Greener Synthesis of Silver Nanoparticles usingPlant Extract Tribulusterrestris and their Antibacterial and Antioxidant Activities

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Abstract: In current years, the sustainable approach of synthesizing silver nanoparticles (AgNPs) has gained much interest amongresearchers. The flora of the Indian field is rich with a variety of medicinal plants thathavethe potential to include various sources of effective, cost-efficient, non-toxic, environmentally safe reducing and stabilizing compounds that can be utilized in synthesizing in AgNPs. Here, in this study, the author investigates an efficient and sustainable route of AgNP preparation from 1 mM aqueous $AgNO_3$ using leaf extracts of *Tribulusterrestris* well enriched for their vast availability and therapeutic property against Urinary tract infections (UTIs) samples. The successful preparation of AgNPswas monitored using UV-Visanalysis at time-basedintervals, and the synthesized nanoparticles were characterized through FT-IR and TEM analysis.The shape of the nanoparticles was found to be spherical, with the particle size ranging from 20-90 nm. However, the particle was agglomeratedas per the data received from TEM. The synthesized nanoparticlesexhibited antibacterial activity against clinically isolatedUTI caused microorganisms.In addition, AgNPs nanoparticleswere also assessed for antioxidant activity, and they were found to be excellent DPPH radicalscavengersin a concentration-dependent manner.

Keywords: Nanoparticle, Antioxidant activity, DPPH assay, Plant extract.

1. Introduction

Silver nanoparticles among all have gained significant interest due to their unique antimicrobial activity, bactericidal activity,anti-inflammatory, and anti-angiogenesis properties[1-3].AgNPswere reported to showminimal toxicity to humans; however, they exhibited higher toxicity against a varietyof microorganisms [4-6].The AgNPswere found to be excellent antimicrobial activity; because of this application,AgNPshave high scope in medical

instruments, biocompatible products such as scaffolds, burn implants, and wounddressing, and in other fields such as water purification columns and agriculture too. Regarding the synthesis of AgNPs, it can be synthesized using various methodologies, for example,physical process, i.e.,ions sputtering,laser ablation, condensation, and evaporation sol-gel. Another method to synthesizesilver nanoparticles is the chemical process, i.e., chemical reduction, which includes hydrazine hydrate, sodium borohydride, greener protocols, etc. [7].Unfortunately, using these expensive and toxic chemicals and harsh and high-temperature conditions leads the researcher todevelop analternative sustainable method of preparing.The source of generation also cannot be neglected.Hence, it is important to emphasize an alternate synthetic route that is cost-effective and environmentally friendly in parallel. The techniques for obtaining nanoparticles using naturally occurring reagents such as plant extracts, sugars, biodegradable polymers,and especially chitosanused as either reductants or capping agents can be effective, simple, and simple reproducible waysto provide more stablenanoparticles[10-12]. Microorganisms can also be utilized to synthesize the silver nanoparticles, but the synthesis rate of synthesiswas quite slowcompared to plant-mediated routes[13]. However, plants as raw materials for the synthesis of silver nanoparticles are still unexplored and need to be studied in detail.

Herein this article,we report the synthesis of silver nanoparticles using the *Tribulusterrestris*leaf extract without using any harmful solvent. The nanoparticle was characterized using UV-Vis, TEM, and FT-IR spectroscopy. The authorhas demonstratedthe potential of the leaf extract-mediated nanoparticle for antimicrobial and antioxidant activities.The synthesized could strive for the possibility of approachingthe edge-level utilities in every aspect of the fieldof medical area.

2. Materials and Methods

2.1. Preparation of plant extract

Tribulusterrestris leaf extract was used to prepare Ag nanoparticles. The preparation method of silver nanoparticle with the leaf extract is as follows The Fresh leaves were collected from Cheyyarin themonth of February and authenticated by Dr. Jayaraman, Father of Taxonomists Tambaram. The plant leaves were cleaned with normal water in order to remove the dust particlesand dried organic matter. In order to make them usable, the plant leaf was cleaned up

withdouble distilled water and dried at room temperature for a duration of 24 h. The leaf was cut into pieces. 20 g of finely cut leaves were kept in a 200 mL beaker, and the distilled water was added to the beaker. The leaf was kept boiling for a duration of 30 min. After 30 min, the extract was cooled at room temperature, filtered with Whatmann filter paper,and stored at four °C.

