

# Green Synthesis, Characterization of Silver Nanoparticles using *Justica Schempria* Leaf Extract and its Antibacterial Activity

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**Abstract:** Antibiotic resistance of microbes to the current commercially accessible drugs is characterized as a cautious thought issue. To alleviate such problems, Ag-NPs were synthesized by reducing Ag<sup>+</sup> to Ag with *Justica Schempria* leaf extract; Silver nanoparticles were synthesized and characterized using visual observation (color change), UV-Vis absorption, and FT-IR; The presence of active phytoconstituents like polyphenols was identified using qualitative screening methods, FT-IR spectroscopy and quantitatively by determine total phenolic content. The formation of silver nanoparticles was affirmed by the color changes from colorless to brown and, finally, deep red. The reduction process was monitored by UV-visible spectroscopy that showed surface Plasmon resonance at 444.53 nm, pointing out the formation of Ag-NPs. Besides this, in Ag-NPs peak shift, decreased intensity and broadness and appearance of new peak compared to *Justica Schempria* leaf extract of FT-IR spectroscopy reveals the formation of Ag-NPs. Antibacterial activities were also tested against two gram-negative and two gram-positive bacteria using the disc diffusion method. The biological findings of this work would be valuable in progressing the viability of restorative drugs, and the *in vivo* might be explored as a continuation of this thought.

**Keywords:** Ag-NPs; green synthesis; phytochemicals; antibacterial activities.

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## 1. Introduction

People have used restorative plants to cure diseases since ancient times, and they are the sources of different drug formulations in all systems of medicine. Different parts supplying low-cost medicine to the population by using the plant have been used in the Ethiopian conventional system of various restorative plants [1,2]. The reason is that plants naturally have the capacity to synthesize beneficial items for us, known as phytoconstituents, that are utilized to perform natural capacities [3,4]. Modern science uses these medicinal plants with proper purification and formulation and given suitable measurements [5].

The rate of multidrug-resistant bacteria is expanding worldwide, and it is the single most common cause of death in both developed and developing countries [6]. To alleviate such problems, the pharmaceutical industry centers on generating metal nanoparticles, which are compelling antimicrobial exercises [7]. Nano-scale materials have a large surface area to volume ratio and size and shape-dependent physicochemical properties, making them prepare poly-molded nanoparticles [8]. These poly-formed nanoparticles have more expected results in

treating bacteria because of their comparative appearance to certain microbes and effectively continually entering the human cell. They take extra consideration in present-day nanotechnology analysts [9].

Later, metals and their oxide nanoparticles are centered due to their surprising properties and various applications [10]. To an awesome degree, the synthesis of metal nanoparticles (M-NPs) has pulled in much consideration in nanotechnology investigations, as there is expanding request in industries and medical sectors such as fillers, disinfectants, optics, antimicrobial agents, drug delivery, and catalytic products [11,12]. Despite numerous metals in nature, some of them, such as gold, silver, palladium, and platinum, are synthesized broadly in nanostructured shape [8,13]. Among the respectable metals of nanoparticles, silver nanoparticles (Ag-NPs) have gotten exceptionally awesome inquiry about consideration for their far-reaching applications in later times [14,15]. Usually, Ag-NPs have been explored broadly due to their prevalent physical, chemical, and biological characteristics, and their predominance stems basically from the size, shape, composition, crystallinity, and structure of Ag-NPs compared to their bulk forms [14,16]. In addition, Ag-NPs have several important applications in antimicrobial agents, capable of purifying drinking water, debasing pesticides, and murdering human pathogenic bacteria [17,18]. Ag-NPs have an assortment of morphological characters (i.e., spherical, triangular decahedral, quasi-spherical, ellipsoidal, hexagonal, nanospheres, nano triangles, nanorods, and polygonal prisms) and are synthesized by diverse synthesizing strategies [19].

