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Antimicrobial Activity of Silver Nanoparticles Stabilized by Liposoluble Extract of *Artemisia terrae-albae*

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Abstract: The global spread of multi-resistant pathogenic microorganisms has significantly complicated the treatment of chronic wounds. The development of novel drugs requires a substantial time investment. Hybrid materials such as nanoparticles stabilized by plant extracts are considered the best for creating efficient antiseptic substances. This paper is the first to discuss quantitative and qualitative analyses of the phytochemical constituents of the medicinal plant *Artemisia terrae-albae*, collected in Kazakhstan. The antimicrobial activity of the extracts, as well as of silver nanoparticles (AgNPs) stabilized by *Artemisia terrae-albae* extract, were evaluated. AgNPs were characterized by an average size of 82 nm or larger with a negative surface charge. TEM analysis of the obtained suspension showed a nonuniform structure of particles synthesized at a low concentration of ethyl acetate solvent in water. The SPR peak of AgNPs@Art aq. extract was detected at 420 nm, while any clear SPR peak was observed for AgNPs@Art ethylacetate extract. Diluted *Artemisia terrae-albae* extracts did not exhibit pronounced antimicrobial activity due to the poor solubility of compounds in water. Nevertheless, the AgNPs@Art aq. and AgNPs@Art EtAc. extracts possessed antimicrobial activity against the *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* (ATCC 8739), and *Candida albicans* ATCC 10231 strains.

Keywords: *Artemisia terrae-albae*; antimicrobial activity; green synthesis; AgNPs; *SPR*; *TEM*

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1. Introduction

The genus *Artemisia* is one of the largest and most extensively distributed genera within the *Astraceae* (Compositae) family. This genus is heterogeneous and consists of over 500 distinct species primarily found in temperate regions across Europe, Asia, and North America. These species include perennial, biennial, and annual herbs as well as small shrubs. The genus *Artemisa* is one of the largest and widely distributed genera in the *Astraceae* (Compositae) family [1,2]. The name 'Artemisia' is derived from the Greek word 'Artemis,' which is employed for women's diseases in folk medicine and corresponds to Diana, a Greek goddess [3].

Artemisia spp. contains various chemical lipophilic and hydrophilic compounds including lactones, terpenoids (e.g., myrcene, germacrene D, camphor, chamazulene), flavonoids, and flavonoid glycosides [4–8].

In traditional folk medicine, *Artemisia* has been utilized for its medicinal properties, including its roles as fever reducer, antiseptic, anthelmintic (used against parasitic worms),

tonic (to promote health and vitality), diuretic (to increase urine production), and for addressing abdominal pain [9].

Substances isolated from *Artemisia* exhibit antibacterial, anti-inflammatory, hepatoprotective, antidepressant, antioxidant [7,10,11], antimicrobial, antiviral [4,12,13], antistress, hepatoprotective, antispasmodic, antimalarial, and antipyretic properties as well as for the treatment of chronic fever [14].

In review 29 *Artemisia* spp. located in Pakistan are discussed; the authors conclude that essential oils and artemisinin are compounds utilized to treat malaria; cancer; stomach, kidney, liver, spleen, and other bacterial fungal diseases; and helminhts parasites, etc. [15].

Earlier very promising antibacterial and anticancer results were obtained for silver nanoparticles (AgNPs) stabilized by *Artemisia quttensis* Podlech aerial parts extract. The AgNPs@*A. quttensis* produced a cytotoxicity effect on HT29 and HEK293 cells [16]. According to another study, AgNPs@*Artemisia oliveriana* extract efficient was for the most part more resistant to the gram-positive bacteria than to the gram-negative bacteria, producing a shown significant cellular toxicity on the A549 cell line [17]. AgNPs@ *Artemisia kopetdaghensis* extract with average size of 22 and 18 nm was potent against *K. pneumoniae* and *S. aureus*, respectively. Moreover, it proved to be anti-proliferatively active for the human hepatocellular cancer cell line [18]. 46-nm AgNPs@*Artemisia absinthium* extract was antimicrobially resistant to all tested bacterial strains compared to the pure extracts [19]. Similar biological activity results were observed for AgNPs@*Callendula off.* and the Callendula off. extract [20].

AgNPs@*Artemisia annua* were efficient for three species with minimum inhibitory concentration (MIC) in the range of 80–120 mg/L. Thus, *Candida glabrata* was significantly sensitive to AgNPs, followed by *Candida tropicalis* and *C. albicans* [21]. AgNPs@*Artemisia annua* of less than 50 nm produced an antiplasmodial effect on parasitized red blood cells in a AgNPs concentration range of 0.6 to 7.5 mg/L [22]. AgNPs @*Artemisia vulgaris* extract with an average size of 7 nm was applied for optical sensor development. It was selective and highly sensitive to Hg^{2+} ions and may be utilized to produce sensors for environment monitoring [23]. Moreover, AgNPs@*Artemisia capillaris* particles with an SPR of 534–543 nm which were dependent on extract concentration particles with an average size of 17 $\pm$ 5.47~30 $\pm$ 9.8 nm were successfully tested in a catalytic redaction [24]. AgNPs@*Artemisia ciniformis (A. ciniformis)* leaf extract with a size in the range of 4–14 nm inhibited the proliferation of AGS human gastric carcinoma cells [25]. AgNPs@*Artemisia monosperma* triggered a significant decrease in MCF-7 cell viability by 13–86%, with an IC_{50} value 32 mg/L [26]. AgNPs@*Artemisia herba-alba* plant extract possessed spherical particles of 9.7 to 36.7 nm and showed an efficiency for the pest *Spodoptera littoralis* larvae [27].

The present study investigates the chemical ingredients for the aerial part of the medicinal plant *Artemisia terrae-albae* grown in the Almaty region of Kazakhstan and the use of the extract as a reducing and stabilizing agent of AgNPs. The extraction method, eluent composition, and other conditions can significantly influence the chemical composition of the extract. Additionally, factors such as the timing of plant collection and the location of cultivation can impact the content of biologically active compounds. Therefore, it is necessary to assess the activity of both the extract and the AgNPs obtained under specific conditions. Antimicrobial activity of AgNPs and their physico-chemical characterization are discussed.

