CHARACTERIZATION OF SILVER NANOPARTICLES USING LEAF EXTRACT OF MEDICINALLY POTENT PLANT *Toddalia asiatica (L.)* Lam

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ABSTRACT

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. The recent development and implementation of new technologies have led to a new era, the nanoparticle of bio molecules in plants can acts as capping and reducing agents and they have investigated in order to find an eco-friendly techniques for production of well characterized. The present investigation was carried out to green synthesis of Agno3 nanoparticles by using the medicinal plant of *T.asiatica*. They were synthesized by mixing ethanol extracts and 1mM of agno3, the formation of nanoparticles was monitored by visualizing color changes and it was confirmed by UV-vis spectrophotometer, FTIR, XRD, SEM, EDX, PSA, ZP the result of various techniques confirmed the presence Agno3 nanoparticles.

KEYWORDS: *T.asiatica*, UV, FTIR, XRD, SEM, EDX, PSA, ZP.

INTRODUCTION

Nanoparticles represent a particle with a nanometer size of 1–100 nm. The nanoscale material has new, unique, and superior physical and chemical properties compared to its bulk structure, due to an increase in the ratio of the surface area per volume of the material/particle. The most widely studied nanoparticle materials are metal nanoparticles because they are easier to synthesize. Moreover, these materials have a wide range of applications: detectors, catalysts, surface coating agents, and antibacterial/antimicrobials,
among many others. Some of the most studied metallic nanoparticles include silver (Ag), gold (Au), platinum (Pt), and palladium (Pd). (Henry F. Aritonang et al., 2019).

One of the fields in which nanotechnology finds extensive applications is nanomedicine, an emerging new field which is an outcome of fusion of nanotechnology and medicine. Medicine is no more physician job exclusively, the materials and devices designed at the level of nanoscale are for diagnosis, treatment, preventing diseases and traumatic injury, relieving pain and also in the overall preservation and improvement of health. Nanotechnology can improve our understanding of living cells and of molecular level interactions. A number of nanoparticles based therapeutics have been approved clinically for infections, vaccines, and renal diseases. Oligodynamic silver having antimicrobial efficacy extends well beyond its virotoxicity and it has lethal effects spanned across all microbial domains. The application of silver nanoparticles in drug delivery, drug discovery, and new drug therapies has declared war on many dreadful diseases and they use the body natural transport pathway and natural mechanism of uptake of the drug by the diseased cells.

Synthesis and characterization of nanoparticles is an important area of research as selection of size and shape of nanoparticles provide an effect control over may of the physical and chemical properties. However, these methods cannot avoid the use of toxic chemicals in the synthesis protocol, Gold, Silver and platinum nanoparticles are widely applied to human contact areas such as shampoos, soaps, detergents, shoes, cosmetic products and tooth pastes as well as medical and pharmaceutical applications. Therefore, there is a growing need to develop Ecofriendly process for nanoparticles synthesis that do not use toxic chemicals.

(Narayanaswamy Krithiga et al., 2015)

Medicinal plants are the richest bioresource of drugs of traditional systems of medicine; modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. As stated by World Health Organization about 80% of the world’s population still depends solely on traditional or herbal medicine for treatment of diseases. Medicinal herbs play an essential role in the prevention and treatment of cancer. Promising efficacy comparable to the often high cost and adverse effects of standard synthetic drug agents. ((https://www.sciencedirect.com › topics › agricultural-and-biological-sciences-2019).
Toddalia asiatica (L.) Lam. (Rutaceae) is commonly called “Forest Pepper” which is found in South Africa, Sri Lanka, South India, Western Nilgiri, Palani hills and Tirunelveli district. All parts of the plant are claimed to have medicinal value. The bioactive compounds like alkaloids, flavonoids, tannins and phenolic compounds are responsible for the medicinal value of the plant which produces a definite physiological action on the body. All parts of the plant are used to treat sprains, contusions, cough, malaria, dysentery, gastralgia and furuncle infections. It is also used in the treatment of paralysis, intermittent fever, dyspepsia, bronchitis, nausea and arthritis. The root bark is bitter, astringent, digestive, carminative, constipating, antidiarrhoeal, and diuretic. The fruits are used for cough and throat pain relief. The extracts of the plant have been reported to possess skin whitening property, antifeedent, spasmolytic, wound healing, anti-microbial, anti-inflammatory and analgesic activities. (Krithiga thangavelu et al., 2015).