Nanoparticles can be synthesized broadly by physical, chemical, and natural strategies [20]. Physical courses for a blend of NPs have some drawbacks. These strategies require high vitality and space and are costly [12,21]. The chemical synthesis strategy of nanoparticles is not appropriate for restorative utilization due to hazardous chemicals authoritative on their surface. Other than items delivered in chemical courses, they are poisonous to the environment [22]. Organic strategies are an elective device for nanoparticles rather than chemical and physical synthesis strategies. The biological synthesizing method uses plant materials (i.e., root, seed, fruit, peels, latex leaves, flowers, and bark), microorganisms (i.e., viruses, bacteria, fungi, and algae), and animal cell cultures as an alternative procedure for preparation of nanoparticles. More vitally, biosynthesis of nanoparticles is eco-friendly, time reasonable, toll viable, and free of perilous fabric on their surface [23]. Moreover, the synthesized nanoparticles may be coated with bioorganic compounds that make them appropriate for therapeutic applications. Plant extracts are particularly important reducing agents because they contain phytochemicals such as polyphenols, flavonoids, terpenoids, and alkaloids present in the plant extract, which play a key role in the synthesis of nanoparticles. In particular, the emerging antimicrobial activities, along with interesting green synthesized nanoparticles, have recently caught the attention of many researchers [24-26]. In this context, phytoconstituents are extracted from different parts of plants to synthesize nanoparticles. However, there is still no report on the green synthesis of silver nanoparticles using *Justica Schemperia* leaf extract and its antibacterial activity.

*Justicia schimperiana* has different local names in Ethiopia, including “dhumuugaa” in Afaan Oromoo, “Sensel” or “Simiza” in Amharic, and “Surpa,” “Kasha,” or “Keteso” in Sidama. It belongs to the family of Acanthaceae [27]. The present study was carried out to synthesize silver nanoparticles from *Justica Schemperia* leaf biosynthetically. Furthermore, the antibacterial activity of *Justica Schemperia* leaf extract and biosynthesized Ag-NPs were

studied. The biosynthesized Ag-NPs exhibit an important antibacterial activity against both gram-negative and gram-positive bacteria.

## 2. Materials and Methods

### 2.1. Lists of chemicals.

All the chemicals used were in analytical grade. The chemicals are those used for extraction, detection, and antibacterial tests, which include silver nitrate, methanol, ethanol, ethyl acetate, hexane, chloroform, ferric chloride, nutrient agar, HCl, NH<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, petroleum ether, Na<sub>2</sub>CO<sub>3</sub>, Folin Ciocalteu reagent (mixture of phosphomolybdate and phosphotungstate), gallic acid, NaOH, Zn powder, iodine, potassium iodide, and glacial acetic acid.

### 2.2. List of equipment and instruments.

A grinder, spatula, electronic balance, flasks, and filter paper were used for plant leaf extraction and to isolate and determine the contents of the extract (column chromatography, TLC sheet, rotary evaporator, pencil, and ruler). Finally, Petri dishes were used for the antibacterial activity test. Instruments are also used for the determination of absorption peak (Ultra violet visible spectroscopy (UV-vis)) and for the determination of functional groups present (Fourier transform infrared spectroscopy (FT-IR)).

### 2.3. Plants leave collection and extraction.

The leaves of *Justica Schempria*, as shown in Figure 1, were collected from Woken around Debark University and thoroughly washed with distilled water three times. The fresh leaves were dried in the nonattendance of sunlight and pounded to create a fine powder. According to [28], the extraction was carried out by mixing 50 g of the pounded *Justica Schempria* leaf with 400 ml of a mixed solvent of water and ethanol with a 1:4 ratio in 1 L Erlenmeyer flask. The mouth of the flask was covered by aluminum foil as soon as the solvent was mixed and allowed to be shaken by a magnetic stirrer for 24 hours at 500 rpm. Finally, the extract was filtered using a 180 mm diameter filter paper, and the solvent was removed using a rotary evaporator. The percentage yield of the crude extract was recorded and stored at 4°C for future utilization.



**Figure 1.** *Justica Schempria* plant leaf.

### 2.4. Phytochemical analysis.

*Justica Schempria* leaf extracts were subjected to the phytochemical screening or qualitative test for alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, and other phytoconstituents [29-31].

