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Synthesis of Silver Nanoparticles from *Oroxylum indicum*

K. A. Madhushree[1,3], Poornima[1,3], R. Mahesh[2,3], Raviraj Kusanur[2,3], and H. G. Ashok Kumar[1,3]

[1]*Department of Biotechnology, R.V. College of Engineering, Bengaluru, India*
[2]*Department of Chemistry, R.V. College of Engineering, Bengaluru, India*
[3]*Visvesvaraya Technological University, Belagavi, India*

In recent years, gold and silver nanoparticles are gaining prominence as antimicrobial agents. Silver nanoparticles have been employed in medicine, bio-sensing and agriculture. The synthesis of silver nanoparticles from plant extracts are gaining importance as it is eco-friendly and safe. In the present study, the aqueous extracts of leaf, root and stem of *Oroxylum indicum* are used as the reducing agents for the synthesis of the silver nanoparticles. Various concentrations (1.0, 2.0, 5.0 or 10 mM) of silver nitrate are used with aqueous extract of *O.indicum* for the synthesis of silver nanoparticles. The synthesized silver nanoparticles are characterized using UV–visible spectroscopy, Fourier-transform infrared spectroscopy, and x-ray diffraction and scanning electron microscopy techniques. The antibacterial activity of these silver nanoparticles is studied against *Escherichia coli* and *Bacillus subtilis*. The maximum inhibitory activity is observed from silver nanoparticle synthesized from 5 or 10 mM silver nitrate with *O.indicum* extract.

В останні роки наночастинки золота та срібла набувають популярности в якості антимікробних аґентів. Наночастинки срібла були використані в медицині, біозондуванні та сільському господарстві. Синтеза наночастинок срібла з рослинних екстрактів набуває важливого значення, оскільки вона є екологічною та безпечною. У цьому дослідженні водні екстракти листа, кореня та стебла *Oroxylum indicum* використовуються в якості відновників для синтези наночастинок срібла. Різні концентрації (1,0, 2,0, 5,0 або 10 мМ) нітрату Арґентуму використовуються з водним екстрактом *O.indicum* для синтези наночастинок срібла. Синте-

зовані наночастинки срібла характеризуються за допомогою спектроскопії у видимій і ультрафіолетовій областях світла, інфрачервоної спектроскопії на основі Фур'є-перетвору, рентґенівської дифракції та методів сканувальної електронної мікроскопії. Антибактеріяльна активність цих наночастинок срібла вивчається проти *Escherichia coli* і *Bacillus subtilis*. Максимальна стримувальна активність спостерігається від наночастинок срібла, синтезованих з нітрату Арґентуму концентрацією у 5 або 10 мМ із екстрактом *O.indicum*.

Key words: *Oroxylum indicum*, silver nanoparticles, FTIR, XRD, antibacterial activity.

Ключові слова: *Oroxylum indicum*, наночастинки срібла, інфрачервона спектроскопія на основі Фур'є-перетвору, рентґенівська дифракція, антибактеріяльна активність.

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1. INTRODUCTION

The pathogenic microorganisms gaining multi drug resistance has become a major problem in health sector [1, 2]. Hence, finding solution for this problem would help in overcoming the new pathological conditions. Nanotechnology is a field that is gaining importance in recent years, which includes manufacturing of materials of 1–100 nm ranged size [3]. The nanoparticles have a special property of behaving as an antimicrobial agent due to its high surface area [2]. Synthesis of nanoparticles can be done using biological, physical and chemical approach. The chemical method of synthesis of the nanoparticles need stabilizing agent called the capping agent. Hence the biological approach is gaining prominence since it is ecofriendly and cost effective compared to chemical and physical approaches [4, 5]. Silver nanoparticles rather than the silver ion are of intense research as these nanoparticles have unique property of low concentration required for minimal inhibition [2, 6, 7, 8].

