

**Biosynthesis of Microbial Resistance Au-nanoparticles from Aqueous Extract of *Tridax procumbens* Leaves****K. Madhu Sudana Rao¹, E. Chandra Sekhar², K. S. V. Krishna Rao^{3*}**

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ABSTRACT

The study highlights the fabrication of gold nanoparticles (GNPs) by fresh leaves of *Tridax procumbens* (TP), an herbal medicinal plant. The bio-reduction of auric chloride ion (Au^{+3}) is quite rapid, readily to be performed at room temperature. This study describes a rapid and eco-friendly synthesis of TP-GNPs are be done using TP leaf extract in a single pot process and is observed when the medium turns into purple color with the addition of aqueous Au^{+3} . The formation of TP-GNPs is verified by ultraviolet-visible spectroscopy. The possible functional groups of TP leaf extract and their changes after treating with aqueous auric chloride are evaluated by Fourier transform infrared spectroscopy. The size of synthesized TP-GNPs is measured by dynamic light scattering analysis, and morphology is examined by transmission electron microscopy in nano range and energy dispersive X-ray spectrum confirming the presence of gold. The average size of TP-GNPs is ~7 nm and monodispersed. Further, we apply anti-bacterial activity on both *Escherichia coli* and *Bacillus subtilis*, and the results suggest that the TP-GNPs suspension exhibits anti-bacterial activity.

Key words: *Tridax procumbens* leaf extract, Gold nanoparticles, Microbial resistance, Bio-reduction.

1. INTRODUCTION

At present, there has been a progressive need to derive an environmentally exonerable nanoparticle (NPs) synthetic process that does not involve any toxic chemicals. Production and applications of inorganic NPs are in the areas such as optics, photo imaging, electronic devices, sensors, biosensors, catalysis, and as antibacterial [1-9]. These NPs have been produced chemically and physically for a long time; however, their biological invention has only been investigated recently. Synthesis of NPs using plant materials, for the most part, has only recently been intended within the past three decades, whereas the production of NPs using existing plants has only been studied in the past half-decade. The synthesis of metal NPs using biological materials has been exposed to produce NPs of the same shapes and sizes as those produced through chemical or physical methods. Currently, the noble metallic NPs and nanostructured materials are increasing interest in recent research, because of their interesting properties exhibit completely new and improved properties as compared to the larger particles of the bulk material

with specific characteristics, such as size distribution and morphology.

Recently, several papers have been published on plant leaf extracts with regard to gold nanoparticles (GNPs) [10-17]. In this study, we have developed TP-GNPs using *Tridax procumbens* leaf extract (TPLE) under mild conditions. TP is known for several potential therapeutic activities such as antiviral, antioxidant, antibiotic efficacies; wound healing activity, insecticidal, and anti-inflammatory activity [18]. Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding and as a hair tonic [19]. GNPs expose many biological applications dependent on their size, surface area, and charge of the metal ion [20-23]. These GNPs can enter cell membranes in a nonendocytotic approach, including fast and large uptake, without toxic effects to the cell if introduced at low concentration [24,25]. Compare to other leaf extracts, the TPLE extract itself acts as an anti-microbial resistance and TP-GNPs formed with controlling size and shape. The developed TP-GNPs

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suspension exhibits enhanced antimicrobial resistance compared to other synthesized NPs.

To date, there is no report of synthesis of TP-GNPs utilizing an aqueous leaf extracts of TP. In this experiment, TP leaf extract in a concentrated aqueous solution of Gold (III) chloride resulted in a reduction of silver ions (Au^{+3}) and formation of TP-GNPs. The schematic representation of biosynthesis of GNPs of GNPs from an aqueous extract of TP leaves is shown in Scheme 1.

2. MATERIALS AND METHODS

2.1. Materials

TP leaves were collected from our university campus (Sri Krishnadevaraya University, Anantapur, India) and Gold (III) chloride trihydrate received from Sigma-Aldrich Chemie Germany. Double distilled water (DDH_2O) and all the reagents were used without further purification.