### 2.5. Determination of total phenolic content.

The total phenolic content of *Justica Schempria* leaf extract was determined using Folin-Ciocalteu's method and gallic acid as a standard [32]. The dried extract (0.5 mg) was dissolved in distilled water (1 ml). The sample solution (50  $\mu$ l) was mixed with 50  $\mu$ l of Folin-Ciocalteu's reagent, followed by 50  $\mu$ l of Na<sub>2</sub>CO<sub>3</sub> (10% w/v). After incubation at 30°C for 60 min, the absorbance will be measured at 765 nm using a UV-visible spectrometer. Total phenolic content was calculated from a calibration curve using gallic acid as a standard and expressed as milligrams of gallic acid equivalent per gram dry weight of the extract.

### 2.6. Synthesis of silver nanoparticles.

Ag-NPs were synthesized from *Justica Schempria* leaf extract as 1.3 g of silver nitrate salt was dissolved in 250 mL distilled water to prepare a 30 mM salt solution, then;

#### 2.6.1. Using the different concentrations of silver nitrate and the same amount of plant extract.

From this, 50 ml (1 mM, 10 mM, 20 mM, and 30 mM) was prepared in different reaction flasks and preheated separately in the water bath at 77.8°C for 10 minutes with occasional shaking, and 2 mL of plant extract was added slowly to each reaction flask. Then each of the mixtures was further heated for 10 minutes to finish the reduction process.

#### 2.6.2. Using different amounts of *Justica Schempria* leaf extract and the same concentration of AgNO<sub>3</sub>.

50 ml of 30 mM salt solution was poured into three different reaction flasks, and they were preheated for 10 minutes at 77.8°C. Then, 1 ml, 2 ml, and 3 ml of leave extract were added to each reaction flask and further heated at 77.8°C for 10 minutes with occasional shaking to finish the reduction process.

### 2.7. Characterization of Ag-NPs.

Formation of Ag-NPs was noticed visibly through a gradual change in the color of the reaction mixture. Then it was subjected to optical measurements using UV-visible spectrophotometer scanning (Sanyo SP65 UV/Visible spectrophotometer) in a 1 cm path quartz cell at a resolution of 1 nm in the range of 200 nm to 800 nm. FT-IR spectrum of the synthesized nanoparticle was recorded on (Perkin Elmer FT-IR spectrometer) ranging from 4000-400cm<sup>-1</sup>.

### 2.8. Antibacterial activity testing.

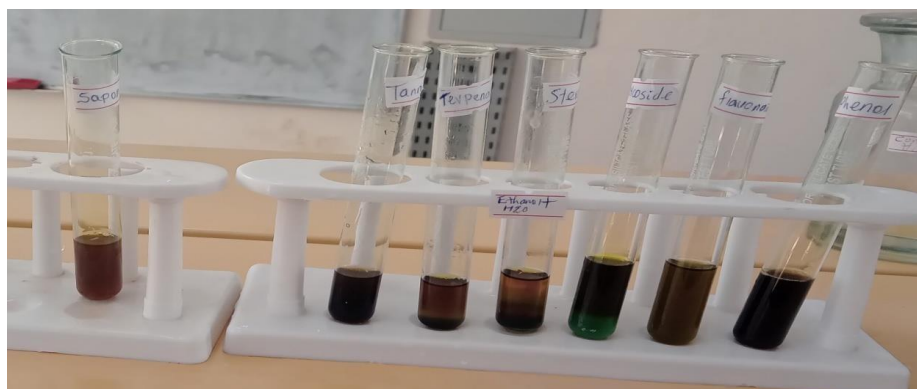
The discs were soaked separately with *Justica Schempria* leaf extract, silver nitrate solution, and a solution containing Ag-NPs of each type. Gentamycin was placed at the center of the plates and used as a positive controller. Then, the discs were air-dried in a sterile condition. The plates containing nutrient agar media were prepared by swabbing them with the microbial cultures two-gram positive (*S. aureus* and *S. pyogens*) and two-gram negative (*E. coli* and *K. pneumoniae*). Previously prepared discs were placed on each part of the plate. The discs were soaked in order of solutions containing plant leaves mediated synthesized Ag-NPs, plant leaf extract, and 30 mM Silver nitrate solution. The plates were incubated at 37°C for 24 hr. Then, the maximum zone of inhibition was observed and measured for analysis against each type of test microorganism. The activity results were calculated as a mean of

triplicates. The antibacterial tests were carried out at Debark University, Department of Biology Laboratory room, Debark, Ethiopia.

