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Biogenic synthesis and antibacterial activity of silver nanoparticles using an extract of *Tinospora cordifolia*.

Kokane Rutuja Sharad*[1], Dr. Rajesh Khathuriya[2]
*[1] Department of pharmacognosy, Pacific Academy of Higher Education & Research University, Udaipur, Rajasthan, India.
[2]Department of pharmacognosy, Pacific Academy of Higher Education & Research University, Udaipur, Rajasthan, India.

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ABSTRACT:
Silver nanoparticles (AgNPs) have gained considerable attention in biomedical research due to their remarkable antimicrobial properties and wide pharmaceutical applications. The present study focuses on the green synthesis of silver nanoparticles using polyherbal extracts of *Tinospora cordifolia* and *Ficus religiosa*. Plant extracts act as natural reducing and stabilizing agents, offering an eco-friendly and cost-effective alternative to conventional chemical synthesis methods. The synthesis of silver nanoparticles was confirmed by the visual colour change of the reaction mixture, indicating the reduction of silver ions. The synthesized nanoparticles were further characterized using suitable analytical techniques to determine their physicochemical properties. The antibacterial activity of the prepared formulation was evaluated against selected pathogenic bacteria using the agar well diffusion method. The results demonstrated significant antibacterial activity of the synthesized silver nanoparticles against both Gram-positive and Gram-negative bacteria. The enhanced antimicrobial effect may be attributed to the small particle size and increased surface area of nanoparticles, which facilitate better interaction with microbial cell membranes. This study highlights the potential of polyherbal-mediated silver nanoparticles as promising antibacterial agents for pharmaceutical and biomedical applications

INTRODUCTION:
Nanotechnology Research and use of materials at a small scale, usually between 1 and 100 nanometres. The prefix ‘nano’ refers to one-billionth of a unit, a meter. At this size, materials often behave differently compared to their larger forms due to quantum-level effects.

This branch of science seeks to harness these unusual properties to design new and improved materials, tools, and systems. Scientists employ advanced techniques, such as scanning probe microscopy and molecular self-assembly, to control the arrangement of atoms and molecules at the nanoscale.

Because of these techniques, researchers have created nanomaterials with properties that are superior to traditional materials. These may include greater strength, better conductivity, or enhanced chemical reactivity. Well-known examples include graphene, carbon nanotubes, and various nanoparticles.

Nanotechnology has especially promising uses in the field of medicine. Through nanomedicine, Nanotechnology is also used in medical imaging and diagnostics.

In the area of clean water, nanomaterials such as nanotubes and nanoparticles are integrated into advanced purification systems, effectively removing pollutants and ensuring safe drinking water.[4]

unknowingly utilized nanoparticles by grinding materials into fine powders to produce vibrant pigments with unique optical effects. Though they lacked scientific understanding of the nanoscale, they were still applying its benefits. [5]
Researchers began investigating their use in drug delivery systems, discovering that nanoparticles could transport medicines more precisely to targeted locations in the body. This improved Therapy effectiveness while reducing side effects.[6]

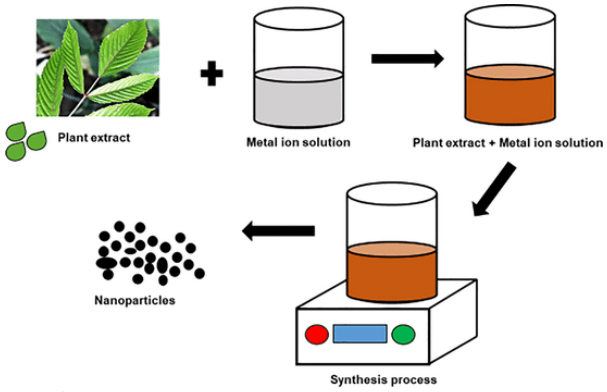

Fig.1: Diagrammatic Representation of Green Nanoparticle Formation

Tinospora cordifolia (Guduchi) is a fast-growing, drought-resistant climbing shrub that can be cultivated with relative ease across various regions of India. Due to its high medicinal value, its cultivation has gained interest among the herbal and pharmaceutical industries.

Guduchi is considered one of the most important herbs in Ayurveda, often referred to as “Amrita”, meaning *divine nectar* due to its rejuvenating properties. It is classified as a Rasayana – promoting longevity, vitality, and overall health. According to Hindu mythology, *Guduchi* is believed to have originated from drops of Amrit (the elixir) spilled during the churning of the ocean (Samudra Manthan), endowing it with divine and immortal qualities.

