

**Original Article**

# INFLUENCE OF PHYSICO-CHEMICAL PARAMETERS ON THE FABRICATION OF SILVER NANOPARTICLES USING *MARANTA ARUNDINACEA* L AND ITS ANTI-MICROBIAL EFFICACY

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## ABSTRACT

**Objective:** To investigate phytosynthesis of silver nanoparticles, factors governing the synthesis of nanoparticles and antibacterial activity of synthesized silver nanoparticles.

**Methods:** The leaf extract of *Maranta arundinacea* L. was used for synthesis of silver nanoparticles which was confirmed by changing the colour of reaction mixture from colourless to brown. Synthesized silver nanoparticles were characterized by UV-vis spectroscopy (UV-vis), Fourier Transform Infra Red Spectrophotometer (FTIR), X-ray Diffraction (XRD), Energy dispersive X-ray diffraction (EDX), Atomic Force Microscopy (AFM), High-Resolution Transmission Electron Microscopy (HR-TEM) and testing the antibacterial activity against *E. coli*, *S. typhi*, *S. aureus* and *B. polymyxa* following well diffusion method.

**Results:** Silver nanoparticles shows characteristic UV-Vis absorption peak at 406 nm. The influence of physico-chemical parameters was studied and optimized to obtain nanoparticles of diverse sizes. The size of silver nanoparticles ranges from 30 to 90 nm and were found to be spherical in shape with crystalline nature. The synthesized silver nanoparticles showed good antimicrobial activity against multi-drug resistant *S. aureus*, *B. polymyxa*, *E. coli* and *Salmonella typhi*. Silver nanoparticles show that higher antibacterial activity was observed in Gram negative bacteria than in Gram-positive bacteria. The highest zone of inhibition  $7.033 \pm 0.033$  mm was observed in *S. typhi* at 250  $\mu$ l and lowest zone of inhibition  $4.100 \pm 0.066$  mm was seen in *B. polymyxa* at 250  $\mu$ l.

**Conclusion:** Silver nanoparticles synthesized using leaf extract of *Maranta arundinacea* L. may exhibit reasonable testing antimicrobial activity against some pathogenic microbes.

**Keywords:** Silver nanoparticles, *Maranta arundinacea* L, Physico-chemical parameters, Antimicrobial activity

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## INTRODUCTION

Nanotechnology provides the tools and technology platform for the investigation and transformation of biological system which offers inspiration models and bio-assemble components to nanotechnology [1]. Nanoparticles are present at a higher surface-to-volume ratio with decreasing size of nanoparticles. They possess unique optical and electronic properties compared to bulk material. It has been reported that since ancient times silver is known to have antimicrobial [2], antiviral [3], anti-plasmodial [4], anti-inflammatory [5], anti-cancer [6, 7] and anti-diabetic [8] activities. The size, shape and surface morphology play an important role in controlling the physical, chemical, optical and electronic properties of these nanomaterials. Several physical properties of silver nanoparticles, such as Surface Plasmon Resonance (SPR), can be tailored for specific application by controlling the size, shape and morphology of the nanoparticles [9-11]. The nanoparticles synthesis generally involves either a "top down" or a "bottom up" approach. The bottom up approach mostly relies on chemical and biological methods of production. The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks.

Synthesis of nanoparticles through biological methods is a good alternative over chemical and physical methods as they are environmental friendly and economic. Since past few decades, it was only the prokaryotes that have been exploited for the synthesis of nanoparticles. But recently, it was found that highly evolved organisms like algae, diatoms, plants and other components of eukaryotes also possess the reducing potential to convert the inorganic metal ions into metal nanoparticles. This approach is an alternate route of synthesizing biocompatible nanoparticles compared to chemical synthesis, which is the latest possible way of linking material science and biotechnology in the emerging field of nanobiotechnology. The extracts of various plants and plant parts such as leaf, fruit, flower, tuber and rhizome were used