2.2. Biosynthesis of GNPs (TP-GNPs)

Leaf extract is prepared by a green process technique, using the standard procedure described by Hariprasad *et al.* [26]. TP leaves are collected and washed with DDH_2O and kept at room temperature in a dark place for 12 h. From this, 20 g of leaves are chopped finely and boiled in 100 mL sterile DDH_2O for 5 min. The obtained extract is filtered and used for further experiments.

2.3. Characterization

Ultraviolet (UV)-visible absorbance spectroscopy: Bio-reduction of the TP-GNPs by auric chloride is monitored using a Series 3000 double beam UV-visible spectrophotometer (UV-Vis Spectrophotometer, Lab India, UV-3092). The UV-visible spectrophotometric readings are recorded at a scanning speed of 0.5 nm intervals and are scanned from 200 to 700 nm. Fourier

transforms infrared (FTIR) analysis: FTIR (Perkin Elmer Spectrum Two, UK) spectrophotometer is measured to TP-GNPs. Dynamic light scattering study (DLS): The zeta sized nano sequence performs size measurements using advancement called DLS spectroscopy measures Brownian motion relates this to the size of the particles. Mean diameter and size circulation of the TP-GNPs are determined by DLS method using a Brookhaven BI-9000 AT instrument (Brookhaven Instruments Corporation, USA). Transmission electron microscopy (TEM): Samples of bio-synthesized TP-GNPs are prepared by placing a drop of the colloidal solution on carbon-coated copper grids, allowing the films on the TEM grids to stand for 2 min removing the excess solution with blotting paper and letting the grid dry before measurement.

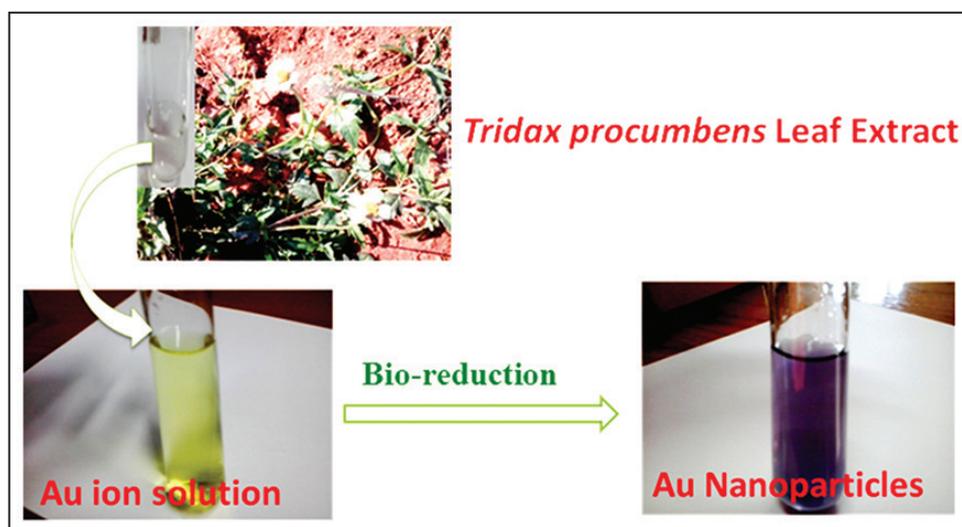
2.4. Antibacterial Activity

Anti-bacterial activity of TP-GNPs is performed by disc method of Bauer-Kirby [27]. Mueller Hinton Agar (M173) plates are prepared for the rapid growth of *Bacillus* (G^{+ve}) and *Escherichia coli* (G^{-ve}) bacteria for this study. 10 μL of each sample that is TP-GNPs solution, Gold (III) Chloride and the pure extract is impregnated onto filter paper discs of 5 mm diameter. The plates are incubated for 18 h, at 37°C in the incubation chamber. Diameter of the inhibition zone around the disc is measured. Anti-microbial activity can be resolute by comparing the zone diameter obtained with the known zone diameter size for susceptibility (Gentamycin).

