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Green synthesis of silver nanoparticles from stem bark extract of *Terminalia tomentosa* Roxb. (Wight & Arn.)

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ABSTRACT

In the present work silver nanoparticles was synthesized from stem bark extract of Terminalia tomentosa Roxb. (Wight & Arn.), a plant widely found in a large region in India, as reducing agent. Terminalia tomentosa Roxb. (Wight & Arn.) is known to be rich in antioxidant molecules which was used as reducing agents. Silver nanoparticles grow in a single-step method, at room temperature, and with no addition of external energy. The nanoparticles have been characterized by ultraviolet-visible spectroscopy and transmission electron microscopy, as a function of the ratio of silver ions to reducing agent molecules. The nanoparticles diameters were found to be in the range of 5 to 50 nm. The synthesized silver nanoparticles from the Terminalia tomentosa Roxb. (Wight & Arn.) stem bark extract, which do not involve any harmful chemicals were well-dispersed.

Keywords: Silver nanoparticles, *Terminalia tomentosa* Roxb. (Wight & Arn.), Antioxidants, Electron microscopy, Green synthesis.

INTRODUCTION

Currently, improving and protecting our environment using green chemistry have become important issues in many fields of research [1]. In the field of nano-science, the use of various biological units instead of toxic chemicals for the reduction and stabilization of metal nanoparticles, has received extensive attention [2]. Biological entities, such as bacteria [3], fungi [4], yeasts [5], algae [6] or plants [7, 8], have been reported as serving as both reducing and stabilizing agents. Among these possible bio- resources, biologically active products from plant resources represent excellent scaffolds for this purpose [7]. Plant extracts, which are rich in bioactive compounds, have recently been used for NPS green synthesis. Many different plant leaves and herbs for the synthesis of nanoparticles have been reported [9]. The mechanism of biosynthesis of nanoparticles in plants may be associated with the phytoremediation concept in plants [7].

Terminalia tomentosa Roxb (Wight & Arn.) (Syn.: *Terminalia alata* Heyne ex.Roth, *Terminalia crenulata* Roth, *Terminalia elliptica* Willd.) belonging to family Combretaceae [10-12]. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. The bark is bitter & stypic, useful in vitiated conditions of pitta, ulcers, vata, fractures, haemorrhages, bronchitis cardiopathy, strangury, wounds, haemoptysis, dysentery, cough, verminosis ,leucorrhoea, gonorrhoea & burning sensation (Ayurveda) [13-14]. The most promising approach for generating new fields in biomedical sciences is the pharmaceutical application of nanoparticles (NPs) [1]. Among metal NPs, nanosilver exhibits outstanding physical, chemical and biological properties [15]. Ag-NPs have potential in treating a variety of diseases, including retinal neovascularization, immunodeficiency syndrome [16], infection [17] and cancer [18]. The growth factors imbalance is involved in the acceleration of several diseases including cancer, ocular, and inflammatory diseases [19].

The present study describes a green method using flowers of *Terminalia tomentosa* Roxb (Wight & Arn.) extracts for the biosynthesis of silver nanoparticles (Ag-NPs) without any additive protecting nanoparticles from aggregating, template shaping nanoparticles or accelerants. The current simple biosynthetic method using precursors from flowers of *Terminalia tomentosa* Roxb (Wight & Arn.) provides high-yield nano-sized materials. The characterization and formation mechanisms of Ag-NPs are discussed.

MATERIALS AND METHODS

$AgNO_3$ was purchased from Merck (Mumbai). Stem bark of *Terminalia tomentosa* Roxb (Wight & Arn.) were obtained from a Karjat, Maharashtra. All the solutions were prepared with double distilled water. Other chemicals were of analytical grade.

Extraction Preparation

The identification of *Terminalia tomentosa* Roxb (Wight & Arn.) was confirmed by a plant taxonomist from Blatter's Herbarium, St. Xaviers College, Mumbai. where a sample was deposited with the voucher specimen number 34516. The stem bark of *Terminalia tomentosa* Roxb (Wight & Arn.) were air-dried in the shade at room temperature. For the production of the extract, ground, stem bark (5 g) was boiled with DDW (100 mL) in an Erlenmeyer flask while being continuously stirred for 30 min. The extract was cooled to room temperature, filtered and used for the synthesis of Ag-NPs.

Synthesis and Characterization of Ag-NPs

For the preparation of silver nanoparticles, $AgNO_3$ aqueous solution (5 mM) was mixed with different volume of plant extract under continuous stirring at 40 °C. Thes color of the solution slowly changed from grayish yellow to dark brown indicating the formation of silver nanoparticles.

