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Biosynthesis of Silver Nanoparticles using *Ajuca ive* Leaf Extract and Assessment of their Activity on E. coli and Streptococcus bacteria

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Abstract

Silver nanoparticle synthesis from plant extracts has been widely used in medicine, particularly as an antibacterial agent. In the present study, *Ajuca ive* leaf extract was performed using an aqueous solution tested for its phytochemical components. The results of the phytochemical analysis of *Ajuca ive* leaf extract had alkaloids, carbohydrates, proteins, flavonoids, phenols, saponins, and coumarins. Synthesis of silver nanoparticles was performed using *Ajuca ive* leaf extract with a 1 mM solution of silver nitrate. The synthesized silver nanoparticles were characterized with UV-visible spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), and Fourier Transform Infra-Red (FTIR) spectrum. SEM analysis revealed the size of the AgNPs of 36 nm, 55 nm, 70 nm, 100, and 300 nm. The EDX study showed that the optical absorption peak was detected at 3 keV, the characteristic peak for the absorbed metallic silver nanoparticles. FTIR analysis identified the possible functional group involved in reducing silver metal ions into silver nanoparticles. In addition, the antibacterial activity of synthesized

silver nanoparticles was also examined and the results showed good antibacterial activities against *E. coli* and *Streptococcus* bacteria.

Keywords: *Ajuca ive* Leaf, Silver Nanoparticles, Antibacterial Activity, E. coli

Introduction

Nanoparticles (NPs) and nanotechnology have attracted the scientific community's attention worldwide. They have emerged as a fast-developing and fascinating area of research. Nanoparticles (NPs) are the most fundamental component in the fabrication of nanostructures and are generally defined as particular matter with at least one dimension that is less than 100 nm or its size ranges from 1 to 100 nm. [1-2] NPs possess unique physical and chemical properties because of their high surface area and nano-scale size. Due to these characteristics, they are appropriate candidates in many areas such as biomedicine, cosmetics, health care, food, drug-gene delivery, the environment, mechanics, optics, chemical industries, electronics, space industries, and energy science.[3] NPs can be generally divided into two; organic nanoparticles, which consist of carbon nanoparticles (fullerenes), whilst inorganic nanoparticles include semiconductor nanoparticles (TiO2 and ZnO2), magnetic nanoparticles, and noble metal nanoparticles (Au and Ag).[4]There is a rising attention in inorganic nanoparticles, especially noble metal nanoparticles, because they afford greater material properties with useful versatility. Silver nanoparticles (AgNPs) have attracted many researchers due to their important properties such as good conductivity, chemical stability, catalytic activity, surface-enhanced Raman scattering, and antimicrobial activity. [6-8] AgNPs are considered safer than their chemically synthesized counterparts. [9] In addition, silver nanoparticles were used in various fields, including food, health care, medical, consumer, and industrial uses, because of their fantastic physical, chemical, and biological properties.[10] The production of silver nanoparticles of a distinct size via a variety of biological systems, including bacteria, fungi, and plant extracts has been reported.[4,11-12] Plants can accumulate amounts of several heavy metals in their diverse parts. In addition, green synthesis of nanoparticles using plant extracts appears to be a very efficient way to create a technology that is quick, safe, non-toxic, and environmentally friendly.[14-16] Green synthesis of silver nanoparticles utilizing phytochemicals as bio reductants is achieving a better impetus.[17] Phytochemicals of plant extract (terpenoid, flavonoid, phenols, and alkaloids) are claimed to be responsible for reducing and stability of nanoparticles.[18-19] However, there is still little known about the behavior of AgNPs in the eco-environment.[20] This study focused on the possibility of

