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ORIGINAL ARTICLE

Biosynthesis and characterization of silver nanoparticles using *Bacillus subtilis*, *Escherichia coli*, and leaf extracts of *Jatropha* and *Ocimum* species

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Abstract
Silver nanoparticles (AgNPs) are used in many fields for various purposes, and the biosynthesis of AgNPs through biological routes has recently gained attention. In this study, *Bacillus subtilis* and *Escherichia coli* isolated from soil within the premises of an abattoir in Minna, Niger State, Nigeria and plant extracts of Tulsi leaves (*Ocimum tenuiflorum*) and Jatropha leaves (*Jatropha curcas*) were evaluated for their ability to synthesize AgNPs. Through visual confirmation and ultraviolet and visible (UV–Vis) spectrum analysis, it was discovered that *Bacillus subtilis* and *Escherichia coli* had absorption peak at 425 and 318 nm, respectively, while *Jatropha curcas* and *Ocimum tenuiflorum* had absorption peaks at 416 and 400 nm, respectively. X-ray diffraction (XRD) analysis revealed that silver nanoparticles synthesized from *B. subtilis* and *E. coli* had a strong peak around 31.66°, which was absent in nanoparticles synthesized with *O. tenuiflorum* and *J. curcas*. High-resolution transmission electron microscopy (HRTEM) revealed that the obtained AgNPs had spherical shape and sizes for silver nanoparticles synthesized using *B. subtilis* and *E. coli* with an average size of 11.10 ± 0.21 and 38.89 ± 0.42, but the spherical shape of silver nanoparticles in *E. coli* was evenly distributed compared to the spherical shape in *B. subtilis.* Nanobars, nanopyramids, nanorods and hexagonal silver nanoparticles were observed in the HRTEM analysis of *J. curcas* with average size of 12.28 ± 0.37, and nanoflowers were observed in the AgNPs synthesized by *O. tenuiflorum* with an average size of 12.99 ± 0.15. These results of the study showed that *Bacillus subtilis* and *Escherichia coli* as well as plant extracts of Jatropha and Tulsi could be used to produce silver nanoparticles for application in various fields.

Keywords Silver nanoparticles · Nanoflowers · Abattoir · Biosynthesis · Biological routine · Nanorods

Introduction

The synthesis of silver nanoparticles from plant extract and microorganisms is an eco-friendly approach [1], in the sense that the end product is water and carbon dioxide which does not smell or pollute the environment but rather add value to it [2]. It is essential to search for original possible approach to synthesize nanoparticles that are useful in medicine, for treatment of wastewater, pharmaceutical industries and public health [3]. In addition, the introduction of nanomaterials with unique properties, particularly metal nanoparticles, offers new expectation to cure and treat diseases [4]. Among the metal nanoparticles, Ag NPs have been widely useful; reducing agents and surfactants are frequently used during the chemical production of Ag NPs, to reduce silver ions and manage the growth of Ag NPs [5]. However, due to the involvement of these poisonous compounds, chemical approach suffer a lot of deficiencies [6]. Therefore, it is important to make more effort to search for environmentally friendly methods for producing Ag NPs. Raman et al. [7] synthesized a facile, eco-friendly, stable in time and non-toxic silver nanoparticles (Ag NPs) using the extract of edible mushroom, *Agaricus bisporus*. Senthamarai et al. [8] also synthesized and characterized zinc nanoparticles (ZnO NPs) by biophysical method using unripe fruit extract of *Aegle marmelos*. There is a lot of increasing interests in

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biosynthetic approach because they avoid toxic chemicals and involve very simple steps [9]. Compared to other chemical methods, biosynthetic methods can synthesize Ag NPs with manageable size and shape without using poisonous substance [10]. Fungi, algae, bacteria, and plants, among others, could synthesize Ag NPs. The plant extract is a better choice among the different bio-tools owing to their abundant components that can act as reducing as well as stabilizing agents [11]. *Bacillus subtilis* and *Escherichia coli* and plant extracts of Tulsi leaves (*Ocimum tenuiflorum*) and Jatropha leaves (*Jatropha curcas*) were used to biosynthesize Ag NPs. Plant extracts of Tulsi leaves (*Ocimum tenuiflorum*) and Jatropha leaves (*Jatropha curcas*) contain numerous polyhydroxy flavonoids with strong antioxidant activity, which can act as reducing agents in the production of Ag NPs. The objective of this study is to develop an easy approach to the biosynthesis of Ag NPs. Ag NPs of different sizes were successfully synthesized using the bacteria broth and a known amount of the plant extract as reducing agents. A series of characterization techniques including ultraviolet–visible (UV–Vis) spectroscopy, X-ray diffraction (XRD) and high-resolution transmission electron microscopy (HRTEM) were used to validate the properties of the biosynthesized Ag NP. This study is meant to develop a cost-effective and environmentally friendly process in the production of Ag NPs.

Materials and methods

Soil sample was collected from Minna modern slaughterhouse in Tayi village, Bosso Local Government Area, Niger state, Nigeria. The soil samples were delivered to the Microbiology Laboratory of the Federal University of Technology, Minna, in a clean polythene bag. The pour plate technique was used for the isolation of bacteria. For 24 h, the plates were kept at 37 °C. On nutrient agar, separate colonies with different morphologies were identified and subcultured repeatedly. The identification of the isolates was confirmed using conventional bacteriological and molecular techniques.