2.2.Synthesis of Ag nanoparticles

80mL of 1mM of $AgNO_3$ solution and 20mL of plant extract were added together in 20 mL of the round bottom flask. The reaction mixture was kept in a rotator shaker fora duration of 3 days. After three days, the color of the reaction mixture changes from pale yellow to dark brown. Once the dark brown color was achieved, the reaction was stopped. The progression of the reaction was also monitored using a UV-Vis spectrophotometer.After completion of the reaction, the unreacted AgNPs from the supernatant were separated by centrifuge at 8000 rpm for 10 min. After centrifugation, the pellets were collected and washed, coupled with distilled water to remove the unreacted reactants, and kept for drying overnight. Later the sample was characterized using a UV-Vis spectrophotometer, TEM, FT-IR, etc.

2.3.Method for the isolation and identification of organisms from UTI samples.

Several biochemical tests were conducted to confirm the species of five microorganisms. The cultural characteristics and biochemical test results are shown in **Table 1**.

Table 1. Biochemical test of different microorganisms

ORGANISMS	GRAM STAINING	MOTILITY	CAPSULE STAINING	Spore staining
Escherichia coli	Gram-negative rod	+	-	-
Staphylococcus aureus	Gram-positive cocci	-	-	-
Klebsiellasp	Gram-negative rod	-	+	-
Enterococcus sp	Gram-positive cocci	-	-	-
Proteus sp	Gram-negative rod	+	+	-

2.4.Characterization of synthesized Ag NPs

UV-Vis spectral analysis was done by using a Shimadzu UV-visible spectrophotometer (UV-1800, Japan). UV-Visible absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One 1 mLof the samplewas taken into the test tube using the micropipette, and subsequently, absorbance was monitored at room temperature using UV-Vis spectroscopy. FT-IR spectra were recorded on Perkin Elmer 1750 FTIR Spectrophotometer. The particle size and surface morphology were analyzed using Transmission electron microscopy (TEM), operated at an accelerated voltage of 120 kV.

2.5.Assessment of antimicrobial assay

The antibacterial assays were done on UTI-isolated pathogens such as *E.coli*,*Pseudomonasaureginosa*, *Klebsiella*, and *Proteus vulgari* using the standard well diffusion method. The selective medium (HiMedia) was used in order to subculture bacteria.Mueller–Hinton agar (MHA) were used to study the antibacterial activity of nanoparticle.The sample cultures wereinoculated and incubated at 37^0 C for 24 h. For all the experiments, freshly prepared cultures (overnight) were taken and spread on the MHA plates to cultivate bacteria. The culture was divided into four wells, and the four wells were cut using gel puncture. The first three wells were added to the sample, and the fourth well was added to the antibiotic. After this, the plates were incubated at 37ºC fora duration of 24 hrs. After incubation, the zones were measured, the readings were tabulated, and the results were mentioned in the result and discussion section.

2.6. Antioxidant Test

0.5 µL of DPPH (80 µM) was added into 29.5 mL methanol in 100 mL of the conical flask. The solution was mixed well and kept in the dark before use. Freshly prepared silver nanoparticle (1 mg/mL) was taken in distilled water and ultra-sonicated for 30 min in order to distribute uniformly. Silver nanoparticles were taken at various concentrations from 10µg/ml to 50µg/ml for antioxidant activity. The volume of the solution was maintained at 1 mL, and all the samples were incubated in a dark place for 30 minduration. The UV-Vis absorbance was recorded after 30 min to assess the DPPH scavenging activity of silver nanoparticles in a concentration-

dependent manner. The percentage of DPPH scavenging activity of AgNPs was calculated by the formula mentioned below.

$$\% \text{ Inhibition} = [(\text{Absorbance of control} - \text{Absorbance of sample})/\text{absorbance of control}] \times 100.$$

The IC_{50}value of the DPPH scavenging activity by AgNPs was calculated, and the graph was plotted and discussed in detail in the result and discussion section.

