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Green synthesis of silver nanoparticles and its effective rolein Antibacterial activity using *Plumeria rubra* L.

Priyanka Chaudhari[1*]
[1*]Department Of Botany, Sheth M.N. Science College, Patan.-384265, Gujarat, India

N.K.Patel[2]
[2]Department of Botany,Sheth M.N. Science College, Patan.-384265, Gujarat, India

***Corresponding Author:** Priyanka Chaudhari

*Department Of Botany, Sheth M.N. Science College, Patan.-384265, Gujarat, India,
E -mail: priyankachaudhari188@gmail.com

Abstract

Metallic nanoparticles are widely used in all areas of science and technology, including the medical sciences, and they continue to pique the interest of researchers interested in discovering new dimensions for their value, which is typically related to their correspondingly small sizes. Due to its distinct relevance, silver nanoparticles have drawn particular attention among other noble metal nanoparticles. The proposed technology produces colloidal silver nanoparticles without the use of hazardous chemicals in an economical and environmentally friendly way. *Plumeria rubra* flower extract is vowed to help lessen and stop the reaction's agents. The produced nanoparticles are analysed by ultraviolet-visible (UV-vis) spectroscopy, Fourier transforms infrared microscopy (FTIR), x-ray diffraction (XRD) nm, and transmission electron microscope to disclose the nature of the nanoparticles (TEM). The nanoparticles have a size range of 33–53 nm. This approach produces homogenous, stable silver nanoparticles. It was discovered that the produced silver nanoparticles had improved antibacterial capabilities and displayed a zone of inhibition against particular Gram-negative and Gram-positive bacteria. Based on the findings, it can be said that plant resources can be effectively exploited to produce AgNPs, which can then be employed in a variety of industries, including biotechnology and nanotechnology.

Key-words:*Plumeria rubra, Silver Nanoparticles, TEM,* Anti-bacterial

INTRODUCTION

The field of research known as nanotechnology studies materials with a size between 1-100 nm. The physicochemical characteristics of metal vary when it shrinks to the nanoscale, and the properties of nanomaterials differ from those of bulk metal. These nanoparticles are utilized in a wide range of industries, including biology, electronics, cosmetics, and coatings. Metal nanomaterial colloidal solutions are transparent due to their optical characteristics, making them suitable for application in packaging, coatings, and cosmetics. Due to their antibacterial, antifungal, larvicidal, and anti-parasitic properties, silver nanoparticles are among the metal nanoparticles with the broadest applicability in industry and medicine.

There is a need to create efficient and dependable experimental techniques for AgNPs synthesis because of the many human-beneficial applications for these materials. Ag, Pt, Au, and Pd are just a few of the different kinds of nanoparticles that have recently been created using chemical, physical, and biological techniques. Chemical processes are widely used, yet harmful substances produce poisonous byproducts when they are synthesised [1]. The high pressure and temperature required for the reaction demand a lot of energy to maintain through physical approaches [2]. The chemical and physical approaches, which are seen as pricy and inappropriate for a sustainable ecosystem [3], thus have their drawbacks.

AgNPs synthesis with biological components is on the rise as natural processes offer "green chemistry" processes that are harmless and suitable for the environment. The physical and morphological characteristics of metal nanoparticles are powerfullyinclined by the solvents and reducing agents. The uses of the nanoparticles were impacted by the diversity in size, shape, and morphology. Reducing precursors determine the shape of AgNPs. These are reducing and stabilising precursors that can be produced either directly or indirectly by bacteria, fungus, algae, or plants [4, 5]. The interaction of these biomolecules has been used in the past for a variety of applications, including bioleaching, bio mineralization, and the recovery of metals [6].

However, the process of creating nanoparticles from proteins has not yet been fully understood, necessitating a lot more testing. Due to its uniqueness and environmentally benign methodology, metal nanoparticle production needs special consideration. Due to its ability to modify metals into their nano-size, nanotechnology is experiencing remarkable growth in the twenty-first century. Plant extracts can be a relatively affordable and environmentally friendly option for the industrial synthesis of nanoparticles [7].