MATERIALS AND METHOD

Preparation of the extracts

The plant, Toddalia asiatica (L.) Lam., were collected from NC division, Hulical(po), Coonoor at Nilgiri district, Tamilnadu, India. The specimen sample was identified and authenticated by Dr.M.Palanisamy, Scientist ‘D’-In-Charge, Botanical Survey of India, Southern Regional Centre, Coimbatore-641 003, Tamilnadu, India. The identification No. BSI/SRC/5/23/2017/Tech. 677. Fresh leaves of Toddalia asiatica (L.) Lam., were collected during march for preparation of the ethanolic leaf extract, washed in tap water, rinsed with distilled water, the leaves were then shade dried for about 1 week and crushed into a coarse powder using a blender, two hundred gram of the coarse powder was kept soaked in ethanol (1L, 95%) for 3 days with occasional shaking, then filtered using filter paper. Finally, the filtrate was evaporated to dryness under reduced pressure using rotary evaporator and then stored until use.

SYNTHESIS OF SILVER NANOPARTICLES USING ETHANOLIC EXTRACT OF Toddalia asiatica (Song, J.Y., Beom et al., 2008) and (Shyam Perugu et al., 2016)

About 1mM silver nitrate solution was prepared using 200 ml of deionised water. To this, 20 ml of ethanolic plant extract was added and placed in dark at room temperature for a time period of 24 hours. After the incubation time period, the synthesized silver nanoparticles was separated and determined by centrifugation at 10,000 rpm for 15 minutes. This process was continual for three times to obtain a pellet. The pellet was dried using petroleum ether for
evaporation. For complete removal of petroleum ether, ethanol was used for multiple washes and further it was allowed to dry to obtain in a powdered form. Then the designed silver nanoparticle of plant extract was stored at -4°C for further studies.

**Characterization of Synthesized Silver Nanoparticles**

The synthesized silver nanoparticles was characterized by using UV-Visible Spectroscopy, Fourier Transform - Infrared Spectroscopy (FT-IR) analysis, X-Ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), Energy Dispersive X- Ray Spectroscopy (EDX), zeta potential (ZP) and Particle size analysis (PSA).

**Uv- Visible Spectroscopy Analysis**

For observing the name of AgNPs, UV-Visible spectroscopy was used as a powerful tool for the characterization of colloidal particles. Noble metal particles are ultimate applicants for the study with UV-Visible spectroscopy, while they exhibit strong surface plasmon resonance (SPR) in the visible region; they are highly sensitive to the surface adaptation. The reduction of Ag⁺ ions was monitored by measuring the UV-VIS spectrum of the solution.

**Fourier Transform-Infrared Spectroscopy (Ft-IR) Analysis**

FT-IR analysis of AgNPs-EETA was carried out on a Shimadzu IR-prestige-21 (Shimadzu Corpn., Japan) in the diffuse reflectance mode operated at a resolution of 4 cm⁻¹ in the range of 400-4000 cm⁻¹ to evaluate the functional groups that might be involved in nanoparticles formation.

