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Research Article

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING SOME MEDICINAL PLANT LEAVES EXTRACT AND STUDY OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

The synthesis of silver nanoparticles (AgNPs) using plant extracts has gained attention due to its eco-friendly approach and antimicrobial properties. In this study, AgNPs were synthesized using leaf extracts of *Ficus religiosa* (Peepal) and evaluated for antibacterial activity against *Escherichia coli* using the well diffusion method. The antibacterial activity varied across different concentrations. The formation of AgNPs was confirmed using UV-Vis absorption spectroscopy (200–600 nm), and their particle size was determined using a particle size analyzer. The results indicate that *Ficus religiosa* is an effective source for AgNPs biosynthesis, with potential applications in various scientific and industrial fields. this protocol as simple, rapid, one step, eco-friendly, nontoxic and an alternative conventional physical/chemical method. Only 24hrs. Were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical.

Keywords: Silver nanoparticles, Well diffusion assay, Medicinal plants, Antibacterial activity, Green synthesis.

INTRODUCTION

The ‘green’ environment friendly processes in chemistry and chemical technologies are becoming increasingly popular and are much needed as a result of worldwide problems associated with environmental concerns (Thuesombat *et al*., 2014). Silver is the one of the most commercialised nano-material with five hundred tons of silver nanoparticles production per year (Larue *et al*., 2014) and is estimated to increase in next few years. Including its profound role in field of high sensitivity biomolecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities (El-Chaghaby & Ahmad, 2011; Veerasamy *et al*., 2011). Conventional chemical methods for AgNP synthesis often involve hazardous chemicals, high energy consumption, and the generation of toxic byproducts, raising environmental concerns. In contrast, biological or "green" synthesis, utilizing natural resources such as microorganisms, enzymes, and plant extracts, offers a sustainable, cost-effective, and eco-friendly alternative (Iravani *et al*., 2014). Plant extracts, rich in various bioactive compounds like polyphenols, flavonoids, terpenoids, and alkaloids, act as both reducing and stabilizing agents in the synthesis of AgNPs, eliminating the need for external toxic chemicals.

Nanoparticles represent completely new and improved properties based on specific characteristics such as size distribution andmorphology (Logeswari and Abraham, 2015). Nanoparticles are synthesized by two different ways: Chemical and Biological. Chemical synthesis of nanoparticles leads to synthesis of environmentally toxic byproducts but production of silver nanoparticles from green extracts including bark, leaf, root etc is nontoxic and has effective stability (Prasad *et al*., 2012). Thus, the best and the most ecofriendly way tosynthesized nanoparticles is a biological method. Biological way is simple, fast and economical. Moreover, this green technology does not involve any toxic chemicals (Zhang *et al*., 2016). The nanoparticles are characterized by using UV-Vis

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spectroscopy. Medicinal plants have been traditionally used for centuries to treat various ailments, including infections, due to their inherent antimicrobial properties. Integrating the antimicrobial potential of medicinal plant extracts with the enhanced antibacterial activity of AgNPs through biosynthesis presents a synergistic approach to develop novel and effective antibacterial agents. This research focuses on the biosynthesis of AgNPs using extracts from selected medicinal plants known for their traditional use in treating infections. The study aims to screen the antibacterial potential of these biosynthesized AgNPs against clinically relevant bacterial pathogens and compare their efficacy with that of the crude plant extracts. In this present study, silver nanoparticles were synthesized using the leaf extract of *Ficus Religiosa*, which is a traditional medicinal tropical plant. *Ficus Religiosa* commonly referred to as Peepal tree, sacred tree or Bodhi tree an evergreen tree belonging to family of Moraceae family (fig or mulberry family). Silver nanoparticles can be inhibitory to various microorganisms.

MATERIALS AND METHOD

Survey and collection of plant leaves

In the present research work, the plant sample was collected from the various regions of the Jabalpur regions such as Govt. M.H. College of Home Science campus of Jabalpur region. The collected leaves were used in the preparation of extracts for the green synthesis of AgNPs, and the evaluation of their biological activities.

Table 1. Select Indian medicinal plant used to treat various kinds of human.