2. Materials and Methods

Petroleum ether 99%, chloroform 99%, ethyl acetate 99%, and butanol 99% were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Ethanol 96% was produced by Talgar ethanol factory (Talgar, Kazakhstan); $AgNO_3$ 99% was acquired from Labrpharma (Almaty, Kazakhstan). The materials for the antimicrobial experiments, including culture media and specific compounds such as nutrient broth, nutrient agar, and tryptic soy broth, were bought from Himedia, India. Additionally, lecithin (containing a minimum of 60% phosphatidylcholine, with specific attributes: negative for Escherichia coli, iodine number

between 60 and 70, maximum peroxide number of 3.0, maximum 3% lysophosphatidylcholine, and maximum 20% non-polar lipids, in accordance with distilled water standards per GOST R 58144-2018) and tween-80 (with a molecular weight of 1310 g/mol) were acquired from AppliChem, Germany. The test strains used, including *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC6538, *Escherichia coli* (ATCC 8739), and *Candida albicans* ATCC 10231, were sensitive strains obtained from the Republican Collection of Microorganisms (RCM) in Astana, Kazakhstan.

2.1. Plant Material

The aerial part of the *A. terrae-albae* plant was collected in June 2023 in the Alakolsky district (GPS coordinates 46.221455, 81.237623) of the Almaty region. The air-dried aerial part of *A. terrae-albae* was cut into small pieces and stored at room temperature until future use.

2.2. Extraction

The dried *A. terrae-albae* plant material, weighing 1.7 kg, was ground into a fine powder and subjected to three consecutive ethanol extractions (each lasting seven days) at room temperature, using a total of 15 L of ethanol. After removing the solvent through vacuum evaporation, 80.30 g of the resulting residue was combined and suspended in water. This mixture was then sequentially partitioned using petroleum ether, chloroform, ethyl acetate (EtAc), and butanol to obtain distinct extracts. The process yielded specific extracts as follows: petroleum ether extract (5.2 g), chloroform extract (25.8 g), EtAc extract (16.2 g), and butanol extract (5.4 g). The quantitative and qualitative compositions of bioactive compounds in the aerial part of the plant were determined using established methods as previously described [4].

2.3. AgNPs Stabilised by A. terrae-albae Extract Synthesis

6.6 g of ethanolic extract of *A. terrae-albae* dissolved in water 1 to 40 was mixed with 13.2 g of 1.5 mM $AgNO_3$ aqueous solution. The pH of the reaction mixture was 4.6 and was adjusted to 9.8 by adding a few drops of 10% ammonia solution. Instantly after pH adjustment the solution darkened. The SPR peak was observed at 445 nm.

To 0.061 g of the ethyl acetate extract of *A. terrae-albae* was added 2.75 mL of 8% solution of ethyl acetate in water. Then, the 45-fold-diluted plant extract solution was mixed with 5.5 mL of 1.5 mM $AgNO_3$ aqueous solution, and the pH was adjusted by 10% ammonia solution to 9.4 Some separation of organic phase was observed. The color of the AgNPs@EtAc *Artemisia* extract ×45 became dark grey. After a 30 min incubation at room temperature in the dark place, the solution was treated in an ultrasonic bath for 2 min at conventional temperature and the UV-vis was recorded.

Analogously to the above description, 0.06 g of the ethyl acetate extract of *A. terrae-albae* was diluted 60 times by 8% solution of ethyl acetate in water. The obtained extract was mixed in a ratio 1 to 2 with 1.5 mM silver nitrate solution, and pH of the solution was adjusted to 9.4 with 10% ammonia solution. The final suspension of AgNPs@ *Artemisia EtAc* extract ×60 appeared to be homogeneous compared to AgNPs@ Artemisia EtAc extract ×45. Non-phase separation was observed. AgNP suspension was prepared using the standard method [20].

An etalon suspension of AgNPs stabilized with citrate was created using a well-established method. In summary, 90 mL of a 1 mM AgNO3 solution was mixed with 5 mL of a 10 mM sodium citrate solution at room temperature. Next, 10 mL (equivalent to 7 mg) of a freshly prepared sodium borohydrate solution was added with continuous stirring for several hours. The resulting AgNPs suspension was then stored at +4 °C for future use [20].

2.4. Zeta Potential Measurement and Size Distribution

An examination of the zeta potential was conducted using the Malvern Zetasizer Nano ZS90 instrument (United Kingdom). This analysis aimed to investigate the stability and dimensions of the produced AgNPs. Zetasizer systems employ electrophoretic (ELS) and dynamic light scattering (DLS) techniques, offering insights into particle mobility, charge, and size in dispersive systems, spanning from nanometers to micrometers [28]. The following configuration involved running 5 scans for each sample run, with a total of 3 runs conducted. The entire experiment was replicated at least 6 times. DLS analysis was executed using the Zetasizer 3000 instrument from Malvern Instruments. The size distribution was established by performing 10 scans for each spectrogram run, with a minimum of 10 runs recorded. The error bars were generated using standard the zeta potential software.

2.5. TEM

A JEOL JEM-1400 plus microscope was used to characterize the AgNPs stabilized by the plant extract, which was dispersed on the carbon coated copper grid, mesh size 200, and left it dry. The images were taken at the following settings with electron acceleration 80 kV and magnification 20,000, 50,000, 120,000 and 400,000 times. The TEM images were analysed using Image J area analysis and following a calculation of the average diameter.

2.6. Assessment of Antibacterial Effects of Artemisia Extracts

To assess the antibacterial properties, we employed a method involving double serial dilutions in an isotonic solution, followed by inoculation onto solid nutritional agar to evaluate the survival of the bacteria after being exposed to antimicrobial agents. The microorganism suspensions were prepared using isotonic saline solution, resulting in an absorbance of 0.056 at a wavelength of 625 nm, which indicated antimicrobial agents. The microorganism suspensions were prepared using isotonic saline solution, resulting in an absorbance of 0.056 at a wavelength of 625 nm, indicating a cell concentration of approximately 8×10^8 CFU/mL. Subsequently, serial dilutions ranging from 1:1 to 1:2048, with a ratio of water extract *Artemisia extract* $\times 40$: isotonic solution, were created using a Repeater M4 (Eppendorf) in a standard 96 well plate for ELISA. As a result, the final cell concentration was anticipated to be approximately 2.6×10^8 CFU/mL. These preparations were then kept at room temperature for 24 h, after which they were plated onto agar broth plates and kept at 37 °C overnight [20]. The growth of bacteria was checked visually (Figure 6).