**X-Ray Diffraction (Xrd) Analysis**

The characterization of purified AgNPs-EETA was carried out by X-ray diffractometer (XRD-600, Shimadzu, Japan) for the crystallographic structural analysis. The nanocrystallite domain size was calculated from the width of the XRD peaks using Debye-Scherrer equation, assuming they are free from non-uniform strains.

\[
D=\frac{0.94\lambda}{\beta\cos\theta},
\]

Where D is the average crystalline domain size perpendicular to the reflecting planes, \(\lambda\) is the wavelength of X-ray, \(\beta\) is the full width at half maximum (FWHM) and \(\theta\) is the diffraction angle.
Scanning Electron Micriscopy (Sem) Analysis
SEM analysis was undertaken to know the size and shape of the AgNPs-EETA and it was examined using JOEL-JFC 6360 SEM. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra particles were removed using a blotting paper. Then the films were placed on the SEM grid and allowed to dry and the image of nanoparticles was taken.

Energy Dispersive X-Ray Spectroscopy (Edx) Analysis
EDX analysis was used for the confirmation of presence of elemental silver. X-ray spectrometer taken benefit of the photon nature of the light. In the X-ray range single photon produces energy; it is enough to produce a measurable pulse X-ray. For the analysis of spectrum, a semiconductor material was used to detect X-ray along with the processing electrons.

Dynamic Light Scattering (Dls) Analysis
The particle size range of the nanoparticles along with its polydispersity was determined using a particle size analyzer (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation). Particle size was arrived based on measuring the time dependent fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion.

Zeta Potential Analysis
The zeta potential of the synthesized nanoparticles was determined by means of zeta potential analyzer (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation, using Zeta plus software). The measurement of zeta potential is based on the direction and velocity of particles under the influence of known electric field.

RESULTS AND DISCUSSION
Visual Observation of Biosynthesized Silver Nanoparticles
Addition of ethanolic extract of *Toddalia asiatica* leaves into the aqueous solution of silver nitrate led to the change in colour of the solution from lightgreen to dark brown following 24 hours of incubation, which is shown in Figure1 a and b.
Figure 1: Synthesis of silver nanoparticles using ethanolic extract of *Toddalia asiatica* (L.) Lam. Leaves.

a- Ethanolic extract of *Toddalia asiatica* leaves

b - Silver nanoparticles of ethanolic extract of *Toddalia asiatica* (Colour changes after adding AgNO₃)

This change in colour might be owing to the occurrence of active molecules present in the ethanolic plant extract. The colour change was a clear evidence for the formation of silver nanoparticles. The change in colour was stable even after 24 hours of incubation and also there was no precipitation of the solution was observed. This suggested that the particles were well diffused in the solution, and there was no obvious agglomeration. This colour variation may be attributed to excitation of surface plasmon resonance vibration of silver nanoparticles.

Further, the formation of silver nanoparticles were appraised and confirmed by obtaining the relevant absorption spectra using UV-Visible spectroscopy and other techniques.

**Characterization of Silver Nanoparticles**

Characterization techniques help to resolve better synthesizing conditions for good quality of silver nanoparticles. Characterization of synthesized silver nanoparticles also provides information about accuracy and efficiency of methods used. Synthesized nanoparticles can be characterized by, UV-Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDX), Particle size analysis (PSA) and zeta potential techniques.
**Uv – Visible Spectroscopy Analysis**

The absorption maxima for silver nanoparticles using ethanolic extract of *Toddalia asiatica* leaves was obtained around 450nm which is shown in Figure 2.

![Figure 2: UV – Visible spectra of silver nanoparticles of ethanolic extract of Toddalia asiatica leaves.](image)

Similar results were also reported by Afrah E. Mohammed *et al.*, (2018), Absorption peaks of the mixture of AgNPs and plant extract showed silver surface plasmon resonance at 420, 425 nm, 430, 440 nm, and 430 nm for AgNPs prepared by aqueous and alcoholic extracts of phoenix dacctylifer, Acacia nilotica, and Ferula asafetida, respectively.

