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№ 8585

Comparison Between Green Synthesis and Chemical Synthesis of Silver Nanoparticles: Characterization and Their Antimicrobial Activities

Mariam Ambaya, Faiza Saeed and Wanisa Mohammed

August 3, 2022

COMPARISON BETWEEN GREEN SYNTHESIS AND CHEMICAL SYNTHESIS OF SILVER NANOPARTICLES: CHARACTERIZATION AND THEIR ANTIMICROBIAL ACTIVITIES

ABSTRACT

*This study was conducted on the preparation of novel silver nanoparticles (AgNPs) by using chemical and green approaches. Sodium benzoate (SB) and O-*acetylsalicylic acid (AS) *are used as capping ligands in the chemical method. While the extract of Teucrium apollinis was used in the green method. Produced AgNPs were stable in room condition up to 3 months. The AgNPs were characterized via using UV-Visible absorption spectroscopy (UV-Vis), dynamic light scattering (DLS), and attenuated Fourier transform infrared (ATR-FTIR). This study confirms the ability of Teucrium apollinis to produce AgNPs with high stability compared with AgNPs stabilized by chemical ligands. The antibacterial activity of AgNPs against Pseudomonas aeruginosa was done as well. The stability of AgNPs were monitored at different pH values (2-12). The use of Teucrium apollinis extract as capping ligand could be good environmentally method to synthesize AgNP that offers a novel and possible alternative to chemically synthesized AgNPs.*

Keywords - Teucrium apollinis; Silver nanoparticles, antibacterial activities, O-acetylsalicylic acid, Aspirin, stability.

1. INTRODUCTION

Among metal nanoparticles, silver nanoparticles (AgNPs) are progressively used in numerous fields, such as medical, food, health care, consumer, and industrial purposes, as a result to their unique chemical and physical properties [1].

A wide range of antibacterial, antifungal, and antiviral activities can be found in silver nanoparticles. Invading bacterial cell walls with silver nanoparticles can alter the structure of cell membranes and even cause cell death. Their effectiveness is a result of both their enormous surface area to volume ratio and their nanoscale size. [2].

The selection of ligands is of utmost importance for the colloidal stability and function of the nanoparticles. Likewise, the selection of ligands utilized in nanoparticles synthesis can affect their final sizes and shapes as well as increasing their colloidal stability [3].

Significant research has shown that green synthesis is constrained by the time and location of production as well as problems with low purity and poor yield due to the complexity in geographical and seasonal distributions of plants and their compositions. Green synthesis, on the other hand, offers alternative development prospects and prospective uses while taking into account present environmental issues and pollution linked to chemical synthesis [4].

It is well mentioned that selecting suitable ligands is very important for either direct synthesis of NPs or modification of a NP surface. The nature and strength of bonding between the capping ligands and NP surface is critical factor to control of the sizes and stability of NPs. For example, carboxyl and hydroxyl groups offered a strong binding affinity for iron oxide NPs, however, thiols showed high affinity to gold surfaces based on the literature observation [3].

The biological activity of AgNPs depends on several factors including surface chemistry, shape, size, size distribution, particle morphology, particle composition, capping agent, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type. The type of reducing agents used for the synthesis of AgNPs are considered to be a crucial factor for the determination of cytotoxicity [1]. This study compared between biocompatible AgNPs dispersed in an aqueous solution and capped by chemical ligands and plant extract.

2. EXPERIMENTAL

2.1 Materials and Methods

All chemicals used in this work were purchased from Sigma-Aldrich, including Silver nitrate AgNO3, sodium borohydride (98.0%) (NaBH4), sodium benzoate, and o-acethylsalsalic acid.