3. RESULTS AND DISCUSSION

3.1. Visual Identification of GNPs

The comprehensive study on the biosynthesis of TP-GNPs is carried out by aqueous extract of TP leaves. Figure 1 shows no significant peak for the pure leaf extract of TP without the addition of HAuCl_4 , and Figure 1 exhibits the HAuCl_4 -treated leaf extract



Scheme 1: Schematic representation of biosynthesis of gold nanoparticles.

incubated at 2-72 h. In the leaf extract of TP, the TP-GNPs synthesis process is completed at 72 h. The color change from yellow to dark purple indicates the formation of TP-GNPs at 74 h of incubation time and confirms the reduction of HAuCl_4 into TP-GNPs using the aqueous extract. The color change is dependent on the incubation time and excitation of the free electrons of phytochemicals present in the aqueous extract. The colloidal solutions of TP-GNPs show a very intense color, which is absent in the bulk material as well as for individual atoms; it is due to surface plasmon resonances [17].

3.2. UV-vis Spectra of TP-GNPs

The fabrication and stabilization of the bio-synthesized TP-GNPs in the colloidal solution are monitored by UV-vis spectrophotometer analysis. TP-GNPs are known to exhibit at maximum in the range of 400-700 nm. Figure 1 shows that there is no significant peak for the TP-GNPs due to the absence of HAuCl_4 and strong characteristic absorption peak (λ_{max}) around 545 nm is noted for the TP-GNPs in the leaf extract, and it is due to the surface plasmon resonance effect. The synthesis of TP-GNPs is monitored at different time intervals such as 2, 6, 12, 24, 48, and 72 h which are clearly shown in Figure 1. Initially, at 2 h, the TP-GNPs are absorbed slowly, and the absorbance is gradually increased up to 72 h. After 72 h, there is no absorbance which indicated that the TP-GNPs synthesis process is completed. The reduction of Au^{+3} ions occurs fairly but the 72 h are found to be very stable in the colloidal suspension.

3.3. FTIR Spectroscopy

The earlier reports [18] show flavonoids and terpenoids are present in the TP leaf. FTIR measurements are carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of TP-GNPs synthesized by the plant

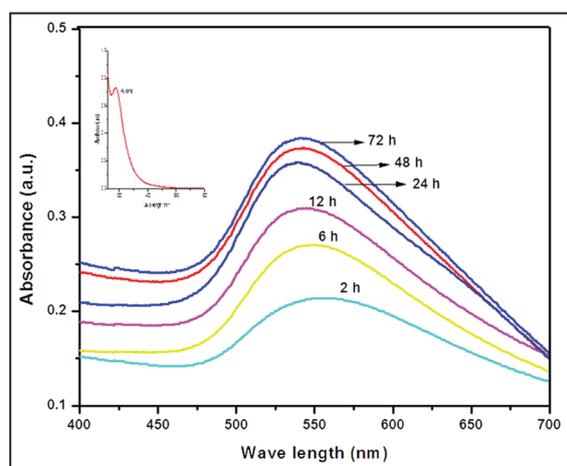


Figure 1: Ultraviolet-visible spectra of *Tridax procumbens* (TP)-gold nanoparticles obtained using leaves extract of TP.

extracts. Figure 2a shows the pure leaf extract of TP. Absorbance bands of TP leaf extract are observed at 3460 cm^{-1} assigned to O–H stretch, 1633 cm^{-1} and 1410 cm^{-1} are assigned to C=O and C–N amide stretching vibrations, respectively. The absorbance bands of TP are observed at 1117 cm^{-1} assigned to C–O alcohol stretch and 655.75 cm^{-1} assigned to C–H alkenes stretch. While in the case of TP-GNPs, these absorbance bands are slight shifts is observed, that is, 1633 is shifts to 1636 cm^{-1} , $1410\text{--}1402\text{ cm}^{-1}$ and $1117\text{--}1123\text{ cm}^{-1}$, respectively. These shifts are due to the rapid excitation of free electrons in the leaf extract of TP. Finally, the TP-GNPs are produced by hydroxyl groups of TP and the O–H peak disappears in Figure 2b.