Collection of materials

From a farm in Maikunkele, Niger State, Nigeria, the leaves were collected from fresh Tulsi leaves (*Ocimum tenuiflorum*) and Jatropha leaves (*Jatropha curcas*).

Preparation of plant extracts

In order to avoid damaging the plant's thermo labile constituents by direct sunlight, the leaves were cleaned with clean water and then air dried for fifteen (15) days at room temperature. The stems were removed and the leaves were ground into a fine powder. A conical flask with a capacity of 1000 ml was filled with 500 ml distilled water, 25 g of the powdered leaves was weighed, and the mixture was cooked for 25 min. A muslin cloth and then filter paper were used to separate the aqueous extracts (Whatman no.1). Refrigerated silver nanoparticles were formed from the filtrate [12]. The plant extracts were analyzed using the procedures of Trease and Evans [15] and Sofowora [16].

Isolation and identification of bacteria

Soil samples within the premises of Minna modern abattoir, Tayi village, Niger State, Nigeria were collected in sterile polythene bags and transported to the Microbiology laboratory of the Federal University of Technology, Minna, Niger State, Nigeria. The soil sample were diluted in sterile saline solution (0.9% w/v) and isolates were obtained by spread plate technique on nutrient agar medium at 37 °C for 24 h. Morphologically distinct colonies were isolated and repeatedly sub-cultured on nutrient agar. Identity of the isolates was affirmed after characterization by standard bacteriological methods and molecular technique.

Molecular identification of test organisms using molecular sequencing

The bacterial isolates were subjected to DNA isolation and 16S rDNA identification using universal forward 27F (5 prime AGAGTTTGATCMTGGCTCAG 3 prime) and reverse 1429 R(5 prime TACGGYTACCTTGTTACG ACTT3 prime) primers. Following the species identification, the strain was outsourced for whole-genome sequencing to Genotypic Technology Ltd., Bengaluru, India. Whole-genome sequencing was done using Illumina MiSeq platform, with a paired end library. A total of 2,546,530 paired end raw reads of 1500 base pairs length on average were sequenced, out of which 2,540,525 high-quality paired end reads, with 90% read length scoring Phred quality score of 35 and above, were assembled into 36 Scaffolds. The assembled genome of Sample A was 99.87% identical to *Bacillus subtilis* MFN 15, while the assembled genome of Sample B was 99.93% identical to *Escherichia coli* UBR 11.

Biosynthesis of silver nanoparticles

Silver nanoparticles were synthesized externally as reported by Siddiqi et al. [13]. Nutrient broth was used to sub-culture the isolated colonies, and incubated at 37 °C for 24 h. The culture supernatant was obtained by centrifuging the broth for 10 min at 8000 rpm. In a 500 ml Erlenmeyer flask, around 200 ml of aqueous solution containing 1 mM silver nitrate was mixed with 100 ml of culture supernatant. Shaking at 150 rpm and in a dark environment kept the mixture

stable. The solution's hue changed as the silver nitrate was reduced, allowing the experimenters to track their progress.

Silver nanoparticles were confirmed by adding 5 ml of *Ocimum tenuiflorum* and *Jatropha curcas* aqueous extracts to a solution of aqueous 1 mM AgNO3 at 70 °C and pH 12 for 60 min.

Characterization of biosynthesized silver nanoparticles

UV–visible spectrophotometer was used to monitor the color changes in the nanoparticles synthesized nanoparticles [14]. S-3400 N, Hitachi instrument, X-ray diffraction and energy-dispersive X-ray (EDX) spectroscopy were used to determine the crystal structure and elemental composition of silver nanoparticles synthesized in this study. Selective area electron diffraction revealed the lattice structure of the silver nanoparticles, which were synthesized in this study using the S-3400N instrument [15].

Results and discussion

Phytochemical components of plant extracts

Ocimum tenuiflorum and *Jatropha curcas* extracts were subjected to phytochemical analysis in an effort to identify the major types of secondary metabolites present. Phytochemical secondary metabolite study of leaves from *J. curcas* and *O. tenuiflorum* was compared qualitatively and quantitatively in Tables 1 and 2. The presence of tannins and saponins in the leaves of *J. gossypifolia* and *J. multifida* was previously reported by Baranwal et al. [16] and was validated in our investigation. Tables 1 and 2 demonstrate that alkaloids, tannins, saponins, flavonoids, and phenols were found in the leaves of the plants studied, although to various degrees of intensity and quality. According to Deshmukh et al. [17], qualitative analysis may help identify bioactive principles and pave the way for the discovery and development of nanoparticles. There were similar findings (Table 1), which demonstrate the relatedness of *Jatropha* and *Ocimum* species studied as well as their potential in nanotechnology as reducing agents during the green syntheses (see below). Particularly in *J. curcas* and *Ocimum tenuiflorum*, tannins were found in high concentrations during the qualitative screening. Alkaloids were also found in the leaves of *J. curcas* and *Ocimum tenuiflorum*, albeit to a lesser extent.

Flavonoids, phenols, and saponins were the most common phytochemicals discovered in the leaves of the species, followed by flavonoids. Alkaloids and tannins were the most abundant compounds in the leaves, although their concentrations varied widely. As far as phytochemical concentrations go, flavonoids were found to be the most prevalent in the leaves of the two plant species studied: *J. curcas* had an 1866.33 mg/100 g concentration while *Ocimum tenuiflorum* had 780.19 mg/100 g (Table 2). This may be due to the solvent used, as reported in the work of De Matteis et al. [18], who also showed the effect of solvent on phytochemical extraction of silver nanoparticle with more extract via aqueous extraction, for example, flavonoids are popular secondary metabolites that have a variety of biological activities at non-toxic concentrations [19].