3. Results and Discussion.

AgNPs were synthesized by a green approach using$AgNO_3$ and *T. terrestris*leaf extract as a starting precursor. The detailed experimental procedure is mentioned in the experimental section.It is really important to note that the formation of Ag nanoparticles can also be monitored using a UV-VIS spectrophotometer. The reaction colour changes from dark brown toyellow. This observation gives us an indication of the successful synthesis of silver nanoparticles. Figure 1 mentions the synthetic scheme of the preparation of nanoparticles using the *TribulusTerrestris*and $AgNO_3$.

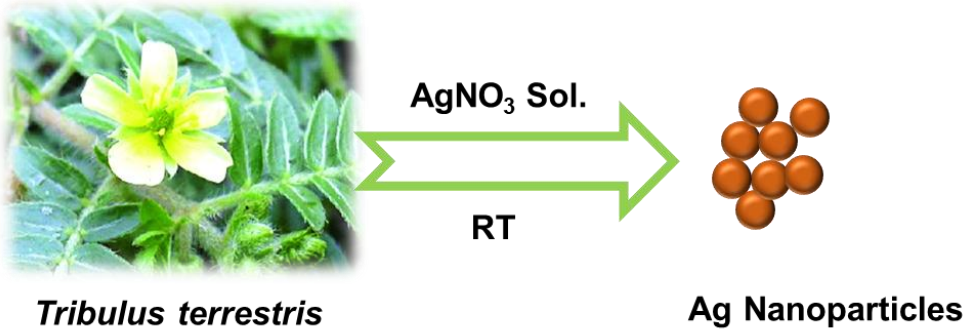

Fig. 1. Representative scheme of synthesis of Ag nanoparticles from *TribulusTerrestris* leaf extract using $AgNO_3$ solution.

The reaction was completed in 25 h as the UV-Vis spectra show the absorbance maximums at 440 nm. Figure 2a shows the graph of UV-Vis spectroscopy. This gave us the conformation of the formation of silver nanoparticles. So, a peak at 440 nm is the characteristic peak ofAgNPs [14].The absorbance spectra ofcontrol leaf extract at starting point (1h)showeda peak at 280nmwhich isthe characteristic peak of the presence of bioactive compounds in the leaf extract. After completion of the reaction, the unreacted $AgNO_3$ solutionwas separated from our final product by centrifuge at 8000 rpm for 10 min.Furthermore, the formation of AgNPs was

againconfirmed by the FTIRspectrum shown in Figure 2b. The IR spectrum of AgNPs at 1625, 1090, 610cm^{-1}correspondsto the -N-H stretch, carbonylstretch (-C-O-C-or -C-O- stretch vibrations of amide linkages),and C=O stretching vibrations, respectively.Furthermore, the peak at 1390 cm^{-1} was due to theCN- aromatic amino groups present in the control sample. The presence of organic functionalities confirms the leaf extract coatingcoated on the surface of the nanoparticles.Furthermore, the shape of synthesized AgNPs were observed through TEM analysis, as shown in Figure 2c.TEM analysis shows that the nanoparticle wasspherical and found in agglomeration form. The avg. size ranges from 20-70nm.The agglomeration may be due to the presence of unreacted leaf extracts.