2.7. Antimicrobial Activity of Plant Extract and AgNPs

The preparation of test strain suspensions involved using 18–24 h cultures of the test strains. In this process, an aliquot of the respective test strain was introduced into a test tube containing 5–6 mL of 1/50 nutrient broth using a sterile loop. The contents were thoroughly mixed to achieve homogeneity. To determine the cell concentration, optical density was measured ensitometrically and found to be 1.2 units, following the McFarland standard. This measurement corresponded to a cell content of 6×10^8 CFU/mL for bacterial suspensions and 6×10^6 CFU/mL for yeast cell suspensions. To create working concentrations, the stock suspension was subjected to 10-fold serial dilutions; a final inoculum of 6×10^5 CFU/mL was reached for each test strain. 0.4 mL of the obtained suspension of the corresponding strain was applied on the hole in the surface of agar. Bacterial growth was evaluated after incubation for 24 h at 30 °C [29].

3. Results and Discussion

3.1. AgNPs Synthesis and Characterization

Recently, great attention has been focused on the utilization of plant extracts for AgNPs synthesis and its following usage as antimicrobial agent. There are a few studies related to *Artemisia* species (*Artemisia quttensis*, *Artemisia oliveriana*, *Artemisia kopetdaghensis*, *Artemisia absinthium*, *Artemisia annua*) extracts with AgNPs [16–19,21]. However, the role of *A. terrae-albae* extract in AgNP formation has not previously been studied.

The surface Plasmon resonance SPR peak of AgNPs@*Art* H_2O at 420 nm confirmed the spontaneous formation of AgNPs (Figure 1A). In order to check the reduction of all silver phytocomplexes to AgNPs, the reaction mixture was additionally treated in an ultrasonic bath for 2 min in standard conditions. Enhancement of the AgNPs SPR peak after the additional treatment, which indicates the completion of the redox reaction, was not observed. Previously, the mechanism of AgNP stabilization by plant extract and the influence of various trigger parameters (UV or microwave irradiation, pH, temperature) on AgNP inducing have been discussed [20]. The research focused on examining the production of silver nanoparticles with sizes ranging from 35 to 60 nanometers, which were created using an aqueous extract derived from *Eclipta prostrata* and subjected to ultrasonic treatment. The resulting AgNPs displayed activity against the fourth-stage larvae of two disease-carrying mosquitoes: *Culex quinquefasciatus say*, which transmits filariasis, and *Anopheles subpictus Grassi*, responsible for spreading malaria [30]. On the other hand, AgNPs@*Art* EtAc extract ×45 and AgNPs@*Art* EtAc extract ×60 did not have a clear SPR peak, because of the wide size distribution of the AgNPs (Figure 2). Nonetheless, TEM and light scattering indicated the presence of AgNPs in samples (Figures 3 and 4).

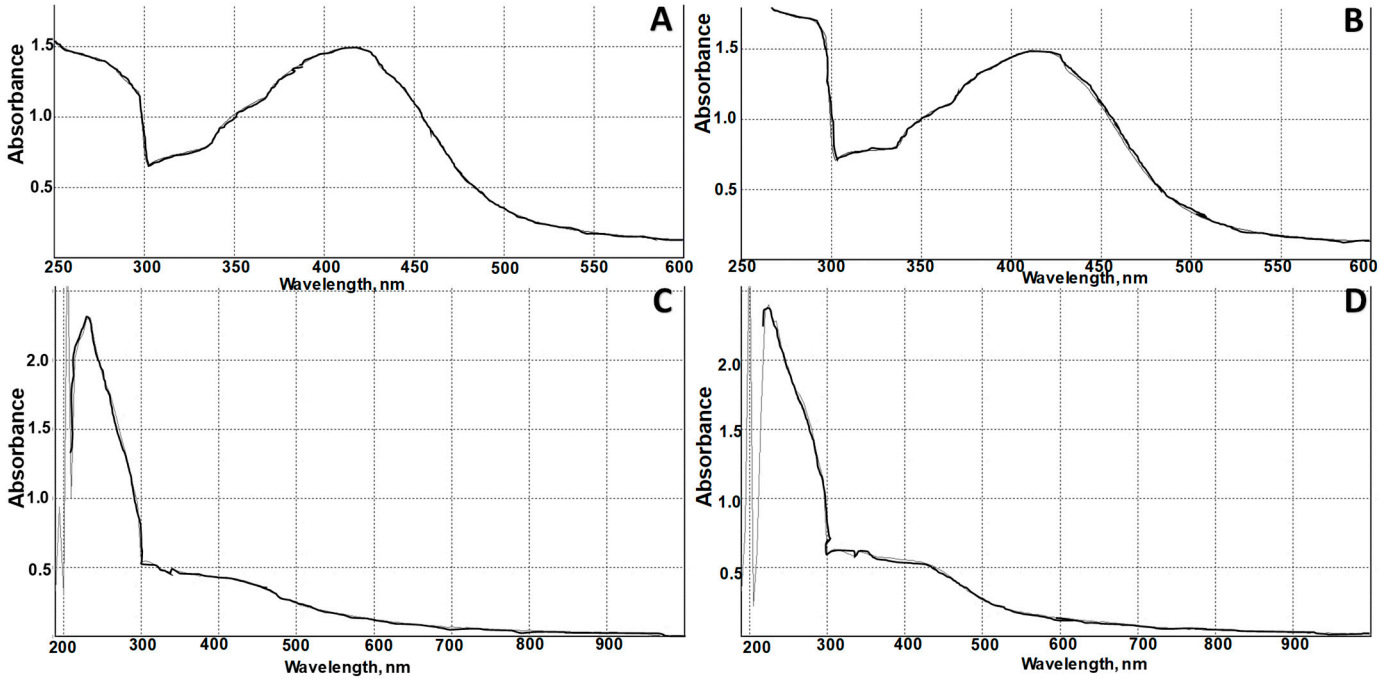

Figure 1. UV-vis spectra of AgNPs stabilized by the Art aqueous extract: (**A**) before ultrasonic treatment Art H_2O ×40 + $AgNO_3$ and (**B**) after ultrasonic treatment Art aq. extract ×40; (**C**) Art aq. extract ×60 before ultrasonic treatment; (**D**) Art aq. extract ×60 after ultrasonic treatment. Suspensions were additionally diluted 10-fold by water before spectra recording.