### 3. Results and Discussion

#### 3.1. Phytochemical test analysis of the extracted *Justica Schempra* leaf.

Successful determination of biologically active compounds from plant material largely depends on the type of solvent used in the extraction procedure [33]. As we have read in different literature, a mixed solvent of water and ethanol with a 1:4 ratio is effective in extracting dominantly poly phenolic phytochemical constituent of *Justica Schempra* leaf, making it capable of reducing the Ag<sup>+</sup> ion because poly phenolic compound contains O-H and N-H donates electrons [34]. Besides this, phytochemical tests were carried out qualitatively on the *Justica Schempra* leaves extract using aqua alcoholic solvent, have constituents of alkaloids, flavonoids, and tannins compounds, as shown in Figure 2 and Table 1.



**Figure 2.** Phytochemical screening test.

**Table 1.** Phytochemical screening test

Phytochemical	Alkaloid	Tannin	Phenol	Flavonoid	Anthraquinones	Terpenoids
Chemical test	Mayer's reagent test	Ferric Chloride test	Ferric Chloride Test	Alkaline reagent test	Bontrager's test	Salkowski's test
Colors and precipitate observed	Brown precipitate	Dark green	White precipitate	Yellow precipitate	Greenish color	Reddish brown
Result	+	++	++	+	-	-

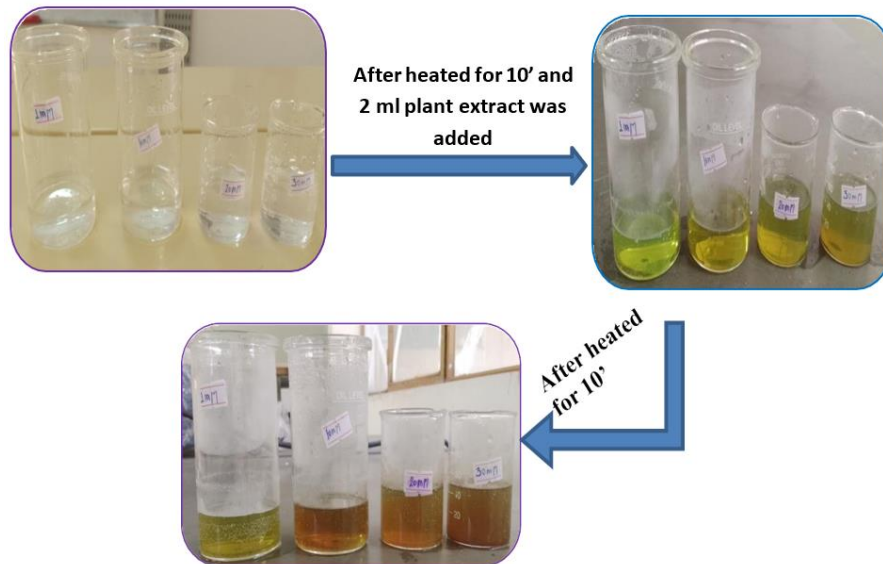
(++) = highly presents (+) = presents (-) = not present.

