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GREEN SYNTHESIS OF SILVER NANOPARTICLE BY USING *TINOSPORA CORDIFOLIA* LEAF EXTRACT AND ITS ANTIMICROBIAL PROPERTY

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Silver nanoparticles, Antimicrobial, *Tinospora cordifolia*, SEM, Green synthesis.

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ABSTRACT: *Tinospora cordifolia* is one of the best medicinal plants holds most prominent place in Ayurveda Rasayana herbs and it also known as "rejuvenate herbs" because of its medicinal properties. It has been found to exhibit anti-microbial properties, its anti-microbial feature found in root, stem, and leaf extracts on pathogenic micro-organisms. Green synthesis of AgNPs can potentially eliminate chemical agents' problems that may have adverse effects, thus making nanoparticles more compatible with the eco-friendly approach. The present study focuses on the green synthesize of silver nanoparticle, which was conducted by *Tinospora cordifolia* leaf extract. Characterization of silver nanoparticle was performed by Scanning Electron Microscopy. Silver nanoparticles exhibit strong anti-microbial effect against gram-positive and gram-negative bacteria such as *Escherichia coli, Pseudomonas syringae, Staphylococcus aureus and Enterococcus faecalis.* Both methanolic and ethanolic extract showed good antimicrobial activity against all the bacteria whereas methanolic extract had wide range of anti-microbial activity than ethanolic extract.

INTRODUCTION: In the field of nano-technology, nanoparticles are the basic essential elements that exhibit advanced characteristics based on size, morphology, and other size-dependent properties. Silver nanoparticles are actively involved in the medical sciences due to their antimicrobial actions on pathogenic bacteria [1]. Population rise, in-adequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs, and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [2]. Developing antibiotic resistance is a big matter of concern. The enormous and irresponsible use of antibiotics, has contributed significantly to the advent of the resistant strains [3]. The biological silver nanoparticle synthesis methods using plants were reported to be clean, nontoxic, cost-effective, and environmentally acceptable compared to nanoparticle synthesis [4]. Plants have been used for medicinal purposes long before prehistoric period. Every part of the medicinal plants such as leaves, stem, root, fruits, foliage, extracts, decoctions, infusions, powders is used in the treatment of different diseases of humans and animals [5].

Leaves of different plants such as *Azadirachta indica, Oscimum tenuiflorum, Ficus benghalensis, Tinospora cordifolia etc,* have been used for the synthesis of silver nanoparticles [6]. The healing properties of these medicinal plants and herbs were well accepted by our ancestors and nowadays also being scientifically proven as well. Phytochemicals are chemical substances or bioactive compound present in medicinal plants produce a definite physiological action on the human body and it has

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protective or disease preventive property [7]. Phytochemicals are grouped as alkaloids, flavonoids, tannin, saponin, sterol, *etc*. These phytochemicals are responsible for the anti-microbial effects of the plant extract *in-vitro* [8]. *Tinospora cordifolia* is one of the best medicinal plants belongs to Menispermaceae family, it is known to produce diverse classes of pharma-cologically compound, it has been used to treat number of diseases like jaundice, anemia, skin disease, allergic condition, diabetes, inflammation, urinary disorder [9]. *Tinospora cordifolia* holds most prominent place in Ayurveda Rasayana herbs and it also known as "rejuvenate herbs" because of its medicinal properties [10].

Tinospora cordifolia has been found to exhibit anti-microbial property, its anti-microbial feature found in root, stem, and leaf extracts on pathogenic micro-organisms [11]. Green synthesis of AgNPs can potentially eliminate the problem of chemical agents that may have adverse effects, thus making nanoparticles more compatible with the eco-friendly approach. Moreover, the synthesized AgNPs enhance the therapeutic efficacy and strengthen the medicinal values of *T. cordifolia* [12]. The present communication reports the synthesis of silver nanoparticle and scientific evaluation of medicinal efficacy of *Tinospora cordifolia* as antibacterial agent and to examine the morphology of *Tinospora cordifolia* by SEM analysis.

MATERIALS AND METHOD:

Preparation of *Tinospora cordifolia* Leaf Extract and Biosynthesis of Silver Nanoparticles for Scanning Electron Microscopy (SEM) Analysis:

Preparation of Leaf Extract: 12.5 gram of *Tinospora cordifolia* leaf powder was weighed and mixed with double distilled water in a clean conical flask, kept in a water bath at 60 ºC for 5 min, heated the extract on low flame by using wire guaze for 20 minutes. The extract was filtered by using Whatman filter paper. After the extraction, filtrate was taken out and stored at 4 ºC for further use [13].