2.2 Synthesis of Silver Nanoparticles by Chemical ligands

2.2.1 Synthesis of AgNPs functionalized by sodium benzoate and O-acetylsalicylic acid

Briefly, (0.0080 g, 0.055 mol) and (0.0054 g, 0.092 mol), from (SB) and (AS) respectively were dissolved in 20 ml of distilled distilled water. Next these mixtures of stabilized ligands were added to silver nitrate solution ($AgNO_3$ 0.003 g, 0.017 mol in 15 ml of distilled water) and left to stir for 2 hours at room temperature. When a reducing agent (prepared $NaBH_4$) (0.0025 g in 12 ml of distilled water) was gradually added to later mixed solutions, the colors of the solutions were changed from colorless to light brown., and yellowish-brown, respectively, confirmed to prduce AgNPs. To ensure that the reactions were complete, the mixtures were stirred for a further two hours. For the consequent characterizations, the samples were washed by (distilled H_2O/EtOH) (2:1) and centrifuging and re-dispersing three times in order to remove the residual chemicals. The samples were kept in accordance with standard laboratory procedures.

2.3 Green Synthesis of AgNP by using Extract of Teucrium apollinis

The aerial parts of Teucrium apollinis were collected from the Slop Mountains of Ras El-Hilal; Cyrenaica region, Libya, during the flowering stage in April 2021. The plant was identified by Mr. Abdossalam Elmogassapi, lecturer in the Department of Botany, the College of Science, University of Benghazi, Libya.

2.3.1 Teucrium apollinis extraction procedure

In brief, the aerial part of the plant was cleaned and dried in the shade. The dry plant was then pulverized into a soft powder. This powder was extracted with petroleum ether (40-60°C) *via* using Soxhlet. Then the solvent was evaporated using a rotary evaporator at 40°C to get the plant extract that used as a capping ligand in this study.

$AgNO_3$ (0.0050 g, 0.029 mol) was dissolved in 25 ml of distilled water. Then 0.0090 g of extract plant was added to the last mixture and left to stir for 2 hours. At this point in time, a light yellowish-green color appeared once 5 ml of the freshly prepared $NaBH_4$ (0.0017 in 15 ml of distilled water) was added step by step to the last mixture, the vigorous stirring was continued for an extra 2 hours as described in Figure 1.

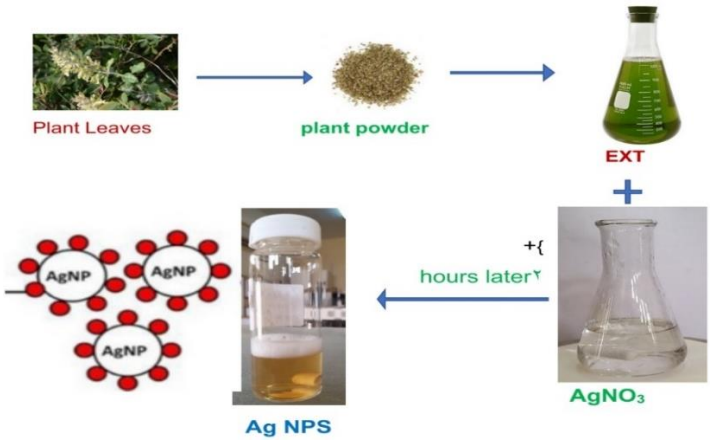

Figure 1- Preparation procedure of AgNP using an extract of Teucrium apollinis as a coating agent

3. RESULTS AND DISCUSSION

Current study illustrates synthesis of AgNP via reduction of aqueous Fe2(SO4)3 by freshly aqueous $NaBH_4$ in the presence various coating agents. The formation of AgNPs was indicated by the appearance of yellowish-brown colour which was usually have wavelength at 350-450 cm^{-1} in the UV-Vis spectra [5].

3.1.1 Study of the Iron Oxide Nanoparticle's Stability

One of the important factors of usability of metal nanoparticles is their stability in time. In order to evaluate the long-term stability of AgNP functionalized by SA, AS, and extract Teucrium apollinis. AgNPs in the presence of these stabilizing agents are shown if Figure 2. Absorption spectra were recorded for up to 12 weeks after synthesis. The intensity of plasmon resonance bands is increased without shift of its position, indicating to big sizes are produced after about 10 weeks, and no evidence for any aggregation may can happened. It can be seen that SB-AgNP has absorbance a 398 nm at initial time which changed to 402 nm after 10 weeks. According to Czechowska-Biskup R et al., changes in the values of absorbance predicts that reduction process still takes place in tested systems throughout the storage period [6].