3.4. DLS and TEM Analysis

The size of TP-GNPs is confirmed by DLS and TEM analysis which illustrates that the TP-GNPs are with size ranging of 7 nm (Figure 4a-d). The alignment analysis using EDAX shows the strong characteristic peaks of gold (Figure 4d). All the peaks except Cu may be attributed to the bio-molecules of TP leaf extract. This clearly shows that aqueous extract of diatom is very much capable of reducing gold ions in the medium and converting them into well-dispersed TP-GNPs.

3.5. Antimicrobial Resistance of TP-GNPs Colloidal Suspension

GNPs show efficient anti-bacterial property compared to other salts due to their tremendously large surface area, which provides better contact with microorganisms [28]. All the tested materials are possessed by variable anti-bacterial activity which is against G^{+ve} bacteria (*Bacillus subtilis*), and G^{-ve} bacteria (*E. coli*) are against Gentamycin as a positive control. Pure TP leaf extract has less inhibition (Figure 5) when compared to gold chloride and

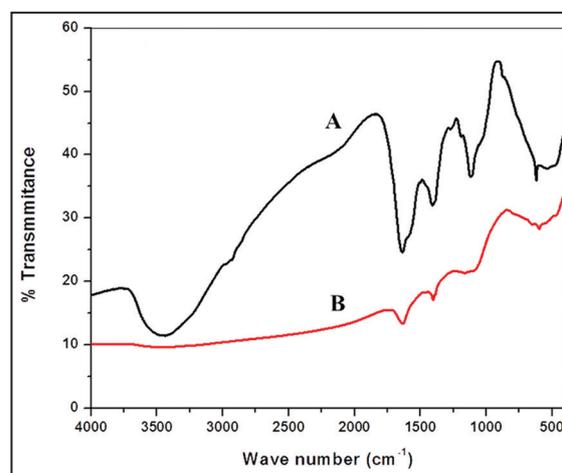


Figure 2: Fourier transforms infrared spectra of (a) *Tridax procumbens* (TP) leaf extract and (b) TP-gold nanoparticles.

TP-GNPs and all the zone inhibition measurements are placed in Table 1. This clearly indicates that

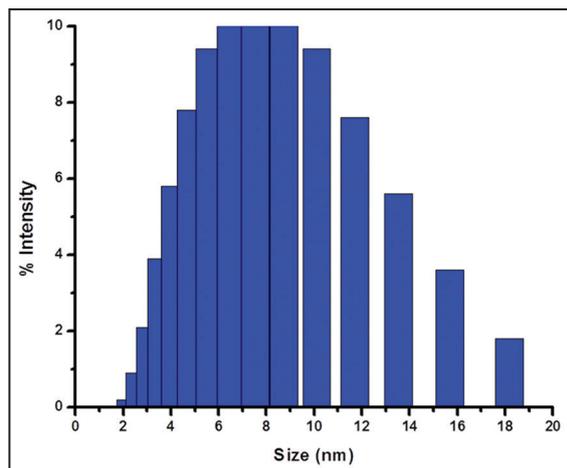


Figure 3: Particle size determination by dynamic light scattering.

the anti-bacterial activity is increased when GNPs are associated with tested bacteria [29]. The exact mechanism for the antibacterial activity of GNPs is not known till now, but theoretically many studies report that the GNPs could bind to the bacterial membrane, occupy the cell and cause appetite of proton motive force which leads to the distraction of bacterial cell by forming pores on the bacterial cell wall [30].

4. CONCLUSION

TP-GNPs synthesized by the green approach have been reported in this study using TP leaf extract. TP leaf extract could act not only reduce the Au⁺³ ions but also to control the size i.e. ~7 nm. The accepted method is well suited with green chemistry principles as the plant extract serves as a dual functional molecule as reductants and a stabilizing agent for the synthesis of TP-GNPs. Further, the TP-GNPs formation is confirmed by UV-visible, FTIR, DLS, and TEM-energy dispersive X-ray spectrum studies.