for the synthesis of silver and gold nanoparticles, with further testing the efficacy of nanoparticles for antimicrobial activity. Among such plants, *Murraya koenigii* [12], *Garcinia mangostana* [13], *Olea europaea* [14], *Tribulus terrestris* [15], *Artemisia nilagirica* [16], *Hibiscus cannabinus* [17], *Alternanthera sessilis* [18], *Aegle marmelos* [19], *Melothria maderaspatana* [20], *Morinda citrifolia* [21], *Syzygium samarangense* [22], *Phyllanthus maderaspatensis* [23], *Hemidesmus indicus* [24], *Leea indica* [25], *Carica papaya* [26] *Lawsonia inermis* (Henna) [27], *Linum usitatissimum* [28], *Lavandula intermedia* [29], *Allophylus serratus* [30], *Bauhinia acuminata*, *Biophytum sensitivum* [31] and *Polygonum glabrum* [32] were employed.

*Maranta arundinacea*, l also known as arrowroot *Maranta*, belongs to family Marantaceae. Arrowroot is a perennial plant growing to a height of between 0.3 m (1 ft) and 1.5 m (5 ft). Twin clusters of small white flowers bloom about 90 d after planting. The plant exhibits various medicinal properties such as being antidysentritic, antidote, aphrodisiac, antipyretic, depurative, and hypocholesterolemic. Its leaves are lanceolate. The edible part of the plant is the rhizome. *Maranta arundinacea*, L is reported for the antioxidant activity and hypolipidemic effect [33, 34]. The biochemical activities of plants are mainly due to the phytochemical components present in it. *Maranta arundinacea*, L. is rich with various phytochemicals such as alkaloids, carbohydrates, cardiac glycosides, proteins, amino acids, phenolic compounds, terpenoids, saponins, flavones and gum [35]. The present investigation is undertaken to study phytosynthesis of silver nanoparticles using leaf extract of *Maranta arundinacea*, L, factors governing the synthesis of silver nanoparticles and antibacterial activity of the synthesized nanoparticles.

## MATERIALS AND METHODS

### Chemicals and plant material

Silver nitrate was obtained from Sigma-Aldrich chemicals. All glassware was washed with distilled water and dried in oven. Fresh

leaves of *M. arundinacea* were collected from Siddapur, Uttarkannad, Karnataka, India.

#### Preparation of leaf extract

Ten g of leaves were thoroughly washed with de-ionized water and were cut into small pieces and boiled in 100 ml of deionized water for 20 min. Extract was filtered with Whatman 41 filter paper and stored at 4 °C to be used for further experiments.

#### Synthesis of silver nanoparticles

Five ml of *M. arundinacea* leaf extract was added into 95 ml of aqueous solution of 1 mmol AgNO<sub>3</sub> for reduction and stabilization of Ag<sup>+</sup> ions into silver nanoparticles. The colour of solution changes from colourless to brown, indicating the formation of silver nanoparticles and further confirmed by characteristic absorption spectra by UV-Vis spectrometer.

#### Different parameters for investigation

The effect of various parameters of reaction mixture on end product was spectrophotometrically investigated.

#### Hydrogen ion concentration (pH)

The pH of reaction mixture was maintained at 4, 5, 6, 7, 8, 9, 10 and 11 respectively.

#### Reaction temperature

The reaction temperature was maintained at 0, 10, 37, 40, 50, 60, 70, 80 and 90°C using water bath.

#### Concentration of leaf extract

The serial dilution of the plant leaf extract varying 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml can be used with the same volume ratio (5 ml extract 95 ml 1 mmol silver nitrate).

#### Concentration of silver nitrate solution

The reaction was monitored by using different concentration of silver nitrate (1, 2, 3, 4 and 5 mmol) solution for the synthesis of nanoparticles.

#### Characterization of silver nanoparticles

The UV-Vis spectrum of the sample was monitored using UV-2450 (Shimadzu) spectrophotometer operated with 1 nm resolution within the range of 300–800 nm. Further characterization was done by Fourier Transform Infra Red Spectrophotometer (FTIR) (F-7000FL) spectrometer. In order to remove any free biomass residue, the residual solution after reaction was centrifuged at 4000 rpm for 20 min and the resulting suspension was re-dispersed in 10 ml sterile distilled water. Centrifugation and re-dispersion were repeated for three times. Finally, the dried samples were palletized with KBr and analyzed using FT-IR. For Energy dispersive X-ray diffraction (EDX) analysis, the reduced silver was dried on a carbon tape placed on a copper stub and performed on a HITACHI SU6600. Dried sample was collected for the determination crystalline structure of Ag nanoparticles by X' Pert pro X-ray diffractometer operated at 40 kv and 30 mA with Cu K $\alpha$  radiation. Morphological characterization of the sample was done by High-Resolution Transmission Electron Microscopy (HR-TEM) (JEOL JSM6701-F), a pinch of dried sample was coated on a carbon tape. It was again coated with platinum in an auto fine coater and then the material was subjected to analysis.

