GREEN SYNTHESIS OF SILVER NANOPARTICLES SYNTHESIZED FROM *TERMINALIA BELLERICA* AND DETECTION OF ANTIBACTERIAL ACTIVITY

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ABSTRACT

In this study, Terminaliabellirica extract is used as a reducing and stabilizing agent during the green production of AgNPs. +Ve and-Vebacteriawas tested for resistance to the produced AgNPs' antibacterial activities. Antibacterial activity was performed against the tested bacterial strains. The study contends that a viable strategy for the creation of novel antibacterial substances for numerous applications is the green synthesis of AgNPsutilizingterminaliabellirica extract. The synthesis method is an economical and sustainable replacement for conventional methods due to its eco-friendliness. In our research study, we found that silver nano particles of terminaliaberllirica showed antibacterial activity against S.mutantsandL.acidophilus.These silver nanoparticleshave significant role to develop drugs against various antimicrobial drugs

Keywords: Terminalia; Silver nano particles; Antibacterial activity; Drug discovery

INTRODUCTION

Nanoscience is a young scientific field having many applications in the fields of electronics, materials, and biomedicine. Many metal nanoparticles (NPs) made in a variety of ways have been widely reported for use in the aforementioned domains. Metal nanoparticles have many benefits, but it is crucial to comprehend the properties of various transition metals and how they interact with biological systems. One of the most active subfields of nanoscience at the moment is the synthesis of nanomaterial. Nanomaterials that enhance human quality of life have received particular attention. The silver nanoparticles (AgNPs), which are recognized for their bactericidal and inhibitory effects, are a notable example. By manipulating shape and size at the nanoscale, nanotechnology is used to create, represent, manipulate, and apply structures.Nanotechnology is the alteration of matter by various physical or chemical techniques to create chemicals with specific purposes. It can alternatively be said to be any microscopic-sized particle with at least one dimension significantly less than 400 nanometers. In view of significant surface to volume ratio and quantity of surface atoms, nanoparticles (NPs) have many useful uses and multi-functional qualities in a variety of sectors, including nutrition, energy, and medicine.

Several researchers have contributed significantly to the domain of nanotechnology. By utilizing the latest technology, silver nano synthesis, gold nano synthesis have been performed by various researchers (Samberg et al. 2010, Sarsar et al. 2013, Tran and Le 2013; Chou and Ren 2000; Sahin et al., 2017; Karikalan et al., 2016; Ghorbanpour,etal., 2018).Throughout, there is a great deal of interest in the use of nano-sized selenium as food additives, particularly for people who are deficient in selenium, as well as a vital therapeutic agent with few side effects in medicine(El-Ramady et al., 2014, Liu et al., 2018, Singh et al., 2019).Liu et al.,(2018) reported the use of Ocimumtenuiflorum leaf extract to produce spherical-shaped SeNPs with a size range of 15-20 nm.Yet, the plant-based nanoparticles offer potential antioxidant functional groups that have higher antioxidant profiles and greater stability, controlled release, and biocompatibility(Khalil et al., 2020).As opposed to their macro-sized counterparts, metal particles with a nanometer-sized size are increasingly found to have radically distinct physical, chemical, and biological characteristics. Metallic nanoparticles possess features that are of relevance to scientists for various uses, according to a recent flurry of studies on nanoparticles (Vilchis-Nestor et al., 2008). Aspergillusfumigatus, Fusariumsolani, Aspergillusflavus, and Aspergillusniger were all treated with fruit extract from Hyphaenethebaica (L.) Mart. CeO2 NPs at a concentration of 2 mg/mL, are found to be effective at inhibiting enzyme catalytic sites and causing the denaturation of enzymes, which stopped translation, protein assembly, and protein folding and induced chitin oxidation (Mohamed et al., 2020).Nanotechnology shouldn't be seen as a singular strategy with a small number of applications (Veisi et al. 2016; Rasheed et al. 2019; Pakzad et al. 2020). Testing for grittiness, pH, rheological properties, emulsion, and stability are among the physicochemical characteristics used to evaluate creams containing silver nanoparticles(Galatage et al., 2020). Cell apoptosis is ultimately caused by ROS-mediated biotoxicity. Until far, the main toxicity mechanism for nanoparticle-based cancer therapy has been explained as ROS production caused by oxidative stress. In order to create zinc, iron, and copper oxide nanoparticles, which are effective biological and pharmacological agents to combat various infections, researchers employed the aqueous extract of Terminaliabellerica fruits (Pithawala and Jain 2021, Akhter et al., 2019).The green synthesis of nanomaterial has received a great deal of interest ,since it offers simple, inexpensive, easily saleable, environmentally acceptable products with little harm to humans (Singh et al., 2015).In countries like Nepal, India, and the United States, the plant Terminaliachebula, which belongs to the Combretaceae family, is used to cure a variety of human illnesses (including fever, TB, indigestion, cardiac and hepatic diseases, and gastrointestinal disorders).In Sri Lanka,China,Vietnamand Malaysia, plant leaves, bark, fruits and roothasfound to be numerous medicinal properties such as antimicrobial, anti-cancerous, antitussive, antispasmodic, antiasthmatic and antihelminthic (Gilani et al., 2008, Ravishankaraet al., 2019; Singh and Kumar, 2013).

