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Biological and Chemical Research (ISSN 2312-0088), Vol.6, 96-103 | Science Signpost Publishing

Synthesis and Antimicrobial Applications of Silver Nanoparticles From *artemisia absinthium plant*

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Received: April 04, 2018 / Accepted: May 02, 2018 / Published: June 25, 2019

Abstract: The widespread use of nano materials leads to more interest in the methods used to obtain these materials. In this study, silver nanoparticles (AgNPs) were synthesized using the extract obtained from the green shells of artemisia absinthium in the case of waste. Reaction solution color change and UV-vis spectra confirmed reduction of silver ions to generate silver atoms. During the synthesis process, functional groups that participated in the reduction and were responsible for the reduction were examined by FTIR (Fourier transform infrared spectroscopy) analysis. SEM-EDX analyzes showed that AgNPs had a global appearance and had a large silver element composition. The XRD results showed a 14.58 nm Crystalline size and the TGA-DTA analysis showed the degradation temperatures of AgNPs. These nanoparticles have been shown to show effective anti-microbial activity of the AgNPs on the gram positive *Staphylococcus aureus*, gram negative *Escherichia coli* and *Candida albicans* yeast. As a resultThe silver nanoparticles (AgNP) showed relatively higher antibacterial activity.

1. Introduction

Nanobiotechnology examines the size, shape and distribution of biological materials with a scale of 1-100 nm nano [1]. AgNPs can be synthesized using physical, chemical and biological methods. However, other methods are more dangerous than biological methods because they contain more toxic chemicals. [2]. Various biological resources are used for biological methods. Plant, alga, virus, bacteria, etc.[1], [3]. Nano particles are used in cosmetic, biomedical, anti-cancer and anti-microbial agents in many different fields including food industry, cleaning materials, bioremidation of wastes. [4], [5], [6].

Today, antibiotic resistance is a serious problem every day. The anti-microbial effect of silver has been known for many years. At this point, AgNPs will contribute greatly to the search for anti-microbial agents. [7]. The fact that plant resources are easy to obtain and cheap makes synthesis with this method more attractive. [8],[9]. Phytochemicals in plant extracts are responsible for reducing Ag^{+} form to Ag^{o} form. Phytochemicals also provide stability during synthesis [10].

In the light of this information, in this study, we have successfully synthesized AgNPs and examined their anti-microbial effects by using a cheap, eco-friendly and simple method of ***artemisia absinthium***.

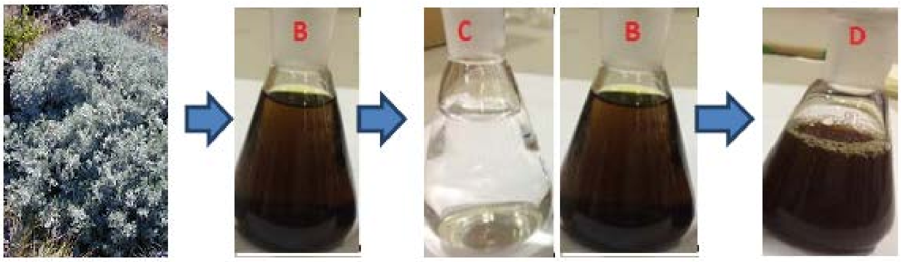

Figure 1. Synthesis of AgNPs using plant extract

A. Green shells of ***artemisia absinthium***, **B.** Extract from green shells, **C.** 1 mM Silver Nitrate solution, **D.** After mixing with C and B (after synthesis)

2. Materials and Methods

2.1. Preparation of extracts and silver nitrate ($AgNO_3$) from green shells of *artemisia absinthium*

Green ***artemisia absinthium*** shells were extracted from hard-shelled walnuts. Washing was done with tap water and then with distilled water. It was allowed to dry at room temperature. After drying, the shells were reduced in size. 20 g of ***artemisia absinthium*** shell were mixed with 500 ml of distilled water and boiled. Cooled in room conditions. First the filter was filtered through a filter paper and then with whatman filter paper. 1 mM aqueous solution of Sigma-aldrich brand 99.8% purity solid (silver nitrate) $AgNO_3$ was prepared.