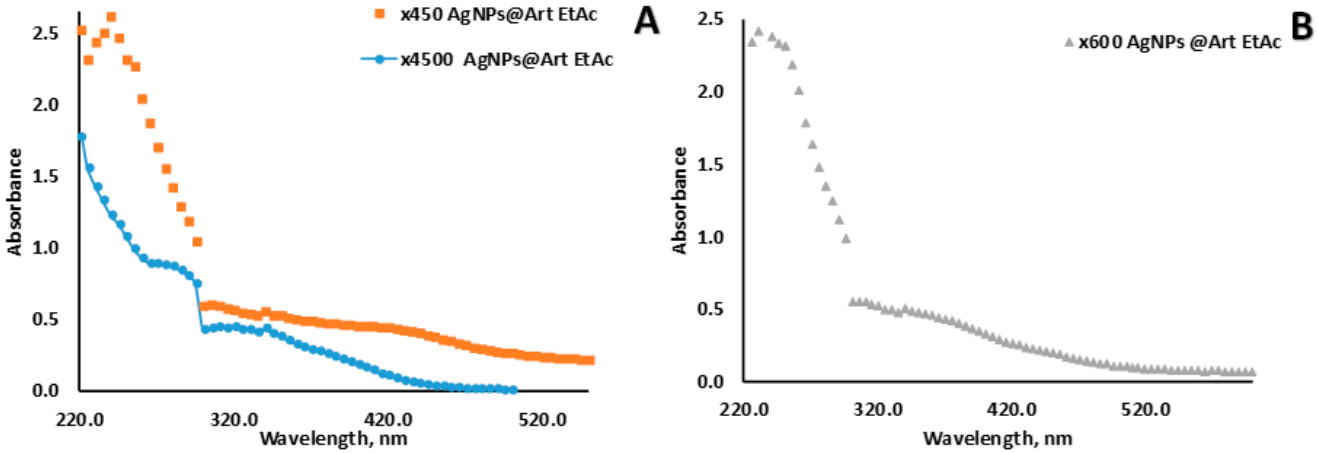

Figure 2. (**A**) UV-vis spectra of AgNPs@Art EtAc extract ×45 and (**B**) AgNPs@Art EtAc extract ×60 additionally diluted 10 and 100 times by distilled water.

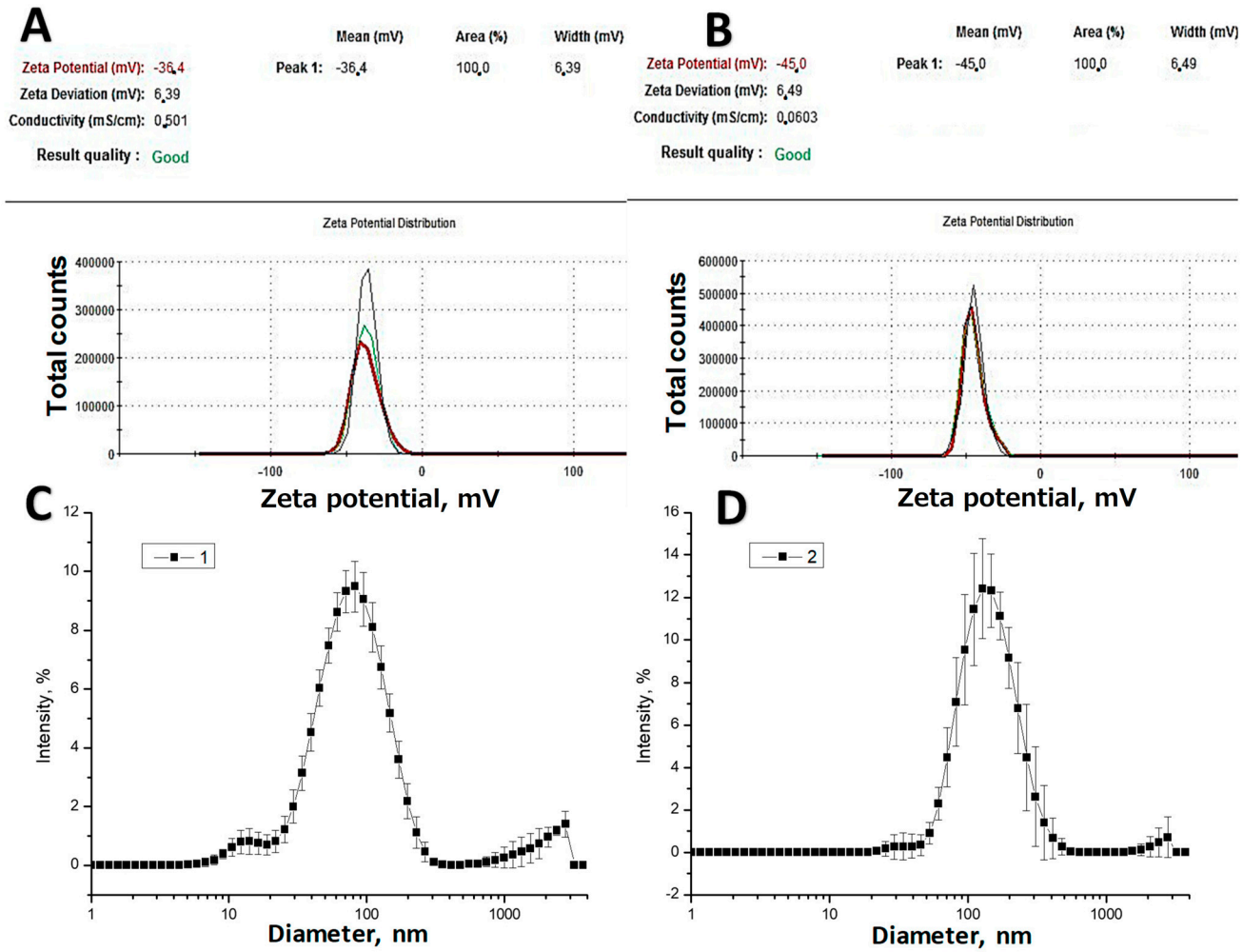

Figure 3. Zeta potential of AgNPs: (**A**) AgNPs@ *Art* EtAc extract ×45; (**B**) AgNPs@EtAc *Art* extract ×60; *AgNPs* size distribution: (**C**) AgNPs@EtAc *Art* extract ×45; (**D**) AgNPs@EtAc *Art* extract ×60, $n = 3$.

