Green Synthesis of Silver Nanoparticles Using Extract of Artemisia absinthium L., Humulus lupulus L. and Thymus vulgaris L., Physico-Chemical Characterization, Antimicrobial and Antioxidant Activity

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Abstract: The novelty of this study is twofold: AgNPs were obtained and characterized using Artemisia absinthium (A. absinthium), Humulus lupulus (H. lupulus), and Thymus vulgaris (T. vulgaris) plants extracts; moreover, a green and environmentally friendly approach for the synthesis of silver nanoparticles (AgNPs) using aqueous extracts was developed. This paper discusses new approaches about the synthesis of AgNPs. T. vulgaris, H. lupulus, and A. absinthium, which are renewable and common plants, perfect as reducing, stabilizing, and capping agent for green synthesis of silver nanoparticles (AgNPs). The extracts and synthesized AgNPs were characterized by various physico-chemical, phytochemical, morphological scanning electron microscope (SEM/EDS) and transmission electron microscope scanning (TEM), and antibacterial activity. The antioxidant activity of extracts and AgNPs were also assessed by 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS**), 2,2-diphenyl-1-picrylhydrazyl (DPPH*), cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and trifluoperazine dihydrochloride (TFPH*) scavenging assays. Extracts/AgNPs showed significant antioxidant activity in all cases. A. absinthium/AgNPs, H. lupulus/AgNPs, and T. vulgaris/AgNPs displayed activities against DPPH* (0.14 ± 0.00; 0.11 ± 0.00 and 0.14 ± 0.00 mmol/g), ABTS** (0.55 ± 0.05; 0.86 ± 0.05 and 0.55 ± 0.05 mmol/g), respectively. TEM analysis confirmed the average particle size, it estimated t A. absinthium/AgNPs–46 nm, H. lupulus/AgNPs size of synthesized particles was 42 nm and T. vulgaris/AgNPs–48 nm. SEM analysis revealed that T. vulgaris/AgNPs showed in solitary cases as snowflake-like, branched, but in a general spheric shape, H. lupulus/AgNPs were wedge-shaped, and A. absinthium/AgNPs were the spherical shape of the synthesized AgNPs. EDS analysis confirmed the purity of the synthesized AgNPs with a strong signal at 3.2 keV. A. absinthium/AgNPs, H. lupulus/AgNPs, and T. vulgaris/AgNPs exhibited higher antibacterial activity against all tested bacterial strains compared to their respective pure extracts. It is concluded that AgNPs synthesized in extracts have a broad range of biological applications, which can be used as an eco-friendly material without having negative effects on the environment.
Keywords: plant extracts; green synthesis; silver nanoparticles; A. absinthium; H. lupulus; T. vulgaris

1. Introduction

Nanotechnology and nanoscience are essential for many industry fields and sciences. Nanosized materials have unique chemical, optical, and physical properties and antimicrobial activity. Silver nanoparticles (AgNPs) have drawn researcher’s attention to their vigorous biological activities, including antimicrobial, antioxidant, wound healing, and anticancer activities. Consistently, green synthesis of NPs has gained a great deal of attention because of its advancement over chemical and physical methods as it is cost-effective, environmentally-friendly, as well as it does not require use of high pressure, energy, temperature, and toxic chemicals [1]. Moreover, various nanomaterials such as gold, copper, zinc, magnesium, titanium, and silver were used for antimicrobial activity against pathogenic microorganisms [2–5]. Previously, many studies reported synthesis of silver nanoparticles using different morphological parts of plants extracts, for instance, leaves, flowers, seeds, or stems [1]. It has been reported that plant metabolites such as terpenoids, phenolics, tannins, flavonoids, terpenoids, alkaloids, and polysaccharides contribute to the reduction of Ag ions to AgNPs [6,7]; therefore, each plant’s phytochemical composition is different; still common similarities determine their unique properties [8–12]. In essence, physico-chemical, and technological parameters, in particular, the size, morphology, and stability of AgNPs, depend on the method of preparation of extract, nature of the solvent, mixing ratio, concentration, pH, and temperature of the reaction mixture [2,4].

Of particular interest, plants with antimicrobial properties hold great potential for the green synthesis of AgNPs against microbial diseases due to the complexity of their phytochemical composition [2,5,13]. Hence, in the present study, A. absinthium, H. lupulus, and T. vulgaris were selected because they were not reported on in the synthesise of silver nanoparticles. The first plant was chosen A. absinthium, which is one of the most widely distributed Artemisia genus species. A. absinthium, a perennial herb, can be found all over the world. In ethnic medicine, it is used as an insecticide and has antiparasitic, antispasmodic, and antibacterial effects [14]. In addition, it is used in the treatment of chronic fevers and inflammation. Secondly, H. lupulus is an industrial and medicinal plant, also known in folk medicine. H. lupulus plays an important role in brewing industry because of its organoleptic features, which are derived from many different bitter organic acids, essential oils, resins, and polyphenol compounds [15,16]. The plant consists of a high amount of α- and β- acids such as humulones, lupulones, and isohumulones. Consistently, they are responsible for organoleptic characteristics and other biological activities, including antioxidant, anti-inflammatory, antimicrobial, and antitumoral effects [16–19]. Besides this, hops are used to manage anxiousness, cough and fever, spasms, inflammation, and toothache as traditional medicine [20–24]. Lastly, T. vulgaris, a member of the Lamiceae family aromatic perennial plant, is widely used in folk medicine for its expectorant, antibroncholitic, antispasmodic, anthelmintic, carminative, and diuretic properties and antimicrobial activities [20] T. vulgaris accumulates up to 2.49 mL/100 g (DW) of essential oils [21].

The present study was designed to synthesize AgNPs using aqueous A. absinthium, H. lupulus, and T. vulgaris extracts (Figure 1). These species are used as antibacterial agents in traditional medicine and are known for their biological activity in the scientific literature. The morphology and structure of particle size of the synthesized AgNPs were accomplished by using a scanning electron microscope (SEM). Antioxidant activity was tested by different methods such as ABTS and TFPH•+ and radical cation scavenging, DPPH• free radical scavenging, CUPRAC, and FRAP assay. Additionally, spectrophotometric studies helped to determine the total phenolic, flavonoid, proanthocyanidin content, and hydroxycinnamic acid derivatives. The antimicrobial activity was investigated against Gram-negative/positive bacteria cultures by the agar disk diffusion test method.
Figure 1. Graphical abstract representing the overview of paperwork with key highlights.