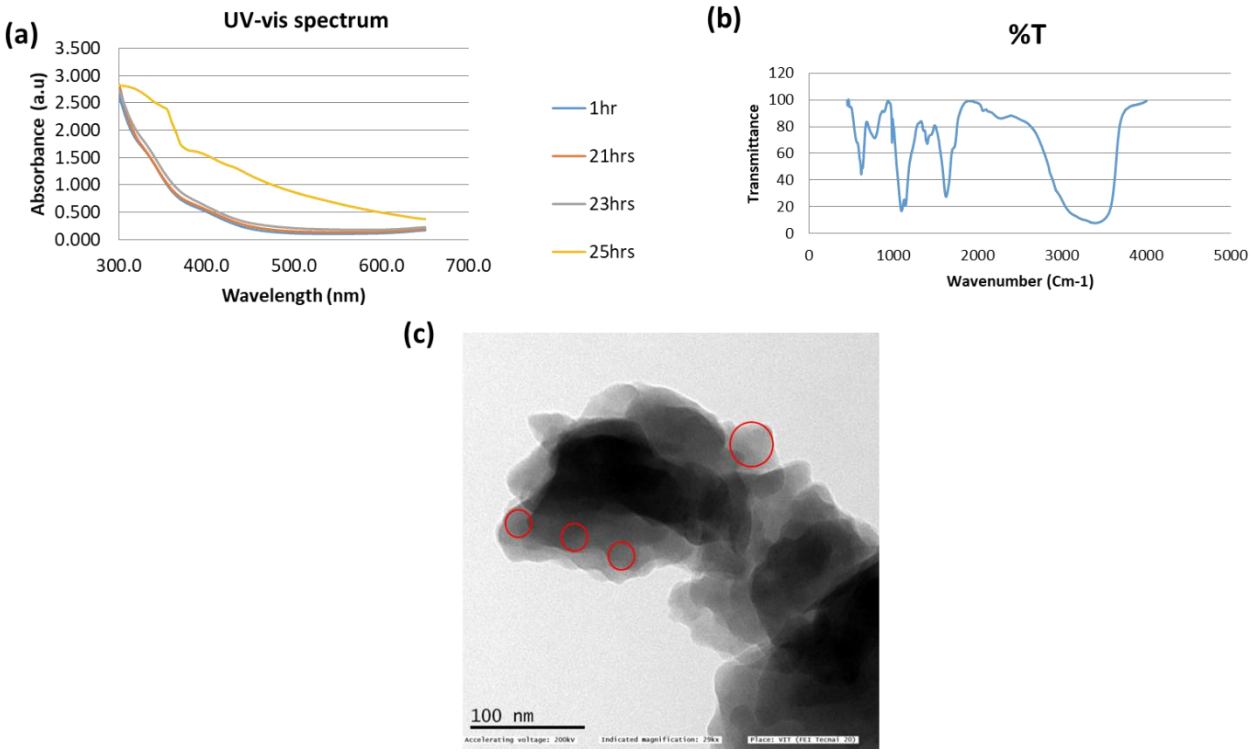

Fig. 2.(a) Monitoring of the synthesis of Ag NPs using UV-Vis Analysis,(b) FTIR spectra of Ag NPs, and (c) TEM image of synthesized Ag nanoparticles.

4. Antimicrobial activity: TheAgNPs nanoparticles were assessed for antimicrobial activity.SynthesizedAgNPsexhibitedsignificant antibacterial activity against Gram-negative bacteria (*E. coli*, *Klebsiellasp*and *Proteus sp*) and Gram-positive bacteria (*S. aureus*,*Enterococcus sp*) via zone inhibition assay as shown in table 2. The Gram -vebacteria*E. coli*, *Klebsiellasp*and *Proteus sp*showed a maximumzone of inhibition compared to the Gram-

positive bacteria. The reason for this can be due to the thinner peptidoglycanlayer that easily allows for the penetrationof silvernanoparticles. The Ag^+ ions released from AgNPs, whichprobably act as reservoirs for thebacterial cells,lead to the structural deformationof bacteria and result in cell death.