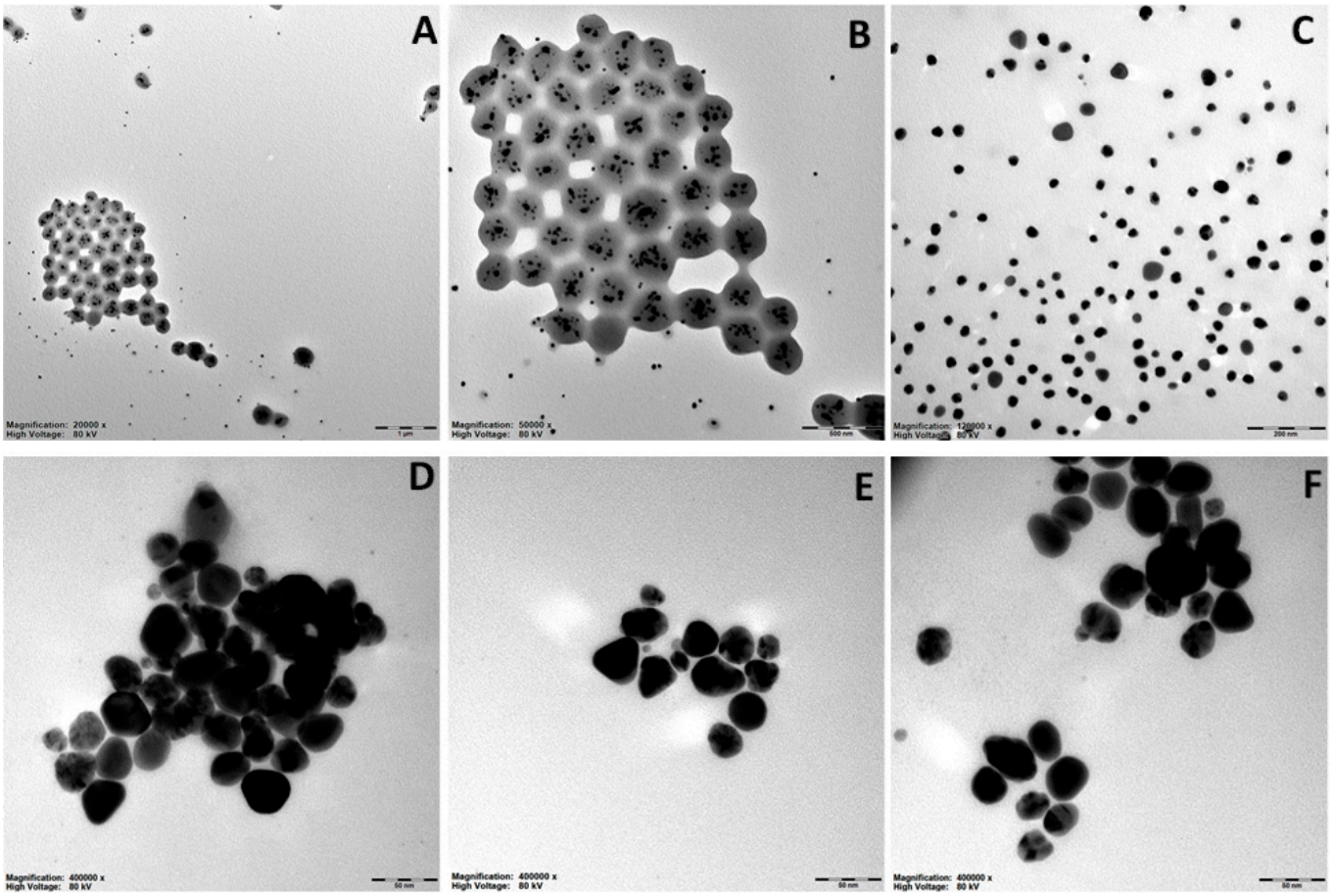

Figure 4. TEM photograph at different magnifications: (**A**–**C**) AgNPs@EtAc *Art* extract ×40; (**D**–**F**) AgNPs@EtAc *Art* extract ×60.

In the formation of AgNPs, phenolic groups of flavonoids participate in the reduction of Ag ions, which in turn become oxidized to quinone functionality [31]. We propose that additionally to the abovementioned, other functional groups such as aldehyde groups of Vanillin or other aromatic aldehyde may participate in the reduction process and following stabilization of AgNPs via carboxyl groups and chelate formation with oxygen and nitrogen containing functional groups.

AgNPs@EtAc *Art* extract ×45 was characterized by size in the range of 80–120 nm, while in AgNPs@EtAc *Art* extract ×60 the main fraction ranged from 80–150 nm (Figure 3C,D). Interestingly, that dilution factor of the plant extract also effects the surface charge of AgNPs, most probably due to a change in the ethylacetate in the solution. The zeta potential of AgNPs@EtAc *Art* extract ×45 and AgNPs@EtAc Art extract ×60 was −36 and −45 mV, respectively. This indicates that molecules chemosorbed on the AgNPs have a negative charge. Previously, the zeta potential of AgNPs-*Calendula off.* possessed a similar value—38—and had good aggregative stability within 9 months of storage at 4 °C [20]. Adoni et al. have demonstrated that AgNPs@*Artemisia Annua L.* aq. extract obtained from leaves is characterized by a particle size within the range of 5–20 nm and a zeta potential of −26 millivolts [32]. MF Baran's study found that AgNPs stabilized with *Artemisia Absinthium* exhibited a maximum absorbance for a SPR peak of 449 nm. Additionally, X-ray analysis revealed that these nanoparticles had a size of approximately 14.6 nanometers [33]. Another study reported that distinctive SPR bands of AgNPs were detected at around 430 nm; these were attributed to AgNPs synthesized using *Artemisia argyi* leaf extract and having an average size of approximately 77.6 nm [34].