2. Materials and Methods

2.1. Plant Material

Finely cut *A. absinthium*, *H. lupulus*, and *T. vulgaris* (manufacturer UAB “Švenčionių vaistažolės”) material were purchased from a public pharmacy, operating in Kaunas, Lithuania. Plant material was ground to a powder using a mill (IKA® A11 basic “Staufen”, Germany). Loss on drying before the analysis was determined by drying powdered raw plant material in a laboratory drying oven to complete evaporation of water and volatile compounds (temperature: 105 °C; the difference in weight between measurements: up to 0.01 g) and by calculating the difference in raw material weight before and after drying. The data were recalculated for dry weight.

2.2. Extraction

Fifty grams of raw material was weighed (exact mass of ground material) and soaked for 2 h in 70 (v/v) ethanol. The soaked raw material was transferred to a percolator, covered with extractant—70 (v/v) ethanol, and left to macerate for 24 h. An ethanolic phase of liquid extract was evaporated using a rotary evaporator. The obtained plant extracts were used as green reductants and capping agents for the biosynthesis of AgNPs.

2.3. Preparation of Silver Nanoparticles

Silver nitrate was purchased in Merck, Germany. An amount of 0.03 g silver nitrate was weighed and dissolved in 5 mL distilled well-mixed water. Next, 30 mL of each of the aqueous extracts—*A. absinthium*, *H. lupulus*, and *T. vulgaris*—was added to a silver nitrate aqueous solution under vigorous stirring (speed 500 rpm) at 25 °C temperature for 2 h, then a 24-h incubation at room temperature was completed. During synthesis, gradual change in color from mild yellow to dark brown in the reaction mixture indicated the presence of silver nanoparticles.

2.4. Spectrophotometric Studies

2.4.1. Determination of Total Phenolic Content

All spectrophotometric measurements were carried out using a M550 (Spectronic CamSpec, Garforth, England, United Kingdom). The total phenolic content in the extracts was determined using the Folin-Ciocalteu method [22]. Gallic acid concentration range was 0.25–4 mg/mL. The total phenolic content was expressed as mg/g gallic acid equivalent (GAE) per gram of dry weight (DW) (mg GAE/g DW).

2.4.2. Determination of Total Flavonoid Content

The total amount of flavonoids was determined using the described methodology by Urbonaviciute et al. (2006). Rutin concentration range was 0–0.85 mg/mL, while the total...
amount of flavonoids was expressed as mg/g rutin equivalent (RE) per gram of dry weight (mg RE/g DW).

2.4.3. Determination of Total Proanthocyanidin Content

Ten microliters of extract was mixed with 3 mL 0.1% DMCA reagent solution in acidulated C₂H₅OH (9:1, v/v) [23]. After 5 min, the absorption was measured at λ = 640 nm. The total content of proanthocyanidins (mg/mL) in the extract was expressed as (−)-epicatechin equivalent (EE) using (−)-epicatechin. (−)-Epicatechin concentration range 0.125–1.3 mg/mL. In dehydrated material, the total content of proanthocyanidins was expressed as mg EE/g DW.

2.4.4. Determination of Total Content of Hydroxycinnamic Acid Derivatives

The total content of hydroxycinnamic acid derivatives was determined using the described methodology [9]. Sample preparation: 2 mL 0.5 M HCl, 2 mL Arnow reagent, and 2 mL NaOH attenuate solution was added to 1 mL of extract and diluted with distilled water up to 10 mL. Blank solution: 2 mL 0.5 M HCl, 2 mL NaOH attenuate solution was added to 1 mL of extract and diluted with distilled water up to 10 mL. Total content of hydroxycinnamic acid derivatives in the extract was expressed as mg/mL of chlorogenic acid equivalents (ChAE) using chlorogenic acid calibration curve, while chlorogenic acid concentration range was 0.0625–1 mg/mL. In dry material, total content of hydroxycinnamic acid derivatives was expressed as mg ChAE/g DW.

2.5. Analysis of Extract Antioxidant Activity

2.5.1. ABTS•⁺ Radical Cation Scavenging Assay

Parental ABTS•⁺ radical solution is prepared by oxidizing ABTS with potassium persulfate. An amount of 2 mM of ABTS was dissolved in purified water and 0.7 mM of potassium persulfate was added. Then, prepared solution was stored for 16–17 h in the dark at a room temperature, until ABTS and potassium persulfate fully reacted. Meanwhile, a stable ABTS•⁺ radical cation formed during this reaction. Parental ABTS•⁺ solution, when protected from light, at room temperature, remained stable for more than 2 days.

Working ABTS•⁺ solution was prepared by diluting a parent ABTS•⁺ solution with distilled water to 0.8 ± 0.03 absorption units at wavelength of 734 nm [24]. Ten microliters of extract was mixed with 3 mL of working ABTS•⁺ solution in a quartz cuvette. After 30 min, a decrease in sample light absorbance was determined using a spectrophotometer. Antiradical activity was expressed as TE based on Trolox calibration curve; Trolox concentration range—8000–24,000 µM.

2.5.2. DPPH• Free Radical Scavenging Assay

The DPPH• free radical scavenging activity was determined using the method proposed by Brand-Williams et al. [25]. Ten microliters of the extract sample was mixed with the DPPH• solution in 96.3 v/v ethanol (3 mL, 6 × 10⁻⁵ M) in a quartz cuvette. Using a spectrophotometer, a decrease in light absorbance was determined at a wavelength of 517 nm, until absorption equilibrium was achieved (approximately after 30 min.). Antiradical activity when applying DPPH• method was expressed as TE based on Trolox calibration curve; Trolox concentration range—400–20000 µM.

2.5.3. CUPRAC Assay

In the meantime, CUPRAC solution included copper (II) chloride dihydrate (0.01 M in water), ammonium acetate buffer solution (0.001 M, pH = 7), and neocuproine (0.0075 M in ethanol) (ratio 1:1:1). During the evaluation, 3 mL of CUPRAC reagent was mixed with 10 µL of extracts. An increase in absorbance was recorded at λ = 450 nm. Reduction activity when applying FRAP method was expressed as TE based on Trolox calibration curve; Trolox concentration range—2000–48,000 µM.
2.5.4. Ferric Reducing Antioxidant Power (FRAP) Assay

Contents of FRAP reagent included sodium acetate buffer solution 300 mM (pH = 3.6), 10 mM TPTZ solution in 40 mM hydrochloric acid, iron chloride hexahydrate 20 mM. The working FRAP reagent solution was prepared by mixing sodium acetate buffer solution, TPTZ solution in hydrochloric acid and iron chloride hexahydrate solution in a ratio of 10:1:1. The working solution was prepared right before the use 10 µL of extract was mixed with 3 mL of working FRAP reagent solution in a quartz cuvette [6]. After 30 min, an increase in light absorbance was determined at a wavelength of 593 nm using a spectrophotometer. Reduction activity when applying FRAP method was expressed as TE based on Trolox calibration curve; Trolox concentration range—400–24,000 µM.