The prepared Ag-NPs were monitored by UV-Vis spectroscopy using a UV-Vis spectrophotometer system in the wavelength range from 200–700 nm. Energy Dispersive X-ray spectroscopy (EDS) was performed to study the composition of the product. The size of the distribution and the average size of 50 nanoparticles were estimated on the basis of three TEM images with the assistance of Sigma-Scan Pro software. The zeta-potentials of Ag-NPs in water were evaluated using CAD. Samples were sonicated for 5 min before measurement to ensure that the particles were well dispersed and that the dispersion was homogeneous.

RESULTS AND DISCUSSION

UV-Vis spectra recorded from aqueous solution of silver nitrate with *Terminalia tomentosa* Roxb (Wight & Arn.) extract. The samples display an optical absorption band peak at about 410 nm (Figure 1), typical of absorption for metallic Ag nanoclusters, due to the Surface Plasmon Resonance (SPR). Effect of the reaction time on AgNPs synthesis was also evaluated with UV-Visible spectra and it is noted that with an increase in time the peak becomes sharper. The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution. The weak absorption peak at 200 nm indicates the presence of several organic compounds which are known to interact with silver ions into solution and suggests a possible mechanism for the reduction of the metal ions presented in the solution.

The exact nature of the silver particles formed can be deduced from the XRD spectrum of the sample. XRD pattern of the plant derived AgNPs (Figure 2) shows four intense peaks in the whole spectrum of 2θ values ranging from 20° to 80°. The silver particles formed were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.45°, 44.48°, 64.69° and 77.62°, corresponding to (111), (200), (220), and (311) planes for silver, respectively. The unassigned peaks could be due to the crystallization of bioorganic phase that occurs on the surface of the nanoparticle. Two small insignificant impurity peaks observed at 68° and 75° are attributed to the presence of other organic sub- stances in culture supernatant. The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nanosizes. The peak broadening at half maximum intensity of the X-ray diffraction lines is due to a reduction in crystallite size, flattening and micro-strains within the diffracting domains.

Further analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of elemental silver. Figure 3 shows the Energy Dispersive Absorption Spectroscopy photographs of derived AgNPs. All the peaks of Ag are observed and are assigned. Peaks for Cu and C are from the grid used and the peaks for S, P and N correspond to the protein capping over the AgNPs. Silver nanocrystallites display an optical absorption band peak at approximately 3 keV, which is typical of the absorption of metallic silver nanocrystallites due to surface.

The TEM image (Figure 4) show that the Ag-NPs formed were well dispersed with different shapes such as hexagonal, pentagonal and spherical structures with particle sizes ranging from 5 to 50 nm. The presence of secondary materials can be seen from the capping with dark shades on the surface of nanoparticles, which may be assigned to the bio-compounds present in the *Terminalia tomentosa* Roxb (Wight & Arn.) extract. The bio-components within the *Terminalia tomentosa* Roxb (Wight & Arn.) not only result in the efficient reduction of silver salts to nanoparticles, but, likewise as an appropriate capping agent, inhibiting them from aggregation [20]. The different bio-compounds present in *Terminalia tomentosa* Roxb (Wight & Arn.) extract such as polysaccharides, polyphenols, and proteins can produce nanoparticles with different shapes [21].

Figure 5 shows selected area electron diffraction pattern (SAED) of the silver nanoparticles. The silver particles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate. SAED spots that corresponded to the different crystallographic planes of face-centered cubic (FCC) structure of elemental silver are seen in Figure 5. The XRD spectrum of silver nanoparticles (Figure 2) exhibiting the characteristic peaks of the silver crystallites observed at 2θ values of 38.5°, 44.48°, 64.69° and 77.62° corresponding to (111), (200), (220) and (311) of the face centered cubic (FCC) silver nanoparticles, is also in agreement with SAED result.

Figure 1: UV-visible absorption spectra of *Terminalia tomentosa* Roxb (Wight & Arn.) Ag-NPs