synthesizing silver nanoparticles with *Ajuga iva* (L) extract. *Ajuga iva* is a member of the *Lamiaceae* family and is known locally in Libya by *Chendghoura*; it has another colloquial name Toutoulba.[21] *Ajuga iva* is widely spread throughout the temperate regions of Asia, North America, Europe, and Africa.[22] This plant has various biological activities, including antidiabetic and anti-inflammatory properties, and is widely used in traditional medicine to treat a variety of diseases.[23-25]Additionally, *Ajuga* has been used for the treatment of joint pain, gout, and jaundice.[26] Moreover, a decoction was made from the aerial portion of *Ajuga. iva* (leaves and flowers) has been used to treat many diseases such as kidney and digestive disorders, diabetes, hypertension, and painful menstruation.[27-29] *Ajuca iva* extracts have been shown to have significant antibacterial, anticancer, antioxidant, and antidiabetic activities.[30-32] Furthermore, a wide range of chemical constituents, including ajugarine, neoclerodane diterpenoids, anthocyanins, essential oils, flavonoids, tannins, terpenoids, steroids, fatty acids, and phenolic acids were found to be in *Ajuca iva* extracts.[24,33] Thus, all of the primary and secondary metabolites found in *Ajuca iva's* leaves and fruits account for the majority of its pharmacological and biological actions.[28] There were many reports on the synthesis of silver nanoparticles of *Ajuca* species such as *Ajuga parviflora*,[34] *Ajuga macrosperma*,[35,] and *Ajuga bracteosa.*[36] However, so far there has been one report on the green synthesis of silver nanoparticles using *Ajuca ive* leaves extract and studied their toxicity and photocatalytic activities.[37] Therefore, the present study aims to investigate the synthesis of silver nanoparticles of *Ajuca ive* leaf extract and to examine their antibacterial activity.

2. Materials and Methods

2.1. Material

Silver nitrate was purchased from BDH Chemicals Itd, and Double-distilled deionized water was used.

Ajuca ive plant was collected from the Lamamra area, Al-Khums, Libya. leaves of the plant were cleaned and washed well with distilled water to remove any remaining dust. Then they were left to dry under shade for about 8-10 days. After that, they were put in an oven at 40°C for 3 hours and next, they were ground into fine powder by the grinder.

2.2. Preparation of leaf extract

To prepare the leaf extract solution, 5 g of fine powder was taken into a 250 ml conical flask and mixed with 100 ml of distilled water followed by boiling at 100 °C for 10 minutes. After that, the leaf extract was collected in a separate conical flask by standard filtration method.

2.3. Photochemical screening

The phytochemical screening methods were carried out according to Harborne.[38]

2.3.1. Test for Alkaloids

A few drops of Wagner's reagent were added to 2-3 ml of plant leaf extract. The formation of a brown-reddish precipitate indicated the presence of alkaloids

2.3.2. Test for Carbohydrate

2 drops of alcoholic α-naphthol were added to 2 ml of leaf extract followed by the addition of a few drops of concentrated H_2SO_4, the result a purplish-red ring indicating the presence of carbohydrates.

2.3.3. Test for Flavonoids

A few drops of H_2SO_4 were added to 2 ml of leaf extract, the orange color indicated the presence of flavonoids in the leaf extract.

2.3.4. Test for Proteins

1ml of leaf extract was mixed with a few drops of concentrated HNO_3 and the result confirmed the presence of proteins by changing the colour to yellow.

2.3.5. Test of Phenols

2 drops of 5% of $FeCl_3$ solution were added to 1 ml of leaf extract, changing the colour into blue or black showed phenols contents in the leaf extract.

2.3.6. Test of Tannins

1 ml of leaf extract was mixed with 2 ml of 10 % NaOH showed a positive result and tannins existence.

2.3.7. Test for Saponins

3 ml of distilled water was mixed with 3 ml of plant extract and shaken vigorously for 15 mins a layer of 1 cm of foam was formed indicating the presence of saponins in the plant extract.

2.3.8. Test for Coumarins

2 mL of 10% NaOH was added to the plant extract sample, and a yellow color was observed confirming the presence of coumarins.

3. Synthesis of silver nanoparticles

1 mM AgNO3 solution was prepared and stored in an amber colour bottle. 50 ml of this solution was taken in a flask containing a magnetic bead, then it was put on a magnetic stirrer. After that, 5 mL of leaf extract was added dropwise to the AgNO3 solution with a little heat at 50-60 °C. A change of colour from yellow to dark brown indicated the formation of silver nanoparticles. Next, the conical flask was incubated at room temperature for 48 hours followed by centrifuged at 6,000 rpm for 15 minutes.

4. Characterization techniques

Ultraviolet-visible Spectroscopy (UV-Vis) was carried out in a (HACH LANGE- DR-3900). The size, morphology, and composition of silver nanoparticles were measured using scan electron microscopy (SEM), energy dispersive X-ray (EDX) analysis using JEOL, and X-ray diffraction (XRD) analysis using Bruker D8 discover. Function groups of silver nanoparticles were performed on FT-IR (Perkin Elmer FT-IR Spectrometer Frontier).