2.5.5. TFPH•+ Radical Cation Scavenging Assay

Preparation of parental solution was accomplished as following. Parental TFPH solution was prepared by dissolving 0.480 g (exact mass) of TFPH in 10 mL of purified water, which remained stable for 2 months when stored in the dark at 4 °C temperature.

Preparation of working solution: 0.5 mL of parental TFPH solution was mixed with 0.1 mL of 100 mM K₂S₂O₈ and 70 mL of 4 M sulfuric acid in a 100 mL volumetric flask. Then 4 M sulfuric acid was added to a total volume of 100 mL, while prepared solution was stored in the dark for 15–20 min. Measured light absorption should be equal to 0.70 ± 0.1 absorption units at wavelength of 502 nm. Working solution remained stable for 2 h when stored in the dark at a room temperature [26].

Ten microliters of extract was mixed with 3 mL of working TFPH solution in a quartz cuvette. After 30 min, a decrease in sample light absorbance was determined using a spectrophotometer. Antiradical activity when applying TFPH method was expressed as TEAC based on Trolox calibration curve; Trolox concentration range—2000–16,000 µM.

2.5.6. Calculation of Antioxidant Activity of the Extracts

The antioxidant activity of the extracts was calculated from the Trolox calibration curve and was expressed as mmol of the Trolox equivalent (TE) per one gram of dry weight (DW). TE was calculated according to the following formula:

\[ TE = \frac{c \times V}{m} \]  

where, \( c \): the concentration of Trolox established from the calibration curve (in µM); \( V \): the volume of the extract (in mL); \( m \): the weight (exact) of the herbal raw material (in grams).

2.6. Microscopy

2.6.1. Scanning Electron Microscopy (SEM)

The particle size and structures were studied from the images obtained by SEM FEI Quanta 200 FEG (FEI Company, Hillsboro, OR, USA) manufacturer, city, state abbreviation, country). The samples were examined in a low vacuum mode operating at 20.0 kV using an LDF detector. The content of AgNPs and chemical analysis is of nanocomposites were performed by the energy dispersive spectroscopy SEM/EDS technique with a BruckerXFlash 4030 detector (accelerating voltage 10 kV, distance between the bottom of the objective lens and the object 10 mm).

2.6.2. Transmission Electron Microscopy (TEM)

Tecnai G2 F20 X-TWIN (FEI, USA) examined the size of the synthesized nanoparticles under a transparent electron microscope (TEM). For the TEM experiment, the diluted samples were deposited drop-wise onto carbon-coated copper TEM grids. The Schottky emission electron source was used, the accelerating voltage was 20–200 kV. The resolution of the microscope ranged from 0.8 to 1.0 nm. EDAX spectrometer with r-TEM detector and 11 MPix ORIUS SC1000B (Gatan Inc., Pleaston, California, USA) manufacturer, city, state abbreviation, country) CCD camera were used. The spot/linear resolution was 0.25/0.102 nm.
2.7. Antimicrobial Activity

The antimicrobial activity was investigated against Gram-negative and Gram-positive bacteria cultures. The antimicrobial activity of extracts was tested via an agar well diffusion assay [27]. For this purpose, a 0.5 McFarland Unit density suspension (~108 CFU/mL) of each pathogenic bacterial strain was inoculated onto the cooled Mueller Hinton Agar (Oxoid, Basingstoke, UK), using sterile cotton swabs. Wells of 6 mm in diameter were punched in the agar and filled with 50 µL of extracts. The experiments were repeated three times, and the average size of the inhibition zones was calculated. The antimicrobial activities against the tested bacteria were determined by measuring the inhibition zones’ diameter (mm). The antimicrobial activity of extracts were determined against *Staphylococcus aureus* (S. aureus), *Staphylococcus haemolyticus* (S. haemolyticus), *Enterococcus durans* (E. durans), *Bacillus pseudomycoides* (B. pseudomycoides), *Salmonella enterica* (S. enterica), *Aeromonas hydrophila* (A. hydrophila), *Aeromonas veronii* (A. veronii), *Acinetobacter baumannii* (A. baumannii), *Acinetobacter johnsonii* (A. johnsonii), *Enterobacter cloacea* (E. cloacea), *Cronobacter sakazakii* (C. sakazakii), *Klebsiella pneumoniae* (K. pneumoniae), *Escherichia coli* (E. coli), and *Pseudomonas aeruginosa* (P. aeruginosa), which were obtained from the collection of the Lithuanian University of Health Sciences (Kaunas, Lithuania).

2.8. Statistical Analysis

The statistical analysis was performed using SPSS 20 software (SPSS Inc., Armonk, NY, USA). All the experiments were implemented in triplicate and the results are conferred as mean ± standard error mean (SEM) of all calculated values.

3. Results and Discussion

3.1. Content of Phytochemicals

In the present work, aqueous extracts of *A. absinthium*, *H. lupulus*, and *T. vulgaris* were used for the synthesis of AgNPs. As shown in Figure 2, the biomolecules present in the extracts are responsible for the reduction of Ag⁺ ions to Ag⁰ in a single step.

![Figure 2. Schematic synthesis of AgNPs and reduction of Ag⁺ ions to Ag⁰.](image)

The phenolic compounds and reducing sugars are responsible for the bioreduction of Ag⁺ ions to AgNPs. Moreover, flavonoids and proanthocyanidins may also cause reduction of Ag⁺ ions to AgNPs and act as a capping agent to prevent agglomeration. Exceeding, flavonoids can also directly scavenge molecular species of active oxygen. All the three tested extracts contained flavonoids, consisting of hydroxyl groups with a stronger ability to bind with silver ions and acted as a reducing agent.