#### 3.2. Total phenolic content.

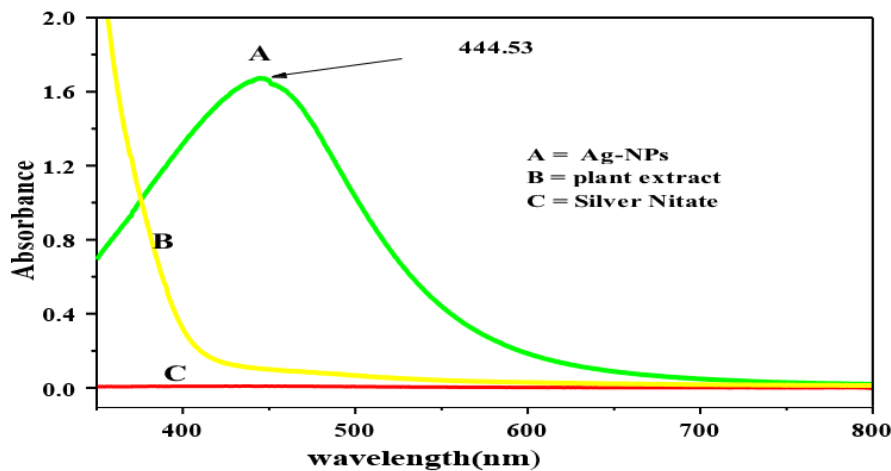
Total phenolic content was measured by the Folin-Ciocalteu method [32] based on the principle of formation of the blue phosphotungstic-phosphomolybdic complex as the result of a reaction between Folin-Ciocalteu and phenolic compounds. The result obtained 451.81±0.32 mg GAE/g dry weight showed that *Justica Schempra* leaf extracts had a high amount of phenolic content in an aqua alcoholic solvent, which is related to the polarity properties of phenolic compounds that are more dissolved in the polar solvents [34,35]. This polyphenolic phytochemical constituent of *Justica Schempra* leaf extracts makes it capable of reducing the Ag<sup>+</sup> ion to Ag by donating electrons, capping, and stabilizing the formed nanoparticles.

### 3.3. UV-VIS spectra analysis.

The formations of silver nanoparticles were confirmed by the change of color when silver nitrate solution was mixed with plant extracts, as shown in Figure 3. A UV-visible spectrophotometer further confirmed the presence of silver nanoparticles in the solution. The UV-visible spectra result showed a distinct strong absorbance at 444.53 nm, suggesting the formation of silver nanoparticles [36], as shown in Figure 4.



**Figure 3.** The color changes observed during the formation of Ag-NPs.



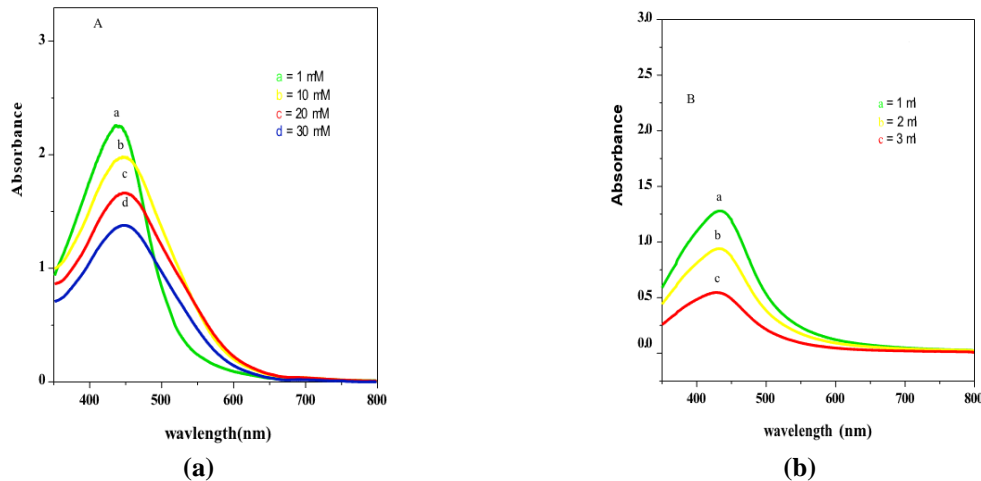
**Figure 4.** UV-visible spectra of synthesized Ag-NPs, plant extract, and AgNO<sub>3</sub>.

#### 3.3.1. Optimization for the biosynthesis of Ag-NPs.

The effect of varying concentrations of silver nitrate and leaf extract on the formation of Ag-NPs was also studied by periodically checking the absorbance of the reaction mixture through UV-visible spectroscopy [37].