The same notes were taken for AS-AgNP and Extract-AgNP, where the wavelength band of ASNPs showed a peak at 401 nm (Figure 2, curve B), which shifted to 408 nm after 11 weeks. This peak became a slight broader, but there is no indication for any aggregation may happen. The shifting of the λmax value towards higher wavelengths with an increase in time shows the formation of agglomeration of smaller particles and then larger particles are produced based on supplementary studies [7]. However, the AgNP functionalized by Teucrium extract (see Figure.2, curve C) showed the biggest size in this work, because of its chemical composition, which contains phenols, flavonoids, and alkaloids [8]. While the stability continues until more than 12 weeks. Herein, there are not highly difference between the stability of all AgNPs synthesized in this study. Otherwise, AgNP synthesized by green methods is preferred as co-friendly environment compared with chemically AgNPs. These observed results confirm that ligands types play significant role to control the sizes, shapes, and stability of metal nanoparticles [9].

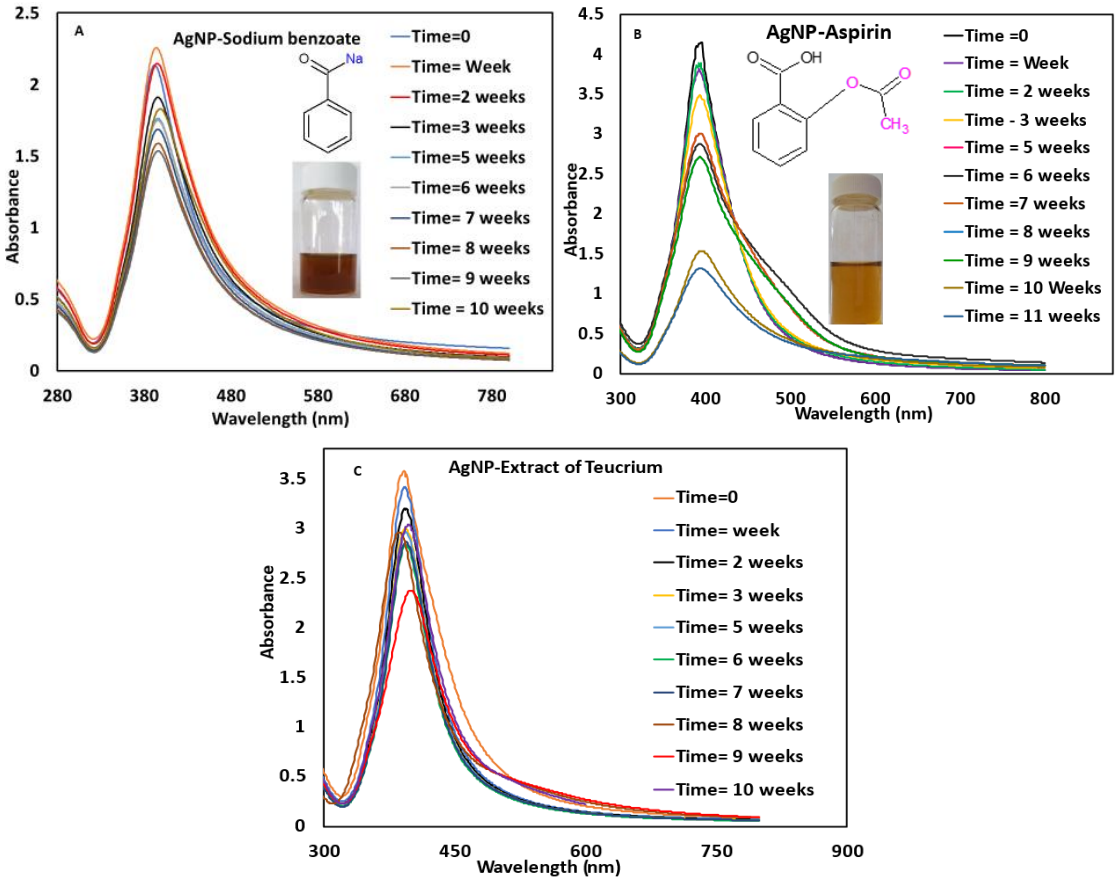

Figure 2- UV-Vis spectra of colloidal AgNPs over a period of 11 weeks. (A= SB-AgNP), (B= AS-AgNP) and (C=extract-AgNP). The peaks of absorption were at a wavelength of 398 nm, 401 nm, and 409 nm respectively at the initial time (time = 0)

