its synthesis, such as by pH, ions, and macromolecules. Their shapes can be relevant to their antibacterial activity [16]. In the past decades, nanotechnology has been used to prepare antioxidant products using minerals including silver, gold, cerium oxide and platinum [17].

The study aimed to synthesize silver nanoparticles using methanolic extract of *L. inermis* (2ml of $AgNO_3$: 0.4ml of extract). Moreover, to Characterize the prepared AgNPs using SEM, UV-Vis spectroscopy. Furthermore, studying and evaluating the antibacterial and antioxidant activity of silver nanoparticles.

2. EXPERIMENTAL

2.1 Collection of *L. inermis* Leaves

Lawsonia inermis ***leaves*** were collected from North West of the city of Nasiriyah in southern of Iraq in march, 2017. The Collected leaves were washed and dried in the shade at room temperature for 10 days, then crushed to obtain powder. The substance was kept in the fridge at 4 °C until use.

2.2 Preparation of *L. inermis* Leaves Extract

Five gram of *l. inermis* leaves powder was weighed and mixed in 100 ml of methanol and the mixture was heated for 10 minutes then filtered through Whattman filter paper No.1.The filtrate was collected and stored at 4˚C for further use.

2.3 Synthesis of silver nanoparticles AgNPs

For synthesis silver nanoparticles, 0.4 ml of *l. inermis* leaves extract was added to 2ml of 1 mM $AgNO_3$ solution in test tube with vigorous shaking for the bio reduction of Ag^+ ions. The color change indicated confirmation for the formation of silver nanoparticles.

Optimization factors

The Effect of pH

pH of the reaction mixture was maintained at 4, 7 and 9, respectively, by using (0.1 N) Hcl and (0.1 N) NaOH. The absorbance of the resulting solutions was measured spectrophotometrically.

The Effect of temperature

To study the effect of temperature on the synthesis of AgNPs, a typical sample was synthesized at 25 °C, 35 °C and 45 °C. Electronic absorption spectra of the aqueous colloidal suspensions were recorded at each temperature range.

2.4 UV-Visible spectrophotometer

The optical property of AgNPs and The reduction of pure Ag + ion were monitored by measuring the UV-Visible spectrum of the reaction mixture. UV-Vis spectral analysis was performed by using UV-Vis spectrophotometer UV-1700 (Shimadzu, Tokyo, Japan) that was operated in the scanning range of 300-800 nm.

2.5 Antibacterial Activity

Antibacterial activity of biologically synthesized AgNPs of *L. inermis* was determined by agar well diffusion method against different pathogenic microorganisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*. Muller Hinton Agar plates were used and swabbed with pathogenic organisms from fresh cultures (105-106 CFU/mL) using a sterile cotton swab. The plates were then incubated at 37 °C for 24 h. At the end of the incubation period, the zones of inhibition were measured to the nearest millimeter [18]. The inhibition zone is the area surrounding the hole with no growth of inoculated microorganisms.

2.6 Antioxidant Activity

The antioxidant activity of AgNPs was determined by DPPH assay method [19]. The capability to scavenge the DPPH radical was calculated using the following equation:

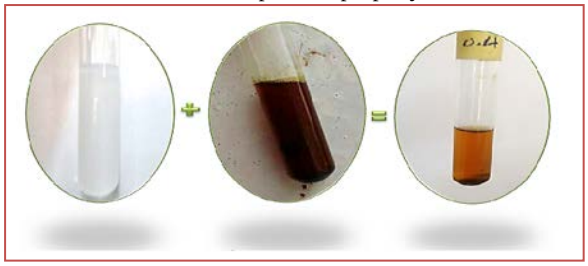
DPPH Scavenged (%) = $\left(\frac{Ac-As}{Ac}\right) x\ 100$

Where Ac and as are the absorbance of the control and test sample, respectively, after 30 min measuring at 517 nm.

3. Results And Discussion

3.1 Visual observation

The color of the *L. inermis* extract was dark brown before its treatment with silver nitrate solution, but after the reaction it turned to yellowish brown (Fig. 1), indicating the formation of Ag-NPs due to reduction of silver ions by active molecules present in the extract. This color is attributed to surface plasmon resonance, which is a size-dependent property of NPs [20].