Using the different concentrations of silver nitrate: as the concentration of AgNO<sub>3</sub> increases from (1 to 30) mM, as shown in Figure 5(A), the intensity of the peak would decrease and become broader due to the increase in a number of the silver ions to be reduced and form particles of larger size. Green synthesis of silver nanoparticles using the phenolic compound as a reducing agent shows the broad UV-visible peaks and shifting to higher wavelengths/redshift/when the concentration of silver nanoparticle was high [38].

Using different amounts of leaf extract: In this work, the synthesis of silver nanoparticles was optimized or checked using (1 ml, 2 ml, and 3 ml); of *Justica Schemperia* leaf extract mixed with fixed 50 ml 30 mM silver nitrate solution. As the extract amount increased, nanoparticle production also increased. This is due to the presence of a sufficient amount of biomolecules from hydro-alcoholic plant extract to reduce  $\text{Ag}^+$  to Ag. Moreover, a decrease in particle size of silver nanoparticles has been observed due to an increase in extract amount, as shown in Figure 5(B) [39].



**Figure 5.** optimization to synthesize Ag-NPs (a) by varying concentrations of  $\text{AgNO}_3$ ; (b) by varying amounts of plant extract.

### 3.3.2. Factors affecting biosynthesis of Ag-NPs.

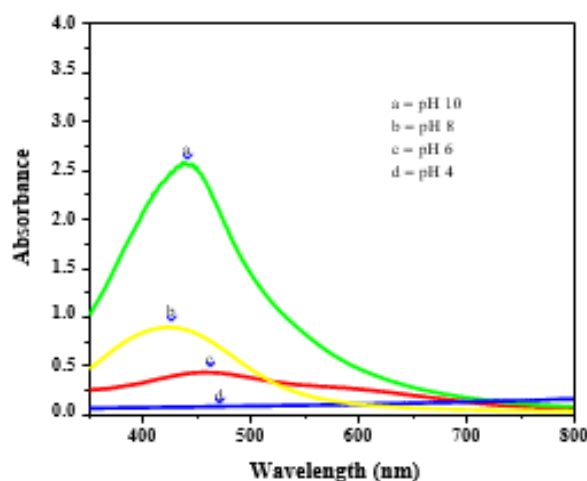
Several factors directly influence the biosynthesis of silver nanoparticles. The difference in reaction temperature, pH level, reaction time, and temperature are some factors that change the UV-visible spectra recorded [40,41].

#### 3.3.2.1. Effect of reaction temperature.

It is evident that the yield of silver nanoparticles has a positive correlation with an increase in temperature [42]. Heating leaf extract with an increase in temperature from 25 to 200°C, an increase in the sharpness of absorption peaks was found for silver nanoparticles. This is because, with an increase in temperature, the reaction rate also increases, which enhances the synthesis of nanoparticles. The sharpness in the absorbance peak depends on the size of the synthesized nanoparticles, as with higher temperatures, the particle size may be smaller, which results in the sharpening of the Plasmon resonance band of silver nanoparticles [41].

#### 3.3.2.2. Effect of pH.

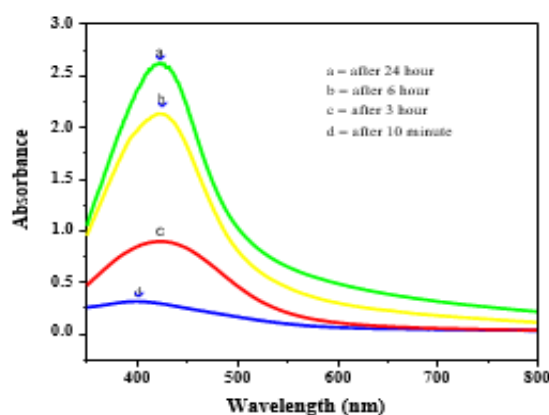
It is obvious from the results of earlier researchers that pH may play a crucial role in the biosynthesis of silver nanoparticles [43]. When the pH of the solution was increased from acidic to basic (pH 4 - 10), adjusted by 0.1 M HCl and NaOH, absorption peak intensity increased, and the spectra became intense and Sharpe, as shown in Figure 6. In the acidic condition, the peak becomes broader since the size of the particle increases. As the pH value increases, the absorption increases and gives a narrow peak. Hence, the basic condition is favored for controlling the particle size.