2.2. Synthesis and Characterization

125 ml of the extract and 500 ml of $AgNO_3$ solution were mixed in 1000 ml erlenmayer and the color change occurred within 40 minutes. Perkin elmer one UV Visible spectrophotometer was used to determine the formation and presence of AgNPs. FTIR analysis was performed with Perkin Elmer Spectrum One and functional groups involved in reduction were evaluated. After centrifugation at 10,000 rpm for 10 min with OHAUS FC 5706, the particles obtained were dried at 75 ^{o}C. RadB-DMAX II computer-controlled X-ray diffractometer, scanning electron microscopy EVO 40 LEQ and SEM-EDAX, the morphology and element composition of the particles were analyzed. The crystal structure and size of the particles by the Model XRD analysis, and the decomposition temperatures of the particles by TGA-DTA analyzes using the Shimadzu TGA-50 device were investigated.

2.3. Determination of Antimicrobial Effects of Silver Nanoparticles

The anti-microbial effects of the particles were evaluated using gram negative Escherichia coli ATCC 25922, pathogenic microorganisms, gram positive Staphylococcus aureus ATCC 29213 bacteria and Candida albicans yeast. The minimum inhibitory concentration (MIC) was determined by microdilution method. Microplate wells were assayed after incubation at 37 ° C by adding a mixture of microorganisms adjusted to the Mc Farland standard 0.5 concentration at appropriate concentrations from the muller Hilton medium, AgNP solution. To compare the activity of AgNPs, vancomycin was used for S.aureus, colistin and flucanous antibiotics were used for E.coli and C. albicans. A 1 mM $AgNO_3$ aqueous solution was again applied for comparison.

3. Findings and Discussion

After changing the extract and 1 mM $AgNO_3$ solution, the resulting color change was observed. This shows the peaks having a maximum absorbance of 449 nm, indicating that AgNPs occur with vibrations on the plasma surface with a dark brown color change in 40 min.[11], [12]. Figure 2. UV-Vis. It contains the data of the analysis.

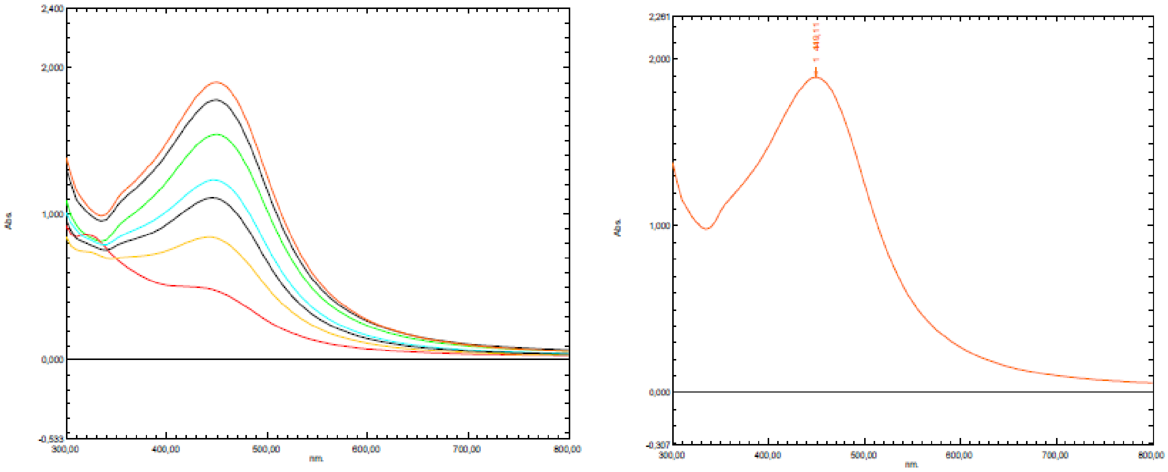

Figure 2. UV-Vis. Analysis Result Data

In FTIR analysis, functional groups responsible for reduction in the synthesis of AgNPs were investigated. The shifts of 3332, 2127 and 1635 cm-1 suggest that the groups -OH, -CN and C = O, respectively, are involved in the reduction (Figure 3). Similar functional groups were evaluated in the studies for the synthesis of AgNPs. In similar studies, functional groups were evaluated. [13], [14].