5. Anti microbial activity

The antibacterial activity of silver nanoparticles against two types of bacteria *Escherichia coli* (Negative-Gram) and *Streptococcus* (Positive–Gram) was studied. The disk diffusion method was used to examine the antibacterial activity of silver nanoparticles. Strained bacteria were spread on the Petri dishes after that, the disks soaked in distilled water as a control, Ag NO_3 solution, and silver nanoparticles were separately put on Petri dishes and were incubated at 37 °C. The zone inhibition of each disk was determined by a ruler after 24 hrs.

6. Results and Discussion

6.1. Phytochemical screening of *Ajuca ive* leaf extract

Phytochemical screening of ***Ajuca ive*** leaf extract was carried out using color-changing and precipitating chemical reagents for examining plant constituent's extract. The results of the testing extract possessed constituents such as alkaloids, carbohydrates, proteins, flavonoids, phenols, saponins, and coumarins as shown in **Table 1.** This result was comparable with previous reports.[39] In addition, it was found that phytochemical constituents might be attributed to its various antimicrobial activity.[40] Furthermore, it is noteworthy that phytochemical components could be the responsible part for the reducing silver ion solution to metallic silver for the synthesis of silver nanoparticles.[41-42]

Table 1. Phytochemical constituents of *Ajuca ive* leaf extract

Chemical Groups	**Observations**
Alkaloids	+
Carbohydrate	+
Proteins	+++
Flavonoids	++
Phenols	+
Tannins	+
Saponins	+++
Coumarins	+++

6.2. Synthesis of silver nanoparticles

The synthesis of silver nanoparticles using *Ajuca ive* leaf extract was performed according to the Linga Rao procedure.[43] The formed silver nanoparticles were indicated by coluor change from yellowish to dark brown after the addition of *Ajuca ive* leaf extract to silver nitrate solution as shown in **Figure 1**. The color change is due to the reduction of ionic silver into metallic silver.[44]

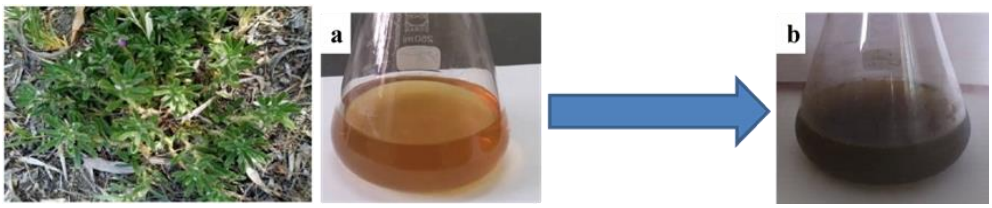

Figure 1. *Ajuca ive* leaf extract **(a)** and synthesized silver nanoparticles **(b)**

6.3. Characterization of silver nanoparticles

6.3.1. UV-Vis Spectroscopy

The synthesized silver nanoparticle was confirmed by UV-visible spectrum that showed the maximum absorbance peak at 430 nm as seen in **Figure 2**. A similar result has been obtained by Thiyagarajan and Kanchana who recorded a characteristic absorption band for silver nanoparticles at 430 nm.[45] This peak occurs due to its specific surface plasmon resonance (SPR) excitation.[46] Moreover, the absorbance peak indicated the formation of spherical and uniform silver nanoparticles.[47-48]

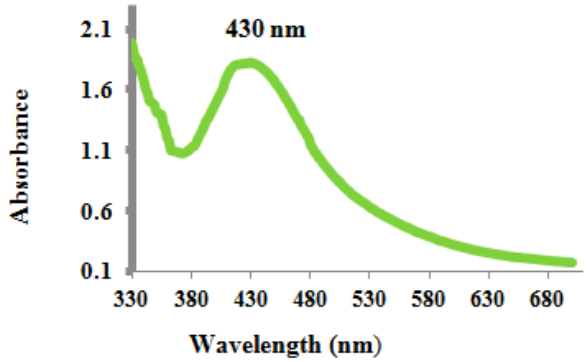

Figure 2. The UV-Vis absorption spectrum of silver nanoparticles synthesized by *Ajuca ive* leaf extract treated with 1mM silver nitrate