The species of *Plumeria rubra* L. is cultivated all throughout the tropical and subtropical world. A total of eight species have been reported from India, but due to certain species' overlapping characteristics, it is difficult to determine their identities [8]. The "red Frangipani," or *Plumeriarubra* L. (syn. *P. acutifolia* Poiret), is one of the species of this genus that is most widely spread in India [9]. The *Plumeria rubra* produces fragrant flowers with five spreading petals that are often red, pink, or purple in colour and have a bright yellow centre. The leaves are lance- or oval-shaped and range in length from 20 to 30 cm.
The biological properties of extracts from various plant components, including flowers, leaves, bark, and latex, include antioxidant, antiulcer, anticancer, antimicrobial, abortifacient, anti-inflammatory, analgesic, anthelmintic, antipyretic, antifertility, and hypolipidemic properties [10–12]. Numerous bioactive substances, such as plumieride, plumeria acid, -sitosterol, Lepel, plumieride, amyrin, fulvoplumierin, plumieridecoumarate glucoside, etc., are found in the flowers [13]. Gold nanoparticles made from frangipani flower extract coupled with conventional antibiotic medications have recently been demonstrated to have antibacterial action [14].
The creation of frangipani silver nanoparticles and analysis of their potential antibacterial properties were the main goals of this study. UV-visible spectroscopy, Fourier transforms infrared spectroscopy (FTIR), X-ray diffraction (XRD), and transmission electron microscopy were used to analyse the produced AgNPs (TEM). In this study, we describe a quick method for producing nanoparticles from plant flower extract. We also characterise the particles' inhibitory effects on Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Flower extraction

Fresh Frangipani flower samples weighing 5g were gathered at the college's botanical garden in Patan, Gujarat, India (coordinates: 23°86'12" N 72°13'03" E). Dr. N.K. Patel, associate professor and head of the botany department at Sheth M.N. Science College in Patan, Gujarat, India, made the identification and authentication of *P.rubra*. Fresh flowers from the collection were cleaned, neatly chopped, and steeped in 100 ml of boiling, double-distilled water for 5 to 10 minutes before filtering through Whatman filter paper no. 1.

Biosynthesis of silver nanoparticles

With a ratio of 1:9 ratio-optimized concentrations (10 ml of flower extract to 90 ml of 1 mM AgNO3), the solution of 1 mM silver nitrate was added drop-wise to the reaction mixture of *Plumeria rubra* flower extract in an Erlenmeyer flask separately. Both were then incubated at 35 °C in the dark for about 24 hours. By changing colour from light yellow to brown and then undergoing spectrophotometric measurement, silver ions (Ag+) were depleted to AgNPs (Ag0) [15].

Characterizations of AgNPs

UV–visible spectral analysis

Using a UV-visible spectrophotometer, the bio-reduction and creation of silver nanoparticles from *Plumeria rubra* flower extract were observed over time (190–1100 nm frequencies at a resolution of 1 nm utilising a UV–visible spectrophotometer- UV-1800 SHIMADZU, Double – beam UV-Vis Spectrophotometer). The created AgNPs were further purified and gathered by repeated centrifugation at 10,000 rpm.

X-ray diffraction analysis (XRD)

XRD analysis was used to determine if the produced silver nanoparticles were crystallised. On a glass slide, a thin coating of AgNPs powder was produced and exposed to monochromatic Cu K radiation at 40 kV and 30 mA. PANanalytical Netherlands' X'pert Pro.

Fourier transform infrared spectroscopy (FTIR)

With the help of FTIR analysis (performed on a Perkin Elmer instrument, USA) over the 4000-400 cm-1 territory, which was acquired from 24 scans with a resolution of 0.4 cm-1, the functional groups of phytocompounds found in *P. rubra* flower extract that are involved in the formation of AgNPs were determined. AgNPs powders were individually combined with potassium bromide (KBr) to create KBr pellets, which were then analysed using FTIR.