A B

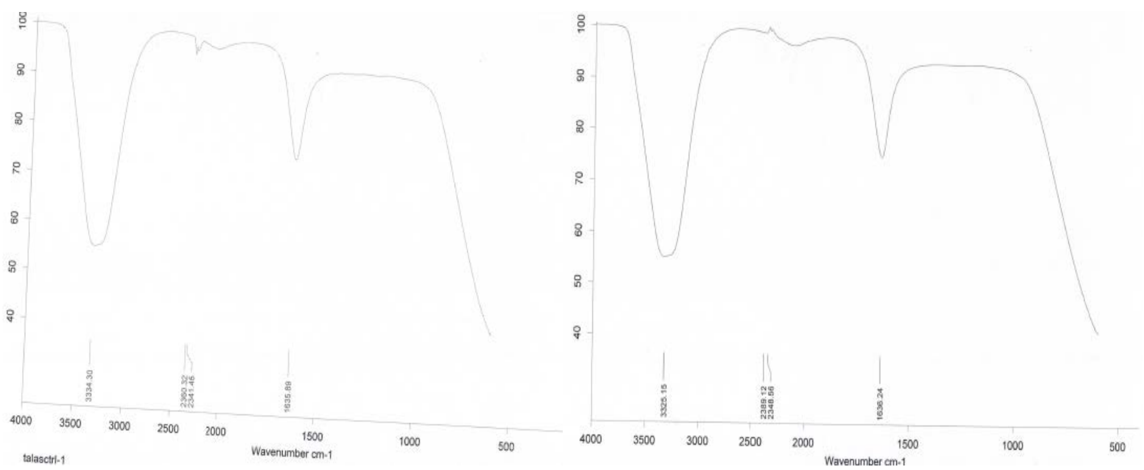

Figure 3. With FTIR Analysis Result Data; A. Shell extract, B. Evaluation of functional groups after synthesis.

In the XRD results, the values of the peaks at 111°, 200°, 220° and 311° in 2θ, were determined as 37.97, 44.11, 64.36, and 77.22 respectively, indicating the characteristic structure of the silver of the peaks of these values (Figure 4). Similar researches have said that these peaks belong to silver. [15],[16]. The crystal size of the obtained AgNPs was calculated using the Debye-Scherrer equation and was found to be 14.58 nm.

$$D = K\lambda / (\beta \cos\theta)$$

In similar studies, size calculation was made using the same formula [17],[10].

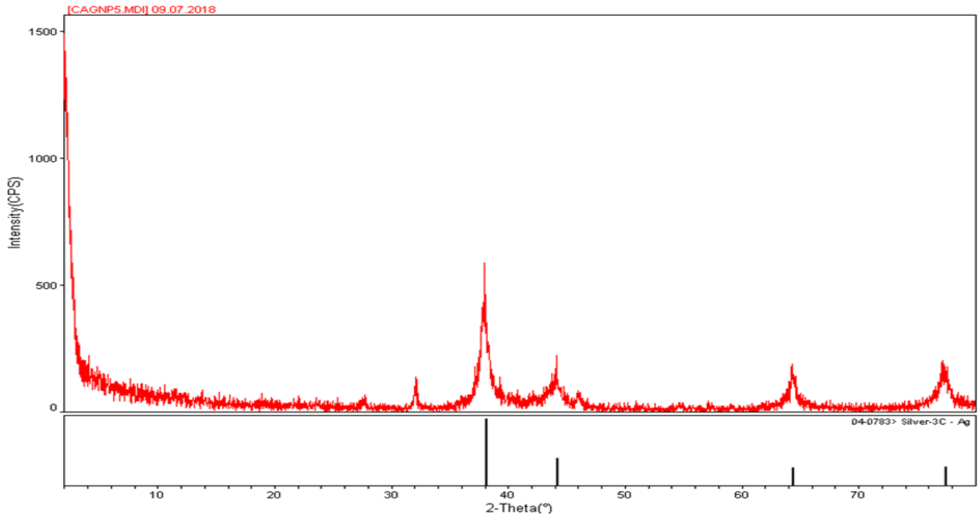

Figure 4. Investigation of crystal structure and silver phases of AgNPs by XRD analysis.