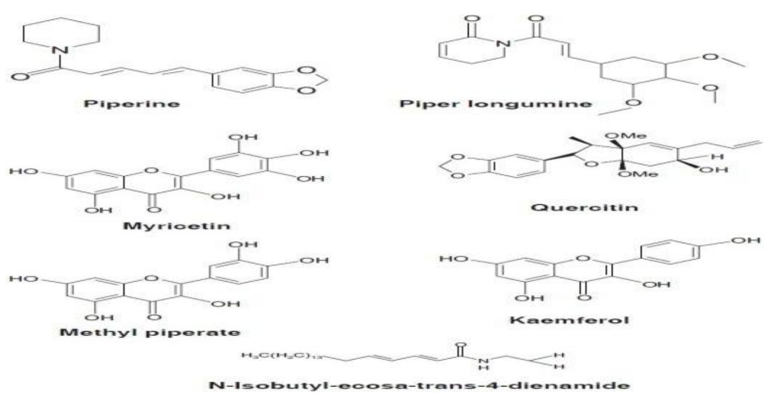
S. No.	Common name	Medicinal plant (Botanical name)	Part of plant used	Ayurvedic or Traditional Uses
1	Peepal Tree	*Ficus Religiosa*	Leaves	Leaves of plant are used to treat Stomach pain, Asthma, Skin diseases, Eczema itching, Blood purification, Liver and spline disease, Swelling in Spline and Hiccups

Phytochemistry

The stem bark of *F. religiosa* are reported phytoconstituents of phenols, tannins, steroids, alkaloids and flavonoids, β-sitosteryl-D-glucoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one. Sheetal *et al*. (2008). The active constituent from the root bark *F. religiosa* was found to be β-sitosteryl-D-glucoside, which showed a peroral hypoglycemic effect in fasting and alloxan-diabetic rabbits and in pituitary-diabetic rats. The fruits contain 4.9% protein having the essential amino acids, isoleucine, and phenylalanine (Oliver *et al*., 1977). The seeds contain phytosterolin, β-sitosterol, and its glycoside, albuminoids, carbohydrate, fatty matter, coloring matter, caoutchoue 0.7–5.1%. (Khare *et al*., 2000) *F. religiosa* fruits contain flavonols namely kaempeferol, quercetin, and myricetin (Figure 1). (Bushra *et al*., 2008) Leaves and fruits contain carbohydrate, protein, lipid, calcium, sodium, potassium, and phosphorus. (Ruby *et al*., 2008) The aqueous extract of dried bark of *F. religiosa* has been reported to contain phytosterols, flavonoids, tannins, furanocoumarin derivatives namely bergapten and begaptol.

Preparation of plant metabolite

Fresh leaves of medicinal plant Peepal (*Ficus Religiosa*) were collected from Govt. M.H. College of Home Science campus of Jabalpur region. The leaves were rinsed with sterilized distilled water & ethanol and air dried under shade for 10 days. Then the plant leaves were grind into fine powder. Then take 5gm of the leaf powder was mixed with 100ml distilled water then boil for 10 min., after boiling, the mixture was cooled and filtered with Whatman filter paper number 1 used for synthesis of silver nanoparticles.



Bio-Synthesis of silver nanoparticles

In the present study, 1mM aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. For synthesis of silver nanoparticles 50 ml filtrate distilled water & ethanol of leaf extract of *Ficus Religiosa* was added for bio-reduction process at room temperature in dark condition for 24 hours. After the proper incubation period the change of color was observed visually (green & dark brown colour).

Agar well diffusion method

The antibacterial bioassay was performed by Agar well diffusion method given by Egrov, (1995) against pathogenic bacteria *Escherichia coli.* The nutrient agars plates were prepared and well of 8mm were made in the plates with the help of a cork borer. Nutrient agar plates were seeded with two sets 20µl (DW) & 40µl (Ethanol) of standardized broth culture of the test bacteria. Each plate was spread evenly on the plate bacterial solution and after 20 min of spreading; the wells were loaded with 100 µl plant metabolites. The Petri plates were then placed in an incubator at 37^0C, and the antibacterial activity of AgNPs was evaluated by measuring the diameter of the zone of inhibition (in cm) surrounding and the wells measured with the help of Hi-media antibiotic zone scale, at Mata Gujri Mahila Mahavidyalaya (Autonomous) Jabalpur., indicating the antimicrobial effectiveness of the silver nanoparticles.