The following substances were studied based on the reviewed literature: phenols, hydroxycinnamic acid, flavonoids, and proanthocyanidin. A great deal of scientific literature have analyzed and discussed the bactericidal properties of plant extracts and their possible application. One of the most important biological effects of plant extracts is high antioxidant activity, which is closely related to anticancer antibacterial, anti-inflammatory, antiallergic, antiviral, hepatoprotective, antithrombogenic, and many other effects [28].

The extracts that were obtained from *A. absinthium*, *H. lupulus*, and *T. vulgaris* material and their phytochemical composition, i.e., phenolic compounds, flavonoids, proanthocyanidins, and hydroxycinnamic acid, was investigated and summarized in Table 1. It was found...
that *H. lupulus* extract contained at most proanthocyanidins from all the tested extracts was at 6.31 ± 0.26 mg EE/g, and similarly to phenolic compounds, this extract contained 10.79 ± 1.44 mg GAE/g. It was reported that methanol extract of hop cones contained 7.4 µg and 7.12 ± 0.09 mg GAE/g total polyphenol [15,29]. Further on, *T. vulgaris* extract contained at most phenolic and flavonoid compounds 55.83 ± 2.55 GAE/g DW and 24.77 ± 0.37 RE/g DW, respectively. Other researchers obtained similar results with phenolic and flavonoid compounds 44.16 ± 1.46 GAE/g DW and 17.89 ± 0.71 RE/g DW, respectively [30].

Table 1. Phytochemical analysis of extracts.

| Compound Name                                      | *A. absinthium* | *H. lupulus* | *T. vulgaris* |
|----------------------------------------------------|-----------------|--------------|--------------|
| The total amount of proanthocyanidins, mg EE/g DW  | 0.99 ± 0.63     | 6.31 ± 0.26  | 1.28 ± 0.55  |
| The total amount of hydroxycinnamic acid derivatives, mg ChAE/g DW | 2.34 ± 0.01  | 3.94 ± 0.13  | 8.12 ± 1.17  |
| The total amount of phenolic content, mg GAE/g DW  | 10.88 ± 0.62    | 10.79 ± 1.44 | 55.83 ± 2.55 |
| The total amount of flavonoids, mg RE/g DW          | 4.42 ± 0.28     | 2.28 ± 0.09  | 24.77 ± 0.37 |

| Compound Name                                      | *A. absinthium/AgNPs* | *H. lupulus/AgNPs* | *T. vulgaris/AgNPs* |
|----------------------------------------------------|-----------------------|-------------------|-------------------|
| The total amount of proanthocyanidins, mg EE/g DW  | 0.86 ± 0.28           | 3.46 ± 0.17       | 0.78 ± 0.21       |
| The total amount of hydroxycinnamic acid derivatives, mg ChAE/g DW | 2.05 ± 0.20 | 3.64 ± 0.09       | 7.14 ± 0.55       |
| The total amount of phenolic compounds, mg GAE/g DW | 8.98 ± 0.24           | 6.76 ± 0.32       | 44.00 ± 4.54      |
| The total amount of flavonoids, mg RE/g DW          | 3.94 ± 0.31           | 1.52 ± 0.08       | 19.04 ± 10.33     |

The results showed that the total amount of proanthocyanidins, hydroxycinnamic acid derivatives, phenolic content, and flavonoids decrease after synthesized silver nanoparticles in all extracts (Table 1).

Notably, *A. absinthium* crude extract and *A. absinthium/AgNPs* total phenolic content in all tested cases decrease about one time. The more significant change of *H. lupulus* was observed, where the total amount of proanthocyanidins and flavonoids decreased ~1.66 times. These changes in phenolic compounds may be associated with the synthesis of AgNPs particles. Therefore these secondary metabolites, specifically phenols, and flavonoids, may interact with AgNPs as capping agents and increase the surface area due to efficient scavenging properties [27].

3.2. Determination of Antioxidant Properties

Epidemiological studies had demonstrated the ability of an antioxidant to reduce or stop the progression of many chronic diseases [31,32]. The antiradical and reductive activity of selected plant sample extracts in vitro is suitable for prospective antioxidant studies. The results, obtained during the studies, will provide a consumer with products rich in antioxidants, valid for the assessment and standardization of the quality of plant raw materials and their products. Plant extracts are natural multicomponent matrices containing various biologically active compounds, the antioxidant activity of which is manifested by different mechanisms. Therefore, plant extracts antioxidant activity cannot be properly evaluated using only one methodology [33]. For these reasons, to fully assess plant extracts antioxidant activity, it is recommended to apply at least two methods to determine antioxidant activity [34]. ABTS, CUPRAC, TFPH, FRAP, and DPPH UV-VIS spectrophotometry methods to the in vitro studies of selected plant extracts’ antioxidant activity were applied. They are often used in antioxidant tests, mostly for natural extracts. The results are summarized in Table 2.
Examination of selected plant extracts using five different in vitro spectrophotometric methods showed that the strongest in vitro antiradical activity, assessed by ABTS and TPHF methods, and in vitro reducing activity, determined by FRAP method, were characterized by extracts of *T. vulgaris* samples. Extracts of *H. lupulus* samples showed the strongest antiradical activity in vitro as measured by the DPPH methodology and in vitro reduction activity by the CUPRAC methodology (Table 2). The total amount of phenolic compounds (TPC) and flavonoid content (TFC) of *T. vulgaris* was significantly higher than *A. absinthium/AgNPs* and *H. lupulus/AgNPs*. In comparison with other studies these are secondary metabolites. These results may indicate the impact of the synthesis of nanoparticles in different plant extract. Similar results were also obtained by other researchers with grapefruit pomace extract/AgNPs and *Thymus citriodorus* and *Thymus vulgaris* [27,35].

### 3.3. SEM Analysis

SEM technique was employed to visualize the morphology, size, and shape of the influence on the reaction medium of AgNPs. Antibacterial activity of AgNPs depends on the nanoparticle morphology: size and shape [3]. The size of synthesized silver nanoparticles might increase their surface area, promoted bacterial cell wall interactions and membrane permeability, and enhance their antimicrobial activity by leakage of bacterial cellular contents [36]. Figure 3 presents typical SEM images of the biosynthesized nanomaterials. Since secondary plant metabolites act as reducing agents and as capping agents for AgNPs, they differ in the phytochemical composition. Different extract biomolecules cap the silver nanoparticles. Microscopic imagines indicate these morphological differences. For *A. absinthium/AgNPs* were spherical-shaped with a size of ~50 nm (Figure 3a). The silver nanoparticles assisted by *H. lupulus/AgNPs* were also spherical and size of ~40 nm. (Figure 3b). The AgNPs assisted by *T. Vulgaris* was spherical-shaped with 45 nm (Figure 3c).