Transmission Electron Microscopy (TEM)

By using a transmission electron microscope, the surface and size morphology of produced AgNPs were determined. The surface of the carbon copper grid was treated with a drop of the AgNPs solution made from *P. rubra* flower extract. The photos were captured at H-7500 Hitachi in Japan with a resolution of 0.36 nm and a working voltage of 40-120 kV.

Antibacterial activity of AgNPs

The antibacterial prospective of AgNPs was investigated against the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilisis*, similarly the gram-negative species *Pseudomonas aeruginosa* and *Escherichia coli*. In test tubes with media, bacterial culture was multiplied and replenished for this (nutrient broth). 1 ml of each broth culture was applied to the nutrient agar media, which was then left in an incubator for 24 hours at 37 °C. The agar well diffusion technique was employed to assess antibacterial activity. The agar plates were covered with 6 mm sterilised filter paper discs that had been dipped in a suspension of synthesised silver nanoparticles (10 g/ml), double distilled water as a negative control, and chloramphenicol (30 g/ml) as a standard. The discs were then nurtured for 24 hours at 25 °C room temperature.

RESULT AND DISCUSSION

UV–Vis spectra analysis

The UV-Vis spectroscopy was used to investigate how the components of the *P. rubra* plant flower extracts affected the decrease of silver ions in the aqueous solution of a silver complex. There was a maximum absorbance peak at 417 nm, showing that the reduction of Ag+ ions in the aqueous AgNO3 solution was what caused the creation of silver nanoparticles (Fig.3). Initial colour change in floral extracts with 1 mM silver nitrate solution was from yellow to brown.

It resulted from the conversion of Ag+ to Ag0 (Fig 1). Subsequently intervals of 30 minutes, three hours, and twenty-four hours from the start of the reaction, the spectra of AgNPs produced from *P. rubra* flower were captured (Fig 3). With an increase in reaction time, the colour intensity was seen to rise (Fig. 2). A peak at 417 nm was visible in the flower extract's absorption spectra. The SPR peak at 435 nm formed in the visible range after 30 minutes and gradually grew more pronounced. The SPR peak intensified with longer reaction times, indicating the biogenic production of silver nanoparticles.

At various reaction durations, the range of 250-700 nm was observed in the absorption spectra of silver nanoparticles produced by the interaction of Neem leaf extract and AgNO3. The colour shift was first noticed after 5 minutes of adding the salt solution to the Neem leaf broth. The absorption maxima is observed at 400 nm, at a higher energy than that achieved by [16] and with olive leaf [17]. When the colour of solution is practically constant after 30 minutes, there is no longer any silver salt available for reaction. The outcomes are in perfect agreement with what was stated by [16Without any movement in peak wavelength, the SPR band's intensity grew over time. Mie's theory [18] states that spherical AgNPs' absorption spectra show a single SPR band, and the number of peaks rises with increasing anisotropy. The SPR band in the current work implies that the produced nanoparticles are spherical in shape, and the SEM examination confirms this.

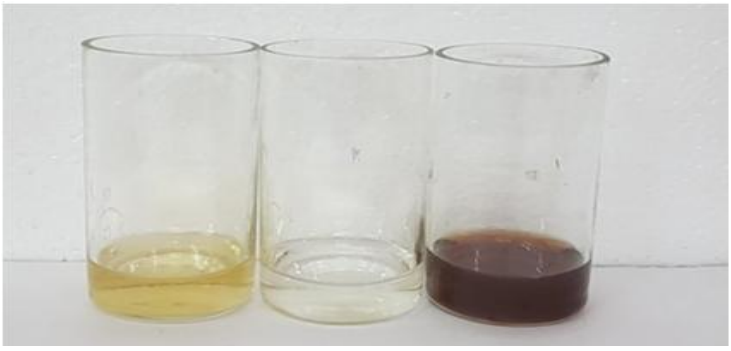


Fig 1: Visual observation (A) *P.rubra* flower extract (B) $AgNo_3$ solution(C) Synthesized AgNPs