RESULT AND DISCUSSION

In the present study, potential of *Ficus Religiosa* leaves extract was used to synthesize AgNPs. The synthesized AgNPs were characterized using UV-visible spectroscopy and the antibacterial activities of these AgNPs were evaluated. Antibacterial activity of the plant metabolites extract was determined by agar well diffusion method against the test *Escherichia coli (MTCC1679)* bacterial strain. The *Ficus Religiosa* plant metabolite was showed the maximum zone of inhibition against *Escherichia coli* (MTCC1679) 24mm in 20µl (DW), 40mm in 40µl (DW), 11mm in 20µl (Ethanol) and 11mm in 40µl (Ethanol) as shown in table. 4.2, graph 4.1 and plate 4.3. Similarly, leaf extracts of *A. officinalis* were examined for its antibacterial potential using five different solvents against some reference strains of human pathogenic bacteria for the crude extract and showed remarkable antibacterial activity with zones of inhibition of 13mm against *Eschericia coli* and 11mm against *Staphylococcus aureus. Fraction* 13(ethyl acetate: methanol 8:2) as the most potent one against with the minimal inhibitory concentration of 30 mm against *E. coli* and 25 mm against *S. aureux* (Bhimba *et al*., 2010).

The *Ficus Religiosa* plant metabolite mixed with in the aqueous solution ion complex, color change from light green to dark brown was observed due to the reduction of silver ions as in Figure 2. The color change is reportedly confirmation of formation of nano-particles. The change of color of silver nanoparticles was observed after the 24 hrs of dark incubation of the sample on the rotary shaker.

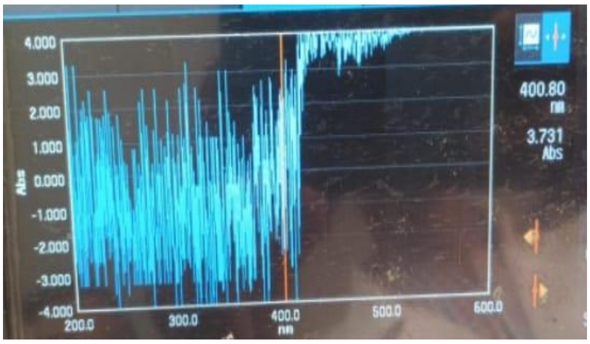
Figure 2. Biosynthesis of silver nanoparticles from plant leaf extract.

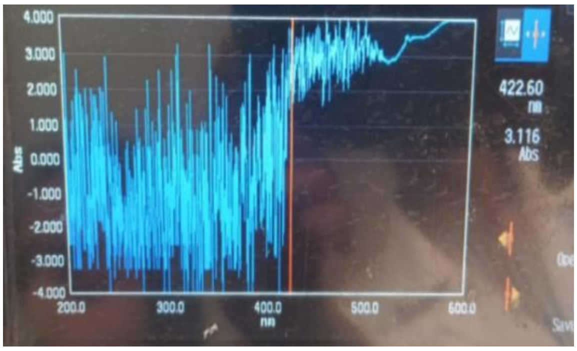
Table 2. Characterization of bio synthesized silver nanoparticles on the basis of colour change.

Name of Bacterial strain isolates	Name of Host plant	Colour change
Escherichia coli (MTCC1679)	*Ficus religiosa*	Brown colour

Table 3. Spectrophotometric analysis of biosynthesized silver nanoparticles on the basis of absorbance (200nm-600nm).

Molar solution of $AgNO_3$ for different plant leaves extract	**Range of wavelength (200-600)**	**Absorbance (in nm)**
1mM $AgNO_3$	422	3.116(ethanol)
(*Ficus Religiosa*)	400	3.731(DW)





Graph 1. UV- Spectroscopy of 1mM AgNO3Ficus Religiosa Biosynthesized silver nanoparticles.

After the biosynthesis of silver nanoparticles by using leaf extract were further characterized by using various approaches such as UV-Visible spectrophotometer to detect the absorbance on the nanoparticles. The findings from the UV-Vis absorption spectrum were considered as a novel technique widely used for structural characterization of nanoparticles. In case reduction of silver nanoparticles during exposure to fungal metabolite was observed as a result of the color change. The color change is due to the SPR phenomenon. The SPR pattern is dependent on the characteristics of the individual metal particles, such as size and shape, as well as the dielectric properties of the medium used for synthesis and the inter-nanoparticle coupling interactions. The intensity of the SPR band increased with reaction time, indicating the synthesis of the AgNPs.

Similarly, the formation of silver nanoparticles synthesized from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* were monitored by UV-vis spectrophotometer analysis. The UV-vis spectra showed maximum absorbance at 420 nm, which increased with time of incubation of silver nitrate with the plants extract. The curve shows increased absorbance in various time intervals (1 h, 24 h and 48 h) and the peaks were noticed at 420 nm corresponding to the surface plasmon resonance of silver nanoparticles. The observation indicated that the reduction of the Ag+ ions took place extracellularly (Logeswari *et al.*, 2015).