Table2.Antimicrobial activity of synthesized AgNPs:Zone of inhibition against the test microorganisms

Organisms	**Concentration**			**Antibiotics (Control)**
	50µL	**100 µL**	**150µL**	
Staph.sp	20 mm	23 mm	26 mm	10 mm
E. coli	20 mm	26 mm	29 mm	8 mm
Enterococcus sp	18 mm	23 mm	25 mm	13 mm
Klebsiella sp.	25 mm	28 mm	30 mm	25 mm
Proteus sp	23 mm	26 mm	32 mm	28 mm

5. Antioxidant activity

We also assessed the antioxidant activity of the AgNPs by free radical scavenging assays, including 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) chemical.A detailed procedure is mentioned in the experimental section.The activity was checked in UV-Vis spectrophotometer at a wavelength of 517 nm [15]. Various concentrations of silver nanoparticles were exposed to the DPPH radical in the dark for 30 min. The activity was compared using a control without the addition of a nanoparticle named a black sample.Ag nanoparticles have shown excellent radical scavenger activity in a concentration-dependent manner. The color of the DPPH solution was changed from deep violet to nearly transparent in the presence of Ag NPs. The absorbance of DPPH at 517 nm gradually decreases with increases of AgNPsgives, further confirming the free radical scavenging ability of AgNPs[16].The percentage scavenging of silver nanoparticles was calculated, and data is shown in Figure 3. 50 µg/mL concentration of Ag NPs showed 34% of DPPH radical scavenging activity.

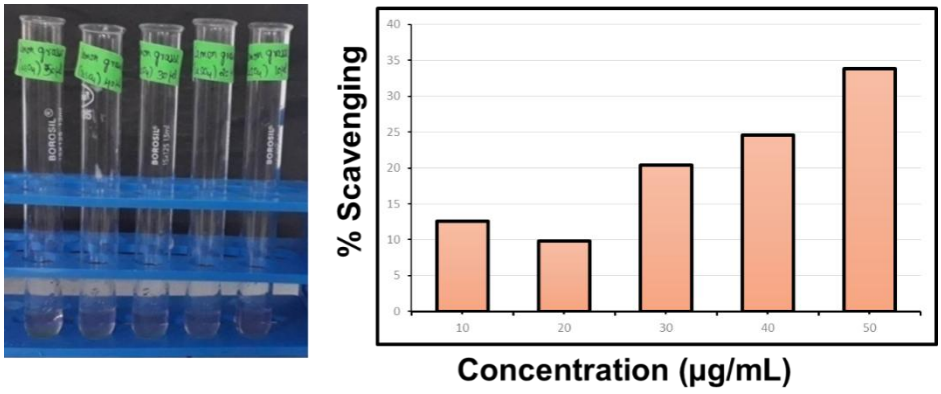

Fig. 3. Antioxidant activity of AgNPs: Free radical scavenging against DPPH radicals.

6. Conclusion

Here in this article, we have successfully synthesized the silver nanoparticle using the *Tribulusterrestris*plant leave and $AgNO_3$ precursor. This synthetic route is greener, cost effective, and more efficient as it excludes external stabilizers/reducing agents. Since the plant extract was used in this synthesis, making it more environmentally safe and friendly. This can also be called a simple one-pot green synthesis of AgNPsusing*Tribulusterrestris*leaf extract at room temperature. The synthesis of nanoparticles was confirmed using UV-Vis, TEM, and IR spectrums.TEMhas shown that the NPs in spherical in shape with little agglomeration. IR showed that the nanoparticles were capped with the organic moiety found in the leaf.UV-VIS also showed the formation of silver nanoparticles. The synthesizedAgNPsalso were being assessed for antimicrobial and antioxidant activity. They have shown efficient antimicrobial activities against both gram +ve and gram -vemicroorganisms.Similarly, the synthesized AgNPsalso has shown efficient radical scavenging capability against DPPH radical. This suggests that this greener protocol could be an alternative to the conventional physical/chemical methods that can be used to synthesize AgNPs, and the nanoparticle was also dual effective for antimicrobial and antioxidant activity.

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data availability statements
The datasets generated during and/or analyzed during the current study are available from the the corresponding author on reasonable request.

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