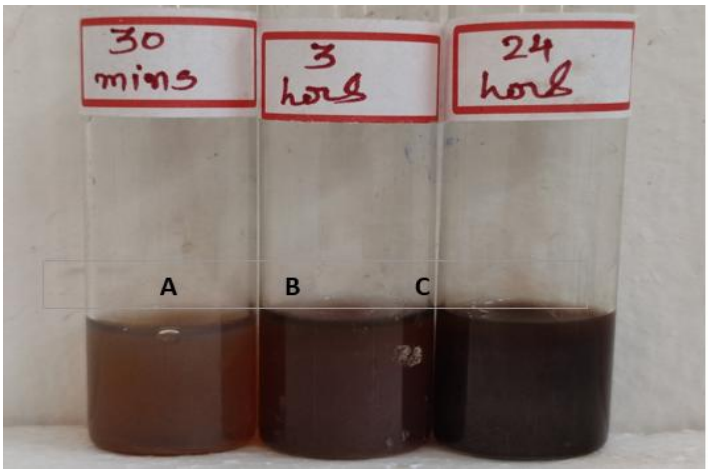


Fig 2: Showing different time interval color intensity of *P.rubra* flower extract with AgNPs

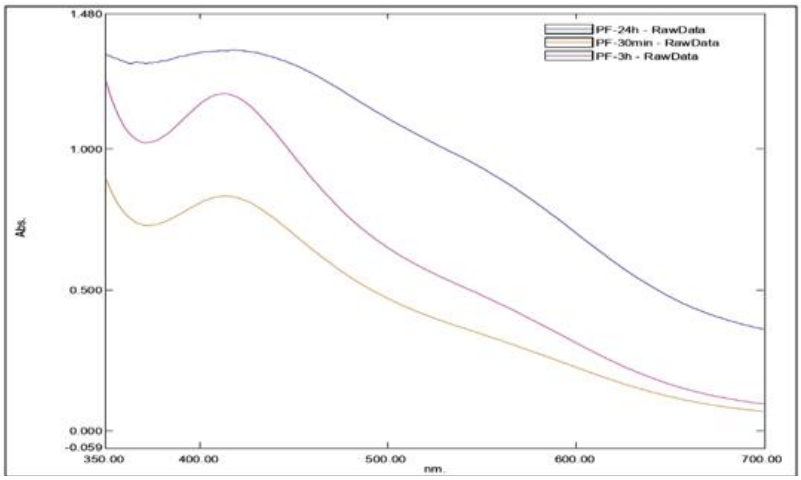


Fig 3: UV-visible spectra of *P.rubra* AgNPs obtained usingflower extract of *P.rubra*at different time interval

X-ray diffraction spectroscopy (XRD)

By employing X-ray diffraction spectroscopy, it is possible to determine the material's crystalline structure, shape, and size (XRD). Typically, the (111), (200), (220), and (311) Bragg's reflections of the face-centered cubic (FCC) structure of metallic silver were measured at 2 = 38.25, 44.43, 64.59, and 77.53, respectively (standard JCPDS card No. 04-0783 or 87-0597). The Debye-formula, Scherrer's d = 0.89/cos, can be used to calculate the crystalline size of the particulate, where d is the particle size, is the X-ray wavelength (1.5406), is the full-width at half maximum of the largest peak (in radians) of the diffraction pattern, and 2 is the Bragg angle [19].According to the Debye Scherrer's equation, the average particle size was 20-40 nm.

These diffraction lines were found at 2 angles and, by comparing them to JCPDS data, they were identified as the (98), (101), (111), and (200) planes of FCC silver. Similar findings were examined by Ondari and Nalini [20] for silver nanoparticles made using *Tridaxprocumbens*. For the (111) and (220) planes, the lattice parameters were 0.4018 nm and 0.4047 nm, respectively. The estimated values agree with the metallic silver standard lattice parameter of 0.40729 nm [21].