6.3.2. XRD analysis of silver nanoparticles

The X-ray diffraction (XRD) was analyzed to examine the crystalline silver of AgNPs of *Ajuca ive* leaf extract. **Figure 3** shows the diffraction peaks at angles of 2θ at 38°, 44°, 67°, and 77° which are related to (111), (200), (220), and (311). These finding results correspond to the face-centered cubic structure of silver metal silver (JCPDS file no. 84-0713 and 04-0783). Whereas other unmarked peaks might be due to the crystallizing of biological organic phases happening on the silver nanoparticles surface. Similar results were reported by Willian and Vanaja et al.[49-50]

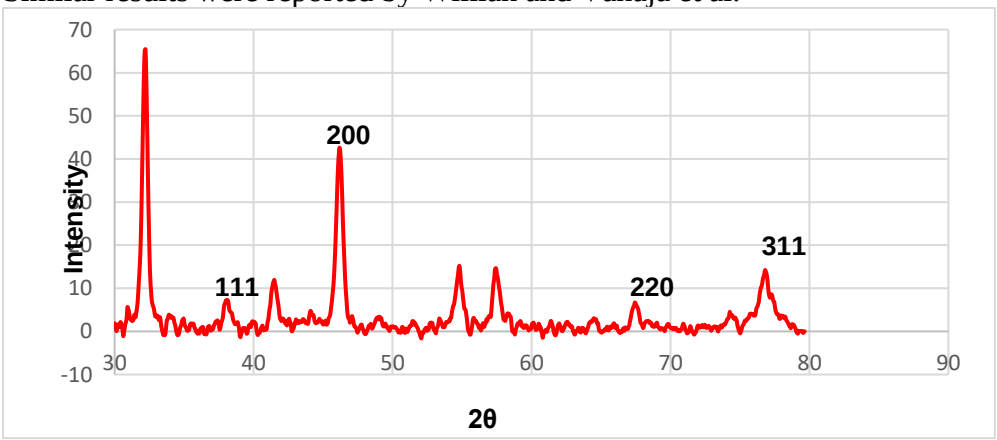

Figure 3. XRD pattern of silver nanoparticle

6.3.3. SEM of silver nanoparticles

The morphology and size of the AgNPs were analyzed using SEM.as seen in **Figure 4**. The images of SEM showed a spherical shape of AgNPs. In addition, the SEM image showed the different sizes of the AgNPs (36 nm, 55 nm, 70 nm,100 nm, and 300 nm). These differences in the shape of the AgNPs might be due to the variety in the chemical structure of

phytochemical constituents which may be responsible for the reduction and stabilization of silver ions into silver metal. Moreover, the increase in the size of AgNPs (300 nm) might be because the two particles come together so in the SEM image appeared one particle. These findings of SEM sizes were parallel with Al Moudani *et al* who found the size of silver nanoparticles of *Ajuca ive* leaf extract were 100-300 nm.[37]

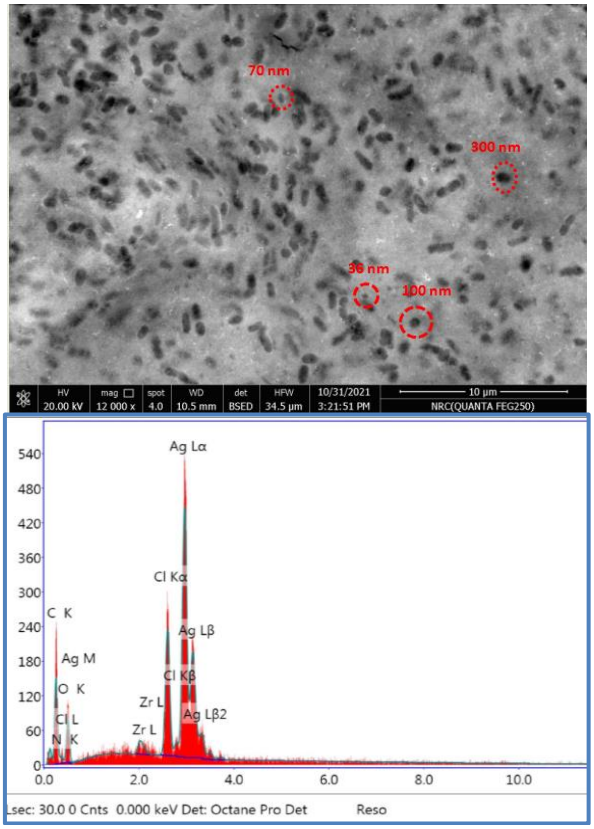

Figure 4. Scanning Electron Micrograph of *Ajuca ive* leaf extract silver nanoparticles (left) and EDX spectrum analysis demonstrating the main peak of silver nanoparticles at 3 keV, which corresponds to silver nanoparticles