![SEM images](image_url)

**Figure 3.** SEM images of silver nanoparticles synthesized using extracts of (a) *A. absinthium*, (b) *H. lupulus*, and (c) *T. vulgaris*.

The assessment of the elemental composition on the surface of the biosynthesized nanomaterials was done using EDS spectroscopy. In general, the optical absorption peak of silver appears at around 3 keV [4,37]. Similar patterns of EDS spectra were reported.
earlier for the AgNPs with an absorption range of 2–4 keV, which may arise because of the possibility of X-ray energy range in different levels [38].

As shown in Figure 3, EDS analysis revealed silver in the nanoparticles formulated for *A. absinthium*, *H. lupulus*, and *T. vulgaris*. Furthermore, the prepared nanoparticles did not exhibit any nitrogen. It indicated the absence of noticeable traces of ions from the AgNO₃ precursor used, which confirms the presence of metallic silver in the sample and evidence of successful reduction of silver ions.

Figure 4 indicates carbon and oxygen atoms in all nanoparticle samples. Once again this confirms the existence of phytochemicals on the surface of the biosynthesized AgNPs since secondary plant metabolites act not only as reducing agents but also as capping agents for AgNPs [36].

Moreover, the presence of magnesium (Mg) in *A. absinthium*/*AgNPs and *T. vulgaris*/*AgNPs extracts samples were indicated. This mineral is a main component of chlorophyll molecule found in green plants. Comparable, in *H. lupulus*/*AgNPs magnesium, was not detected. For *A. absinthium* and *T. vulgaris* extracts stem, and leave morphological parts were used. Meanwhile, *H. lupulus* extract was made with only dried fins.

![A. absinthium EDS spectrum](image1)

![H. lupulus EDS spectrum](image2)

**Figure 4. Cont.**
3.4. TEM

Figure 5 presents typical TEM images of the biosynthesized nanomaterials. These micrographs show the presence of individual nanoparticles with almost spherical shape and roughly, which is characteristic of AgNPs [36]. The particle size and size distribution of the synthesized AgNPs using plant extracts were estimated under TEM micrographs using ImagineJ software. *A. absinthium*/AgNPs size were found to be about 46 nm, and polydispersity of 38.2, *H. lupulus*/AgNPs size of synthesized particles were 42 nm with a polydispersity of 41.0. Consistently, *T. vulgaris*/AgNPs picture the size of particles 48 nm and the polydispersity of 46.1.

Results indicate the nanoparticulate nature of the synthesized materials regarding the successful formation of colloidal particles from silver ions in the presence of aqueous plant extracts. In addition, all the ImageJ graphs described a Gaussian distribution peak at around the average sizes estimated for each nanoparticle formulation (Figure 5), which showed decent particle size distribution as observed with polydispersity values below 50%.

3.5. Antimicrobial Activity

Three plant extracts and AgNPs synthesized in these extracts were investigated to evaluate their antibacterial activity against 15 different pathogenic and opportunistic bacteria strains using the disc diffusion method. Evaluation of antibacterial activity of these plant extracts and AgNPs was recorded in Table 3. The results revealed that all plant extracts were potentially effective in suppressing microbial growth bacteria with variable potency. Results showed that significant antibacterial activity was observed against *S. aureus* with a zone of inhibition to 13.3–25.6 mm of AgNPs synthesized in tested extracts. Other researchers obtained similar results with AgNPs against *S. aureus* where the zone of inhibition was 23–27 mm [39]. *A. absinthium*/AgNPs inhibit all tested bacteria strains-growth with the inhibition zone size of 9 to 19.3 mm, but not all bacteria tested were insensitive to pure *A. absinthium* extract.
Figure 5. TEM micrographs and size distribution of AgNPs obtained using *A. absinthium* (a), *H. lupulus* (b), and *T. vulgaris* (c) extracts.
Table 3. Inhibition zones of the extracts against pathogenic opportunistic microorganisms.

| Pathogenic and Opportunistic Bacteria Strains | Samples          | A. absinthium | H. lupulus | T. vulgaris |
|---------------------------------------------|------------------|---------------|------------|------------|
|                                             | Pure             | AgNPs        | Pure       | AgNPs      | Pure       | AgNPs      |
| S. aureus                                   | 6.3 ± 0.1        | 13.3 ± 0.6   | 20.3 ± 0.6 | 25.6 ± 0.3 | 0.0 ± 0.0  | 18.3 ± 0.4 |
| S. haemolyticus                             | 0.0 ± 0.0        | 13.2 ± 0.4   | 14.2 ± 0.4 | 17.2 ± 0.5 | 5.0 ± 0.8  | 16.2 ± 0.8 |
| P. aeruginosa                               | 0.0 ± 0.0        | 15.6 ± 0.5   | 0.0 ± 0.0  | 15.6 ± 0.4 | 0.0 ± 0.0  | 17.9 ± 0.3 |
| E. coli                                     | 4.3 ± 0.2        | 13.6 ± 0.5   | 13.4 ± 0.4 | 18.1 ± 0.7 | 6.7 ± 0.5  | 16.6 ± 0.3 |
| B. pseudomycoides                           | 0.0 ± 0.0        | 15.1 ± 0.4   | 14.5 ± 0.9 | 21.4 ± 0.6 | 15.3 ± 0.2 | 16.7 ± 0.2 |
| K. pneumoniae                               | 0.0 ± 0.0        | 12.2 ± 0.6   | 0.0 ± 0.0  | 14.3 ± 0.4 | 0.0 ± 0.0  | 13.4 ± 0.1 |
| A. hydrophila                               | 0.0 ± 0.0        | 11.3 ± 0.8   | 0.0 ± 0.0  | 9.1 ± 0.2  | 0.0 ± 0.0  | 13.4 ± 0.1 |
| A. veronii                                  | 0.0 ± 0.0        | 9.3 ± 0.1    | 0.0 ± 0.0  | 12.1 ± 0.7 | 0.0 ± 0.0  | 11.6 ± 0.3 |
| A. baumannii                                | 0.0 ± 0.0        | 11.5 ± 0.8   | 6.0 ± 0.8  | 18.1 ± 0.2 | 0.0 ± 0.0  | 16.3 ± 0.2 |
| A. johnsonii                                | 8.4 ± 0.7        | 17.6 ± 0.7   | 10.0 ± 0.3 | 19.2 ± 0.3 | 7.2 ± 0.6  | 18.2 ± 0.6 |
| E. cloaca                                   | 3.4 ± 0.5        | 9.0 ± 0.1    | 8.0 ± 0.4  | 17.2 ± 0.1 | 0.0 ± 0.0  | 11.6 ± 0.4 |
| C. sakazakii                                | 6.7 ± 0.1        | 19.3 ± 0.7   | 7.0 ± 0.4  | 21.3 ± 0.6 | 2.8 ± 0.4  | 20.4 ± 0.3 |
| K. oxytoca                                  | 0.0 ± 0.0        | 13.3 ± 0.6   | 0.0 ± 0.0  | 13.7 ± 0.4 | 0.0 ± 0.0  | 14.3 ± 0.6 |
| K. pneumoniae                               | 0.0 ± 0.0        | 12.1 ± 0.0   | 0.0 ± 0.0  | 17.3 ± 0.5 | 0.0 ± 0.0  | 14.5 ± 0.7 |
| E. coli                                     | 3.0 ± 0.7        | 13.3 ± 0.4   | 7.0 ± 0.8  | 18.5 ± 0.3 | 3.4 ± 0.5  | 15.6 ± 0.4 |