The pattern clearly demonstrates the strong peaks associated with the (111), (220), and (311) planes at (2h) 38.19, 64.56, and 77.47, respectively. The traditional design of bio-created silver nanoparticles is discovered to have an FCC structure when compared to JCPDS-file no: 89-3722. Using the Debye-equation, Scherrer's the average crystalline size of the silver nanoparticles was calculated [22].

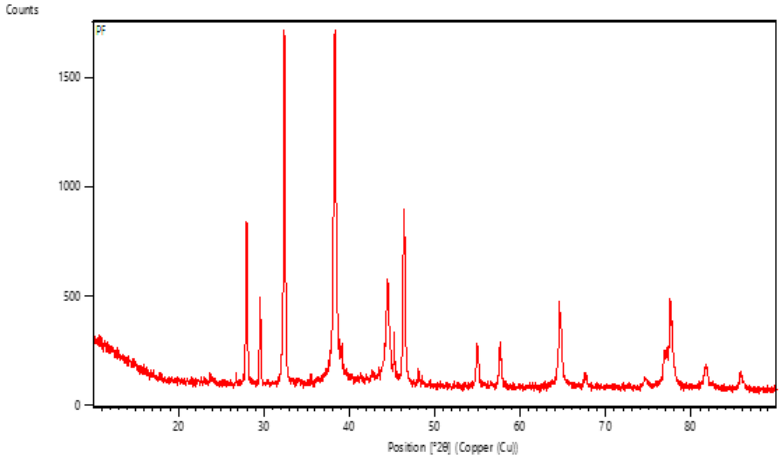


Fig 4:XRD pattern of AgNPs synthesized using the leaf extract of *P.rubra*

Fourier transform infrared microscopy (FTIR)

The functional groups of *P. rubra* flower extract that are involved in the creation and stability of silver nanoparticles were the subject of an FT-IR investigation. In fig. 5, the spectra are displayed. It offers several absorption peaks, which reflects the extract's complexity. FTIR provides information on the functional groups contained in the produced AgNPs to help understand how different phytochemicals found in flower extracts cause them to change from simple inorganic AgNO3 to elemental Ag. To categorise the functional groups of the Plumeria flower extract, FTIR analysis was used.

Stretching the -OH bond of alcohol groups leads to a peak at 3449.88 cm-1, which denotes a bonded hydroxyl (-OH) group. The functional groups in silicon compounds could be the cause of the absorption peak at 2084.02 cm-1. The Primary amine is indicated by the peak at 1630.89 cm-1. The existence of C-N Aromatic amine may be the cause of the absorption peaks at 1383.80 cm-1. The C-O Primary alcohol may be the cause of the band at 1069.34 cm-1. 831.51 cm-1 is the peak caused by C-C stretch, while 612.51 cm-1 is caused by C-Br Halide. The hydroxyl (-OH), carboxyl

(-C=O), and amine (-NH) groups of the caumarins, tannins, or alkaloids of the P. rubra flower are represented by FTIR analysis.