Biochemical and morphological characteristics of bacterial isolates

The microscopic and biochemical tests for identification of bacterial isolates from soil are shown in Table 3

Molecular characteristics of test organisms

Figure 1 demonstrates the integrity of amplified genes on agarose gel and shows the molecular properties of the bacterial isolates with the highest capacity to produce silver nanoparticles. After electrophoresis, the gel-documented pictures of the bacteria were isolated, DNA showed at 1500 kb, indicating that the isolates were pure.

Table 1 Qualitative components of phytochemicals in *Ocimum tenuiflorum* and *Jatropha curcas*

Taxa	Plant part	Alkaloids	Tannins	Flavonoids	Saponins	Phenols
J. curcas	Leaves	+	+	+	+	+
O. tenuiflorum	Leaves	+	+	+	+	+

Keys: + = present, − = absent

Table 2 Quantitative components of phytochemicals present in *Jatropha curcas* and *Ocimum tenuiflorum* leaves (mg/100 g).

		(mg/100 g)				
Taxa plant part		Flavonoids	Phenols	Saponins	Tannins	Alkaloids
J. curcas	Leaves	1866.33	384.86	294.38	109.84	86.73
Ocimum tenuiflorum	Leaves	780.19	562.63	202.03	162.41	82.98

Table 3 Biochemical tests for identification of bacteria isolated from soil

Test	*E. coli*	*Bacillus* species
Gram reaction	−	+
Cell morphology	Rod	Rod
Catalase	+	+
Citrate	−	+
Spore formation	−	+
Urease	−	−
Starch hydrolysis	−	+
Voges Proskauer	−	+
Indole	+	+
Methyl red	+	−
Oxidase	−	−

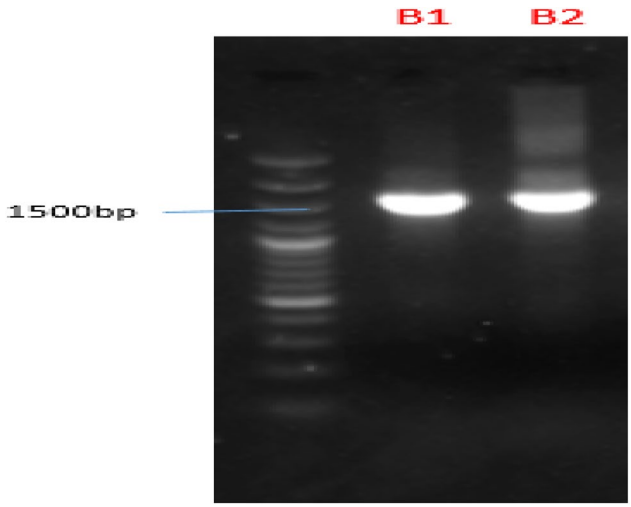

Fig. 1 Agarose gel electrophoresis of *Bacillus subtilis* and *E. coli*

Keys:
B1 = *Bacillus subtilis*
B2 = *Escherichia coli*

Biosynthesis of silver nanoparticles

Bacillus subtilis and *Escherichia coli* were used in the extracellular production of silver nanoparticles. The emergence of a color shift in the reaction mixture (Figs. 2, 3) originally suggested the production of silver nanoparticles, which was later verified by UV–visible spectroscopy (surface plasmon resonance). During the two days of incubation, the supernatant's color went from yellow to dark brown, and its intensity increased as the amount of Ag+ in the solution was reduced to AgO. No color change was observed in the control when incubated for the same length of time and

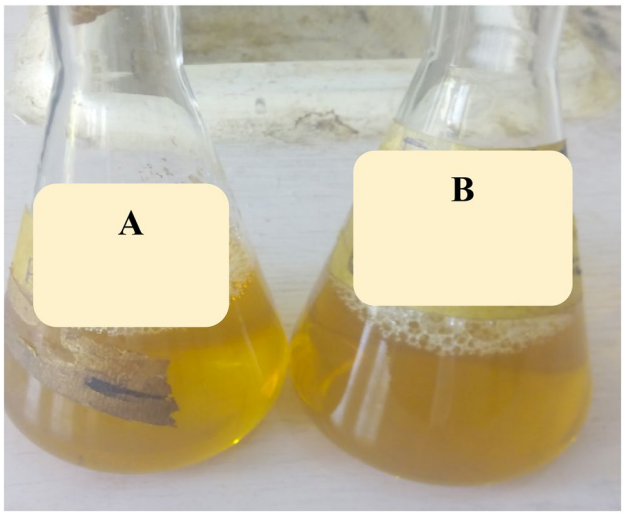

Fig. 2 Initial color during bio-reduction of silver nitrate at 0 h

conditions. Changes in surface plasmon may be responsible for the color shift from a light yellow to a dark brown [20].

Jatropha and *Ocimum* leaf extracts were employed to manufacture silver nanoparticles in the green synthesis. It transformed from yellowish brown to dark brown after 2 days of incubation, when Jatropha and Tulsi leaf extracts were combined separately in the aqueous solution of silver ion complex (Figs. 2, 3). Silver nanoparticles in aqueous plant extract solution have a yellowish brown to dark brown hue because surface plasmon vibrations in silver nanoparticles are excited. Alternatively, the color shift might be due to a reduction of silver ion (Ag+ ions to Ag0), which indicates the creation of silver ions [21]; When compared to high molecular weight proteins from bacterial biomass, flavanone and terpenoid elements of Jatropha and Tulsi leaf extracts may stabilize the synthesis of nanoparticles [21]. Silver ions (Ag+) are mostly reduced and silver nanoparticles are stabilized by polyol and water-soluble heterocyclic components [22].