Aqueous extract of microbial cultures and plants are used for the synthesis of silver nanoparticles has been reported in several research findings. This is due to the phytochemicals that exhibits antioxidant property of plants and in enzymes of microorganisms, where the metal compounds are reduced to form nanoparticles. The green synthesis of nanoparticles refers to synthesis of nanoparticles from natural sources such as aqueous extracts of plants. Several research finding have already been published which explains methods of nanoparticles synthesis from the aqueous extracts of *Acalypha indica* [4], *Ocimum sanctum* [9], *Catharanthus roseus* [10], *Citrullus colocynthis* [11], *Olea europaea* [12], *Pterocarpus santali-*

nus [13], *Rumex hymenosepaluus* [14], *Vitis vinifera* [3], *Zingiber officinale* [15], *Afzelia quanzensis* [16], *Lantana camara* [17], *Chamomile* [5], and *Cucumis prophetarum* [18].

The *Oroxylum indicum* (L.) (Vent.) is an important medicinal plant of family *Bignoniaceous*. This plant contains various secondary metabolites such as flavonoids, tannins, alkaloids and terpenoids [19]. Plants generally have reactive oxygen species that scavenge the oxygen radicals. Such reactive oxygen species are found in bark and roots of *O.indicum* which adds special characteristics such as anti-inflammatory, antimicrobial properties to this plant. Fruit extract has been used in treating heart, throat and lung related disorders. Leaf extract is used in treating ulcers and root extract is used in treating dysentery [19].

In the present study, silver nanoparticles were synthesized from leaf, stem and root extract of *O.indicum*, and its antibacterial activity was investigated.

2. EXPERIMENTAL

2.1. Synthesis of Silver Nanoparticles

Different parts of *O.indicum* such as leaf, root and stem were collected and dried completely and powdered using mortar and pestle. Aqueous extract was prepared by boiling 5 g of the powder in a 250 ml beaker containing 100 ml double distilled water. The dissolved solution was subjected to centrifugation at 5000 rpm for 15 min, then, the supernatant was collected and used for the preparation of the silver nanoparticles. Silver nitrate concentration of 1 mM, 2 mM, 5 mM or 10 mM were prepared by the extract obtained from *O.indicum*. This was incubated in dark and further the solution was subjected to centrifugation for 20 min at 10000 rpm, and then, pellet was collected, dried for 48 hrs, used for antibacterial activity.

2.2. Characterization of the Silver Nanoparticles

The biosynthesized silver nanoparticles were characterized using UV–visible spectroscopy, Fourier-transform infrared spectroscopy, x-ray diffraction and scanning electron microscopy.

UV–Visible Spectroscopy Analysis. Double beam UV–visible spectrophotometer (SL164, ELICO, India) was used for measuring the optical property of the synthesized silver nanoparticles. The silver nitrate of 1 mM, 2 mM, 5 mM and 10 mM at various time intervals of 0, 24, 48, 72 hrs were measured in the spectrum range of 200 nm to 800 nm.

Fourier-Transform Infrared Analysis. Fourier transform infrared analysis is carried out in the range of 3600–600 cm^{-1} using Perkin Elmer Spectrum version 10.4.00.

X-Ray Diffraction Analysis. X-ray diffraction analyser with settings of 40 kV/40mA was used for characterizing the *O.indicum* extract silver nanoparticles. The data for thus synthesized nanoparticles were collected in the angular range of 2θ. The size of these particles was obtained using Scherrer formula.

Scanning Electron Microscopy Analysis. The scanning electron microscopy analysis gives the size of the nanoparticles and the topological view of these particles. Copper coated grid was covered with thin film of the silver nanoparticles and further analysed at a set accelerating voltage of 12 kV (Hitachi su 1500).

2.3. Antimicrobial Screening of Silver Nanoparticles

Disc plate method was used for checking the antibacterial activity of the silver nanoparticles against *B.subtilis* and *E.coli.* Sterile discs were placed on the nutrient agar plates that were swabbed with the pure culture of *B.subtilis* or *E.coli*. The 20 µl of colloidal solution of silver nanoparticles was added to sterile discs, plant extract was also loaded on to one of the sterile disc. Silver nitrate solution and the antibiotic were used as positive control and the plant extracts alone acts as negative control.