Tinospora cordifolia belongs to the Menispermaceae family. This plant has high effectiveness in treating a variety of health conditions, including malaria, cough, digestive problems, colitis, diabetes, and even cancer. Traditionally, it has been used to manage ailments like jaundice, rheumatism, inflammation, bone fractures, and scabies. It is helpful to reduce excessive thirst, improve appetite, strengthen the immune system, and eliminate internal body heat.

From a phytochemical perspective, *Tinospora crispa* contains numerous bioactive compounds. One key constituent, berberine, has shown strong antibacterial activity against *Helicobacter pylori* and several other harmful bacteria. The plant also contains various notable phytochemicals, among them N-cis-feruloyl tyramine, secoisolariciresinol, methyl 3,4-dihydroxybenzoate, and apigenin. The isolation of these compounds has led to studies confirming their important antibacterial qualities.

Phytochemicals have many biological and medicinal activities, as documented. Pharmacists are intrigued by these substances due to their medicinal efficacy and low toxicity. The therapeutic potential of secondary metabolites in *Tinospora cordifolia* was examined and documented, showcasing the phytochemical

characteristics. Terpenoids, along with gums and mucilages, were identified in the aqueous leaf extract but were absent in the methanolic extract. Conversely, proteins, leucoanthocyanins, and glycosides were observed in the methanolic extract, whereas these constituents were absent in the aqueous extract. Additionally, coumarins and phlobaphenes were identified as potentially present in the plant.

MATERIALS AND METHODS:

2.1 Materials:

All chemicals used were of analytical grade and were not subjected to further purification before use. Silver nitrate ($AgNO_3$) was procured from the mine chem laboratory, Mumbai. Leaves of *Tinospora cordifolia* were collected from Bori Kh, Pune, Maharashtra, for the preparation of the extract. Gram-positive (S. aureus) and Gram-negative (E. coli) bacterial strains were obtained from Sharadchandra Pawar College of Pharmacy, otur, Pune. The aqueous phase was prepared using Milli-Q water.

2.2 Preparation of *Tinospora cordifolia* leaf extract:

The stems of *Tinospora cordifolia* were dried for 7–10 days under shade and subsequently pulverized using an electric grinder. The powder drug was extracted with an Alcohol like methanol–acetone solvent system (70:30 v/v) using a Soxhlet apparatus. Extraction was performed in four successive cycles, each with 4000 mL of solvent, maintained at 35–40 °C for 16 hr. To obtain a dry, concentrated sample, the extract was subjected to vacuum rotary evaporation.

2.3 Biogenic synthesis of silver nanoparticles (AgNPs):

Silver nitrate ($AgNO_3$) (molar mass- 169.87 g/mol) was prepared in different concentrations. This concentration of $AgNO_3$ was liquified in 100 mL of distilled water. The synthesis of silver nanoparticles, designated as AgNPs, was performed using a bio reduction method with *Tinospora cordifolia* extract as the reducing and capping agent. A reaction medium was prepared by introducing 10 mL of the extract into 90 mL of a 1 mM aqueous silver nitrate solution. The mixture was heated to 80 °C and subjected to continuous stirring for three hours. The bio reduction of Ag+ ions to metallic Ag atoms was observed as the solution underwent a notable color shift from pale yellow to deep, dark brown. The nanoparticles were then harvested by centrifugation at 15,000 ×g for 20 minutes. This washing and centrifugation cycle was repeated two additional times to ensure the complete removal of any unbound silver ions. The purified nanoparticles were subsequently freeze-dried and stored at 4 °C for preservation.

2.4 Characterization technique

The synthesized silver nanoparticles (AgNPs) underwent rigorous characterization using a combination of microscopic and spectroscopic instrumentation. UV–Visible spectroscopy (SHIMADZU, UV1800) was the primary method for confirming the SPR band and assessing optical properties over the 200–800 nm spectrum. The associated functional groups were mapped using FT-IR spectroscopy (BRUCKER OPTICS, ALPHA-T). For reliable size metrics, including the Polydispersity Index (PDI), Dynamic Light Scattering (DLS) (Horiba, SZ100) was utilized. The crystalline phase was thoroughly studied using X-ray diffraction (XRD) across the 2θ range of 15∘° –80 °. Morphological verification of the nanoparticles' shape and size distribution was carried out by employing both Scanning Electron Microscopy (SEM) and High-Resolution Transmission Electron Microscopy (HRTEM).