Silver nitrate $AgNO_3$; L. inermis extract ; Silver nanoparticles AgNPs

Fig 1. Synthesis of silver nanoparticles by Visual observation indicator.

3.2 UV-Visible spectrophotometer

Monitoring the process of the bio reduction of silver ions to AgNPs was applied in this study by UV-vis spectroscopy. UV-vis spectroscopy might be used to detect the size and shape-controlled NP in aqueous suspensions [21]. UV-Vis spectroscopy was used to follow the reactions process and characterize the optical properties of produced nanoparticles. The formation and optimization of Ag NPs was monitored using UV–Vis spectroscopy by measuring the absorbance in the range of 300–800 nm, by varying the temperature and pH. UV–Vis absorption measurements in the range 300–800 nm can provide deep insight into the optical properties of the formed nanosized silver particles. The change in color indicates the formation of Ag NPs which was further confirmed by the appearance of the SPR band between 400 to 500 nm [22]. The UV-VIS Spectral analysis of the green synthesized nanoparticles was observed a sharp peak around 386 – 450 nm indicates the formation of silver nanoparticles, which was identified as “surface Plasmon resonance band” and this band is ascribed to excitation of valence electrons. The position of absorption band also mainly depends upon dielectric constant of the medium and surface-adsorbed species. The shape of the band was symmetrical, suggesting uniform scattering of spherical shape nanoparticles [23]. There are factors affecting the intensity absorption band and thus affect the synthesis of nanoparticles. This effect is explained as below:

The effect of temperature

The affect Temperature is one of the factors influencing the synthesis of silver nanoparticles. This was confirmed by studying the UV-Vis spectra shown from fig.2 at three different temperatures 25°C, 35°C and 45°C. Where we observe an increase in the rate of formation of silver nanoparticles with increasing temperature.

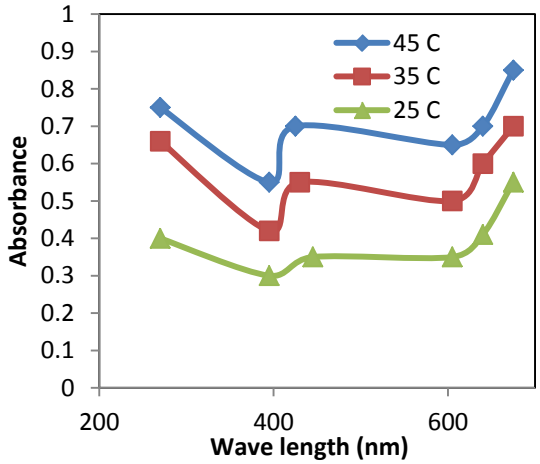


Fig. 2: The effect of temperature on wavelength and absorbance for AgNPs

UV spectra indicated that the wavelength was higher at lower temperature, but the wavelength shifted to a lower value at higher temperature resulting in the formation of smaller silver nanoparticles at higher temperature, whereas at higher wavelength the size of silver nanoparticles increased. The maximum absorbance was observed at 470, 452 and 431 nm at 25, 35 and 45 ^{O}C, respectively. It means at higher temperature, reactants are consumed rapidly, resulting in the formation of smaller nanoparticles [24].

The effect of pH

The effect of pH was studied in three different conditions including acidic, neutral and basic forms. The fig 3 shows the effect of changes in pH on UV-Vis spectra of silver nanoparticles synthesized in pH = 9.0, 7.0 and 4.0, respectively. The highest

color intensity of the reaction mixture was observed at pH 9.there was no reaction at a pH value equal to 3, but monodispersive silver nanoparticles were obtained at pH equal to 9[25].