The inhibition zone formed after incubation at 37°C for 48 hr was measured.

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of Silver Nanoparticles

Various parts of *O.indicum* such as leaf, root and stem was used for the synthesis of silver nanoparticles, and this extract acts as a reducing agent. With addition of varying concentration of silver nitrate to the extract, colour change was observed (Fig. 1, *a*–*c*). Optical characteristics of the nanoparticles were reason for this colour change, this was because of the phenomenon known as surface plasmon resonance. Similar result, which indicates the silver ion reduction leading to the formation of the silver nanoparticles from the extracts, was reported in *Rumex hymenosepalus* [14]. The antioxidants of the plants reduce the silver ion of the silver nitrate into atom, which nucleates to form nanoparticles. The size of the nanoparticles was influenced by the concentration of the silver salts and reducing agent in the solution [14].

3.2. Characterization Studies

The synthesized nanoparticles were characterized using UV–visible spectroscopy, SEM, XRD and FTIR. The optical property, topology, shape, size, capping of the particle and other important properties of the silver nanoparticles were analysed.

UV–Visible Spectroscopy Analysis. The synthesis of the silver nanoparticles was confirmed using UV–visible spectroscopy (Fig. 2). This was due to the property of Plasmon resonance of the nanoparticles [20]. The size of the particles and the absorption spectra were proportional [9]. There was a secondary peak observed due to particle destabilization in the solution.

As the concentration of silver nitrate increases, there was increase concentration of silver nanoparticles being formed hence intensity of the peak increases. But at the concentrations of 5 mM and 10 mM of silver nitrate, there was no significant peak difference being observed. The intensity of peak increases with incubation period but remains unaltered after 72 hr. Hence, it can be conclud-

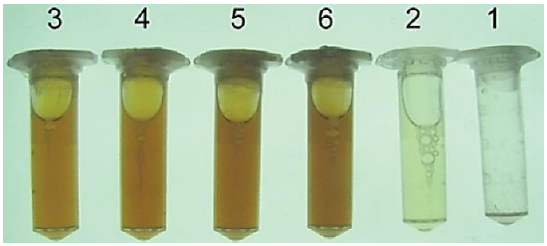


Fig. 1. Silver nanoparticles synthesized using extracts of leaf of *O.indicum*. 1—Silver nitrate solution; 2—plant extract; 3—Ag-NPs in 1mM $AgNO_3$; 4—Ag-NPs in 2 mM $AgNO_3$; 5—Ag-NPs in 5 mM $AgNO_3$; 6—Ag-NPs in 10 mM $AgNO_3$.

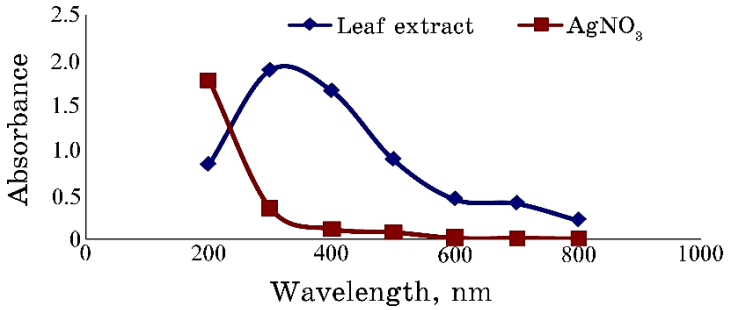


Fig. 2. UV–visible spectra of silver nanoparticles synthesized using the extracts of leaf of *O.indicum*.

ed that the equilibrium was reached at 72 hr. Hence, for further study, silver nanoparticles prepared from 5 mM silver nitrate with an incubation period of 72 hr were used for maximum nanoparticles production. Further, it was also observed that the intensity of the peak was highest for the leaf extract silver nanoparticles (Fig. 2).