The antibacterial efficacy of the silver nanoparticles was confirmed by the formation of a zone of inhibition around the well (Figure 5). The antibacterial activity of biosynthesized silver nanoparticles from the plant leaves extract of Ficus Religiosawas studied against bacterial strain *Escherichia coli (MTCC1679).* The silver nanoparticles of *Ficus Religiosa*was showed the maximum zone of inhibition against *Escherichia coli (MTCC1679)* as shown in figure 3, table 4, graph 2. Similarly (Pranab *et al.*, 2024) studied the La_2O_3 and CeO_2 nanoparticles were successfully synthesized using an aqueous extract of *Ficus religiosa* leaves. UV-Visible, FTIR, and PXRD analysis confirmed the optical behaviour, vibrational modes, and size of synthesized La_2O_3 and CeO_2 NPs. The antibacterial activity of synthesized nanoparticles was performed against overnight grown bacteria to view its inhibited growth zone. The antioxidant activity of synthesized NPs exhibits good results and may have potential applications in the field of biomedicine and material science.

Table 4. Zone of inhibition in mm against *Escherichia coli (MTCC1679).*

Plant sample	Zone of inhibition *Escherichia coli (MTCC1679)*			
	20µl (Distilled Water)	40µl (Distilled Water)	20µl (Ethanol)	40µl (Ethanol)
Ficus Religiosa	24mm	40mm (Bacterial growth inhibit)	11mm	11mm

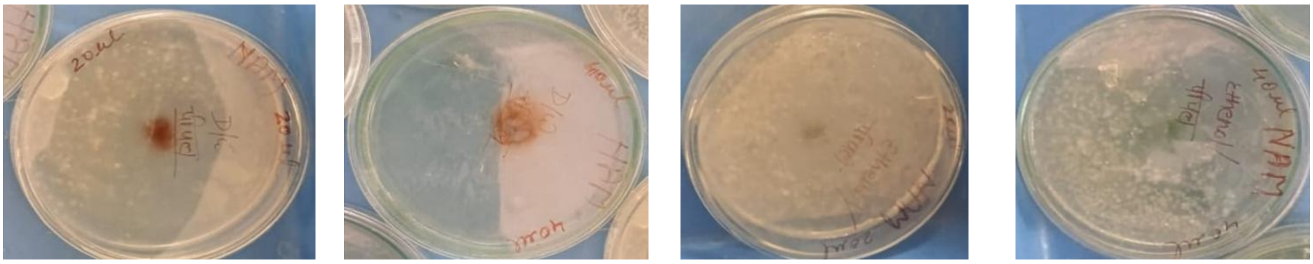
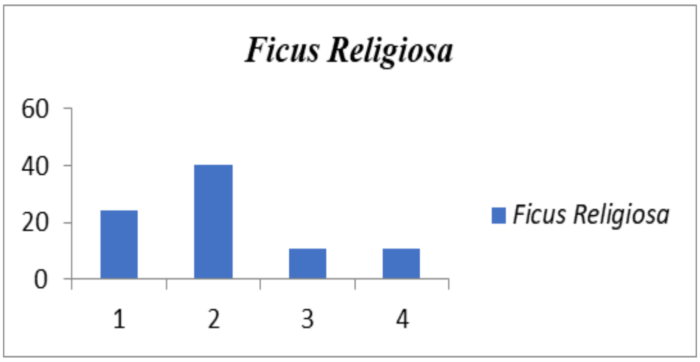


Figure 3. Screening of Silver Nanoparticles for Antibacterial Activity.



Graph 2. Antibacterial activity of biosynthesized silver nanoparticles.

CONCLUSION

The green synthesis of silver nanoparticles offers a potentially, ecofriendly, non-toxic, and cost-effective approach for the synthesis of nanoparticles. Different plant extracts can be used for the synthesis of silver nanoparticles. In the present research work the plant extract of *Ficus Religiosa* was used for the synthesis of silver nanoparticles. The antibacterial activity was examined against the test bacterial strain *Escherichia coli (MTCC 1679).* It is understood that different types of natural compounds present in plant extracts can act as reducing and stabilizing agents in the synthesis of silver nanoparticles. Furthermore, silver nanoparticles generated by green synthesis have potential applications, especially as antibacterial agents of certain microorganisms for which their efficiency has been scientifically proven, in biomedicine as therapeutic agents and wastewater treatment, in agriculture, in food safety and in food packaging. Therefore, the green synthesis of silver nanoparticles from *Ficus Religiosa* leaves extracts has several advantages such as eco-friendly, biocompatibility and cost-effectiveness. It is concluded that due to these unique properties, silver nanoparticles will have a key role in many of the nanotechnology-based processes.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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