The SEM-EDX analysis results show that the AgNPs are in a global appearance, while the EDX results show that the composition of the silver element is high. In a similar study, it was said that AgNPs were in spherical appearance. [17]. n another study, the peaks of the EDX silver were evaluated [18].

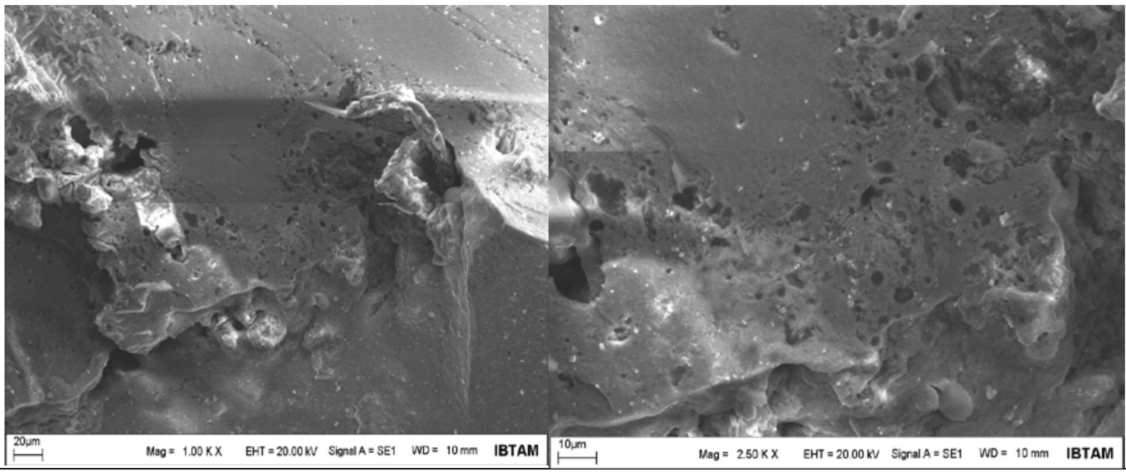

Figure 5. Evaluation of morphology of AgNPs in SEM results.

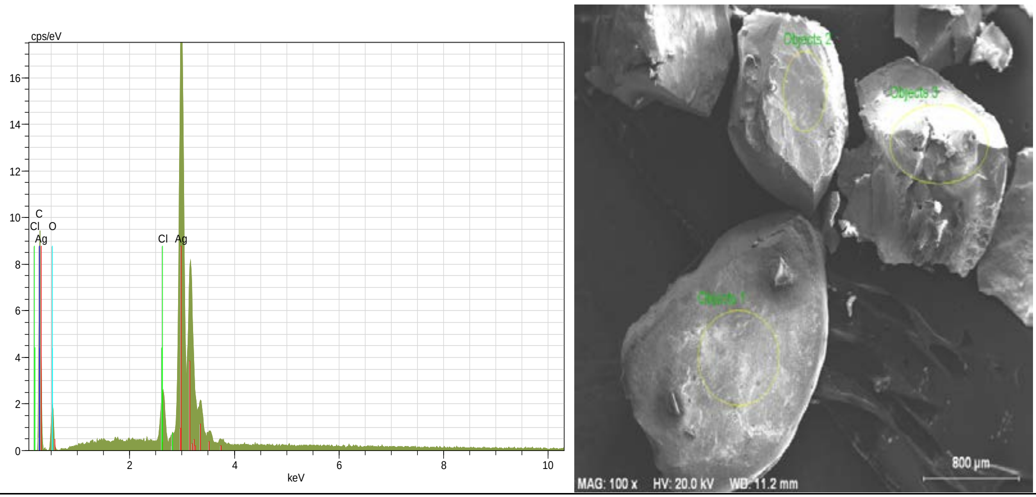

Figure 6. Analysis of the elemental composition by the EDX analysis of AgNPs

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed in an inert N_2 atmosphere from 25 ^oC to 1000 ^oC with a constant heating rate of 10 ^oC. minute^{-1} (Fig. 6.) [19] When TGA and DTA curve is examined, it indicates mass loss of AgNPs against temperature(fig 7.) [20] [21].