The size distribution data obtained by TEM analysis of the AgNPs agree well with the evaluation of the hydrodynamic diameter of the particles analyzed by using light scattering (Figures 3, 4 and S1). As expected, a significant dilution of EtAc extract with water decreases the solubility of the lipophilic compounds and their aggregation due to hydrophobic interactions. This causes the decline of the soluble molecules in water, which may participate in the stabilisation of AgNPs resulting in the formation of larger particles AgNPs@EtAc *Artemisia extract* ×60. The shape of the AgNPs@EtAc *Artemisia* extract ×40 is mostly spherical, whereas AgNPs@EtAc *Artemisia* ×60 is characterized by more a randomized shape (triangle) and includes tetrahedral shape particles. Moreover, AgNPs@EtAc *Artemisia extract* ×60 has less aggregative stability, which leads to their aggregation (Figure 4D–F). According to TEM images analysis the main fraction of AgNPs@EtAc *Artemisia* extract ×60 was in the range of 13–34 nm, whereas the size of AgNPs@EtAc *Art extract* ×40 varied in the range of 18–33 nm (Figure 5).

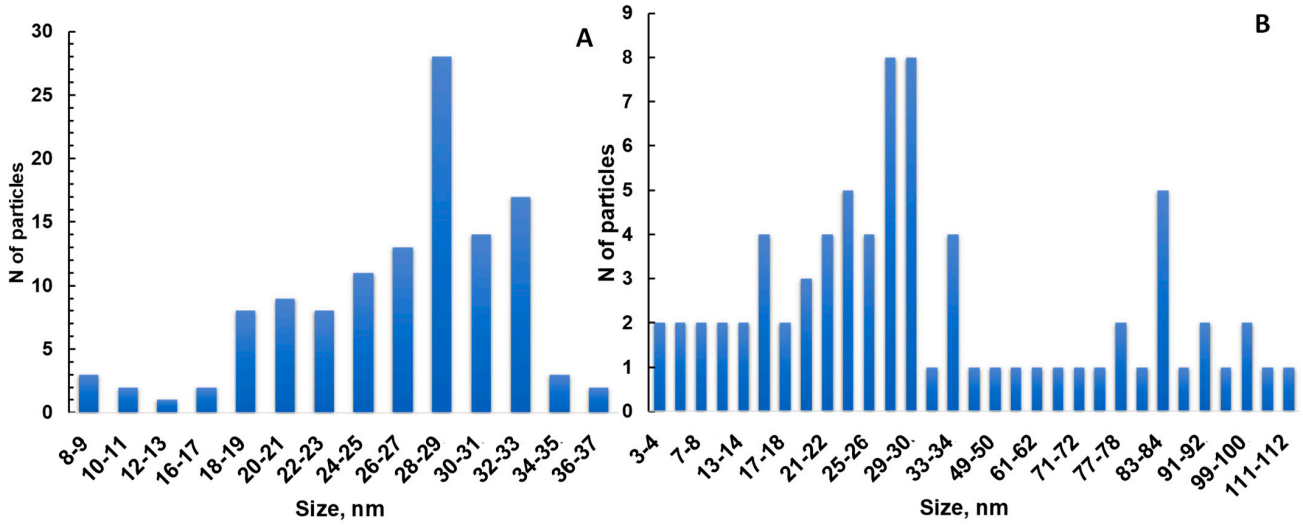

Figure 5. Size distribution of AgNPs using Image J TEM images analysis: (**A**) AgNPs@EtAc *Art extract dilution* ×40; (**B**) AgNPs@EtAc *Art extract dilution* ×60.

3.2. Antimicrobial Activity of AgNPs and Extracts

The screening of various dilutions of *Artemisia* extract did not illustrate the antimicrobial activity against *E-coli* and *S. Aurous* (Figure S2). Previous research conducted by several research groups has extensively elucidated the antimicrobial mechanisms of AgNPs [35]. It was revealed that the primary mechanism involves the generation of reactive oxygen species that disrupt the bacterial cell wall. However, we currently lack the necessary equipment for such a comprehensive investigation. The diluted solutions of extracts of *Artemisia* (butanol extract ×40; ethylacetate extract ×40 and aqueous extract ×20) were not effective for the bacteria inhibition of *E. coli* (ATCC 8739 and *S. Aureus* (ATCC 25923) (Figure 6). The contact time of bacterial suspension with AgNPs was over 24 h. The evaluation of AgNPs stabilized by the *Artemisia terrae-albae* extract aerial part was performed by agar diffusion.

AgNPs citrate and AgNPs@EtAc extract ×60 illustrated comparable antimicrobial activity against *Escherichia coli* (ATCC 8739) *Pseudomonas aeruginosa* ATCC 9027 *C. albicans* (ATCC 10281)(Table 1).

AgNPs citrate did not manifest activity *S. aureus* (ATCC 6538), but it was effective against other strains (Table 1 and Figure 7). *Artemisia EtAc* extract diluted ×15 times and *Artemisia EtAc* extract diluted ×8 times possessed a similar moderate level of efficiency for *Escherichia coli* (ATCC 8739). AgNPs@*EtAc* extract ×60 was active against all the studied strains, and the highest sensitivity was against *E. coli* and *C.Albicans. Artemisia EtAc* extract with dilutions 8, 12, 15, and 16 exhibited activity to *C.Albicans. Artemisia herba-alba*