**Figure 6.** pH effect on synthesis of nanoparticles.

### 3.3.2.3. Effect of incubation time.

Earlier works suggested that contact or incubation time also affects the synthesis of nanoparticles [44]. The intensity of the absorbance of UV-visible spectra increases with increasing the reaction time, as shown in Figure 7. The observed increase of absorbance intensity from 10 minutes to 24 hours obtained narrow peak gaps suggests the stability of synthesized Ag-NPs.

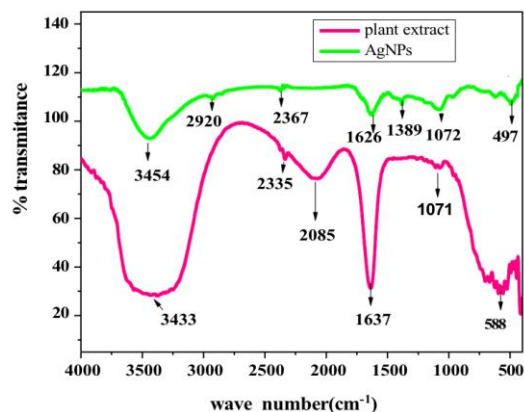


**Figure 7.** Incubation time effect on synthesis of nanoparticles.

### 3.4. FT-IR spectra analysis.

FTIR is one the most important characterization techniques for detecting the functional group in plant extract and silver nanoparticles. FT-IR absorption spectra of green synthesized AgNPs and *Justica Schembria* in the range of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$  are shown in Figure 8. From a plant extract, the broad and intense band at  $3433\text{ cm}^{-1}$ , owing to O–H and N–H stretching, represents the presence of a polyphenolic group essential for reducing  $\text{Ag}^+$  to Ag [45]. The weak absorption at  $2335\text{ cm}^{-1}$  is due to  $\text{C}\equiv\text{C}$  stretching vibration. The peak that was observed at  $2085\text{ cm}^{-1}$  represents the asymmetric stretching of the C–H bond in aromatic compounds [46]. The peak observed at  $1637\text{ cm}^{-1}$  in the *Justica Schembria* leaf extract could be attributed to  $\text{C}=\text{C}$  in aromatic compounds. The bands located at  $1071$  and  $588\text{ cm}^{-1}$  represent the C–N and N–H stretching vibrations of amines, respectively. A peak in the FT-IR spectrum of AgNPs appeared in the lower energy region  $497\text{ cm}^{-1}$ , attributed to the metal-oxygen/Ag–O/bond. The absorption band at  $3454\text{ cm}^{-1}$  is observed, corresponding to the O–H stretching of the surface adsorbed water molecule, and the band at  $2920\text{ cm}^{-1}$  shows the presence of C–H

species. The peak at  $2367\text{ cm}^{-1}$  corresponds to  $\text{C}\equiv\text{N}$  stretching, and the peak found at around  $1626\text{ cm}^{-1}$  corresponds to carbonyl stretch vibrations from  $-\text{COO}$ . The peak observed at  $1389\text{ cm}^{-1}$  corresponds to  $\text{C}-\text{N}$  stretching vibrations of aromatic and aliphatic amines. The peak at  $1072\text{ cm}^{-1}$  is responsible for the  $\text{O}-\text{H}$  deformation/ $\text{C}-\text{O}$  stretch of phenolic and alcoholic groups. The observed peak shift, decreased intensity, and broadness of AgNPs FT-IR spectrum compared to *Justica Schempria* leaf extract reveal the interaction of bulk  $\text{AgNO}_3$  with those phytoconstituent groups of plant extract. The appearance of a new peak in the FT-IR spectrum Ag- NPs shows the formation of a new bond between the reducing agent and silver metal [47].