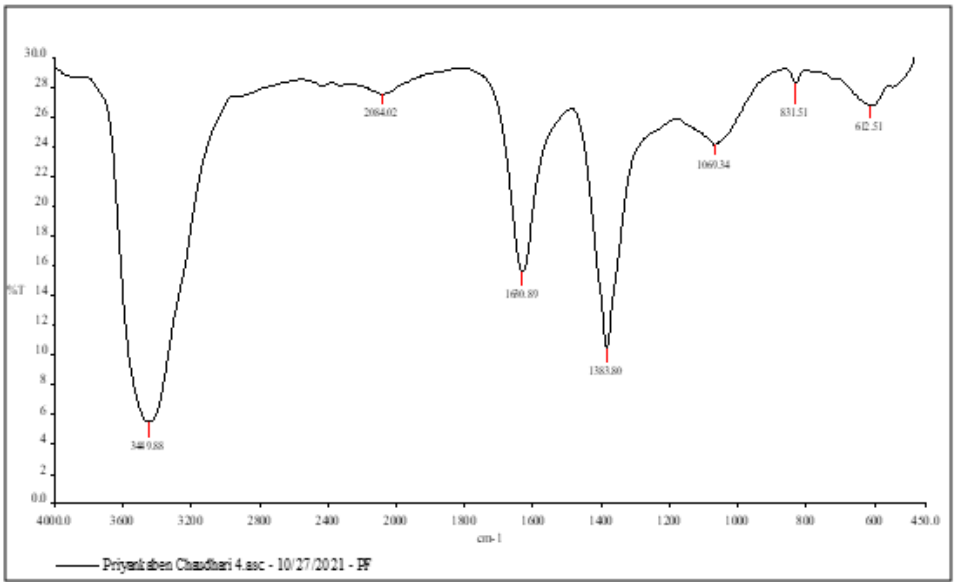


Fig 5: FTIR spectra of synthesized AgNPs of *P.rubra* flower extract

The amide I bands of proteins in the leaf extract and the solid, powerful peaks at 1382 cm-1 are C-N stretch vibrations, respectively [23]. The flavanones may be captivated on the surface of metal NPs as specified by the durable bands at 1074 cm-1, which are initiated by ether linkages [24]. In the plant extract, the phenolic groups elaborated in the ion replacement retort are located in the 1315–1036 and 1455–1600 cm-1 areas [25]. The occurrence of the aromatic group is confirmed by the exciting band at 1592 cm-1, which results from C=C stretching in the aromatic ring [26]. Another study [27] supports the hypothesis that water-soluble flavonoids are involved in the reduction of metal ions utilising plant extracts.

Transmission electron microscope (TEM)

In TEM examination, an electron beam is passed through an incredibly thin sample. As the electrons interact with the sample, they become elastically scattered. According to the density of the scattered electrons are focused by a number of electromagnetic lenses and then anticipated onto a screen to harvest electron diffraction, a phase-contrast image, or a shadow image with changing degrees of darkness. With an atomic or sub-nanometer spatial resolution, transmission electron microscopy techniques can directly image the specimen and offer information on its chemical composition, diffraction, and spectroscopy either concurrently or sequentially

According to the TEM pictures, the particle sizes were between 33 and 53 nm (Fig. 6). The particles have a round shape. Compared to the size determined by DLS analysis, the size determined by TEM analysis was smaller. Histograms are created in the 20, 50, 100, and 200 nm ranges (Fig. 7).