Synthesis of Silver Nitrate Nanoparticle: 1 mM (0.0169 g) of silver nitrate was weighed and dissolved in 100 ml of distilled water; obtained solution was transferred to an amber colored bottle to prevent antioxidation of silver.

Preparation of *Tinospora cordifolia* Leaf Extract with Silver Nitrate Solution: 40 ml of silver nitrate solution and 100 ml of *Tinospora cordifolia* leaf extract was taken in a clean conical flask, stirred the solution for half an hour using magnetic stirrer till the color changes from yellow to brown and heated in water bath for 20 min at 80º till the color changes to dark brown. Change of color indicates the formation of silver nanoparticle [14].

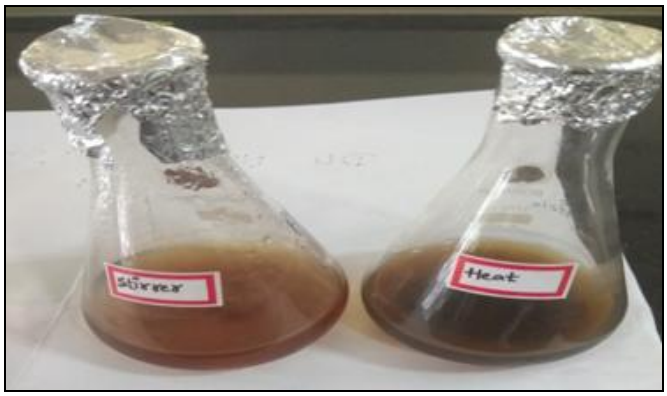


FIG. 1: GREEN SYNTHESIS OF SILVER NANO-PARTICLE

Antimicrobial Test of Silver Nanoparticle against *Pseudomonas syringae:* We have carried out well diffusion method to identify the antimicrobial activity of *Tinospora cordifolia* against *Pseudomonas syringae*. 2 mg/ml of methanol, ethanol, silver nitrate, silver nitrate + *Tinospora cordifolia* leaf extract, and Streptomycin was used as a reference.

SEM Analysis of Silver Nanoparticles: SEM analysis was done by using Zeiss EVO 18-EDX special edition machine compatible with EDX machine. Silver nanoparticles were centrifuged at 10,000 rpm for 30 min and the pellet was re-dispersed in 10 ml ethanol and washed 3 times with sterile distilled water to obtain pellet. Pellet was dried in the oven, and thin films of dried samples (2 mg/ml) were prepared on a carbon-coated copper grid and analyzed for size determination. The particle size and texture of nanoparticles can be analyzed by using image magnification software compatible with SEM and helps in determining the presence and formation of silver nanoparticles.

Antimicrobial Test of *Tinospora cordifolia* Leaf Extract: Antimicrobial activity of methanol and ethanol extract of *Tinospora cordifolia* leaf was carried out by agar well diffusion method [15].

100 µl of 10^{-4} diluted test microorganisms were spread on agar plates. Wells of 6 mm diameter were punched into the agar and filled with 5 µl, 10 µl, 15 µl of plant extract of 50 µg/ml, 100 µg/ml, 150 µg/ml concentrations respectively and 15 µl of ampicillin at 10 µg/ml as a reference standard.

RESULTS: The result of antimicrobial test was found that methanolic extract of *Tinospora cordifolia* was most effective against all bacteria. Maximum antimicrobial activity was found against *S. aureus* (9.6 mm), and the lowest activity was detected against *E. faecalis* (2 mm).

Antimicrobial effect of *Tinospora cordifolia* is due to the presence of secondary metabolites and a variety of active compounds present in the plant.

TABLE 1: ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *TINOSPORA CORDIFOLIA* LEAF EXTRACT