in combination with AgNPs exhibited significant larvicidal toxicity against mosquitoes. The LC50 (a lethal concentration with 50% mortality) of AgNPs against Indian strains was 9.7 mg/L for *Anopheles stephensi*, 10.7 mg/L for *Aedes aegypti*, and 11.4 mg/L for *Culex quinquefasciatus*. In the case of Saudi Arabian strains, the LC_{50} for AgNPs was 33.6 mg/L for *Aedes aegypti* and 38 g/L for *Culex pipiens*. In adult mosquito experiments, the *Artemisia herba-alba* extract alone indicated LC_{50} values ranging from 293 to 450 mg/L, while the LC50 values for AgNPs possessed activity from 8 to 27.4 g/L. This suggests that AgNPs significantly enhanced the adulticidal activity against mosquitoes compared to the *A. herba-alba* extract alone [36]. AgNPs synthesized with *Artemisia tournefortiana Rchb* ethanolic extract had an average size of 23 ± 14.8 nm. Their impact on in vitro cytotoxicity was assessed using normal human embryonic kidney (HEK293) cells and human colon adenocarcinoma cancer (HT29) cells [37]. AgNPs @*Artemisia absinthium* L. (2 mM) with a plant extract (10 mg/mL) at various ratios had a significant impact on the size, stability, and yield of AgNPs. When the extract-to- $AgNO_3$ ratio was 6:4 *v*/*v*, it resulted in the highest conversion efficiency of $AgNO_3$ to AgNPs, producing particles with an average size of less than 100 nm [38]. AgNPs stabilized by *Artemisia afra* have an average size of approximately 30.7 nm. These AgNPs were synthesized within 30 min at a reaction temperature of 80–90 °C using an aqueous leaf extract of *Artemisia afra*. The synthesized AgNPs exhibited SPR in the wavelength range of 423–438 nm [39]. Hu et al. reported that AgNPs stabilised by *Artemisia argyi* leaf extract exhibited a strong antimicrobial activity of 1 µg/mL for *Acinetobacter baumannii*, and 2 mg/L for *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Additionally, the minimum biofilm eradication concentration at 50% for these AgNPs against these strains was 2 µg/mL for *Acinetobacter baumannii*, 4 µg/mL for *Staphylococcus aureus*, 0.5 µg/mL for *Escherichia coli*, and 2 µg/mL for *Candida albicans* [34].

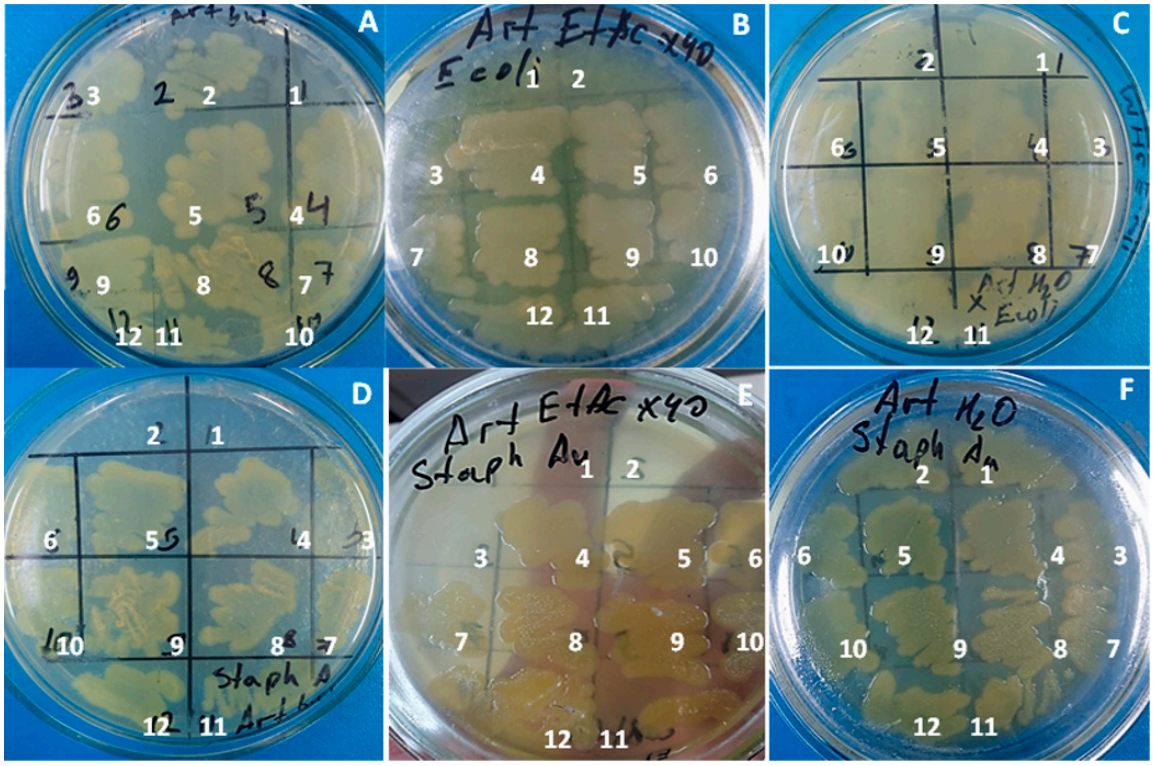

Figure 6. Bacterial growth on agar plate [*n* = 3] after exposure to different dilutions Art extract obtained using different solvents: (**A**) *Art* butanol extract ×40 in 50% ethanol against of *E. coli* (ATCC 8739); (**B**) *Art* EtAc ×40 in 50% ethanol against of *E. coli* (ATCC 8739); (**C**) *Art* aq. extract ×20 against of *E. coli* (ATCC 8739); (**D**) *Art* butanol extract ×40 in 50% ethanol; (**E**) *Art* EtAc extract ×40 in 50% ethanol against of *S. aureus* (ATCC 25923); (**F**) *Art* aq. extract ×20 against of *S. aureus* (ATCC 25923).

Table 1. Zone of bacterial growth inhibition under action of AgNPs and *Artemisia* plant extract at different dilution factor.