Key:
A: *Bacillus subtilis* synthesized silver nanoparticle solution
B: *Escherichia coli* synthesized silver nanoparticle solution

Key:
A—*Jatropha* sp. synthesized silver nanoparticle solution
B—*Jatropha* sp. synthesized silver nanoparticle solution
C—*Ocimum* sp. synthesized silver nanoparticle solution
D—*Ocimum* sp. synthesized silver nanoparticle solution
E—*Escherichia coli* synthesized silver nanoparticle solution
F—*Escherichia coli* synthesized silver nanoparticle solution

Fig. 3 Color changes during bio-reduction of silver nitrate after 48 h

G—*Bacillus subtilis* synthesized silver nanoparticle solution

H—*Bacillus subtilis* synthesized silver nanoparticle solution

Characterization of silver nanoparticles

Particle size distribution, transmission electron microscope (TEM), energy-dispersive X-ray (EDX), X-ray diffraction spectroscopy (XRD) and selective area electron diffraction (SAED) were all used to analyze the biosynthesized nanoparticles.

UV–visible spectrophotometer

A UV–visible spectrophotometer is used to easily study the optical characteristics, validate andquantify silver nanoparticles, Sekar et al. [23]. Figure 4 shows the development of *Bacillus subtilis* and *Escherichia coli* at absorption peaks of 425 and 398 nm, respectively. The broad nature ofthe absorbance peak showed the polydispersed nature of the synthesized silver nanoparticles, Sekar etal. [24]. The silver nanoparticle surface plasmon resonance was responsible for the peak generation [25]. *Bacillus subtilis* and *Escherichia coli* culture supernatants were used to synthesize silver nanoparticles in a manner that is comparable to that of enterobacteria and *Klebsiella pneumoniae* [26]. With respect to the wavelength ranges seen in *Bacillus subtilis* and *Escherichia coli*, these peaks match those reported by Gong et al. [27].

The leaf extracts were analyzed using UV–Vis spectroscopy in order to determine the nanoparticles' size and form. Figure 4 depicts the UV–Vis spectra of the reaction medium after 2 days. Jatropha and Tulsi leaves were found to have silver nanoparticles that had an absorption peak at 416 and 400 nm, respectively, while *Bacillus* and *E. coli* synthesized silver nanoparticles had absorbance peak at 425 and 420 nm.

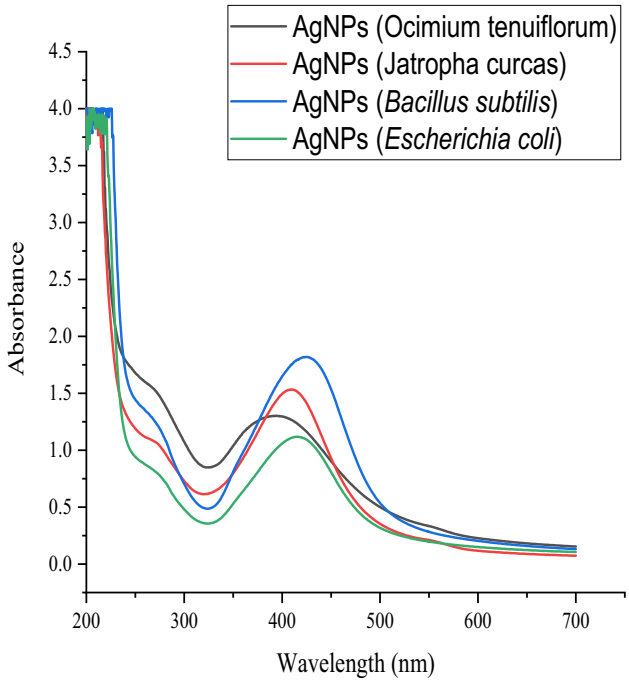

Fig. 4 UV–Vis absorption spectra of silver nanoparticles synthesized using bacteria cultures and plant extracts

Particle size distribution for biosynthesized silver nanoparticles

The average particle size of nanoparticles generated by bacteria growth is 4–4.8 nm (Fig. 5) and 9.402.35 and 31.619.72 nm for *O. tenuiflorum* and *J. curcas*, respectively, are comparable with previous results (Table 1; Fig. 6). Modal et al. [28] stated that *Shewanella oneidensis* MR-1 was used to produce Ag NPs with an average diameter of 4–1.5 nm. The particle size of nanoparticles made from bacteria culture is smaller than those made from plant extract, according to Shanmuganathan et al. [29]. To some extent,