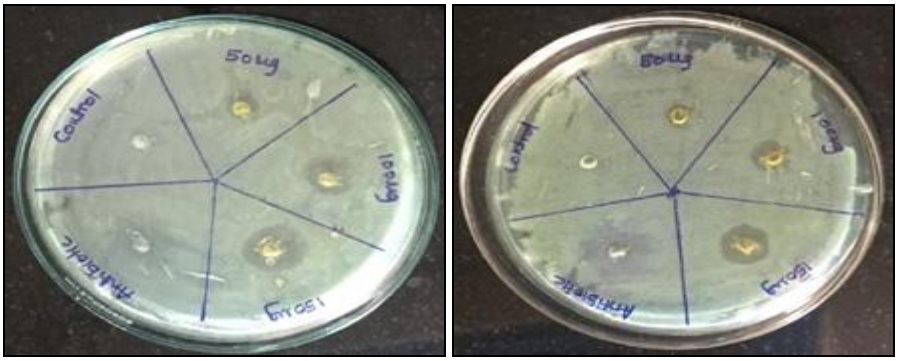
Bacteria	Concentration (µg)	Methanol Extract	Ethanolic Extract	Drug
		Zone of Inhibition (mm)		
	50	3	3	
E. coli	100	5	4	
	150	6	6	12
E. faecalis	50	1.2	Nil	
	100	2	Nil	
	150	6.6	Nil	12
S. aureus	50	3.6	1.2	
	100	4.3	3	
	150	7.6	5	12.6

TABLE 2: ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLE WITH PLANT EXTRACT

Bacteria	Test Compound	Zone of Inhibition (mm)
P. syringae	Methanol extract	Nil
	Silver nitrate + extract	12
	Drug	15
	Ethanol extract	Nil
	Silver nitrate + extract	12
	Drug	13

E. faecalis ***E. coli*** ***S. aureus***

FIG. 2: ZONE OF INHIBITION FROM METHANOLIC EXTRACT OF *T. CORDIFOLIA*



E. coli ***S. aureus***

FIG. 3: ZONE OF INHIBITION FROM ETHANOLIC EXTRACT OF *T. CORDIFOLI*

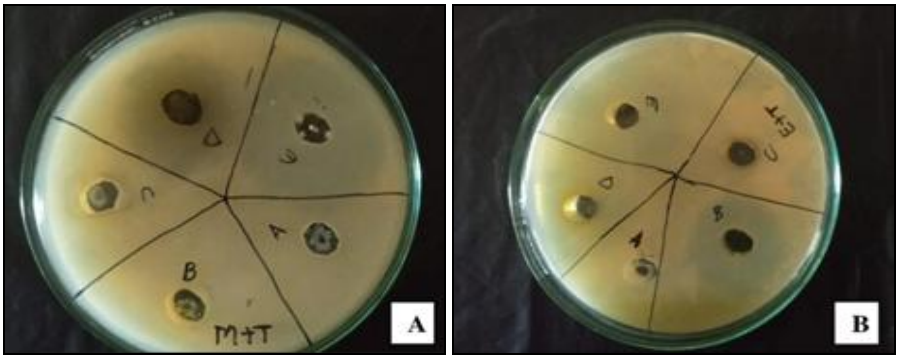


FIG. 4: ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLE AGAINST *PSEUDOMONAS SYRINGAE*

Fig. 4 showing antimicrobial activity of *Tinospora cordifolia* leaf extract with silver nanoparticle against *Pseudomonas syringae* with (A) methanolic extract and (B) ethanolic extract. SEM analysis was utilized to observe the morphology of nanoparticle; figure 5 shows the morphology of nanoparticles obtained at varying distance and magnification (A) distance 100 µm, magnification: 100X, (B) distance 100 µm, magnification: 1.00K X, (C) distance 2 µm, magnification: 3.00K X. **Fig. 6** showing morphology of silver nanoparticle at 300 nm distance and 20.00K X magnification, **Fig. 7** at 300 nm distance and 20.00 K X magnification. Morphology of silver nanoparticle was clear in heat synthesis, whereas it is not clear and appeared in a clumsy manner in heat and stir synthesis.