**Ft-Ir Analysis of Silver Nanoparticleces**

![Figure 3: FT-IR patterns of synthesized silver nanoparticles in the Ethanolic extract of Toddalia asiatica leaves.](image)
Table 1: Possible functional groups and compounds present in silver nanoparticles of Toddalia asiatica leaves.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Wave number (cm⁻¹)</th>
<th>Group</th>
<th>Possible compounds present</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3416</td>
<td>-OH, -NH, -C≡H</td>
<td>Alcohol, phenol, aldehydes, amides, amines and alkynes</td>
</tr>
<tr>
<td>2</td>
<td>2920</td>
<td>-OH, -CH, -CH₂, -CH₃</td>
<td>Hydroxyl groups of carboxylic acids, Aliphatic groups</td>
</tr>
<tr>
<td>3</td>
<td>1629</td>
<td>-C=O, C=C, -NH₂, C=N</td>
<td>Amides, amines, amino acids, unsaturated aliphatics, aromatics and unsaturated heterocycles</td>
</tr>
<tr>
<td>4</td>
<td>1046</td>
<td>-S=O, -NO₂, -CH₂, -CH₃</td>
<td>Sulfate, nitro compound, alkanes and alkenes</td>
</tr>
</tbody>
</table>

The strong band at 3416cm⁻¹ may result from the N-H stretching band of amino groups and indicative bond of –OH hydroxyl group and presence of -C≡H group attributed to alkynes. The well known band at 2920cm⁻¹ can be assigned as absorption bands of -OH of carboxylic acids and – CH-, -CH₂ and -CH₃ of aliphatic groups respectively. Band at 1629cm⁻¹ corresponds to C=O, C=C, -NH₂ and –C=N represents amides, amines, amino acids, unsaturated aliphatics, aromatics and unsaturated hydrocycles. Band around 1046 cm⁻¹ corresponds to –C=O, -C-N, Si-O, -PO represents primary amine, organo silicon and phosphorous compounds. Peak at 730 cm⁻¹ corresponds to –S, C-Cl, -CH and –NH stretch represents disulphide, alkenes, aromatic compounds and aliphatic amines. The band at 601cm⁻¹ corresponds to C-Br and C-Cl represents sulphide, C-Halogen and aromatic compounds.

Parthiban et al., (2018) characterized silver nanoparticles of Annona reticulate leaves using FT-IR analysis and the results exposed that the presence of polyphenols, carbonyls, flavonoids, aromatic compounds and proteins. These reports were similar to our results.

X – RAY DIFFRACTION ANALYSIS

Figure 4 shows numbers of Bragg reflections with the four diffraction peaks at 32.89°, 46.88°, 65.15°, 78.10° in the experimental diffractogram have been identified to be silver metal related to (111), (200),(220) and (311) planes of face centred cubic (fcc) structure of silver (JCPDS, file no. 04 – 0783). The appearance of this sharp peak could have occurred from crystallization of silver nanoparticles which might be due to the reducing and stabilizing agents present in EETA.
Figure 4: XRD pattern of silver nanoparticles from ethanolic extract of *Toddalia asiatica* leaves.

The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the **Debye-Scherer’s formula**:

\[
D = \frac{0.94 \lambda}{\beta \cos \theta}
\]

Where \( D \) is the average crystallite domain size perpendicular to the reflecting planes, 
\( \lambda \) is the X-ray wavelength, 
\( \beta \) is the full width at half maximum (FWHM) 
\( \theta \) is the diffraction angle

By using Debye-Scherrer’s equation, the size of the synthesized silver nanoparticles from *Toddalia asiatica* leaves extract was found to be in the range of 10-30nm and the average size of the particle was established to be 19.06nm.

Similar results were also reported by Shankar *et al.*, (2017) and the results indicate that the obtained width of the prominent peak was found to be 19 nm. These results were very closely conforming with our results. Thus the XRD pattern clearly indicates that the synthesized silver nanoparticles are crystalline in nature.