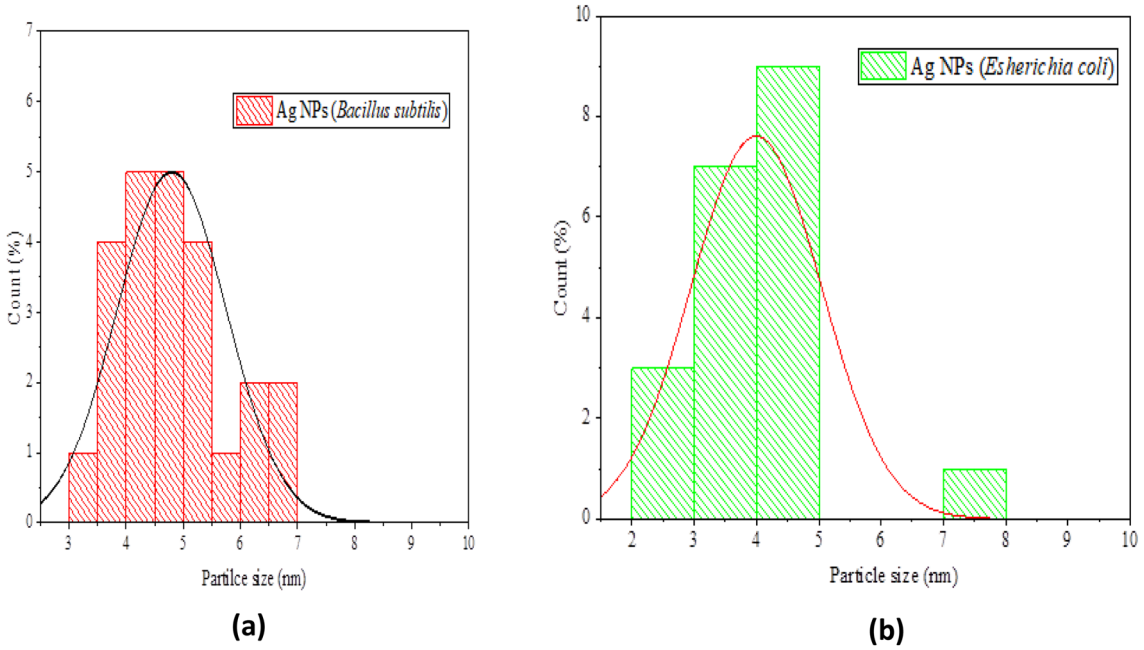

Fig. 5 Particle size for **a** *Bacillus subtilis*, **b** *Escherichia coli*

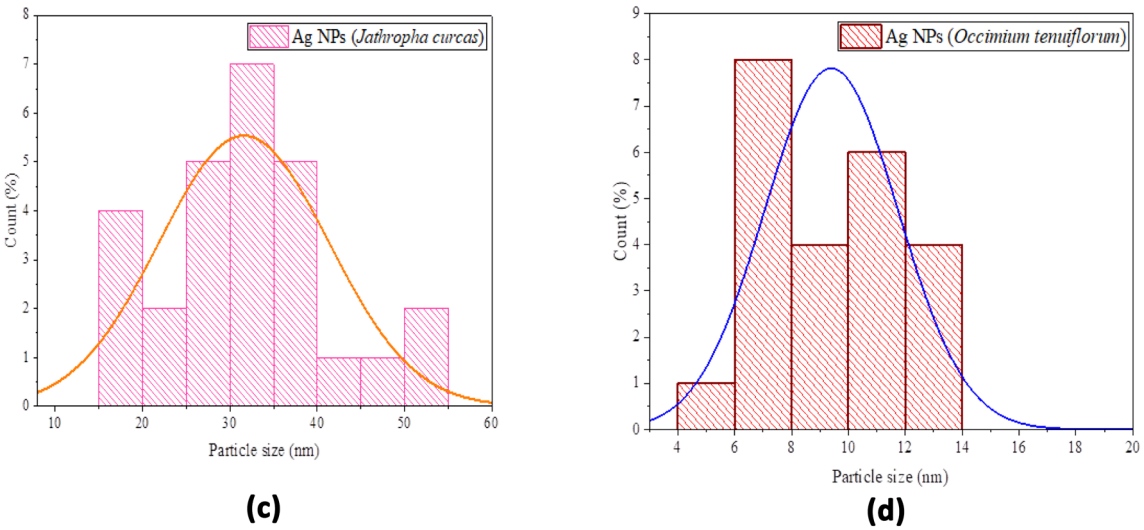

Fig. 6 Particle size for **c** *Jatropha curcas*, **d** *Ocimum tenuiflorum*

the different particle sizes produced by utilizing bacteria culture and plant extracts in the present study may be explained by the use of bacterial culture supernatant.

High-resolution transmission electron microscopy of the biosynthesized silver nanoparticles

Using elevated transmission electron microscopy (HRTEM), the morphology of produced silver nanoparticles was examined. As shown in Fig. 7a, silver nanoparticles generated using *B. subtilis* and *E. coli* had an average size of 11.100.21 and 38.890.42 by HRTEM analysis, but the even distribution of silver nanoparticles in *E. coli* was different from that in *B. subtilis* (Fig. 7b). Although *J. curcas*' silver nanoparticles, which had an average size of 12.28 0.37 nm in the HRTEM study, had nanobars, nanopyramids, nanorods, and hexagonal silver nanoparticles, those produced by *O. tenuiflorum* had nanoflowers, which had an average size of 12.99 0.15. A study published in 2019 by Tenase et al. [30] (ethylene glycol