#### Antibacterial activity

Antimicrobial activity was analyzed with synthesized silver nanoparticles by well diffusion method against Gram positive bacteria Viz. *Staphylococcus aureus* (MTCC3160), *Bacillus polymyxa* (MTCC 190) and Gram-negative bacteria Viz. *Escherichia coli* (MTCC 723, 1554) and *Salmonella typhi* (MTCC3216). Bacterial species were brought from Institute of microbial technology (CSIR-IMTech), Chandigarh. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for

18 h. Agar Muller Hinton media were poured into plates and wells were made. Each plate was inoculated with 18 h old cultures (100  $\mu$ l, 10<sup>4</sup>cfu) and spread evenly on the plate. After 20 min, the wells were filled with the tested nanoparticles at different volumes. All the plates were incubated at 37 °C for 24 h and the diameter of inhibition zone were noted.

#### Statistical analysis

The values were expressed as the mean  $\pm$  standard error of the mean, and all measurements were made in triplicate. The collected data were statistically analysed using a one-way analysis of variances with SPSS 20 software. A p-value < 0.05 was considered statistically significant.

#### RESULTS AND DISCUSSION

When leaf extract of *M. arundinacea* was added to aqueous silver nitrate solution, a dark brown colour appeared within 2 min (fig. 1B). Our results collaborate with the silver nanoparticles synthesized by leaf mucilage of *Sesamum lacinatum* Klein ex Willd., where colour change occurred within 2 min [36]. The colour change was arising due to the property of quantum confinement, which is a size-dependent property of nanoparticles which affects the optical property of the nanoparticles [37]. The absorption spectra showed an intense peak at 406 nm due to the surface plasmon resonance band of silver nanoparticles and broadening of peak indicated that the particles are polydispersed. Different parameters were optimized including pH, temperature, leaf extract concentration and silver nitrate concentration were identified as factors affecting the synthesis of silver nanoparticles.



Fig. 1: (A) Habit of *M. arundinacea* (B) Visual observation a) AgNO<sub>3</sub> b) leaf extract c) leaf extract+AgNO<sub>3</sub>

#### Factors influencing the synthesis of nanoparticles

##### Effect of pH

The pH plays a vital role in synthesis of nanoparticles and in controlling their size and shape. Acidic condition hinders the formation of nanoparticles and forms a larger-sized nanoparticle, whereas small and highly dispersed nanoparticles were formed at basic pH which enhances the formation of nanoparticles. At basic pH large numbers of functional groups are available for silver binding which facilitated a greater number of silver nanoparticles to bind and subsequently form a greater number of nanoparticles with smaller diameters. The raising in pH value at basic condition enhances the repulsive barrier and keeping the nanoparticles physically apart from each other. The excess of H<sup>+</sup> ions at low pH, on the contrary, depresses ionization of the active reducing agent and gates electron transfer due to protonation [38]. The optimum pH for the synthesis of silver nanoparticles is pH=7 (fig. 2).