In our research study, we synthesized ecofriendly silver Nano particles using silver nitrate solution and detection of antibacterial activity of *terminaliabellerica*Nanoparticles.

MATERIALS AND METHODS

In our research study, we used various chemical AgNO3 to synthesis the silver nano particles,fromRathanaScientificSolutions, Chennai, Tamilnadu. Mac Conkey agar and amoxicillin,*In our research study we used Streptococcus mutans* and *Lactobacillus acidophilus* pure isolates were obtainedfrom Himedia Laboratories.

METHODOLOGY

Purification of biosynthesized silver nanoparticles

The TerminaliaBellerica (Fig. 1) seed kernel-derived manufactured nanoparticles had any extra silver ions removed. After centrifuging the silver colloids at 10,000 rpm for 15 minutes, they were washed three times with distilled water. For further characterisation, a dried powder of silver nanoparticles was produced by freeze drying in a lyophilizer.

Preparation of Stock solution

Silver nanoparticles derived from Terminaliabellerica have been produced. Stock solutions (1 mg/mL) have been dissolved in DMSO and serially diluted to the appropriate concentrations (10, 20, 40, 80, and 100 g/mL) in complete growth medium (Fig. 2). Only the vehicle (DMSO 1%) has been applied to untreated control culture samples.

Fig. 1 ***TerminaliaBellerica*** **Fig. 2 AgNPs synthesis**

Bacterial strains and media

Himedia Labs provided the pure isolates of Lactobacillus acidophilus and Streptococcus mutans that were used in this study. Using MacConkey Agar plates, each sample was grown twice to ensure purity. To establish the identity of the working strains, Gram staining and colony morphology were used (Figs. 3 and 4).

Antimicrobial susceptibility testing

Preparation of impregnated discs

AgNps (PGE) impregnated in sterilised 6 mm blank discs with 20, 40, and 60 l of the substance. As a substitute for mouthwash, distilled water-loaded discs were utilised as a negative control. Prior to being applied to bacteria, all impregnated discs were made sure to be properly dried in a 45°C incubator for 18 to 24 hours. Amoxicillin (10 g) is the common antibiotic disc used as positive controls for all strains.

After the discs had fully dried, they were placed on the inoculated Mac Conkey agar and impregnated with AgNps (PGE) using sterile forceps. To establish consistent contact with the agar surface, the discs were lightly pushed. The plates were then turned over and kept incubating for 24 hours at 37°C. For the purpose of evaluating the antibacterial activity, the diameter of the inhibition zone either around the treated discs or surrounding the control discs was measured.

Their diameters were calculated to the nearest whole millimetre with a ruler to determine whether inhibition was present. To ensure accuracy and dependability, each test was run three times. The average of the three replicates for each volume of AgNps(PGE) and antibiotic was then calculated (Fig. 5).