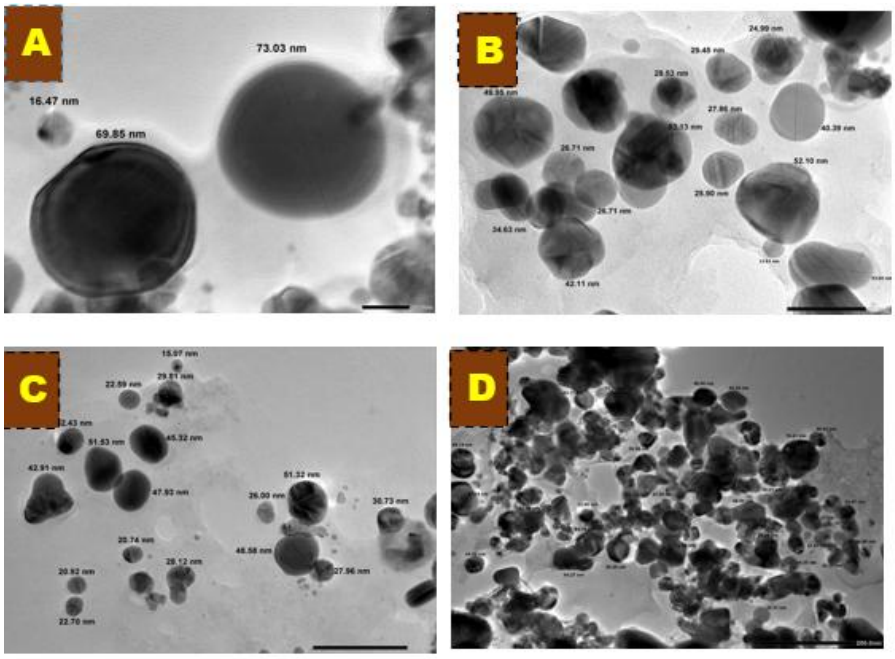


Fig 6. Transmission electron microscopy (TEM) images of AgNPs by *P.rubra* (A) 20 nm (B) 50 nm (C) 100 nm (D) 200 nm

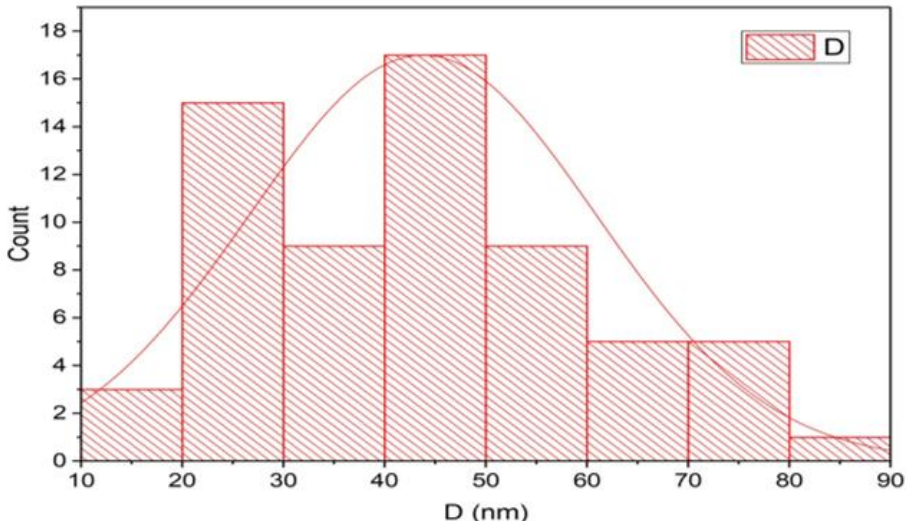
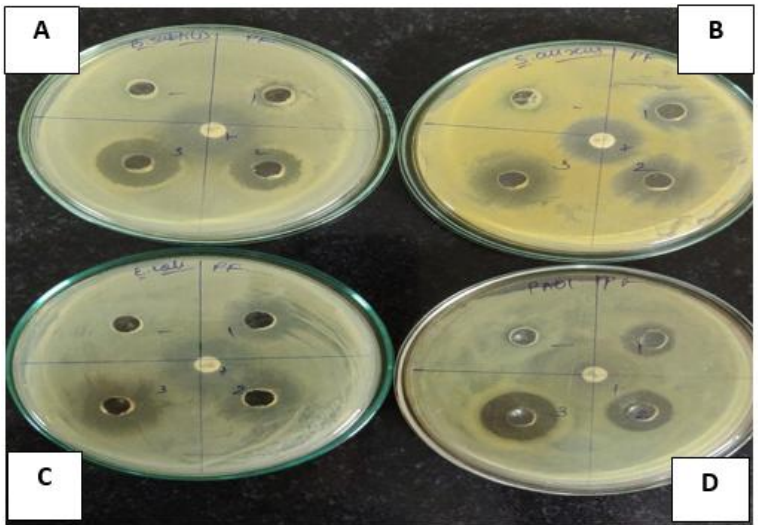


Fig 7:TEM histogram of *P.rubra* AgNPs- Average particle size- 43.76 nm

Antibacterial activity

These synthetic Ag nanoparticles were tested against the following four microorganisms for their antibacterial activity: *Pseudomonas aeruginosa* and *Escherichia coli* are two-gram negative bacteria, while *Bacillus subtilis* and *Staphylococcus aureus* are two-gram positive bacteria. The bacterial strains were cultured for an overnight period at 37°C in complete darkness after applying the suspension of Ag-nanoparticles in various wells. Clear restriction zones were seen all around the wells after 24 hours. According to Table 1, these four bacteria's growth was suppressed to varying degrees by these biosynthesized Ag nanoparticles.