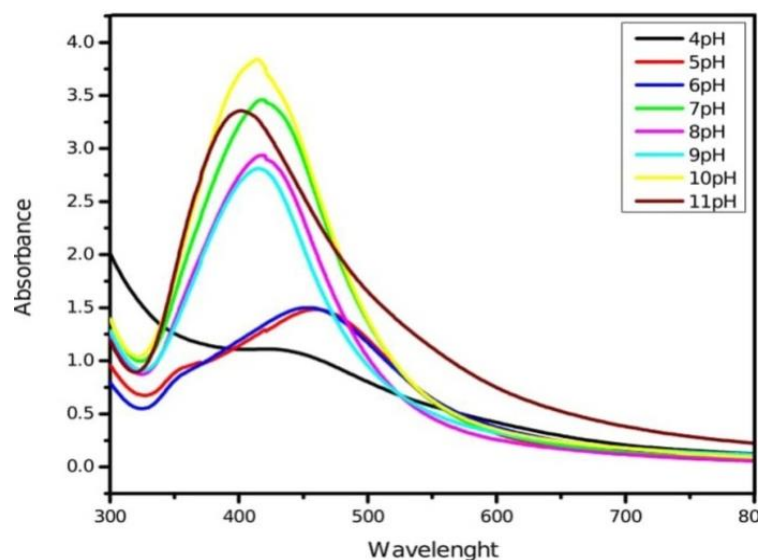


Fig. 2: UV-Vis absorption spectra of silver nanoparticles synthesized by leaf extract of *M. arundinacea* at different pH

#### Effect of temperature

The synthesis of silver nanoparticles depends on the temperature, increased in temperature increases the rate of formation of silver nanoparticles. The size is reduced initially due to the reduction in aggregation of the growing nanoparticles. The peak sharpness

increases with an increase in the reaction temperature. It is evident that increase in reaction rate of conversion of the metal ion to nanoparticles occurs at higher temperature [39]. The optimum temperature for the synthesis of silver nanoparticles is 80 °C (fig. 3). Reaction temperatures play an important role to control the nucleation process of nanoparticles configuration.

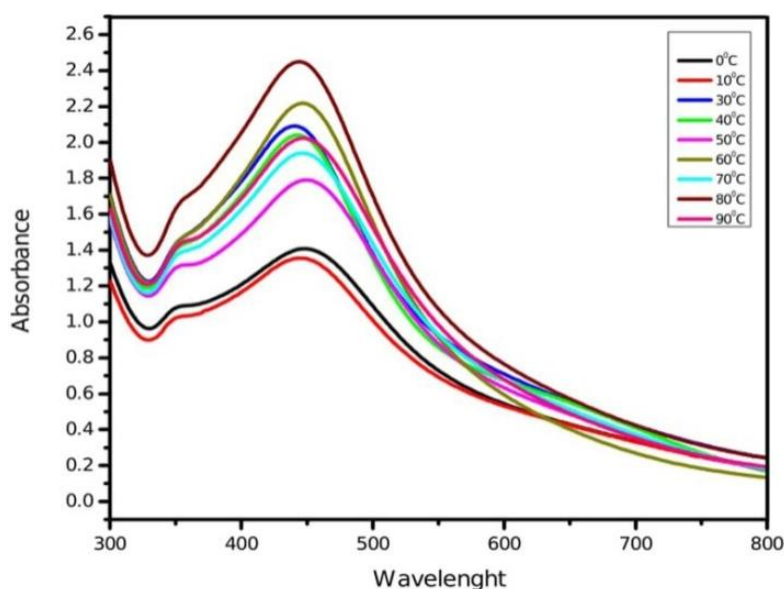


Fig. 3: UV-Vis absorption spectra of silver nanoparticles synthesized by leaf extract of *M. arundinacea* at different temperatures

#### Effect of leaf extract concentration

The quantity of leaf extract plays an important role in the complete conversion of silver ions into silver nanoparticles. Fig. 4 depicts a series of absorption spectra recorded from the solution of silver nanoparticles at different leaf extract concentrations of experimental plants. The optimum condition for the synthesis of silver nanoparticles is 8 ml (fig. 4). The sharpness of the absorption peak is dependent on the quantity of leaf and fruit extract, which gets sharpened at higher quantity up to a certain limit further, increase in quantity showed broader absorption peak. Increase in the leaf extract quantity decreases the size of silver nanoparticles [40].