3.2 Dynamic Light Scattering (DLS) Analysis

Highly compatible stable AgNPs are produced with narrow size distributions, which were in the range of (9.5-12.5 nm, 12-16.2 nm, and 15.2-20.1 nm) accordioning on the stabilized ligand SB, AS, and extract of teucrium apollinis used, respectively. AgNP stabilized by O-acetylsalicylic acid (aspirin) displayed bigger size than AgNP stabilized by sodium benzoate may be due to present acetyl function group The acetyl is known as a withdrawing group. This withdrawing group decreases electron density on COOH causing increases the electrophilic character of the carbonyl carbon, The electron-withdrawing groups have inductive and resonance effects with benzene ring. Generally, these effects cause to increase the acidity of the carboxylic acid. Acetyl group in ortho-position has strong effect which called (*ortho*-effect), and that due to a combination of both steric and electronic factors and then reducing an affinity toward surface of nanoparticles as a similar thought was made by previous reports [10-12].

The results of DLS of all AgNPs and UV-Vis have confirmed each other in this study where AgNPs remained monodisperse and stable event after 3months from prepared time. The size of the AgNPs increases when stabalized by extract plant ligand because of present phenolic compounds. This confirms that ligands are attributed the effect of modifying the growth phase as a similar observation was made by earlier reports [13].