**Scanning Electron Microscopy (Sem) Analysis**

The SEM analysis of synthesized silver nanoparticles of ethanolic extract of *Toddalia asiatica* were visualized under various magnifications of x22,000, x33,000, x35,000 and x55,000. From the Figure5 it was noted that the particles were predominantly spherical in shape, well dispersed with close compact arrangement and the size of silver nanoparticles were ranged from 30-50nm.
Figure 5: Scanning electron micrographs of silver nanoparticles synthesized by ethanolic extract of silver nanoparticles of *Toddalia asiatica* leaves

Similar results were also reported by Khan *et al.*, (2018) The SEM images of *Coriandrum sativum* leaf extract exhibited that majority of the AgNPs are typically spherical in shape bearing smooth surface and well dispersed, and the average particle size was found to be around 6.45 nm.

ENERGY DISPERSIVE X – RAY SPECTROSCOPY (EDX)

Figure 6 shows a signal peak for silver (57.84%) at 3 keV, which confirm the formation of AgNPs due to the surface plasmon resonance of AgNPs, since leaves AgNPs always exhibit absorption peak at 3 keV. The spectrum also exhibits weak signal peak for magnesium (1.27%), chlorine (17.16%) and potassium (3.38%), which may be originating from the biomolecules present in the ethanolic extract of *Toddalia asiatica* that are capping the surface of AgNPs. The stronger peak indicates that the biosynthesized nanoparticles were tremendously made up of silver and its higher percentage of silver indicating the purity of the synthesized sample.
Figure 6: EDX Spectrum of elemental composition of silver nanoparticles from ethanolic extract of *Toddalia asiatica* (L.) Lam. leaves.

Khan *et al.*, (2018) carried out EDX analysis of silver nanoparticles of *Origanum vulgare* and the results indicated the intense sharp signal at 3 keV confirms the presence silver nanoparticles.

**Particle Size Analyzer (Psa)**

AgNPs size measured from the dynamic light scattering (DLS) intensity obeys Raleigh scattering and is proportional to the radius of the particle raised to the sixth power, then a small number of large particles can contribute in the increase of DLS size, resulting in recording an average size of 50 nm.

Figure 7: Particle size of elemental composition of silver nanoparticles from ethanolic extract of *Toddalia asiatica* (L.) Lam leaves.
Similar results were also reported by Shyam Perugu et al., (2018) Size distribution analysis by DLS. The particle size distribution revealed that the particles range from 10–100 nm. The average particle size was found to be 50 nm.

Zeta Potential Analysis

Zeta-potential varies by dissociation of surface groups in a wide range of electrolyte conditions. The zeta potential value for green AgNPs was -36 mV.

![Zeta Potential Analysis](image)

**Figure 8: Zeta potential analysis of silver nanoparticles from ethanolic extract of Toddalia asiatica leaves.**

Similar results were also reported by Afrah E. Mohammed et al., (2018) AgNPs prepared by aqueous and ethanolic extract of phoenix dactylifer showed a mean zeta potential of -35mV respectively.

It was found that the aqueous silver ions exposed to the ethanolic extract of Toddalia asiatica leaves were reduced and nanoparticles were synthesized. The change in the colour of the solution containing the AgNO$_3$ solution and ethanolic plant extract of Toddalia asiatica leaves is consistent with the formation of the silver nanoparticles. The UV-Visible spectroscopy established the formation of silver nanoparticles. The FT-IR spectra showed the possible functional groups and compounds present in silver nanoparticles. The XRD analysis showed various diffraction peaks and crystalline nature of the silver nanoparticles of ethanolic plant extract. SEM analysis showed that the particles are spherical in shape. EDX analysis of the silver nanoparticles showed the presence of elemental silver. PSA of silver nanoparticles showed the presence of average particle size found to be 50nm. Zeta potential
measurement of synthesized AgNPs potential value of -36 mV. This environment friendly method could be a reasonable substitute to the predictable physical and chemical methods used for synthesis of silver nanoparticles and thus promising approach to be used in biomedical and therapeutic applications.

CONCLUSION
Biosynthesis of Ag NPs using plant extract of T.asiatica was carried out for the first time in this study. This study confirmed T.asiatica capability for the biosynthesis of Ag NPs. The characteristics of the biosynthesized Ag NPs were measured by different equipments.

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REFERENCES