Similar results were also obtained in the study of green synthesis of AgNPs from endangered medicinal plant *Withania coagulans*, in which *S. aureus* and *E. coli* bacterial cultures inhibition zone 10.66 ± 0.88 mm 4 ± 0.57 mm, respectively [40].

Other plant extracts, such as pure *H. lupulus*, showed the most potent antimicrobial activity against all tested bacterial species. Nonetheless, *H. lupulus*/AgNPs synthesized in this extract showed robust antimicrobial activity with an inhibition zone size of 9 to 26 mm. These results corroborate with those obtained by other researchers, which tested green synthesized AgNPs antimicrobial activity against Gram-negative bacteria strains [41,42]. Beside this, AgNPs obtained by the green synthesis method using aqueous plant extract showed very similar antimicrobial activity, where the maximum growth inhibition zone against *E. coli* and *S. aureus* are 12.46 mm and 12.82 mm in size, respectively [43].

This antimicrobial activity can be explained by the fact that the silver nanoparticles were obtained in the smallest size in the case *H. lupulus*/AgNPs. Weaker antimicrobial activity was obtained of pure *T. vulgaris* extract was strong effective against *B. pseudomycoides* where inhibition zone 15.1 mm and *T. vulgaris*/AgNPs show a broad spectrum of antimicrobial activity against all tested pathogens. In general, all tested samples showed higher antibacterial activity against *S. aureus* when compared to *E. coli*. This could be attributed to the difference in the cell wall composition between Gram-positive and Gram-negative bacteria.

AgNPs are widely known for their broad antibacterial activity [28]. The antimicrobial activity of silver nanoparticles is because of the attachment between the Ag/NPs and the bacteria’s cell wall. Consequently, this attachment leads to the build-up of envelope protein precursor that causes protein denaturation, proton motivation force loss, and eventual cell death [44]. In essence, the size of the synthesized AgNPs affects antimicrobial activity. If the size is smaller than 50 nm, it is easier for the nanoparticles to attach to the negatively charged bacterial cell wall and rupture it. Which leads to denaturation of protein and, finally, cell death. Continuously, AgNPs have a larger surface area for interaction with bacterial cells. It accelerates the generation of reactive oxidative species that causes more damage to the cellular constitution and, in the end, results in cell death [40].

4. Conclusions

This study demonstrated the eco-friendly, fast, economic-inexpensive, and green nano synthetic route compelling synthesis of silver nanoparticles using aqueous extracts of *A. absinthium*, *H. lupulus*, and *T. vulgaris* plant species. All tested extracts contain hydroxycinnamic acid, flavonoids, and phenolic acid derivatives that provide antimicrobial and antioxidant activity. Extracts/AgNPs showed significant antioxidant potential activity in all cases. *A. absinthium*/AgNPs, *H. lupulus*/AgNPs, and *T. vulgaris*/AgNPs displayed
activities against DPPH* (0.14 ± 0.00; 0.11 ± 0.00 and 0.14 ± 0.00 mmol/g), ABTS** (0.55 ± 0.05; 0.86 ± 0.05 and 0.55 ± 0.05 mmol/g), respectively. SEM characterization techniques confirmed the successful formation of spherical AgNPs. *T. vulgaris, A. absinthium, and H. lupulus* plants serve well as reducing, stabilizing, and capping agents to synthesize silver nanoparticles. Besides this, synthesized silver nanoparticles demonstrated good bactericidal properties with inhibition zones in the range 9.0 ± 0.1 to 20.4 ± 0.3 mm against all tested pathogenic and opportunistic bacteria strains. These microorganisms are commonly involved in infections; therefore, the extracts/AgNPs may be beneficial for the future application.