6.3.4. **EDX analysis** of **silver nanoparticles**

EDX analysis also showed a very strong signal in the silver region, confirming the formation of silver nanoparticles as seen in **Figure 4**. The optical absorption peak was observed at 3 keV, which is typical for the absorption of metallic silver nanoparticles because of surface Plasmon resonance.[51] In addition, other peaks for Cl, O, C, and N were observed which may be due to proteins or enzymes present in the culture supernatant.[52]

6.3.5. IR of silver nanoparticles

FTIR spectra were carried out to identify the biosynthesized silver nanoparticles of *Ajuca ive* leaf extract. The IR spectra of *Ajuca ive* extract and biosynthesized silver nanoparticles were compared. The IR of *Ajuca ive* leaf extract shows absorption bands in the range of 3286.53 and 878.90 cm^{-1} as shown in **Figure 5**. Whereas the AgNPs of Ajuca *ive* leaf extract show absorption bands in the range of 3287.88 and 824.73 cm^{-1} as seen in **Figure 6**. The broad band at 3286.53 cm^{-1} of *Ajuca ive* leaf extract is shifted to 3287.88 cm^{-1} with less intense in AgNPs. Previous reports indicated that this absorbed band corresponds to the OH group of alcohols and phenolic compounds.[53] The vibration band at 2918.87 is shifted to 2921.14 cm^{-1} which is attributed to the stretching vibration of C-H aliphatic compounds. The sharp peak located at 2850.45 in *Ajuca ive* extract is assigned to C=C stretching mode, after the formation of AgNPs this band becomes small and less intense. In the free extract, the absorption band of C=O is at 1730.88 cm^{-1} which is associated with the presence of carbonyl-containing compounds whereas in the AgNPs this band disappeared suggesting the involvement of C=O compounds in the bioreduction of the silver ions.[54]The bands at 1603.01 cm^{-1} and 1416.54 cm^{-1} are mainly attributed to aromatic C=C and C–N stretching vibration bonds of amine functional groups.[55] In the AgNPs, the new band at 1372.62 cm^{-1} is formed that may be associated with N-O symmetry stretching of the nitro compound.[52,56]Likewise, the small band at 1240.47 cm^{-1} and 1008.56 in the free extract is related to the presence of C–O and C–H stretching of polysaccharide.[57] These bands shifted to one absorbed band at 1023.07 cm^{-1}. The bands at 878.90 cm^{-1} and 824.73 cm^{-1} represent the presence of C−H in aromatic compounds in the *Ajuca ive* extract and the biosynthesized AgNPs. The results of infrared spectra of the obtained AgNPs demonstrated the presence of characteristic functional groups such as alcohols, phenols, aldehydes, flavonoids, and amines, that may be responsible for the bioreduction and stabilizing of the synthesized silver nanoparticles.[58-59]