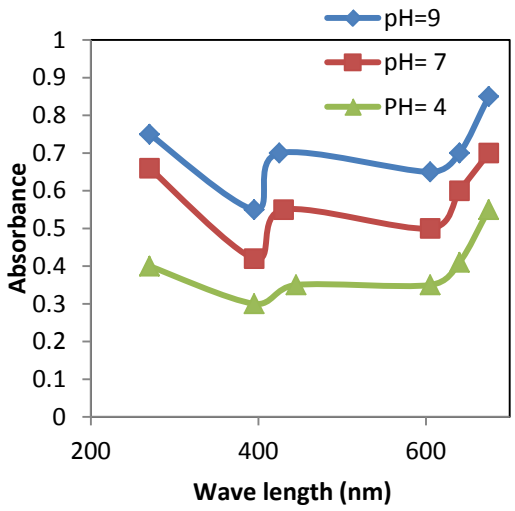


Fig.3: The effect of pH on wavelength and absorbance for AgNPs

3.3 DPPH radical-scavenging activity

In the present study, In order to determine the extent of scavenging effect, antioxidant activity of *L. inermis* extract and AgNPs were comparatively studied by DPPH method. The results were expressed as % RSA (radical scavenging activity) [26]. The DPPH reducing ability of the AgNPs was assessed by observing color change and the control does not show any color change , which produced violet color in methanol solution.it was reduced to yellow colored product, diphenyl picrylhaydrazine [27]. DPPH scavenging assay exhibited effective inhibition activity of AgNPs at pH=9 when compared with AgNPs at pH=7, extract and the standard Ascorbic acid. The antioxidant property of *L. inermis* lawsonia leaves extract was found to be less effective. Our silver particles have shown much better activity. AgNPs synthesized using *L. inermis* leaves extract have the highest recorded radical scavenging activity of .72.57% at pH=9, 65.34% at pH=7, methanolic extract showed antioxidant activity 59.67% as compared to standard ascorbic acid 62.67%, at a concentration of 100 µg/mL. As showing in fig.4

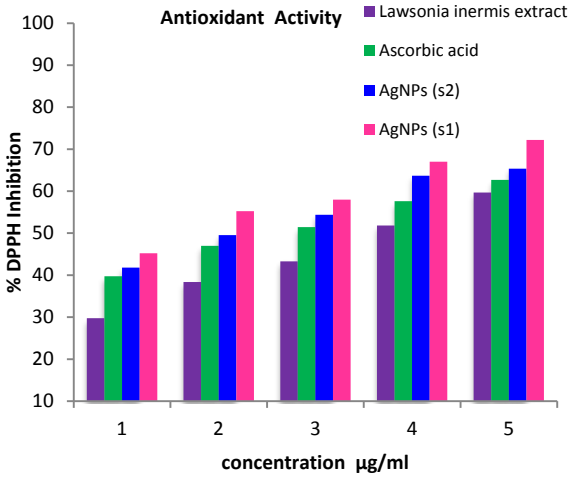


Fig.4 The free radical scavenging activity of AgNPs at different concentration when compared to the standard ascorbic acid and *L. inermis* extract.

3.4 Antibacterial Activity

The potential antibacterial activity of biologically synthesized AgNPs was detected and compared with the antibacterial activity of antibiotic (ciprofloxacin) because it is a strong antibiotic and showed good antibacterial activity against tested bacteria. However, the antibacterial activity was species-dependent. DMSO solvent was used because it showed no efficacy on all the bacteria strain used. The susceptibility of bacteria to plant extracts varied according to bacterial strains and species, which was well documented [28].

The disc diffusion method, a most commonly used technique to access the antimicrobial activity, has been employed by many researchers to confirm antibacterial action of the AgNPs solution. The results of AgNPs showed higher ability to suppress the microbial growth than methanolic extract. The maximum inhibition of AgNPs was recorded against *S. aureus* (22) mm, (20) mm for *E.coli*, (21) for *P. aeruginosa*, (17) mm for *K. pneumoniae* and (21) of *B. subtilis* for AgNPs, and in comparison with antibiotic (ciprofloxacin) showing synergistic effect, the Inhibition zones of ciprofloxacin antibiotic discs were 0,0,30,30,28,22 mm for *P. aeruginosa*, *E.coli*, *B. subtilis*, *S. aureus*, *K. pneumoniae* and *S. pneumoniae* respectively as showed in table 1.