Fig 2: Schematic representation of the biosynthesis of *Tinospora cordifolia* capped $AgNO_3$

2.5 Bacterial assay of AgNPs:

The antibacterial efficacy of the synthesized formulation was determined using the agar well diffusion method. After preparing and sterilizing nutrient agar, it was poured into Petri plates and allowed to solidify under aseptic conditions. The agar surface was then inoculated with *Staphylococcus aureus* and *Escherichia coli*. Wells were bored into the medium, and the test samples were introduced. The plates were subsequently incubated at 37 °C

for 48 hours. The resulting antibacterial activity was quantified by measuring the diameter of the zones of inhibition with an antibiotic zone scale.

RESULT AND DISCUSSION:

XRD structural analysis:

To characterize the silver nanoparticles (AgNPs), XRD analysis was performed, yielding data on the material's phase purity.

In the XRD patterns of silver nanoparticles of *Tinospora cordifolia*, it was observed that there is a reduction in the peak intensity in the XRD pattern of the formulation. This diminished peak suggests the conversion of the drug into an amorphous form It was observed that there is a reduction in the peak intensity in the XRD pattern. studies showed a diminished peak, which suggests the conversion of crystalline drug into amorphous form. This marked reduction in peak intensities explains the significant increase in the dissolution rates by formulation.

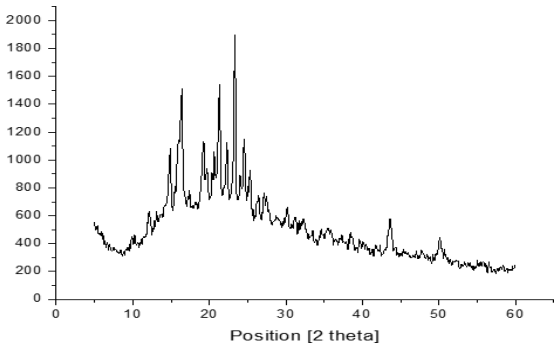

Fig 3: XRD analysis of *T.cordifolia*

3.2 SEM and TEM microscopic analysis

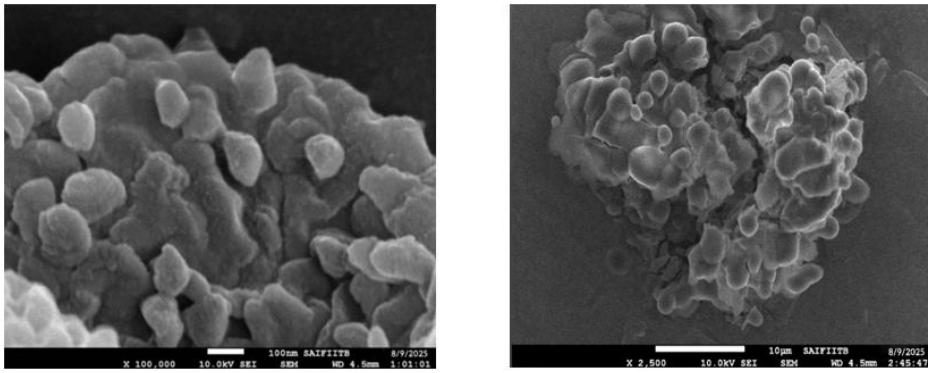

Fig 4: SEM analysis

The surface morphologies of samples A, B were analyzed using SEM Images. While Sample A exhibited poor structural quality due to extreme and dense agglomeration, Sample B had better morphologies. Their structures were defined by macroscopic aggregates composed of distinct, nanosized silver nanoparticles.

The morphologies were further investigated by TEM image analysis.
Based on TEM imaging, the synthesized AgNPs were found to be spherical, with sizes predominantly distributed between 10 and 18 nm. This size range yields an average diameter of 14 nm for the nanoparticles.

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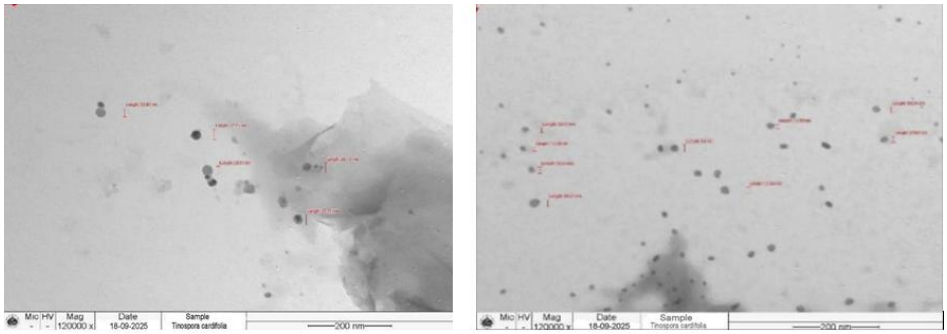