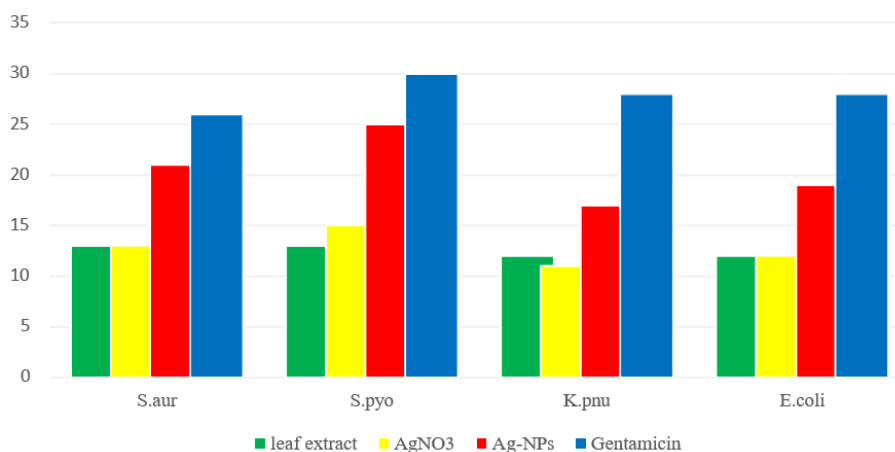
**Figure 8.** FT-IR spectra of plant extract and synthesized Ag-NPs.

### 3.5. Antibacterial activity testing.

Synthesized silver nanoparticles have a broad-spectrum antibacterial effect on gram-positive and gram-negative organisms. Synthesized Ag-NPs cause the interruption of ATP molecules and prevent DNA replication or the formation of reactive oxygen species. It forms pits in the cell walls of gram-negative and positive organisms, causing increased permeability and cell death [48].

**Table 2.** In vitro antimicrobial activity of some human pathogenic bacteria on Ag-NPs by disc diffusion assay.

Compounds	Antibacterial activity (mean IZ diameter (mm)±SD)			
	<i>S.aur</i>	<i>S.pyo</i>	<i>K.pnu</i>	<i>E.coli</i>
Coffee leaf extract	$13 \pm 0.021$	$13 \pm 0.043$	$12 \pm 0.011$	$12 \pm 0.017$
$\text{AgNO}_3$	$13 \pm 0.015$	$15 \pm 0.024$	$11 \pm 0.045$	$12 \pm 0.003$
Ag-NPs	$21 \pm 0.005$	$25 \pm 0.03$	$17 \pm 0.027$	$19 \pm 0.00$
Gentamicin	$26 \pm 0.035$	$30 \pm 0.026$	$28 \pm 0.023$	$28 \pm 0.032$



**Figure 9.** Antibacterial activities of leaf extract,  $\text{AgNO}_3$ , synthesized Ag-NPs, and gentamicin, respectively.

Synthesized Ag-NPs had a greater effect on gram-positive strains than gram-negative bacterial strains because the cell walls of gram-positive bacteria bind larger quantities of metals than the gram-negative bacterial strains. In general, Ag-NPs had a more significant antibacterial action on gram-positive and gram-negative bacteria than leaf extract, as shown in Table 2 and Figure 9. This is due to the large surface area of the nanoparticles; it could be tightly adsorbed on the surface of the bacterial cells to disrupt the membrane. Smaller particles with a larger surface area available for interaction have more bactericidal effects.

#### 4. Conclusions

In this work, silver nanoparticles were synthesized using *Justica Schempria* leaf extract as a reducing, stabilizing, and capping agent, which is simple and cost-effective, and the resultants are exceedingly steady and reproducible. The synthesized silver nanoparticles were characterized using visual observation (color change), UV-Vis absorption, and FT-IR, which confirmed the formation of the silver nanoparticles using *Justica Schempria* leaf extract. The UV-visible spectra result showed a distinct strong absorbance at 444.53 nm, suggesting the formation of silver nanoparticles besides the color change from colorless to brown and deep red. The strong peaks at 497  $\text{cm}^{-1}$  in FTIR spectroscopy appointed to the Ag-O bond indicate the formation of Ag-NPs. The synthesized Ag-NPs were observed to have better antimicrobial activities in all tried pathogens. Thus, the overall biological findings of this work would be valuable in progressing the viability of restorative drugs. Based on the perceptions, the *in vivo* might be explored to continue this thought.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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