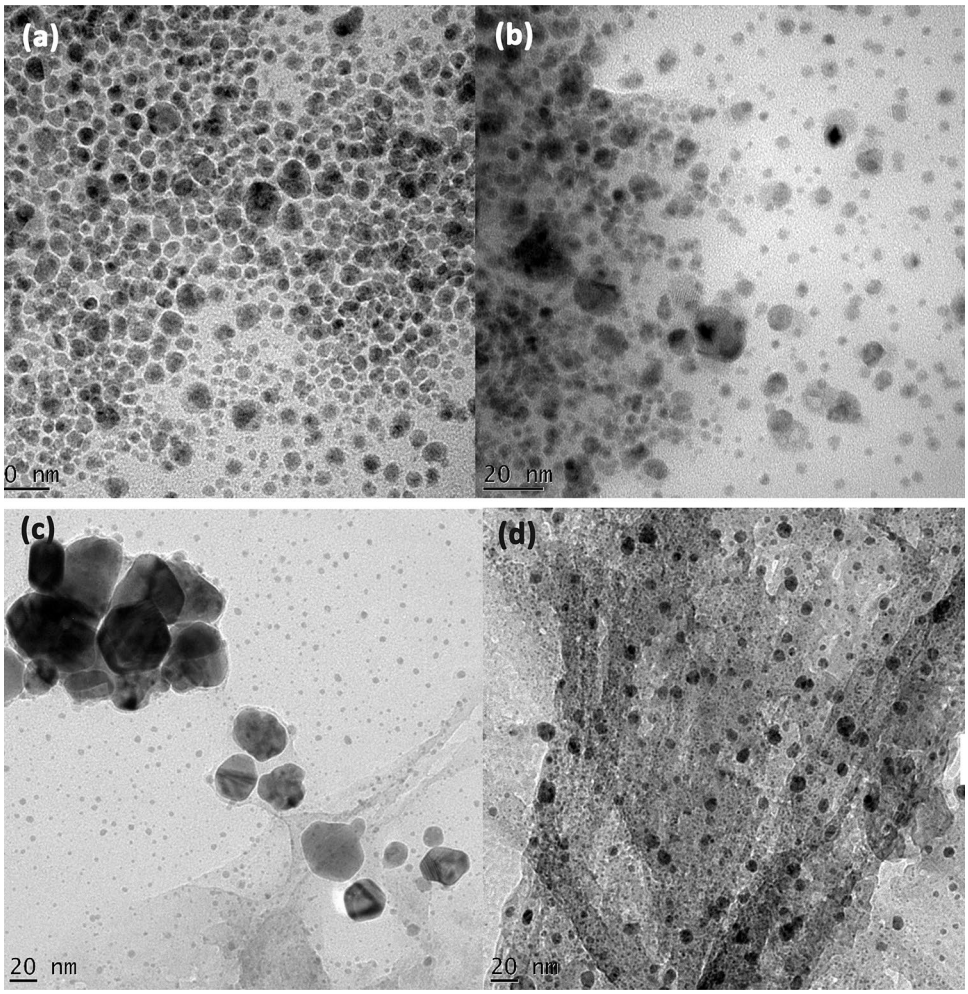

Fig. 7 High-resolution transmission electron microscopy images of silver nanoparticles synthesized by **a** *Bacillus subtilis*, **b** *Escherichia coli*, **c** *Jatropha curcas*, **d** *Ocimum tenuiflorum*

monoethyl ether) described the creation of nanoprisms. Silver nanoparticles have been synthesized by Torras and Roig [31], which suggests that the present results are in line with prior findings.

Energy-dispersive X-ray spectroscopy micrograph of biosynthesized silver nanoparticles

As seen in Fig. 8, a chemical signal for metallic silver is clearly present in the AgNPs generated by EDX analysis [32]. This might be because proteins or enzymes in the bacterial biosurfactant or the plant extracts used in the synthesis emit other elements, such as C, O, Na, and N, as seen in Fig. 8's elemental composition [33]. The EDX spectra of AgNPs produced ex vivo has been studied before [34, 35]. Furthermore, the release of phytobiotics such as proteins, enzymes, carbohydrates, sugars, and amino groups may be responsible for the existence of N in nanoparticles generated from *B. subtilis* and *E. coli* cultures.

Selected area electron diffraction (SAED) images of biosynthesized silver nanoparticles

The AgNPs' polycrystalline structure is seen even more clearly by the SAED ring pattern (Fig. 9). In the SAED patterns, the rings may be ascribed to the diffraction from planes 111 through 222 of a face-centered cubic spherical structure (FCC).

X-ray diffraction spectra of biosynthesized silver nanoparticles

The crystal formation of the generated silver nanoparticles was examined using X-ray diffraction (XRD) analysis (Fig. 10). There were four peaks at 2 matching to 111, 200, 220 and 311 Bragg's reflections that indicated the crystallization of AgNPs. The crystallite sizes of the AgNPs were calculated using Debye–Scherrer's equation. Balasubramanian et al. [36] also synthesized nanocrystals with Braggs reflection peaks at 38.42, 44.36, 64.40 and 77.49 °C that conforms to (111), (200), (220) and (311) lattice planes. Nanoparticles synthesized from