Fig 5 : TEM analysis of *T.cordifolia*

3.3 Formulation:

Solvent evaporation was applied to create nine different formulations of transdermal patches, each with varying quantities of PVA, chitosan, PEG & drug. 10 ml of hot water was used to dissolve PVA after it was accurately weighed. The several formulations were supplemented with chitosan in 1.5% acetic acid, and everything was thoroughly mixed. Drop by drop, maleic anhydride solution was added. The mixture was then thoroughly stirred for thirty minutes. PEG is used as a plasticizer. pour the sample into Petri plates. Cover the Petri plate with an inverted funnel to avoid evaporation. dry the patches for 24 hrs and store them in desiccators.

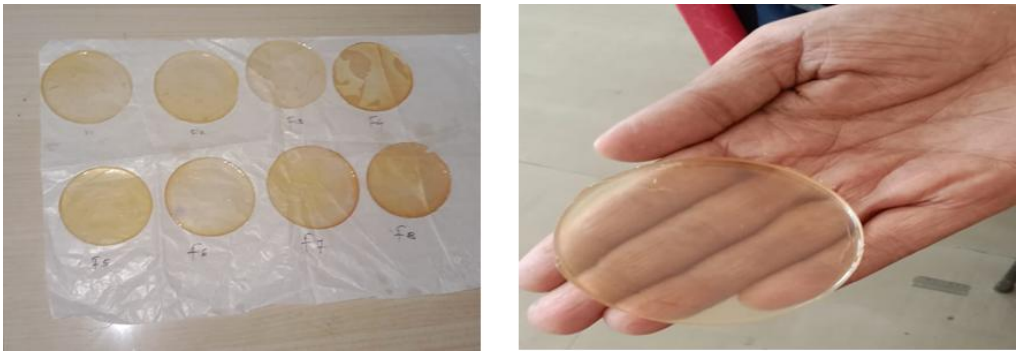

Fig 6: Patch preaparation

3.4 In vitro Antibacterial study:

The antibacterial efficacy of the synthesized formulation was determined using the agar well diffusion method. After preparing and sterilizing nutrient agar, it was poured into Petri plates and allowed to solidify under aseptic conditions. The agar surface was then inoculated with *Staphylococcus aureus* and *Escherichia coli*. Wells were bored into the medium, and the test samples were introduced. The plates were subsequently incubated at 37 °C for 48 hours. The resulting antibacterial activity was quantified by measuring the diameter of the zones of inhibition with an antibiotic zone scale.

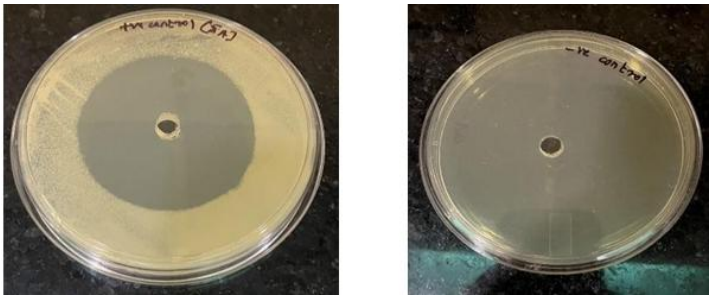

Fig 7: In vitro antibacterial activity of *Tinospora cordifolia*

Table 1: MIC of *Tinospora cordifolia*

	Conc. No.	Concentration (µl/10ml)	Zone of Inhibition (MIC)	
			S. aureus(mm)	E.Coli (mm)
Sample Name (*Tinospora cordifolia*)	S1	400	9.5	8.7
	S2	600	12.4	10.3

CONCLUSION:

The present study confirmed the successful green synthesis of silver nanoparticles (AgNPs) using Tinospora cordifolia extract as a reducing and stabilizing agent. Characterization studies (UV–Vis, FT-IR, XRD, SEM, and TEM) verified the formation of stable, spherical nanoparticles with an average size of about 14 nm.

The synthesized AgNPs showed significant, concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Incorporation into transdermal patches further demonstrated their potential for biomedical applications.

Overall, the study highlights that Tinospora cordifolia-mediated AgNPs are eco-friendly, effective, and promising for antimicrobial drug delivery systems.

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