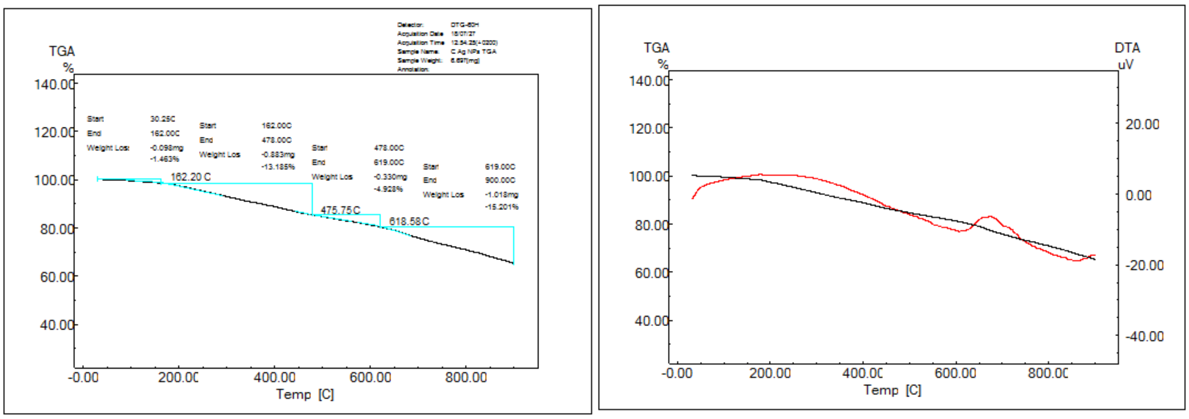

Figure 7. TGA and DTA curve of biosynthesized AgNPs.

The step from 30 to 162 ^oC in the TGA curve of the sample indicates the evaporation of free water; the sample contained 1.46% weigth of water. The two different steps from 162 to 478 ^oC the sample contained 4.92 % phenolic compounds in plant extracts and from 478 to 900 ^oC in the TGA curve refers to the decomposition of organic stabilizing agents that cover the fine surface of nanoparticles; the quantity of organic materials was 15.20 %. It is possible to decrease the organic materials by washing the sample further with distilled water. The total weight loss of the analyzed AgNPs was 25.92 %, which showed that the metallic core (nanoparticles) was surrounded by biomolecules. Metallic silver was approximately 74.08%. It appears to be compatible with similar studies [22].

The anti-microbial effects of AgNPs are becoming more and more important as microorganisms develop resistance to the antibiotics used. AgNPs obtained in our study showed significant anti-microbial effect at lower concentrations compared to silver nitrate and antibiotics on the organisms used. Other studies have provided close values for MIC [23], [24].

Table 1. MIC values of synthesized silver nanoparticles (AgNPs) (mg mL-1), silver nitrate solution and vancomycin, fluconazole, colistin antibiotics on S.aures, S. Albicans and E. coli microorganisms.

ORGANISM	AgNPs	Silver Nitrat	Antibiotic
S. aureus **ATCC 29213**	0.15	0.50	0.50
C. albicans	0.03	0.50	0.50
E. coli **ATCC25922**	0.05	1.00	0.12

4. Result

It is becoming more and more of a focus of interest every day due to reasons such as synthesis with biological resources that include eco-friendly synthesis methods, ease of processing, cheapness of the material used for extracting and lack of toxic chemicals in the synthesis process. We have easily synthesized AgNPs with the extract obtained from the green peel of the ***artemisia absinthium***. AgNPs obtained by environmentally friendly method have the maximum absorbance of 449 nm, X-ray results have 14.58 crystal dimensions and SEM-EDX results have a global appearance and a large amount of silver element composition. Compared with antibiotics used and $AgNO_3$ solution were found to be more effective. This material method can be further developed and used in the medical industry.

Considering that the usage areas of AgNPs are quite high, the product obtained can be used in many areas such as coating industry, cosmetics, extending the shelf life of foods and removing waste.

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