Table1. The inhibitory ability of silver nanoparticles against bacteria strains. (Zone of inhibition)

Name of bacteria's	Bacteria strain	AgNPs	ciprofloxacin	DMSO
Klebsiella pneumoniae	Gram Negative	17	28	0
Pseudomonas aeruginosa	Gram Negative	21	0	0
Escherichia coli	Gram Negative	20	0	0
Staphylococcus aureus	Gram positive	22	30	0
Streptococcus pneumoniae	Gram positive	19	22	0
Bacillus subtilis	Gram positive	21	30	0

Generally, biologically synthesized AgNPs showed good antibacterial capability against Gram-positive than Gram-negative bacteria. The difference in the effect of the antibacterial is due to the difference in the structure of the cell wall, where the cell wall of the gram positive bacteria contains a single layer, while the cell wall of Gram-positive bacteria composed of a rigid thicker multiple layer of peptidoglycan, as it prevented the nanoparticles from entering into cell wall. The difference in the structure of the cell wall is due to its cell wall containing compounds such as lipopolysaccharides, lipoprotein and protein lipid for gram-negative bacteria while Gram-positive bacteria wall, have a lipid content that makes them more permeable and therefore more effectively to gram-negative bacteria [29].

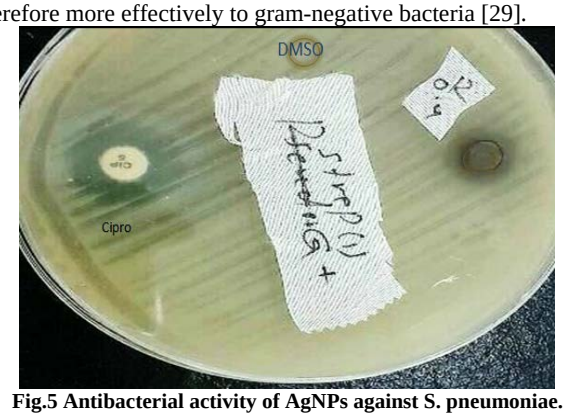


Fig.5 Antibacterial activity of AgNPs against S. pneumoniae.

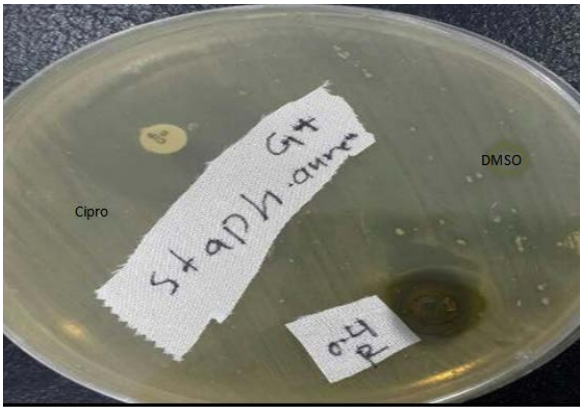


Fig. 6 Antibacterial activity of AgNPs against S . aureus.

Fig 7. Antibacterial activity of AgNPs against E.coli.

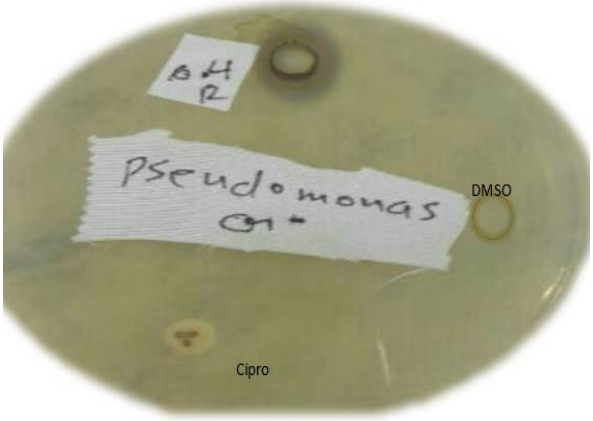


Fig.8 Antibacterial activity of AgNPs against P. aeruginosa.

Fig.9 Antibacterial activity of AgNPs against B. subtilis.

Fig. 10 Antibacterial activity of AgNPs against K. pneumoniae.

CONCLUSION:

The antibacterial activity was examined against six types of strain bacteria, Gram positive bacteria (*S. aureus, B. subtilis and S. pneumoniae*) and Gram negative bacteria (*E.coli ,P. aeruginosa and K. pneumoniae*) by using the agar disk diffusion method. The results showed that silver nanoparticles had a strong effect against Gram positive bacteria and it is more than Gram negative bacteria.

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