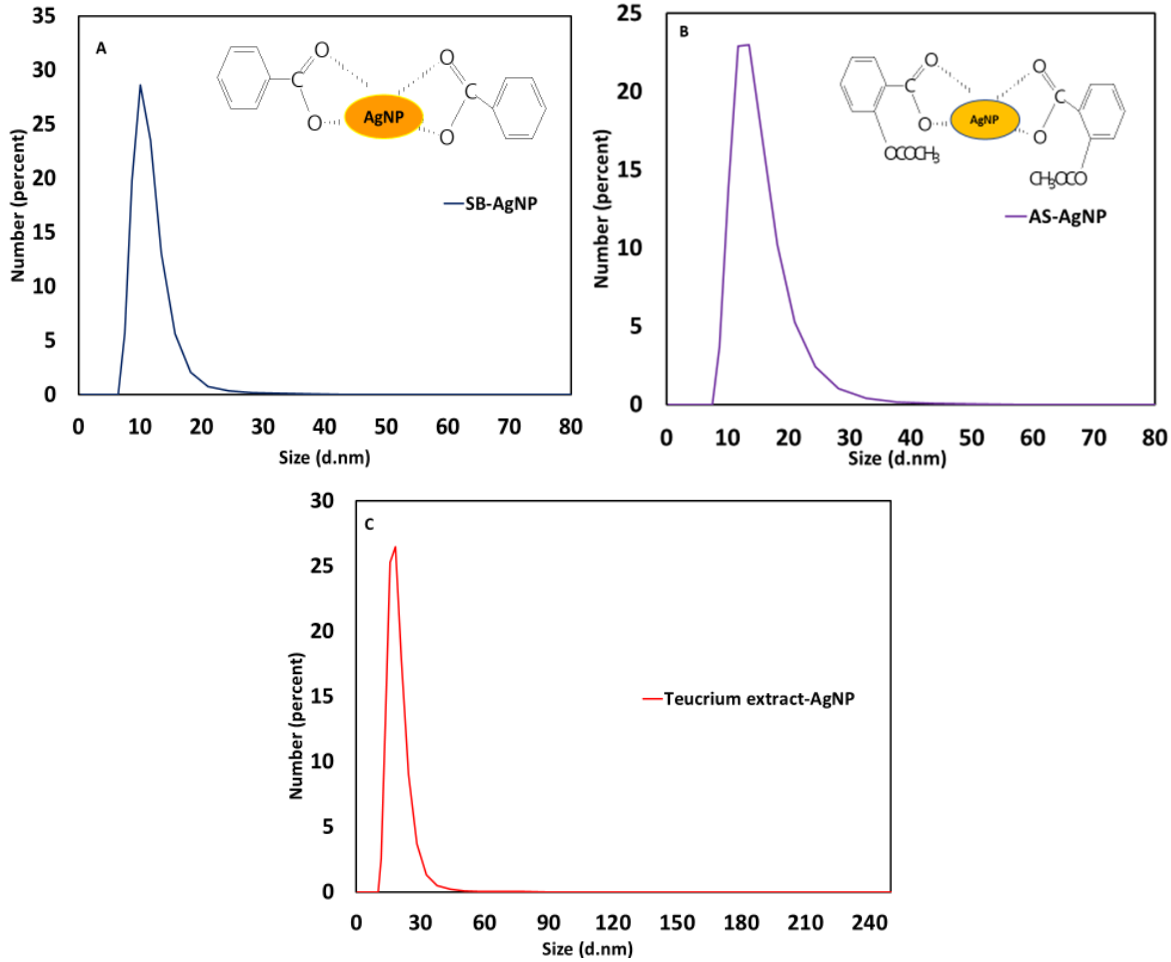

Figure 3- DLS diagrams of AgNPs functionalized by SB, AS, and extract of teucrium apollinis (A, B, and C) respectively. The sizes of AgNPs where A= SB-AgNP 11± 1.2 nm, B = AB-AgNP 14 ± 1.9, and C = Teucrium apollinis extract-AgNP 18 ± 2.7 nm

3.4 Attenuated total reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR)

Figure 4 (A to C) shows the ATR-FTIR spectra of AgNPs functionalized by SB, AS, and Teucrium apollinis extract and their comparison with these ligands, respectively. The first curve (black line) refers to AgNP. Whereas the second curve (red line) illustrates the IR bands of pure stabilized ligands. It is confirmed that ligands contain carboxyl groups were adsorbed onto the nanoparticle surface through its carboxyl groups [13]. From Figure 4(A), the band at 1720 cm^{-1} could be attributed to the C=O stretching in SB ligand which shifted to 1690 cm^{-1} in the case of SB-AgNP.

In addition, the (C=O) peaks for *O*-acetylsalcylic acid appeared at 1643 cm^{-1} and 1620 cm^{-1}, for (OCOCH3) and (COOH) respectively. The (C=O) peaks in COOH is disappeared in SA-AgNP sample, confirm interaction between SA ligand and surface of AgNP. The peak at 1604 cm^{-1} in this ligand indicated (C-O), which shifted toward 1526 cm^{-1} in AS-AgNP. The peaks at 3464 cm^{-1} and 3364 cm^{-1} in *p*-aminobenzoic acid are due to the (NH_2) group. The OH group have a range from 3248 cm^{-1} to 3595 cm^{-1} in the case of the AS ligand, which also shifted after functionalized AgNP to the range from 3200 cm^{-1} to 3564 cm^{-1}.

Additionally, the (C=O) peak for *Teucrium apollinis* appeared at 1705cm^{-1}, which shifted to 1650 cm^{-1} in the case of AgNO functionalized by this ligand. The peak at 2917 cm^{-1} in the *Teucrium apollinis* extact curve is due to an aromatic ring. This peak has been shifted to 3116 cm^{-1} in AgNP sample functionalized by *Teucrium apollinis*, indicating the interaction between them. The broad peak in the range of 3100–3500 cm^{-1} in both the AgNPs curve spectra indicates the presence of –OH groups as shown in Figure 4(curve C).