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**References**

1. Hembram, K.C.; Kumar, R.; Kandha, L.; Parhi, P.K.; Kundu, C.N.; Bindhani, B.K. Therapeutic Prospective of Plant-Induced Silver Nanoparticles: Application as Antimicrobial and Anticancer Agent. *Artif. Cells Nanomed. Biotechnol.* 2018, 46, S38–S51. [CrossRef]  
2. Mahadevan, S.; Vijayakumar, S.; Arulmozhhi, P. Green Synthesis of Silver Nano Particles from *Atalantia Monophylla* (L) Correa Leaf Extract, Their Antimicrobial Activity and Sensing Capability of H_{2}O_{2}. *Microb. Pathog.* 2017, 113, 445–450. [CrossRef]  
3. Liao, C.; Li, Y.; Tjong, S.C. Bactericidal and Cytotoxic Properties of Silver Nanoparticles. *Int. J. Mol. Sci.* 2019, 20, 449. [CrossRef]  
4. Singh, H.; Du, J.; Singh, P.; Yi, T.H. Ecofriendly Synthesis of Silver and Gold Nanoparticles by Euphoria Officinalis Leaf Extract and Its Biomedical Applications. *Artif. Cells Nanomed. Biotechnol.* 2018, 46, 1163–1170. [CrossRef] [PubMed]  
5. Ahn, E.-Y.; Park, Y. Anticancer Prospects of Silver Nanoparticles Green-Synthesized by Plant Extracts. *Mater. Sci. Eng. C* 2020, 116, 111253. [CrossRef]  
6. Benzie, I.; Strain, J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Anal. Biochem.* 1996, 239, 70–76. [CrossRef] [PubMed]  
7. Jain, S.; Mehata, M.S. Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and Their Enhanced Antibacterial Property. *Sci. Rep.* 2017, 7, 15867. [CrossRef] [PubMed]  
8. Naeini, A.; Khosravi, A.R.; Chitsaz, M.; Shokri, H.; Kamalinejad, M. Anti-Candida Albicans Activity of Some Iranian Plants Used in Traditional Medicine. *J. Mycol. Med.* 2009, 19, 168–172. [CrossRef]  
9. Fraisse, D.; Felgines, C.; Texier, O.; Lamaison, J.L. Caffeoyl Derivatives: Major Antioxidant Compounds of Some Wild Herbs of the Asteraceae Family. *Food Nutr. Sci.* 2011, 2. [CrossRef]  
10. Falcão, S.; Bacém, I.; Igrejas, G.; Rodrigues, P.J.; Vilas-Boas, M.; Amaral, J.S. Chemical Composition and Antimicrobial Activity of Hydrodistilled Oil from Juniper Berries. *Ind. Crop. Prod.* 2018, 124, 878–884. [CrossRef]  
11. Cermak, P.; Paleckova, W.; Houska, M.; Strohal, J.; Novotna, P.; Mikyska, A.; Jurkow, M.; Sikorova, M. Inhibitory Effects of Fresh Hops on Helicobacter Pylori Strains. *Czech J. Food Sci.* 2016, 33, 302–307. [CrossRef]  
12. Zheljazkov, V.D.; Kacaniowa, M.; Dinceva, I.; Radoukova, T.; Semerdjieva, I.B.; Astatkie, T.; Schlegel, V. Essential Oil Composition, Antioxidant and Antimicrobial Activity of the Galbuli of Six Juniper Species. *Ind. Crop. Prod.* 2018, 124, 449–458. [CrossRef]  
13. Selvan, D.A.; Mahendiran, D.; Kumar, R.S.; Rahman, A.K. Garlic, Green Tea and Turmeric Extracts-Mediated Green Synthesis of Silver Nanoparticles: Phytochemical, Antioxidant and in Vitro Cytotoxicity Studies. *J. Photochem. Photobiol. B Biol.* 2018, 180, 243–252. [CrossRef]  
14. Mughees, M.; Samim, M.; Ahmad, S.; Wajid, S. Comparative Analysis of the Cytotoxic Activity of Extracts from Different Parts of *A. Absinthium* L. on Breast Cancer Cell Lines and Correlation with Active Compounds Concentration. *Plant Biosyst. Int. J. Deal. All Asp. Plant Biol.* 2019, 153, 569–579. [CrossRef]  
15. Keskin, S.; Sirin, Y.; Çakir, H.E.; Keskin, M. An Investigation of *Humulus lupulus* L.: Phenolic Composition, Antioxidant Capacity and Inhibition Properties of Clinically Important Enzymes. *S. Afr. J. Bot.* 2019, 120, 170–174. [CrossRef]  
16. Alonso-Esteban, J.L.; Pinela, J.; Barros, L.; Cirić, A.; Soković, M.; Calhelha, R.C.; Torija-Issasa, E.; de Cortes Sánchez-Mata, M.; Ferreira, I.C.F.R. Phenolic Composition and Antimicrobial and Anticytotoxic Properties of Hop (*Humulus lupulus* L.) Seeds. *Ind. Crop. Prod.* 2019, 134, 154–159. [CrossRef]  
17. Yamaguchi, N.; Satoh-Yamaguchi, K.; Ono, M. In Vitro Evaluation of Antibacterial, Anticollagenase, and Antioxidant Activities of Hop Components (*Humulus lupulus* Addressing Acne Vulgaris). *Phytomedicine* 2009, 16, 369–376. [CrossRef] [PubMed]
18. Abram, V.; Čeh, B.; Vidmar, M.; Hercezi, M.; Lazić, N.; Bucik, V.; Možina, S.S.; Košir, I.J.; Kač, M.; Demšar, L.; et al. A Comparison of Antioxidant and Antimicrobial Activity between Hop Leaves and Hop Cones. *Ind. Crop. Prod.* 2015, 64, 124–134. [CrossRef]

19. Candiracci, M.; Citterio, B.; Piatti, E. Antifungal Activity of the Honey Flavonoid Extract against Candida Albicans. *Food Chem.* 2012, 131, 493–499. [CrossRef]

20. Al-Bayatti, F.A. Synergistic Antibacterial Activity between *Thymus vulgaris* and *Pimpinella anisum* Essential Oils and Methanol Extracts. *J. Ethnopharmacol.* 2008, 116, 403–406. [CrossRef]

21. Baranauskienė, R.; Venskutonis, P.R.; Viškelis, P.; Dambrauskienė, E. Influence of Nitrogen Fertilizers on the Yield and Composition of Thyme (*Thymus vulgaris*). *J. Agric. Food Chem.* 2003, 51, 7751–7758. [CrossRef] [PubMed]

22. Bobinaitė, R.; Viškelis, P.; Venskutonis, P.R. Variation of Total Phenolics, Anthocyanins, Ellagic Acid and Radical Scavenging Capacity in Various Raspberry (*Rubus Spp.*) Cultivars. *Food Chem.* 2012, 132, 1495–1501. [CrossRef] [PubMed]

23. Heil, M.; Baumann, B.; Andary, C.; Linsenmair, E.K.; McKey, D. Extraction and Quantification of “Condensed Tannins” as a Measure of Plant Anti-Herbivore Defence? Revisiting an Old Problem. *Naturwissenschaften* 2002, 89, 519–524. [CrossRef] [PubMed]

24. Re, R.; Pellegrini, N.; Prottegente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* 1999, 26, 1231–1237. [CrossRef]

25. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT Food Sci. Technol.* 1995, 28, 25–30. [CrossRef]

26. Asghar, M.N.; Khan, I.U. Measurement of Antioxidant Activity with Trifluoperazine Dihydrochloride Radical Cation. *Braz. J. Med. Biol. Res.* 2008, 41, 455–461. [CrossRef] [PubMed]