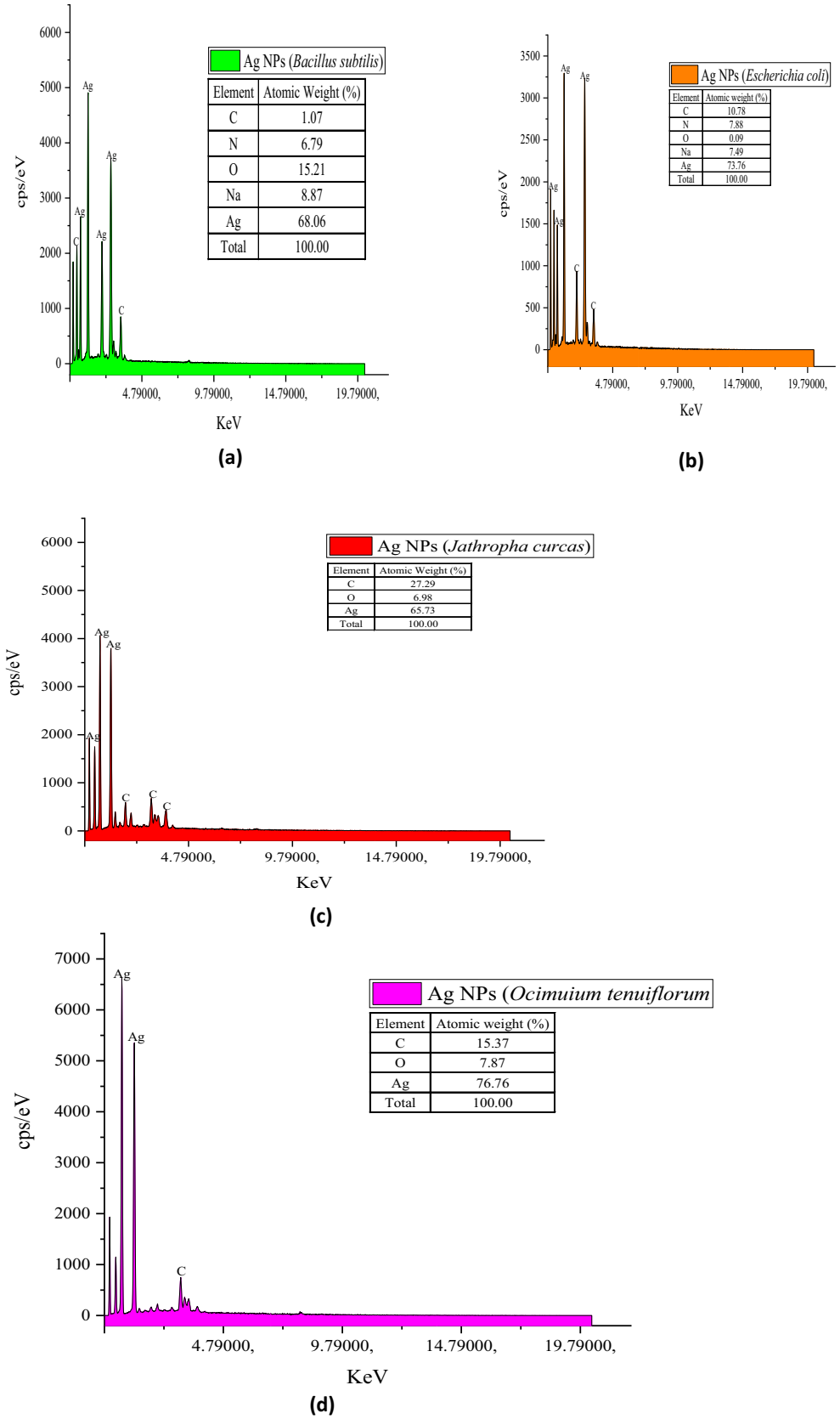

Fig. 8 Energy-dispersive X-ray spectroscopy micrograph of silver nanoparticles synthesized by **a** *Bacillus subtilis*, **b** *Escherichia coli*, **c** *Jatropha curcas*, **d** *Ocimum tenuiflorum*