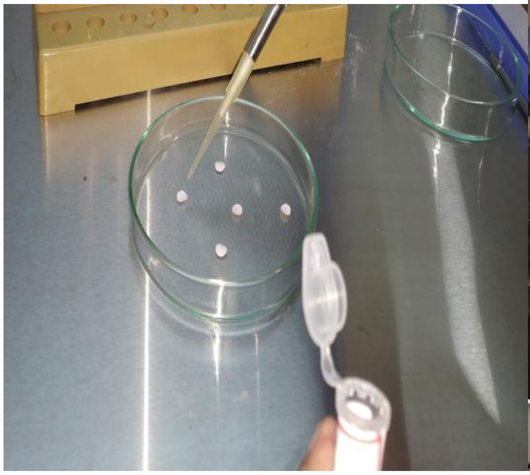
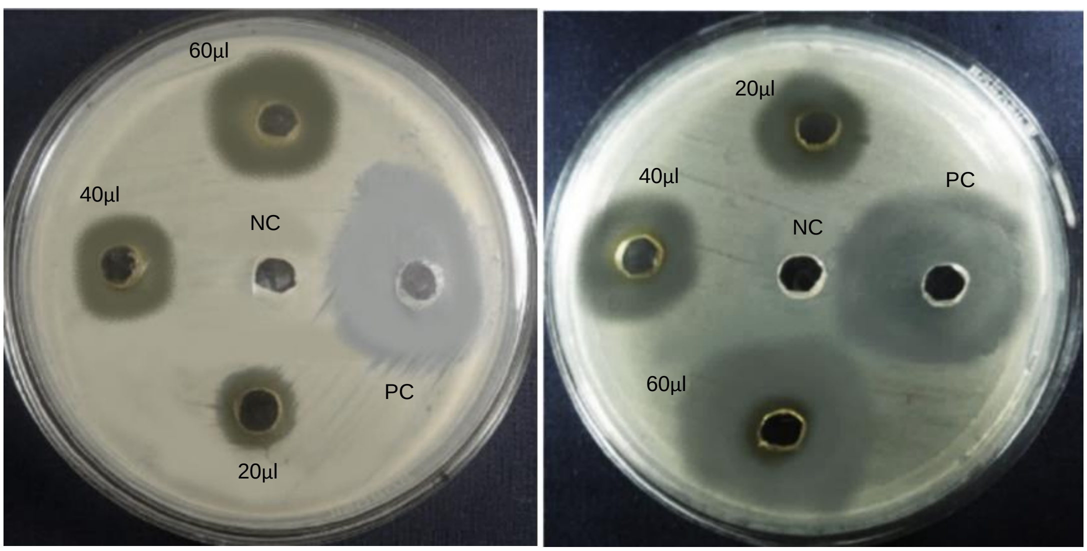


Fig. 3 Plates under incubation **Fig. 4 Addition of AgNps(PGE) to the disc**



(a) S. mutans **(b) L.acidophilus**

Fig. 5 Zone of inhibition

Statistics

To contrast the results of the data's statistical analysis, one way analysis of variance (ANOVA) was used. The threshold for statistical significance was P 0.05. SPSS was used to examine the data (version 11).

Table 1 Antibacterial activity of AgNps(PGE)

Conc (µg/ml)	**Zone of Inhibition (mm)**	
AgNps(PGE)	*S. mutans*	*L. acidophilus*
20 (µl)	7.3 ± 0.01*	8.05 ± 0.54*
40 (µl)	10.5 ± 0.99**	12.56 ± 1.1**
60 ((µl)	12.1 ± 1.1***	15.5 ± 1.3***
Positive control	16.4 ± 1.2***	18.0 ± 1.0***
Negative control	NI	NI

NI means no inhibition zone. Each value is expressed as mean ± SD (n = 3). *p<0.01 and ***p<0.001 statistically significant as compared with negative control.

RESULTS AND DISCUSSION;

Samples was collected and prepared to perform the silvernanoparticles synthesis.Silver nitrate solutionwas used to synthesis the silver nanoparticles. After synthesis of this silver nitrate solution, performed the antibacterial activity using this plant based silver nano particles.ANOVA was used to evaluate the significance of antibacterial activity. Statistical significance was accepted at a level of P < 0.05. Data were analyzed using SPSS (version 11).