27. Saratate, G.D.; Saratate, R.G.; Kim, D.-S.; Kim, D.-Y.; Shin, H.-S. Exploiting Fruit Waste Grape Pomace for Silver Nanoparticles Synthesis, Assessing Their Antioxidant, Antidiabetic Potential and Antibacterial Activity Against Human Pathogens: A Novel Approach. *Nanomaterials* 2020, 10, 1457. [CrossRef] [PubMed]

28. Kumar, V.; Singh, S.; Srivastava, B.; Bhadouria, R.; Singh, R. Green Synthesis of Silver Nanoparticles Using Leaf Extract of Holoptelea Integrifolia and Preliminary Investigation of Its Antioxidant, Anti-Inflammatory, Antidiabetic and Antibacterial Activities. *J. Environ. Chem. Eng.* 2019, 7, 103094. [CrossRef]

29. Maliar, T.; Nemeˇcek, P.; Ürgeov, A.; Maliarov, M.; Nesvadba, V.; Krofta, K.; Vulganová, K.; Krosłak, E.; Kraic, J. Secondary Metabolites, Antioxidant and Anti-Protease Activities of Methanolic Extracts from Cones of Hop (*Humulus lupulus* L.) Cultivars. *Chem. Pap.* 2017, 71, 41–48. [CrossRef]

30. Habashy, N.H.; Serie, M.M.A.; Attia, W.E.; Abdelgaleil, S.A.M. Chemical Characterization, Antioxidant and Anti-Inflammatory Properties of Greek Thymus Vulgaris Extracts and Their Possible Synergism with Egyptian Chlorella Vulgaris. *J. Funct. Foods* 2018, 40, 317–328. [CrossRef]

31. Hajhashemi, V.; Vaseghi, G.; Pourfarzam, M.; Abdollahi, A. Are Antioxidants Helpful for Disease Prevention? *Res. Pharm. Sci.* 2010, 5, 1–8.

32. García-Sánchez, A.; Miranda-Díaz, A.G.; Cardona-Muñoz, E.G. The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases. *Oxid. Med. Cell. Longev.* 2020, 2020, 2082145. [CrossRef]

33. Li, W.; Hydama, A.; Lowry, L.; Beta, T. Comparison of Antioxidant Capacity and Phenolic Compounds of Berries, Chokecherry and Seabuckthorn. *Cent. Eur. J. Biol.* 2009, 4, 499–506. [CrossRef]

34. Schlesier, K.; Harwat, M.; Böhm, V.; Bitsch, R. Assessment of Antioxidant Activity by Using Different In Vitro Methods. *Free Radic. Res.* 2002, 36, 177–187. [CrossRef] [PubMed]

35. Taghouti, M.; Martins-Gomes, C.; Felix, L.M.; Schäfer, J.; Santos, J.A.; Bunzel, M.; Nunes, F.M.; Silva, A.M. PolypHENol Composition and Biological Activity of Thymus Citriodorus and Thymus Vulgaris: Comparison with Endemic Iberian Thymus Species. *Food Chem.* 2020, 331, 127362. [CrossRef]

36. Kambale, E.K.; Nkanga, C.I.; Mutonkole, B.-P.I.; Bapolisi, A.M.; Tassa, D.O.; Liesse, J.-M.I.; Krause, R.W.M.; Memvanga, P.B. Green Synthesis of Antimicrobial Silver Nanoparticles Using Aqueous Leaf Extracts from Three Congolese Plant Species (*Brillantaisia patula*, *Cyperus fleriboga* and *Senna siamea*). *Heliyon* 2020, 6, e04493. [CrossRef]

37. Ismail, V.; Khan, M.I.; Khan, S.B.; Akhtar, K.; Khan, M.A.; Asiri, A.M. Catalytic Reduction of Picric Acid, Nitrophenols and Organic Azodyes via Green Synthesized Plant Supported Ag Nanoparticles. *J. Mol. Liq.* 2018, 268, 87–101. [CrossRef]

38. Escárciga-González, C.E.; Garza-Cervantes, J.A.; Vazquez-Rodriguez, A.; Montelongo-Peralta, L.Z.; Treviño-Gonzalez, M.T.; Barriga, C.E.D.; Saucedo-Salazar, E.M.; Morales, R.M.C.; Regalado-Soto, D.I.; Treviño-Gonzalez, F.M.; et al. In Vivo Antimicrobial Activity of Silver Nanoparticles Produced via a Green Chemistry Synthesis Using Acacia Rigidula as a Reducing and Capping Agent. *Int. J. Nanomed.* 2018, 13, 2349–2363. [CrossRef] [PubMed]

39. Sulaiman, G.M.; Mohammed, W.H.; Marzoog, T.R.; Al-Amiery, A.A.A.; Kadhum, A.A.H.; Mohamad, A.B. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using Eucalyptus chapmaniana leaves extract. *Asian Pac. J. Trop. Biomed.* 2013, 3, 58–63. [CrossRef]

40. Tripathia, D.; Modib, A.; Narayanb, G.; Rai, S.P. Green and cost effective synthesis of silver nanoparticles from endangered medicinal plant Withania coagulans and their potential biomedical properties. *Mater. Sci. Eng.* 2019, 19, 152–164. [CrossRef]
41. Singh, J.; Singh, N.; Rathi, A.; Kukkar, D.; Rawat, M. Facile Approach to Synthesize and Characterization of Silver Nanoparticles by Using Mulberry Leaves Extract in Aqueous Medium and its Application in Antimicrobial Activity. *J. Nanostruct.* 2017, 7, 134–140.

42. Lia, R.; Pana, Y.; Lia, N.; Wanga, Q.; Chena, Y.; Phisalaphongb, M.; Chen, H. Antibacterial and cytotoxic activities of a green synthesized silver nanoparticles using corn silk aqueous extract. *Colloids Surf. A* 2020, 598, 124827. [CrossRef]

43. Yan-yu, R.; Hui, Y.; Tao, W.; Chuang, W. Green synthesis and antimicrobial activity of monodisperse silver nanoparticles synthesized using Ginkgo Biloba leaf extract. *Phys. Lett.* 2016, 45, 3773–3777.

44. Alsammaraie, F.K.; Wang, W.; Zhou, P.; Mustapha, A.; Lin, M. Green Synthesis of Silver Nanoparticles Using Turmeric Extracts and Investigation of Their Antibacterial Activities. *Colloids Surf. B Biointerfaces* 2018, 171, 398–405. [CrossRef] [PubMed]