N of Sample	*S. aureus* (ATCC 6538)	*Escherichia coli* (ATCC 8739)	*Pseudomonas aeruginosa* ATCC 9027	*C. albicans* (ATCC 10281)
AgNPs citrate etalon suspension	0	15 ± 3	12 ± 2	18 ± 3
EtAc extract ×15	11 ± 2	12 ± 2	12 ± 2	12 ± 2
AgNPs@EtAc extract ×45	11 ± 3	0	11 ± 2	11 ± 2
AgNPs@EtAc extract ×60	12 ± 3	20 ± 2	11 ± 2	20 ± 3
EtAc extract ×8	0	11 ± 2	0	11 ± 2
EtAc extract ×12	0	0	12 ± 3	11 ± 2
EtAc extract ×16	0	0		11 ± 2

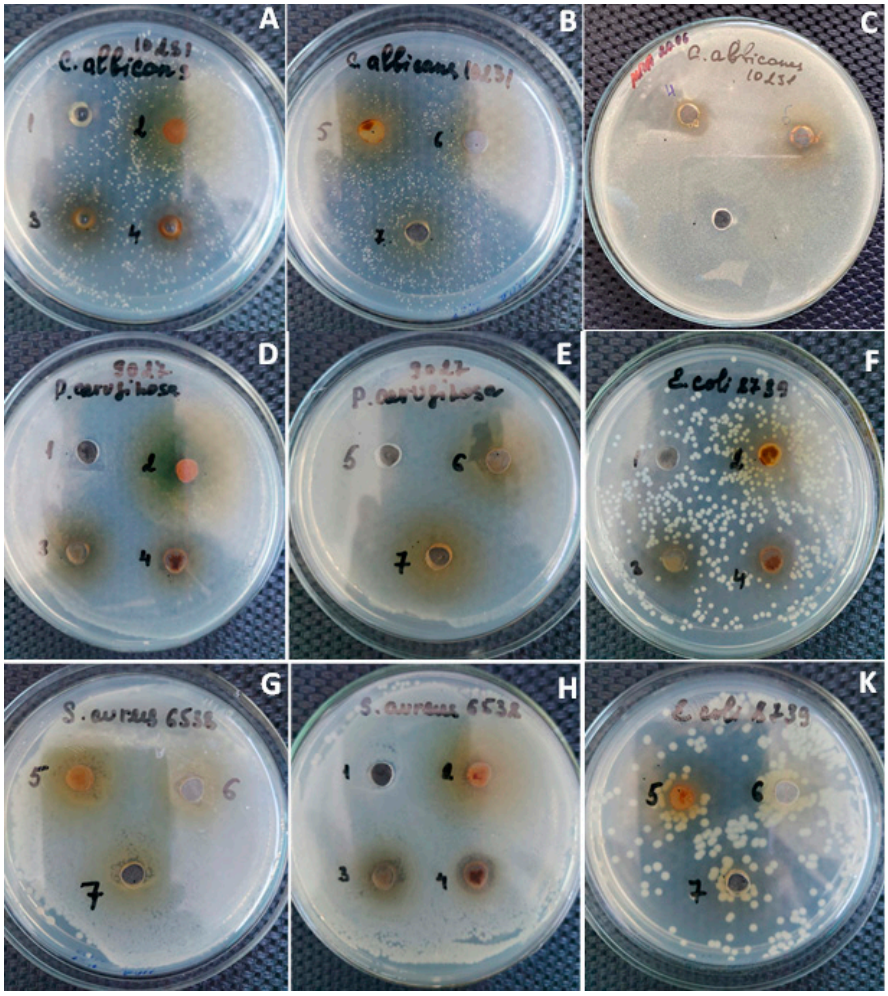

Figure 7. Growth of bacterial strain after exposure to different dilutions plant extract s: (1) control blank; (2) Art EtAc extract ×15; (3) AgNPs@*Art* EtAc extract ×45; (4) AgNPs@*Art* EtAc extract ×45; (5) Art EtAc extract ×8; (6) Art EtAc extract ×12; (7) Art EtAc extract ×16; against (**A**–**C**) *C. albicans* (ATCC 10281); (**D**,**E**) *Pseudomonas aeruginosa* ATCC 9027; (**G**,**H**) *S. aureus* (ATCC 6538) (**F**,**K**) *Escherichia coli* (ATCC 8739).

4. Conclusions

Several *Artemisia terrae-albae* extracts were obtained. The appropriate aqueous and ethylacetate extracts were acceptable for the following evaluation of antimicrobial activity. Moreover, the extracts were utilized to reduce silver ions to AgNPs as well as to serve as a stabilizing agent. The zetta potential measurement of the AgNPs had a negative surface charge. The decrease of the ethylacetate extract solution in water for the *Art @EtAc* extract led to less stable AgNPs and following aggregation with average size in the range of 80–150 nm. Using a diluted ethylacetate solution allowed dissolving the lipophilic compounds and allowing for participation in the AgNP stabilization. Moreover, the preliminary attempt of the ethanolic solution of the Artemisia antimicrobial test illustrated activity only for the high content of ethanol, and it was unclear what is responsible for the activity. Then, EtAc allows the observation of the true values of antimicrobial activity. The dissolution of poorly soluble ethanolic and EtAc *Artemisia terrae-albae* extracts in water using a method of serial dilutions ×40, ×80, ×160 etc. did not reveal activity of *E. coli* (ATCC 8739) and *S. aureus* (ATCC 25923); however, it was difficult and unpractical to prepare a solution at higher concentration. EtAc Art extract diluted 15 and 8 times demonstrated comparable moderate levels of activity against *Escherichia coli* (ATCC 8739). AgNPs@*EtAc* extract ×60 exhibited activity against all the strains studied, with the highest sensitivity observed against *E. coli* and *C. Albicans*. Additionally, *Artemisia terrae-albae* EtAc extract at dilutions 8, 12, 15, and 16 exhibited activity against *C. Albicans*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11103041/s1, Figure S1: TEM photograph: (A–D) AgNPs@EtAc Art extract ×40; Figure S2: Bacterial growth on agar plate after exposure to Art extract obtained using different solvents: (A) Art butanol extract ×40 in 50% ethanol against of E. coli (ATCC 8739); (B) Art EtAc x40 in 50% ethanol against of E. coli (ATCC 8739); (C) Art aq. extract ×20 against of E. coli (ATCC 8739); (D) Art butanol extract ×40 in 50% ethanol; (E) Art EtAc extract ×40 in 50% ethanol against of S. aureus (ATCC 25923); (F) Art aq. extract ×20 against of S. aureus (ATCC 25923).

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