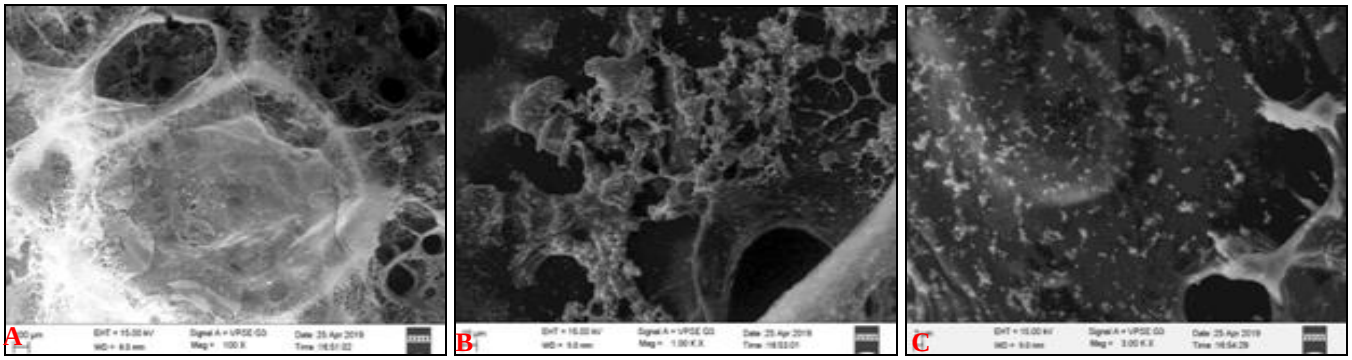


FIG. 5: SEM ANALYSIS OF *TINOSPORA CORDIFOLIA*

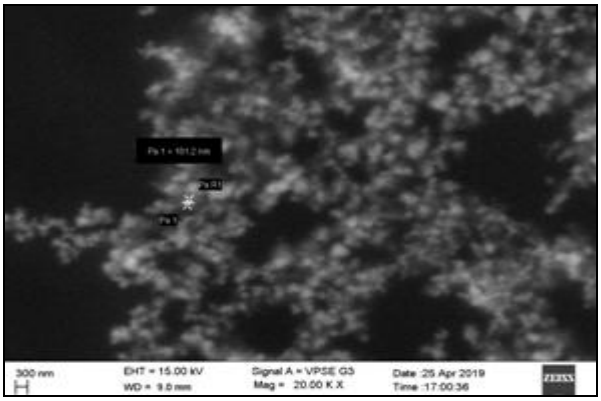


FIG. 6: *TINOSPORA CORDIFOLIA*: HEAT

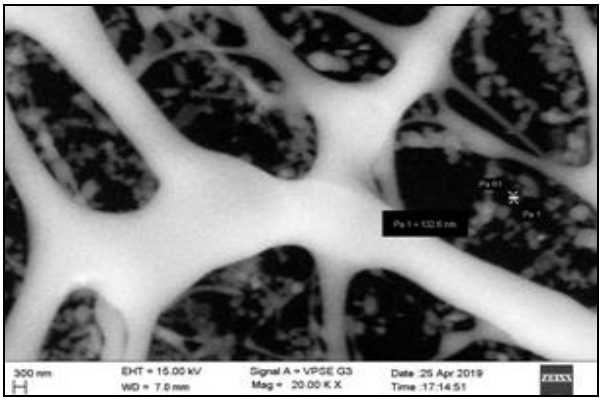


FIG. 7: *TINOSPORA CORDIFOLIA*: STIRRER + HEAT

DISCUSSION: Nanoparticles, compared to bulk materials, exhibit improved characteristics due to their size, distribution and morphology and are widely used in numerous scientific fields. Due to physio-chemical and antimicrobial properties, silver nanoparticles (AgNPs) are very important among metallic nanoparticles, which help in therapies, molecular diagnostics, and in devices used for medical procedures [16]. Chemical and physical methods can synthesize the silver nanoparticle, but due to the huge usage of toxic chemicals and high-temperature conditions, it becomes a mandate to find an alternative method [17]. Synthesis of nanoparticles by biological

methods, using micro-organisms, enzymes, plant extract, and panchakavya, has been suggested as possible eco-friendly alternatives to chemical and physical methods [18]. The plants or plants extract, which act as reducing and capping agents for nanoparticle synthesis, are more advantageous over other biological processes because they eliminate the elaborated process of culturing and maintaining of the cell and can also be scaled up for large-scale nanoparticle synthesize [19]. This green synthesis approach appears to be a non-toxic, cost-effective, simple and eco-friendly alternatively to the conventional methods and would be suitable for developing a biological process for large scale production. Green synthesized silver nanoparticles are found to have enhanced antimicrobial activity against different pathogenic bacteria [20]. The green approach for the synthesis of silver nanoparticles, especially for antibacterial purposes against human pathogens, opens a new path in antibacterial drug discovery [21].

CONCLUSION: In this study, we have done the green synthesis of silver nanoparticles using *Tinospora cordifolia* leaf and conducted the Scanning Electron Microscopy analysis to observe the morphology of nanoparticle. Antibacterial activity of *Tinospora cordifolia* was conducted against *E. coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*, antibacterial activity of silver nanoparticles was conducted against *Pseudomonas syringye*. SEM analysis reveals that morphology of silver nanoparticle was clear in heat synthesis whereas it is not clear and appeared in a clumsy manner in heat and stir synthesis. *Tinospora cordifolia* leaf extract and silver nanoparticle showed a good inhibitory effect on pathogenic bacteria.

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