AgNPs mediated by *P. rubra* flower extract were found to significantly suppress bacterial growth, and among the studied pathogens, *S.aureus* showed the highest level of sensitivity. According to Table 1, the ZOI increased as AgNPs concentrations rose.Gram-positive *S. aureus* (28.58±0.44mm) is inhibited to the greatest extent by AgNPs made from *P. rubra* flower extracts, followed by Gram-negative *P. aeruginosa* (22.43±0.21mm) and*E. coli* (25.39±0.10mm) and Gram-positive*B.subtilis* (21.48±0.41mm) at 60 µl concentration as shown in the table 1.



Note: 1= Negative Control, 2= Plant extract, 3=$AgNO_3$, 4= AgNPs

Fig 8: Antibacterial activity of *P.rubra* -AgNPs prepared by *P.rubra* flower extract against (A) *Bacillus subtilis* (B) *Staphylococcus aureus* (C) *Escherichia coli* (D) *Pseudomonas aeruginosa*

Bacteria Class	Name of the organism	Concentrations (µl)	of Inhibition (mm)		
			Plant Extract	**Silver nitrate ($AgNO_3$)**	**Silver nanoparticles**
Gram negative	***Escherichia coli***	**50 µl**	11.49±0.41	15.67±0.50	19.81±0.21
		60 µl	14.30±0.74	16.59±0.60	23.67±0.66
		70 µl	17.55±0.70	18.91±0.13	25.39±0.10
	Pseudomonas aeruginosa	**50 µl**	14.56±0.40	11.53±0.31	19.64±0.44
		60 µl	17.89±0.68	16.61±0.92	20.60±0.70
		70 µl	19.29±0.27	20.47±0.34	22.43±0.21
Gram positive	***Bacillus subtilis***	**50 µl**	11.78±0.31	14.83±0.90	18.41±0.51
		60 µl	12.48±0.84	17.53±0.21	19.82±0.42
		70 µl	13.47±0.42	20.43±0.64	21.48±0.41
	Staphylococcus aureus	**50 µl**	12.67±0.42	18.34±0.57	24.39±0.67
		60 µl	16.33±0.05	19.34±0.42	25.61±0.56
		70 µl	19.57±0.38	22.64±0.14	28.58±0.44
Positive control	Chloramphenicol	**15 mg**	16.12±0.44		
	Penicillin	**5 mg**	14.77±0.62		
Negative control	Water		-		
CV		0.820			
CD (1%)		0.421			
CD (5%)		0.341			

(NOTE: Values are the mean of three replicates ± standard error.)

Table 1: Antibacterial activity of AgNPs synthesized by using *P.rubra* flower extract

CONCLUSIONS

The creation of dependable and environmentally benign methods for creating metallic nanoparticles is a top priority in the arena of nanotechnology. By using a simple procedure, the current study achieves its goal of "Green" synthesis of silver nanoparticles. We have created a quick, eco-friendly, straightforward, and affordable method for producing stable silver nanoparticles by bio-reducing silver nitrate solution using *P. rubra* aqueous extract. Using UV-Vis, FTIR, XRD, and TEM analytical techniques, the properties of the produced AgNPs were investigated.According to the experimental findings, the produced silver nanoparticles have an average size of between 33 and 53 nm and are stable. It has been established that the produced nanoparticles exhibit antibacterial activity and have been shown to be effective against gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus* as well as gram-negative bacteria like *Pseudomonas aeruginosa* and

Escherichia coli. Due to their antibacterial qualities, green produced silver nanoparticles could be highly helpful in the medical field.

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