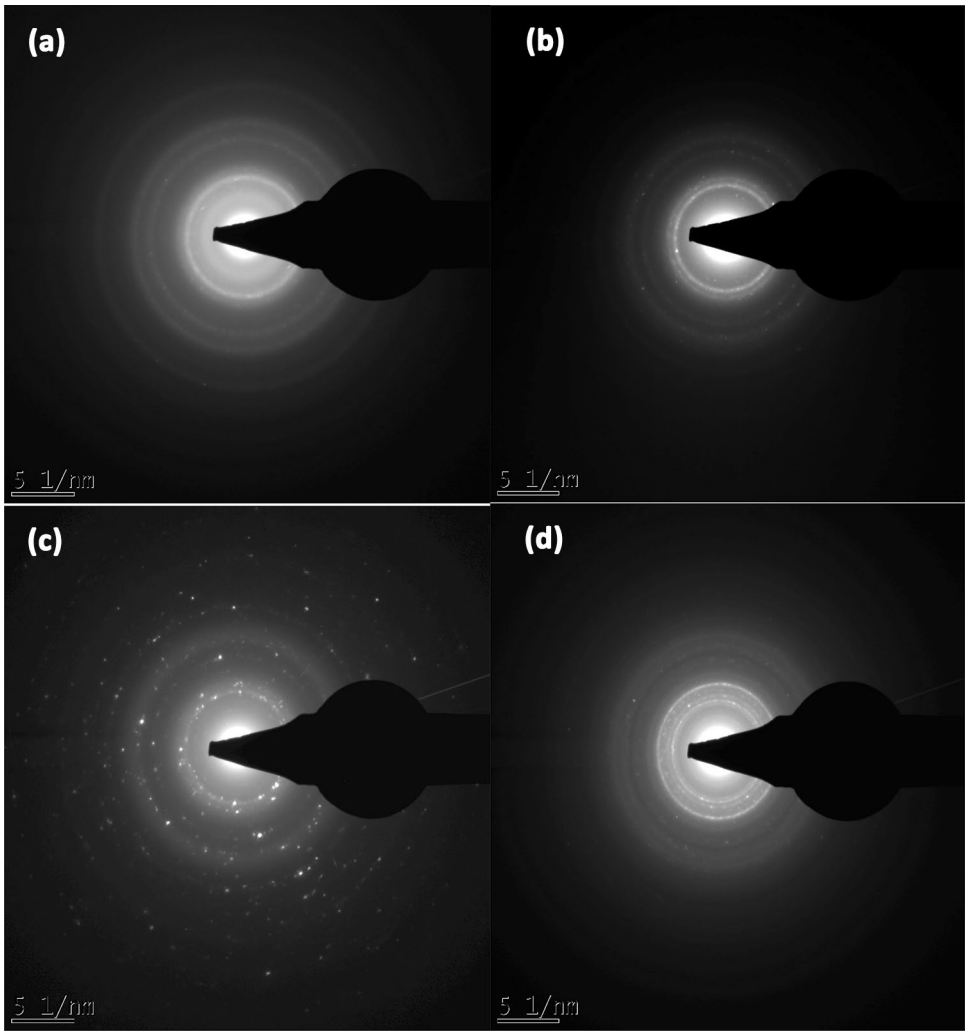

Fig. 9 Selected area electron diffraction images of silver nanoparticles synthesized by **a** *Bacillus subtilis*, **b** *Escherichia coli*, **c** Jatropha leaf extract, **d** Tulsi leaf extract

B. subtilis and *E. coli* displayed a prominent peak at 31.66°, which is missing in nanoparticles produced using *O. tenuiflorum* and *J. curcas*. AgNPs were found to be crystallites based on the XRD spectrum from four separate Bragg's reflection peaks. According to JCPDS file no. 04-0783, these values are in agreement with the JCPDS reference data. It is possible that biomolecules present in the culture supernatant cap the AgNPs, as seen by the unassigned peaks in the Ag NPs' XRD spectrum [37]. *B. subtilis* and *E. coli* cultures were used to create biosynthesized Ag NPs that were spherical in form with mean crystallite sizes of 11.100.21 and 38.890.42 nm, respectively. Using plant extracts, we were able to produce nanoparticles with a crystalline size of 12.280.37 nm for *J. curcas* and *O. tenuiflorum* (see Figs. 5, 6).

Conclusion

Bacillus subtilis and *Escherichia coli* culture supernatant and extracts from Jatropha and Tulsi plants were used in this study to produce silver nanoparticles. Extracellular synthesis of silver nanoparticles in the 4–4.8 nm size range produced spherical particles. Hence, these biocompatible silver nanoparticles can be used to remove pollutants from wastewater, and they are also useful in medicine and pharmaceutical industries. The developed silver nanoparticles are easy to synthesize, contamination free, ecofriendly, consumes less energy and cost effective because of their natural or biological source. The developed silver

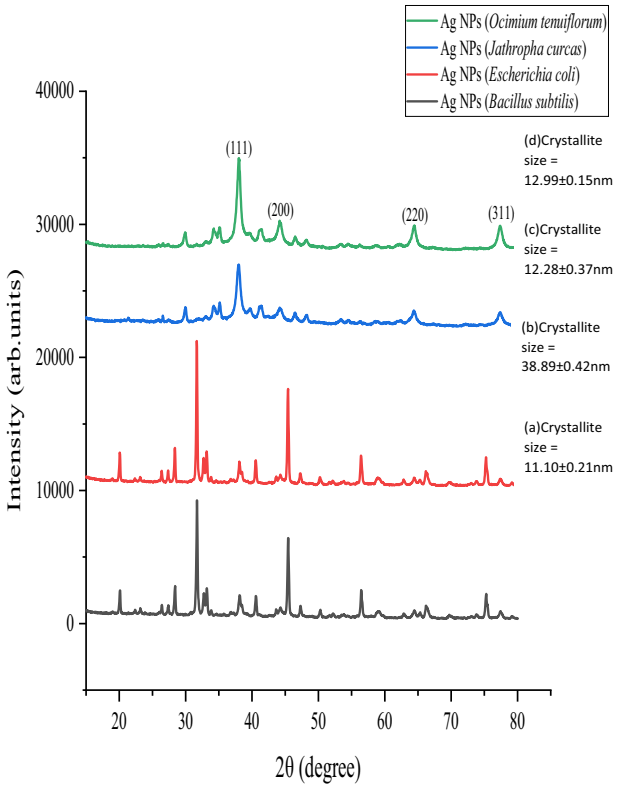

Fig. 10 X-ray diffraction spectra of silver nanoparticles synthesized by **a** *Bacillus subtilis*, **b** *Escherichia coli*, **c** Jatropha leaf extract, **d** Tulsi leaf extract

nanoparticles are preferred over commercial drugs in biomedicine owing to their unique antimicrobial characteristics, adjustable size and shape, enhanced stability of surface bound nucleic acids, high-density surface ligand attachment, transmembrane delivery without harsh transfection agents. All these unique properties of the developed silver nanoparticles make them a promising tool over commercial drugs in medicine and pharmaceutical industries. These findings showed that silver nanoparticles can be produced using isolated *Bacillus subtilis* and *Escherichia coli*, as well as plant extracts from Jatropha and Tulsi.

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Declarations

Conflict of interest There is no any conflict of interest in the manuscript.

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