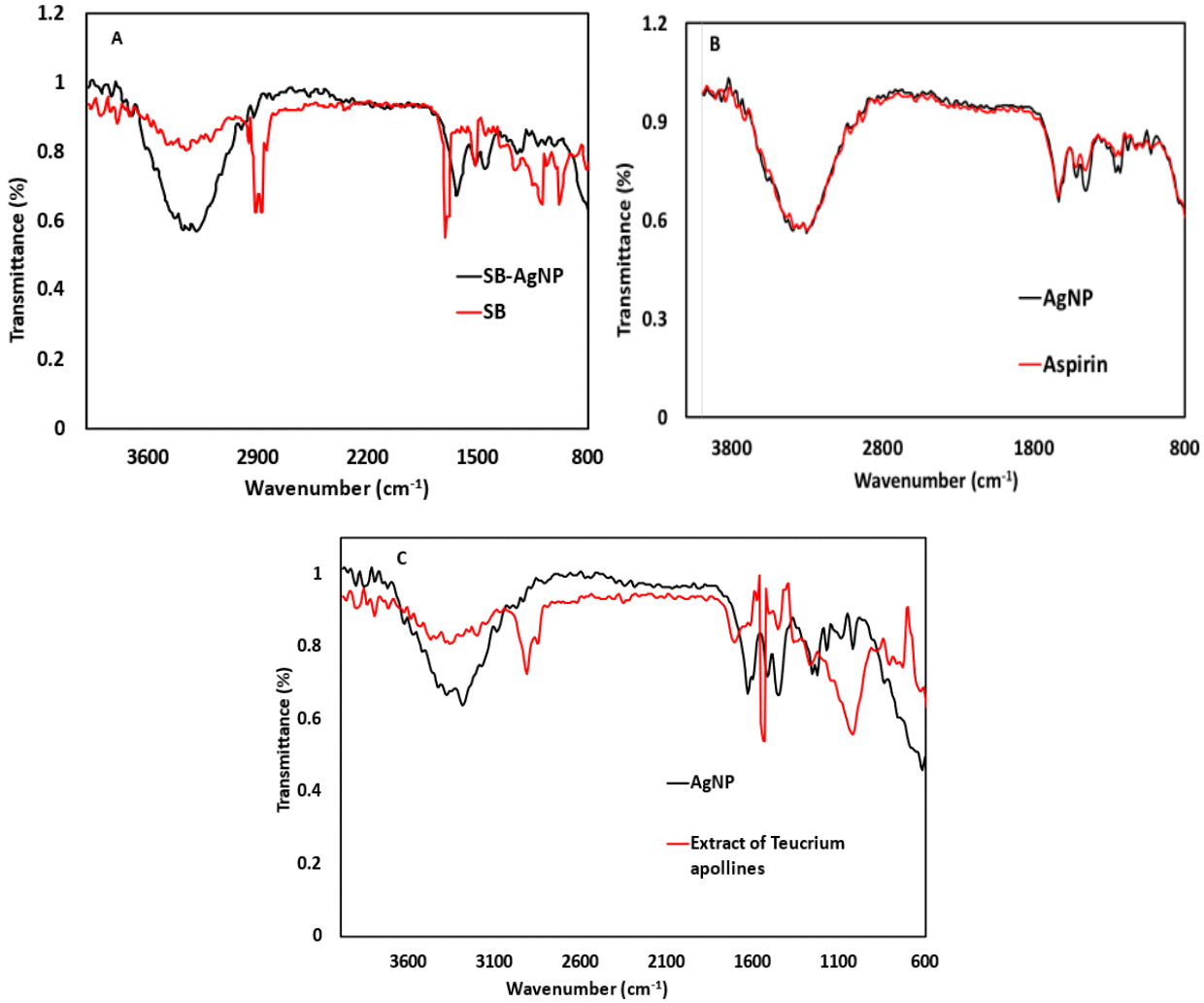

Figure 4 – ATR-FTIR of AgNPs stabilized by (SB (A)), (AS (B)), and extract of Teucrium (C) respectively

2.3. pH stability study of AgNPs

According to previous literature, it was found that the addition of acid and base to the AgNPs can be changing the ionic strength of the dispersion. For example AgNPs were not stable in dispersion with a high concentration (above 0.1M) of a NaCl added and instantly lead to the aggregation [14].