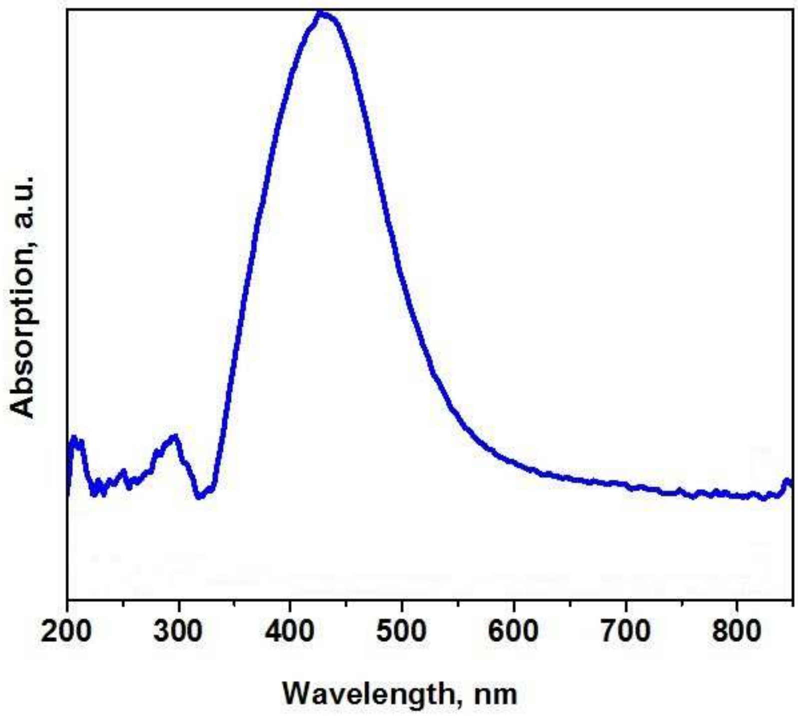


Figure 2: XRD spectrum of AgNPs synthesized from the extract of *Terminalia tomentosa* Roxb (Wight & Arn.) Ag-NPs

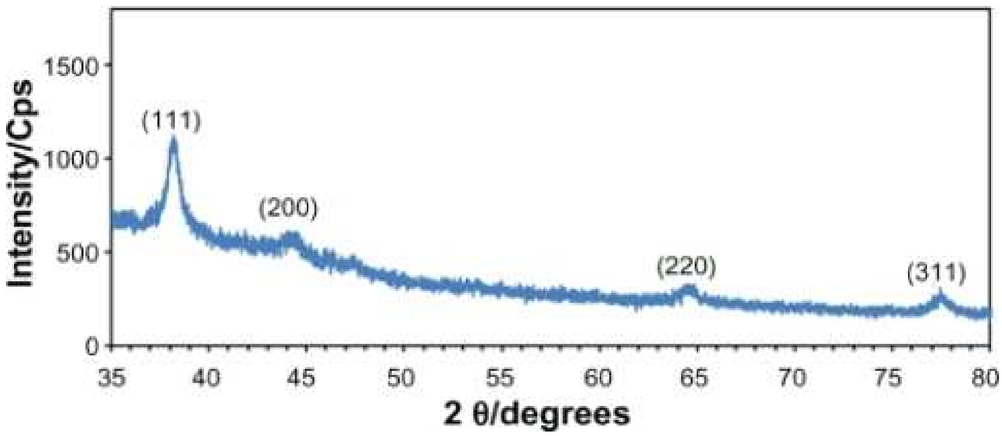


Figure 3: EDAX spectra of extract of *Terminalia tomentosa* Roxb (Wight & Arn.) Ag-NPs

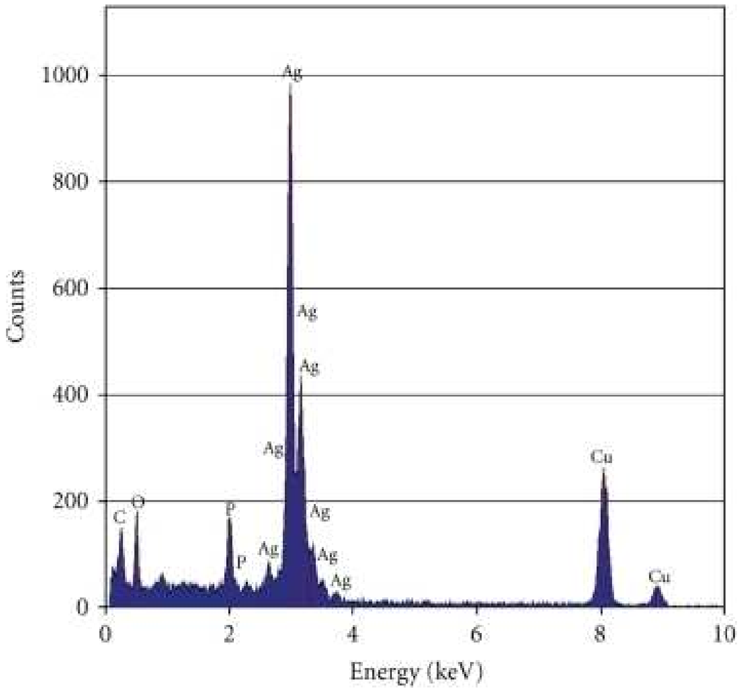


Figure 4: TEM micrograph of biosynthesized *Terminalia tomentosa* Roxb (Wight & Arn.) Ag-NPs