#### Effect of salt concentration

Variation in silver nitrate concentration (1-5 mmol) causes variation in the size of nanoparticles. 1 mmol silver nitrate solution is suitable to yield more number of small-sized silver nanoparticles with sharp intense absorption peak (fig. 5). The absorbance intensity of the solutions increased with higher concentration of silver nitrate due to an enhancement in oxidation of hydroxyl groups by silver ions [41]. The spectrum can exhibit a shift towards the red end or the blue end depending upon the particle size, shape, state of aggregation and the surrounding dielectric medium [42]. Physico-chemical conditions of reaction mixture such as pH, reaction temperature, leaf extract

concentration and silver nitrate concentration have been standardized to obtain sharp intense absorption peaks at 406 nm

in *M. arundinacea* respectively, which indicate the formation of small-sized mono-dispersed silver nanoparticles.

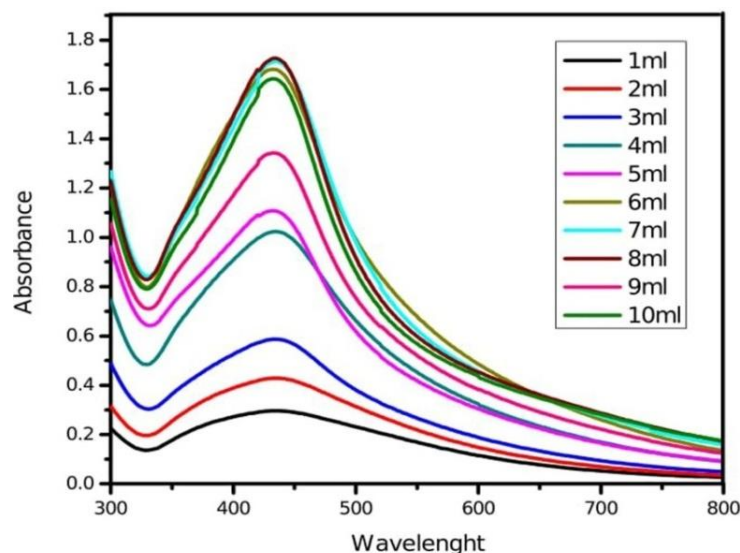


Fig. 4: UV-Vis absorption spectra of silver nanoparticles synthesized by different leaf extract concentration of *M. arundinacea*

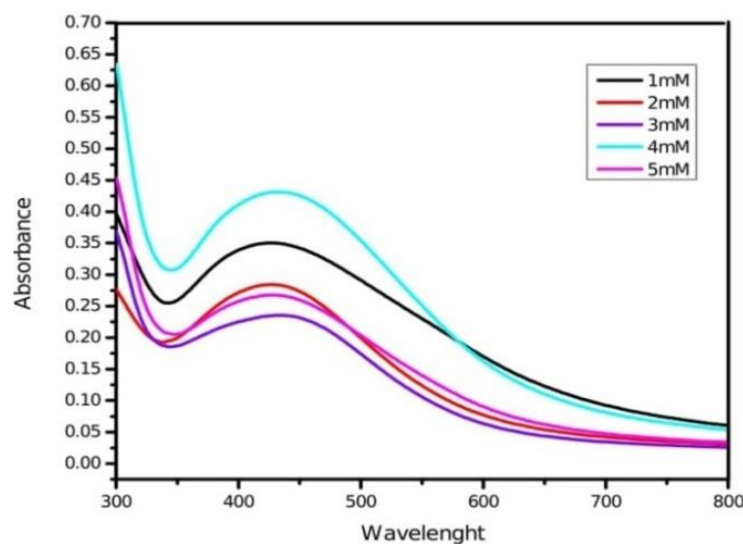


Fig. 5: UV-Vis absorption spectra of silver nanoparticles synthesized by leaf extract of *M. arundinacea* at different silver nitrate concentration

## FTIR

FTIR measurements were carried out to identify the possible reducing biomolecules in the plant (leaf) extract responsible for the formation of silver nanoparticles. Fig. 6 (a and b) and table 1 show that the FTIR spectra of *M. arundinacea* leaf extract and silver nanoparticles, respectively. The band at  $\sim 3400\text{ cm}^{-1}$  arises due to O-H stretching of phenols. The intensity of band was increased in silver nanoparticles compared to plant extract, and this may be due to the binding of silver ions with hydroxyl groups. The band shift in the hydroxyl groups and increased band intensities for carbonyl groups in FTIR spectra confirm the oxidation of these functional groups during synthesis both hydroxyl and carbonyl groups are involved in the reduction and capping of silver nanoparticles. The spectra of silver nanoparticles synthesized by *M. arundinacea* showed two small bands occurring at  $\sim 2900$  and  $2800\text{ cm}^{-1}$  assigned to the methylene antisymmetric and symmetric vibration of hydrocarbons present in the protein; similar peaks were observed in *T. asperellum*