The role of pH in the green synthesis of AgNPs is investigated. The effect of HCl or NaOH addition on the nature of extract Teucrium apollinis-AgNPs is systematically studied as shown in Figure 5 (A, B). The aqueous solution of AgNPs prepared at pH 7-8 values. However, with pH changing The AgNP displayed different surface plasmon resonance (SPR) behavior. This is because to change size and size distribution of AgNP and that supported earlier findings [7,15].

2.3.1. Study of the stability of Teucrium apollinis-AgNPs- in acid and basic conditions of 1M HCl and NaOH

The size of the silver nanoparticles was controlled by changing the pH values of the reaction system. At moderate pH (7-8), smaller size silver nanoparticles were obtained compared to low and high pH values which confirmed via absorbance of the UV result. For instance, at pH 8, the Teacrium extract-AgNP has surface plasmon peak at a wavelength of 409 nm. However, it is increased to 422 nm when the pH increased to 12. The same change was shown at low pH (4-2) value. This difference can be attributed to the difference in the chemical and physical properties of AgNPs [7].

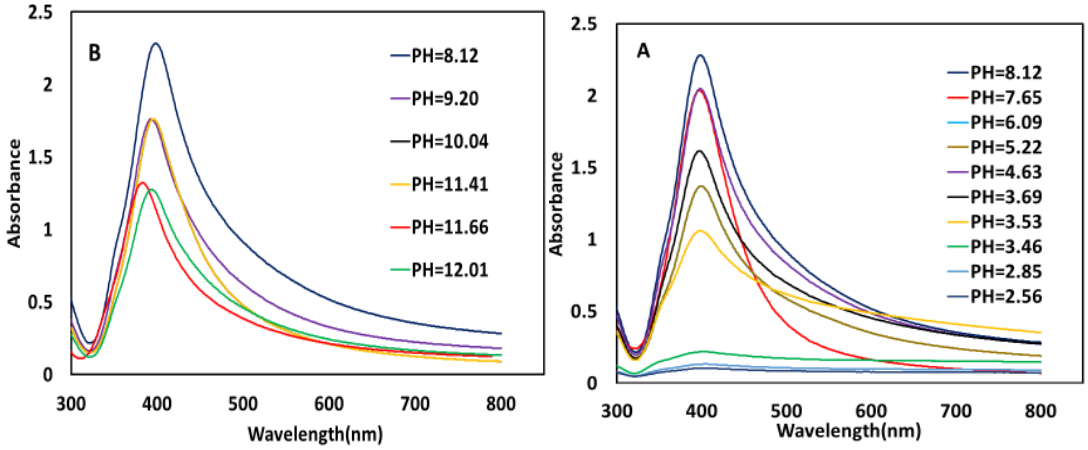

Figure 5- Data collected through UV-Vis across the pH range produced via adding HCl to the Teucrium apollinis - AgNP solution, pH < 7.5, where the HCl additions were from (5-30 µL). and that the peak of the absorption shift towards 409 nm as will describe in detail in the following sections

2.4 Antibacterial activities of Silver Nanoparticles

Nanoparticles have been widely used today, especially in the latest developed science materials. Silver nanoparticles, one of the most popular antimicrobial materials had been generally utilized in the textile industry and medical engineering [1].

Pseudomonas aeruginosa is considered to be the primary causative agent of the majority of babies born infections which leads to significant therapeutic challenges to treatment of these infections [16]. This kind of bacteria was taken as a model in this study as a result to its prevalence and significance in human diseases [17].