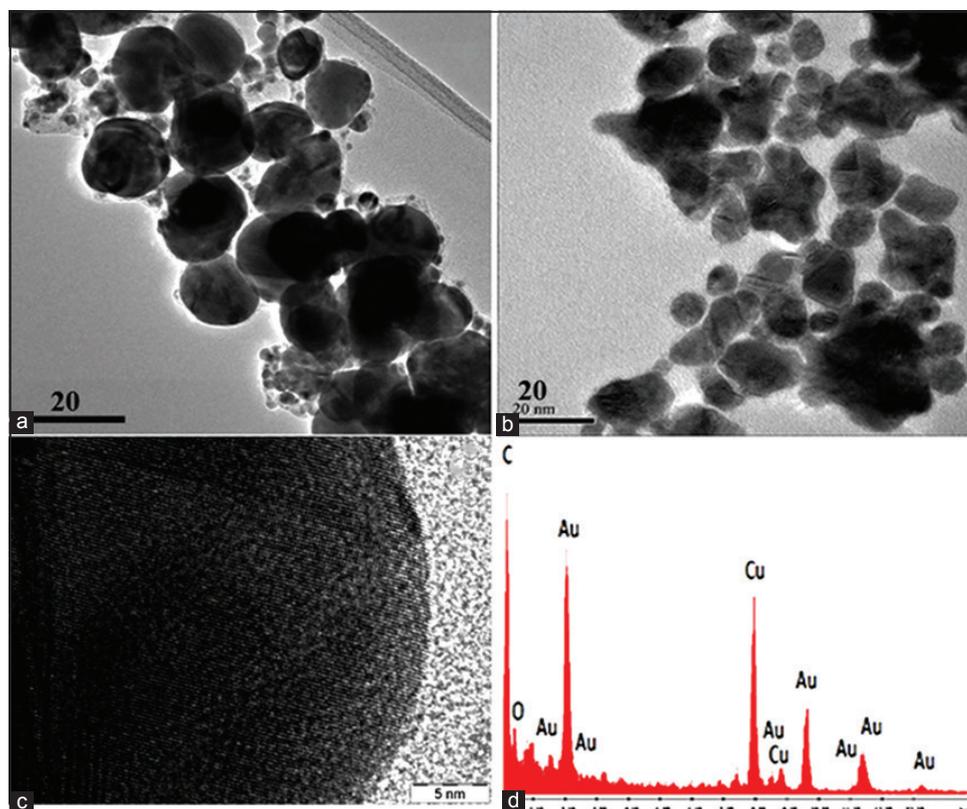


Figure 4: (a-d) Transmission electron microscopy micrograph of the *Tridax procumbens*-gold nanoparticles, (d) Energy dispersive X-ray spectrum confirming the presence of gold.

Table 1: Results of anti-bacterial activity of TP leaf extract, aqueous gold chloride solution, and TP-GNPs.

S. No.	Tested material (10 μ L)	<i>E. coli</i> (ATCC 25922) (mm)	<i>Bacillus</i> (ATCC 6633) (mm)
1	TPLE	12	13
2	HAuCl ₄	16	15
3	TP-GNPs	17	16

TPLE: *Tridax procumbens* leaf extract, TP: *Tridax procumbens*, GNPs: Gold nanoparticles, *E. coli*: *Escherichia coli*

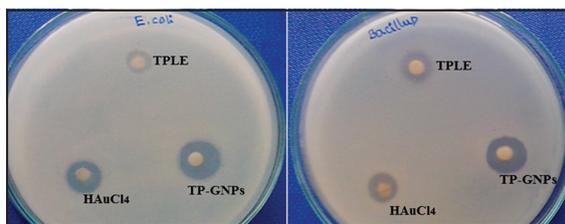


Figure 5: Anti-bacterial activity of *Tridax procumbens* (TP) leaf extract, Gold (III) chloride and TP-gold nanoparticles against *Escherichia coli* and *Bacillus subtilis* pathogenic strains.

The antibacterial activity of TP-GNPs suspension shows the enhancement of the activity toward *E. coli* and *Bacillus*. As a result, it is observed that a fine tuning process of variables may give the end product with typical physical characteristics. In future, we will also study the anti-cancer and anti-HIV activity of TP-GNPs suspension.

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