Fourier-Transform Infrared Spectroscopy Analysis. This was done to know the stability of the synthesized nanoparticles and the bond linkage. Figure 3, *a*–*c* shows the results of the FTIR indicating the presence of active functional group in the nanoparticles since there were a lot of absorption bands. The band at 3240–3300 cm^{-1} corresponds to the functional alcoholic group stretching in polyphenols, proteins and in polysaccharides. The stretching characteristics of the methyl group, secondary amine or aldehydic C–H bond stretching gives a peak at 2920–2928 cm^{-1}. The amide bond of protein, *i.e.*, carbonyl stretching, gives a peak at 1595–1626 cm^{-1}. The peak at 1018–1030 cm^{-1} indicates the presence of alcohol, acidic, ether, ester, carboxylic and aliphatic amine groups. The intensity of the spectra always directly related to the changes in the dipole, *i.e.*, due to the vibrations. This data was supported by the observations in *Zingiber officinale* [21]. Spectra results support the presence of the reducing sugar in solution, *i.e.*, responsible for the reduction of the

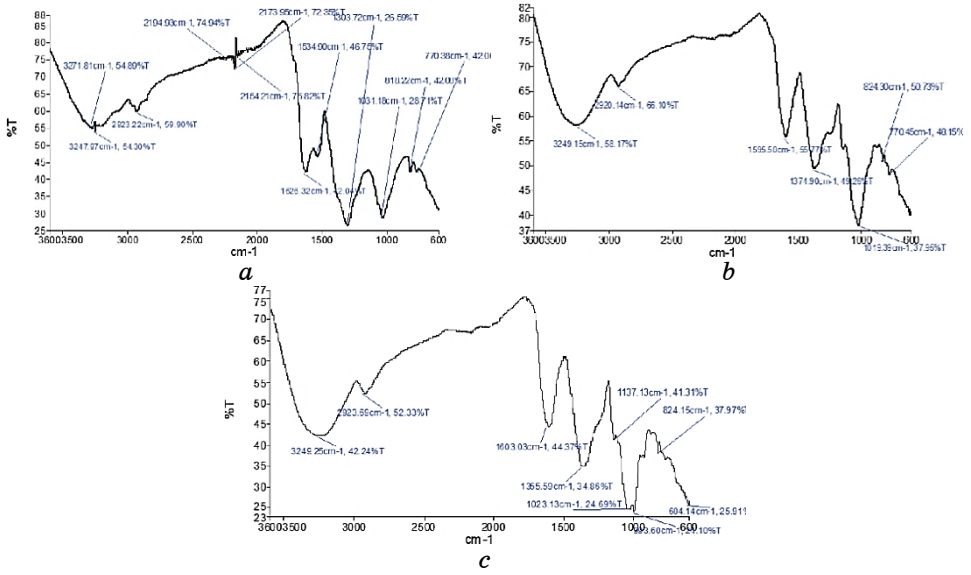


Fig. 3. FTIR spectra of silver nanoparticles synthesized using extracts of *O.indicum*. *a*—FTIR spectrum of Ag-NPs synthesized using leaf extract; *b*—FTIR spectrum of Ag-NPs synthesized using stem extract; *c*—FTIR spectrum of Ag-NPs using root extract.

metal ions and synthesis of the metal nanoparticles.