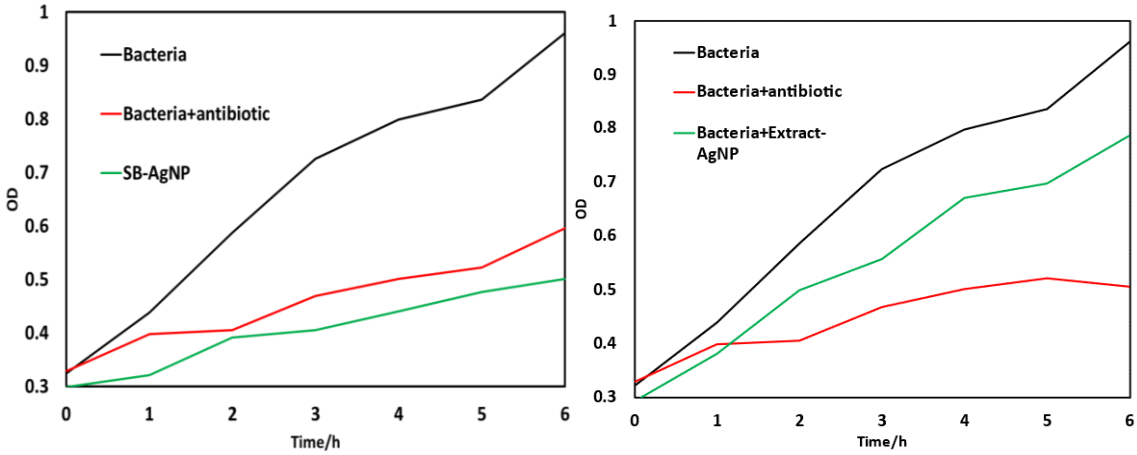

Figure 6- Shows growth curves of pseudomonas aeruginosa bacteria treated with (left = SB-AgNP), and (right = Teucrium extract-AgNP) with antibiotic imipenem10 ug (green curve) at various times in comparison to IO-NPs (P < 0.05). The control sample (black curve) is bacteria without NPs and is used as a reference

In this work, the bioactivity of AgNPs against Pseudomonas aeruginosa bacterial strains was investigated. The technique was carried out in accordance with a predetermined process [18].The samples were incubated for six hours at 37°C. The optical density at 600 nm (OD600) of the samples was evaluated at various time intervals of incubation from 0,1,2,3,4,5, 6 hours by using a UV-Vis spectrophotometer (Jenway model 6305, UK). The antibiotic (imipenem10µg) is used to be compared with IO-NPs samples. The GRAPHPAD PRISM V5.00 software (the ANOVA test ($p < 0.05$) is used to analyze the produced data. Figure 8 displays the antibacterial activity of the produced AgNPs against multi-drug resistant pseudomonas aeruginosa. The bacteria persisted in growth in the presence of SB-AgNPs (1-2 h) at the start of incubation. However, after two hours, this growth significantly reduced (3-6 hours).

In contrast, AgNP showed a significant effect when extract of Teucrium was used as capping agent (see Figure 6, right curve). The OD data in the case of Teucrium-AgNP illustrates inhibition growth of pseudomonas aeruginosa bacteria through incubation (2-6 h). This is because of phenolic compounds that present in the Teucrium extraction which offer high activity against the bacteria compared with chemical ligands that contain carboxyl groups. Likewise, as a result to its high affinity toward bacterial cells, so it can easier interact with the membrane of bacterial cells and inhibit them compared with previous data [19]. With regards to the bioactivity of green synthesized Ag NPs, AgNPs offered higher effective and microbial growth inhibitor against pseudomonas aeruginosa bacteria than chemically AgNPs [19].

4. CONCLUSION

AgNPs in this work prepared with high stability without showing any signs of decomposition, such as particle growth or loss of stability. AgNPs were produced by reduction of Ag (I) to Ag (0) using $NaBH_4$ and functionalized by compounds containing carboxylate groups in their structures. Also, comparison with green synthesis were taken in consideration in this research. These ligands and plant extraction are indeed provided the AgNPs with excellent stability, which is evidenced by using UV-

Vis and DLS results. The effect of the pH (2-12) on the produced *Teucrium apollinis*-AgNP was carry out as well. The stability observation for 12 weeks at normal laboratory conditions displayed that the AgNPs were highly stable with these ligands and no remarkable shift of the maximum peak was noted. AgNP functionalized by extract plant showed higher bioactivity than those functionalized by chemical ligands. But generally, all these AgNPs showed a promising application in the development of antibacterial products which predicts to be an appropriate choice in medical fields in the future. For this reason, synthesis of monodispersed and size controlled of AgNPs is consequently required.

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