Wu Yiling(2021) exploredthe possible use of generated CeO2-NPs for the treatment of SCI. Also, when green produced CeO2-NPs were tested on paediatricleukaemia (CCRF-SB) cells, it was found that they increased cytotoxicity, indicating their potential as a future therapeutic for the treatment of paediatric malignancy. On the other hand, the present work also emphasises CeO2-NPs' potential influence for nanotechnology-based SCI treatment.Yixia Zhang etal., (2010) cytotoxicity is not evidently present, it is reported. Even at very low concentrations, AgNPs@AV hybrids exhibit superior antibacterial action against E. coli when compared to Vera gel and Ag NPs (washed from Veragel) alone.When nanoparticles are created utilising environmentally friendly methods, they demonstrate potent photocatalytic activity against methylene blue when exposed to sunlight and have use in both catalysis and antibacterial treatment (Sharma etal.,2021).

Prashant, (2021)addressed a wide variety of research on silver nanoparticles to better understand their synthesis, modes of action, characterization of physicochemical properties, and applications. The distinct physicochemical characteristics of these nanoparticles are influenced by a number of AgNP variables, including size, surfactant, and structural form. AgNPs can be produced in a number of methods, but green synthesis employs safe chemicals and natural agents to produce a high yield that is biocompatible. In that article, they discussed the environmentally friendly production of silver nanoparticles using Thujaorientalis and Aloe vera leaf extracts, as well as the procedure to assess their antibacterial and antifungal activities (Ngoan, etal., 2022). Maarij(2022) provided a thorough discussion of some of these important mechanisms, such as DNA fragmentation, disruption of the electron transport chain, degradation of the chromosomal assembly, mitochondrial damage, inhibition of ATP synthase activity, inhibition of enzyme catalytic sites, disruption and lipid peroxidation of the cell membrane, and inhibition of numerous cellular pathways. The potential uses of GS-CeONPs in the medical industry to enhance and improve medical precision, as well as to successfully inform illness diagnostic and treatment approaches were also discussed. Shaheen Husain (2023) addressed the modern expansion of AgNP synthesis, characterization, and mechanism, as well as global applications of AgNPs and their limitations.Bindhu etal., (2020) discussed the acquired antibacterial and optical properties and the application of generated AgNPs in water purification.Muhammad Ikram et al., (2021)gives current details on the mechanistic methods used by SeNPs to carry out their assigned jobs, which may aid in the development of precision medicine and the creation of individualized healthcare for the sick population.Hira Munir etal., (2021) described the plant-mediated green synthesis of nanoparticles. Furthermore, various plant parts that have 30 been widely used for the biosynthesis of these NPs with a variety of sizes and shapes via biological 2 31 techniques are briefly described. In conclusion, it is mention that the safer and 32 more practical greener synthesis techniques enabled the bulk production of nanostructured particles. Muhammad Rafique (2017), processed the green synthesis for Ag-NPs along with a selection of the materials' diverse uses. Also, it compares environmentally friendly techniques of synthesis to physical and chemical ones that are equally effective. This information is important for choosing the best approach to create Ag-NPs.

EderleyVélez etal.,(2018) described the procedures for separating and recovering AgNPs from Hg. Nada E. Eisa (2020) investigated the possible health advantages of titanium nanoparticles produced through green synthesis (TiO2NPs). Lupin bean extract and titanium(IV) isopropoxide were used to create TiO2NPs. By using dynamic light scattering, the manufactured particles were analysed to determine their average particle size

Conclusion:

Silver nanoparticles synthesized from Terminaliabellirica are a promising area of research due to the unique properties of the plant extract and the potential applications of the synthesized nanoparticles. Terminaliabellirica is a medicinal plant that contains several bioactive compounds such as tannins, flavonoids, and phenolic compounds that can act as reducing and stabilizing agents in the synthesis of silver nanoparticles.The synthesized silver nanoparticles have demonstrated significant antibacterial activity against a range of bacteria, including Gram-positive and Gram-negative bacteria. This antibacterial activity is attributed to the small size of the nanoparticles, which enables them to penetrate bacterial cell walls and membranes, leading to cell death. The antibacterial activity of silver nanoparticles synthesized from Terminaliabellirica extract has the potential for various applications in medicine, particularly in the development of novel antimicrobial agents for the treatment of infectious diseases. Additionally, the use of plant extracts in silver nanoparticle synthesis offers an eco-friendly and sustainable approach for the development of nanoparticles with diverse applications.Overall, the synthesis of silver nanoparticles from Terminaliabellirica extract and their antibacterial activity represent a promising area of research that offers numerous opportunities for further investigation and potential applications in various fields.

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