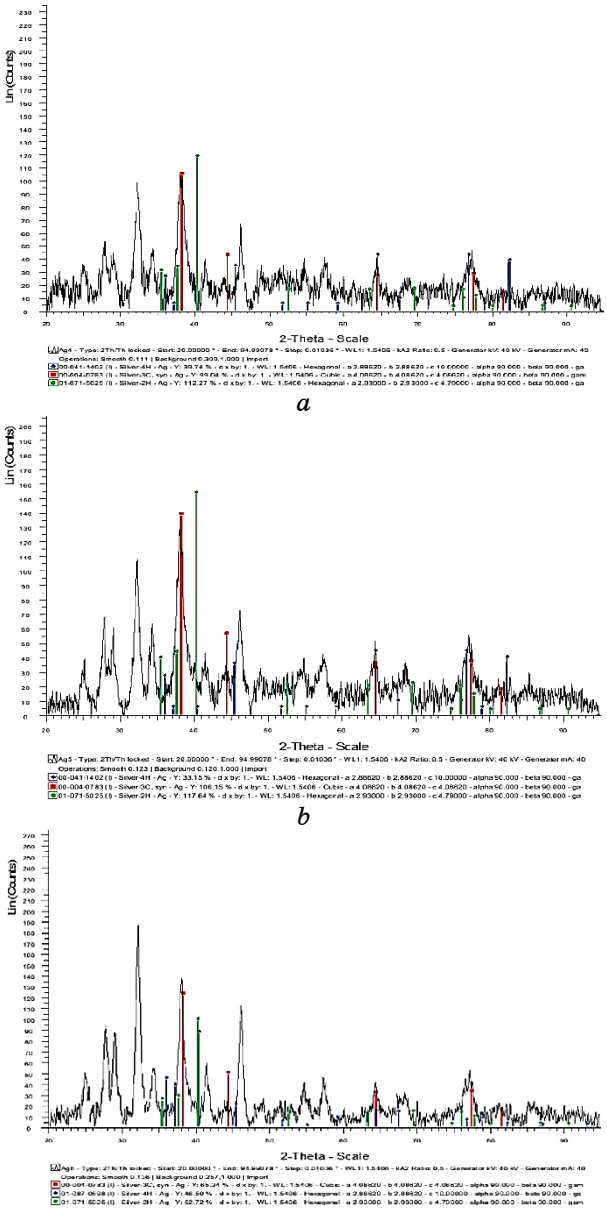


Fig. 4. XRD patterns of silver nanoparticles synthesized using extracts of *O.indicum*. *a*—XRD patterns of Ag-NPs synthesized using extract of leaf; *b*—XRD patterns of Ag-NPs synthesized using extract of stem; *c*—XRD patterns of Ag-NPs synthesized using extract of root.

Fig. 5. SEM images of silver nanoparticles synthesized using stem extract of *O.indicum*.

The silver nanoparticles interact with the proteins and forms larger particles; this was due to the functional group of the protein or the metabolite.

X-Ray Diffraction Analysis. The x-ray diffraction method was used to analyse the structure of the nanoparticles. In the present investigation, the peaks indicate the presence of the cubic and hexagonal crystalline structure of nanoparticles (Fig. 4, *a*–*c*). The peaks at 77°, 67.5°, 64.5°, 45.3°, 44°, 38° correspond to 311, 112, 220, 103, 200, 111 in the spectrum of 2θ. The crystalline nature of biosynthesized nanoparticles were also reported in *Vitis vinifera* [3] and *Afzelia quanzensis* [16]. The broaden peak was observed for smaller particles. The multiple beam of x-rays was used for obtaining the three dimension image of the crystalline structure. The size of silver nanoparticles synthesized from the extract of *in vitro* grown *O.indicum* roots, leaves and stem is found to be 86 nm, 74 nm and 63 nm, respectively. These results were found to fall in the range of 1–100 nm.

Scanning Electron Microscopy Analysis. The highly variable morphology of the nanoparticles was obtained (Fig. 5). Figure further shows the clustered and individual particles. The optical and electrical property was related to the change in the shape of the nanoparticles [12, 22].