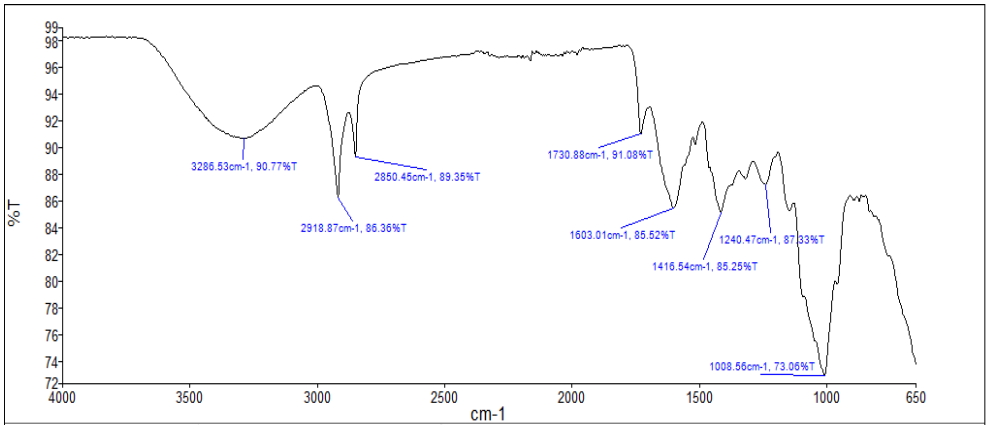

Figure 5. FTIR Spectrum of *Ajuca ive* leaf extract

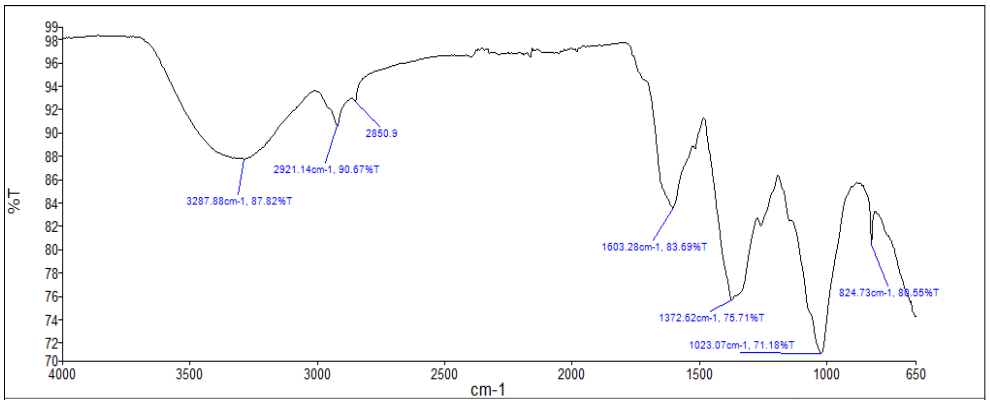

Figure 6. FTIR Spectrum of synthesized silver nanoparticles of *Ajuca ive* leaf extract

6.4. Anti-bacterial activity

The antibacterial activity of the synthesized AgNPs from *Ajuca ive* leaf extract has been investigated. The antibacterial effect of synthesized silver nanoparticles was studied on two types of bacteria *E. coli* (Gram-negative) and *Streptococcus* (Gram-positive). The disc diffusion method was used as an antibacterial susceptibility testing method.[60] The AgNPs of *Ajuca ive* leaf extract **(A1)** were compared with $AgNO_3$(Ag) as shown in **Figure 7.** The findings illustrated that the disc diffusion method proved the antibacterial activity of the AgNPs. The inhibition zones showed a maximum value of 12 mm **(A1)** against *E. coli* and 7 mm **(A1)** against *Streptococcus.* as seen in **Figure 7.** In this study, The AgNPs of *Ajuca ive* leaf extract **(A1)** show better antibacterial activity for both types of bacteria. Similar results were observed for AgNPs against *E coli*.[61]It was reported that the silver nanoparticle size affects the inhibition to a better extent.[62]

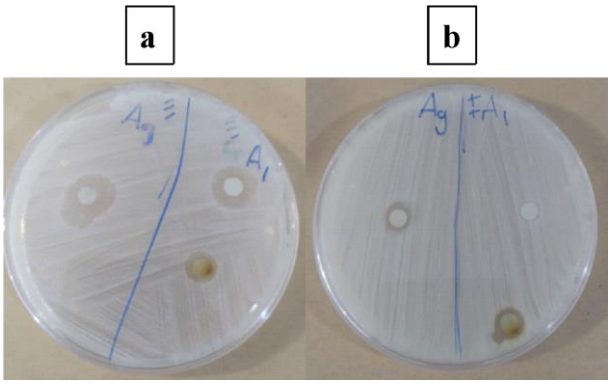

Figure 7. Anti-bacterial activity of formed silver nanoparticles from *Ajuca ive* leaf extract against, *E coli* (a), *Streptococcus (b)*.

Conclusion

In summary, the green synthesis of silver nanoparticles of *Ajuca ive* leaf extract was performed. The formation of silver nanoparticles was characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX), and Fourier Transform Infra-Red (FTIR). In addition, they showed a good antibacterial activity which might be used in the further as an antibacterial drug.

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

Data Availability: All data are included in the content of the paper.

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