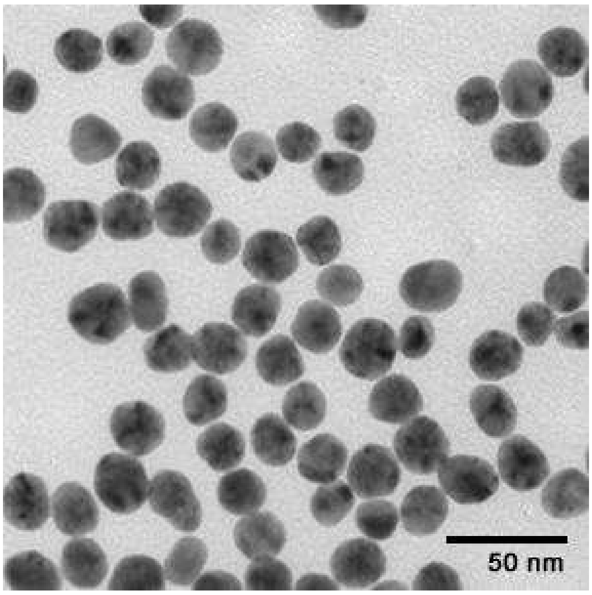
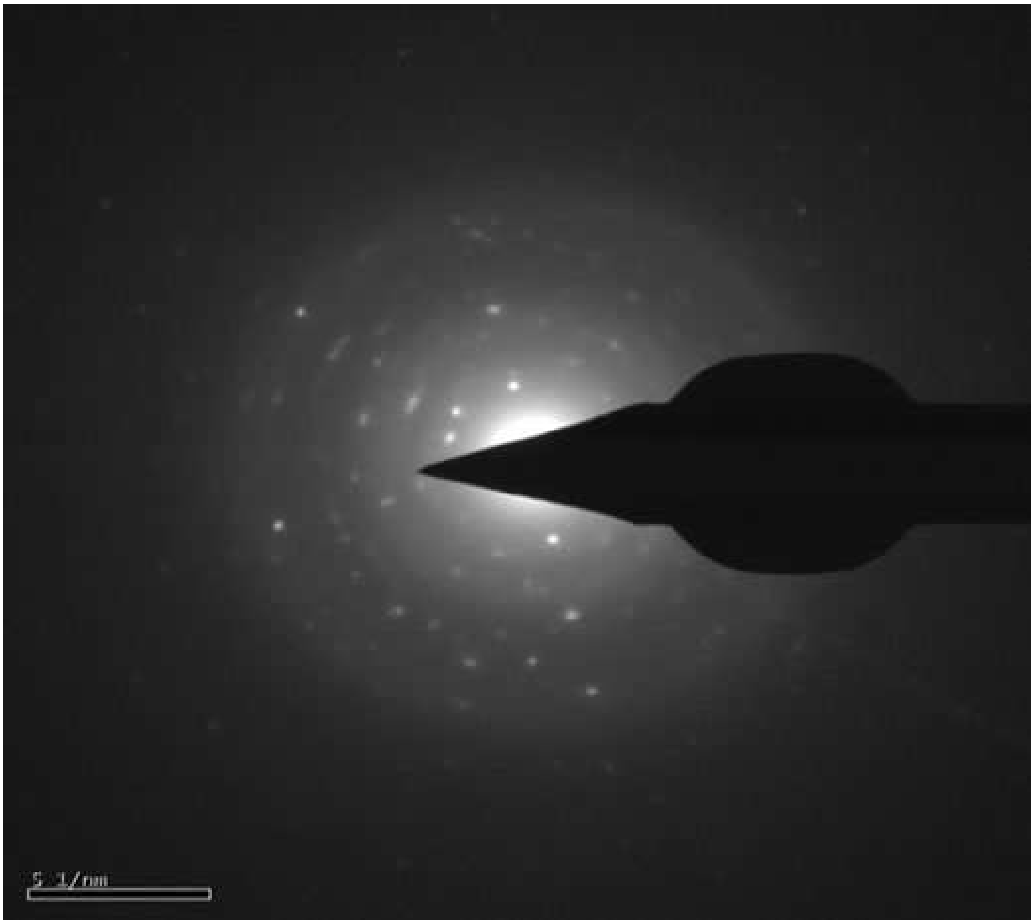


Figure 5: Selected area electron diffraction showing the characteristic crystal planes of elemental silver



CONCLUSION

The rapid synthesis of stable silver nanoparticles of average size ~12 nm using *Terminalia tomentosa* Roxb (Wight & Arn.) extract was demonstrated. Achievement of such rapid time scales for synthesis of silver nanoparticles makes it more efficient as a biosynthetic pathway, though there still remains some scope for further decreasing the reduction time periods to make it a viable alternative to chemical synthesis methods. Probably the biomolecules responsible for the reduction and stabilization of AgNPs are phenols. The phenolics in pineapple exhibit excellent antioxidant activity and these phenols can react with a free radical to form the phenoxyl radicals. Therefore, the use of natural anti- oxidants for the synthesis of AgNPs seems to be a good alternative which can be due to its benign composition. The plant material responsible for the reduction and stabilization of NPs needs further study including extraction and identification of the compounds presented in the extract.

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