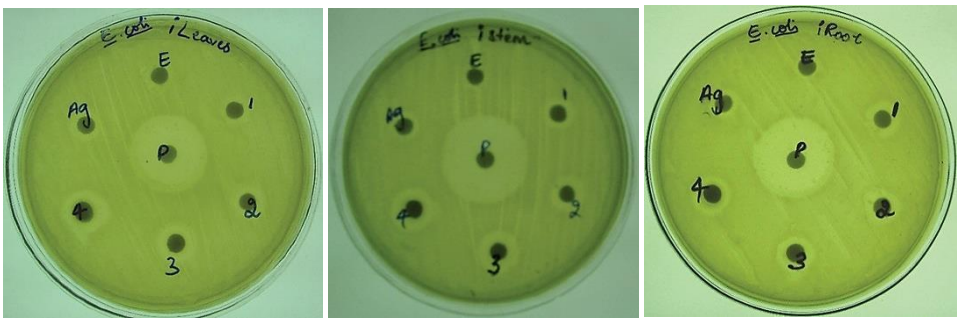
3.3. Antibacterial Activity of the Synthesized Silver Nanoparticles

In this study, the antibacterial activity was studied against *E.coli* and *B.subtilis*.

Figures 6 and 7 show the inhibited growth, when silver nanoparticles are used.

a *b* *c*

Fig. 6. Antibacterial activity of silver nanoparticles synthesized using extracts of *O.indicum* at different concentrations against *B.substilis*. *a*—Antibacterial activity of Ag-NPs synthesized using leaf extract; *b*—antibacterial activity of Ag-NPs synthesized using stem extract; *c*—antibacterial activity of Ag-NPs synthesized using root extract. 1—Ag-NPs containing 1 mM $AgNO_3$; 2—Ag-NPs containing 3 mM $AgNO_3$; 3—Ag-NPs containing 5 mM $AgNO_3$; 4—Ag-NPs containing 10 mM $AgNO_3$. P—positive control (Ampicillin); Ag—silver nitrate; E—extract.



a *b* *c*

Fig. 7. Antibacterial activity of silver nanoparticles synthesized using extracts of *O.indicum* at different concentrations against *E.coli*. *a*—Antibacterial activity of Ag-NPs synthesized using leaf extract; *b*—antibacterial activity of Ag-NPs synthesized using stem extract; *c*—antibacterial activity of Ag-NPs synthesized using root extract. 1—Ag-NPs containing 1 mM $AgNO_3$; 2—Ag-NPs containing 3 mM $AgNO_3$; 3—Ag-NPs containing 5 mM $AgNO_3$; 4—Ag-NPs containing 10 mM $AgNO_3$; P—positive control (Ampicillin); Ag—silver nitrate; E—extract.

The diameter of the inhibition zone increases with increase in the concentration of the nanoparticles (Table). The silver nanoparticles synthesized using root extract with 10 mM concentration of silver nitrate showed a zone of inhibition with a diameter of 14 mm against *B. subtilis* (Fig. 6).

TABLE. Inhibitory effect of silver nanoparticles synthesized using extract of *O.indicum* on *B.subtilis* and *E.coli*.

	Bacillus subtilis			*Escherichia coli*		
	In vitro stem, mm	*In vitro* leaf, mm	*In vitro* root, mm	*In vitro* stem, mm	*In vitro* leaf, mm	*In vitro* root, mm
Positive control	22	21	22	27	21	25
Silver nitrate	9	7	9	8	9	8
Extract	—	—	—	7	9	7
1 mM*	10	7	10	10	10	11
2 mM*	11	10	11	11	10	11
5 mM*	13	11	13	13	11	12
10 mM*	13	13	14	15	14	13

Note: *Silver nanoparticles synthesized at particular mM concentration.

The silver nanoparticles synthesized using stem extract with 10 mM concentration of silver nitrate showed a zone of inhibition with a diameter of 15 mm against *E.coli* (Fig. 7). This antibacterial property was due to the electrostatic attraction of these particles with the cell membrane leading to the damage bacterial cell membrane and disruption of DNA replication [23]. Few reports also suggest the binding of the silver particle to thiol group of enzymes in bacteria [12, 24].

4. CONCLUSION

In present investigation, the silver nanoparticles were synthesized using the aqueous extracts of stem, root and leaf of *O.indicum*. The synthesized nanoparticles were characterized and studied for its antimicrobial activities against *E.coli* and *B.subtilis*. Silver nanoparticles synthesized from 5 or 10 mM silver nitrate showed maximum inhibitory activity.

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