[43]. The absorption band at  $\sim 1455\text{ cm}^{-1}$  was observed in *M. arundinacea* was attributed to methylene scissoring vibration. The band at  $\sim 1098\text{ cm}^{-1}$  was observed in the spectra of silver nanoparticles by *M. arundinacea* corresponds to C-O, C-N or C-C stretching of amino acids. The shift in the band positions  $1637\text{--}1640\text{ cm}^{-1}$ ,  $1384\text{--}1401\text{ cm}^{-1}$  suggest that these functional groups are oxidized during the reaction period and responsible for the formation of silver nanoparticles. The leaf extract shows the absorption peaks at  $1314$ ,  $795$ ,  $712\text{ cm}^{-1}$  was due to the  $\text{-NO}_3$  stretching from silver nitrate, C-Cl stretching of alkyl halides and C-H stretching of strong aromatic mono-substituted benzene. The absorption band at  $1102$  and  $800\text{ cm}^{-1}$  observed in the leaf extract and silver nanoparticles of *M. arundinacea* arises due to OH out-of-plane deformation of ascorbic acid and bending vibration of C-H groups of phenyl rings. The spectrum of silver nanoparticles shows band at  $738$  and  $653\text{ cm}^{-1}$  arises due to carbonyl group (C=O) and alpha-glucopyranose rings deformation of carbohydrates and peaks were formed after the bioreduction of nanoparticles. The carbonyl



groups from the flavonoid and polyphenolic compound have a strong ability to bind silver ion, so that the flavonoid and polyphenolic compound could form a coat over the metal nanoparticles to prevent gathering particles. The linkages between

carbonyl groups and metal ion give a well-known signature in the FTIR spectrum. After the bioreduction of  $\text{Ag}^+$ , shift in the peak at  $1740\text{ cm}^{-1}$  is attributed to the binding of carbonyl group with nanoparticles.

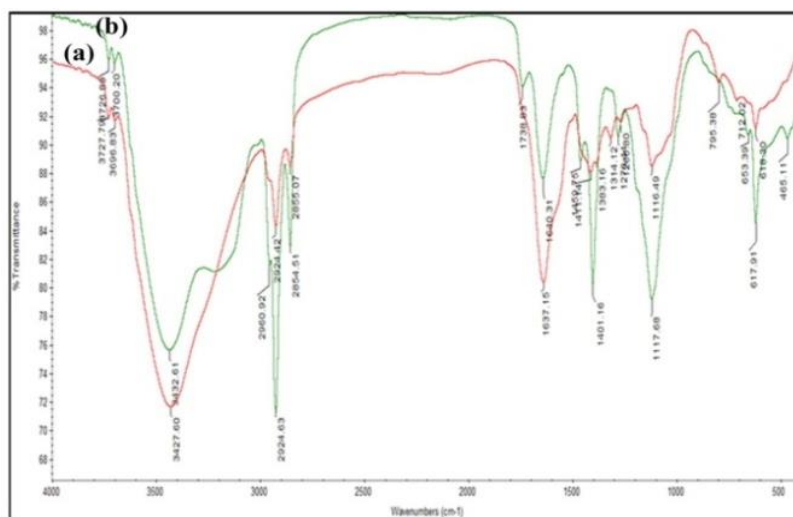


Fig. 6: FTIR spectra of silver nanoparticles synthesized by leaf extract of *M. arundinacea* (a) leaf extract (b) silver nanoparticles solution

Table 1: FTIR absorption peaks and their associated functional groups present in leaf extract and silver nanoparticles synthesized by *M. arundinacea*

S. No.	Leaf extract $\text{cm}^{-1}$	Silver nanoparticles $\text{cm}^{-1}$	Functional groups
1	3427.60	3432.61	N-H/O-H stretching
2	2924.42	2924.63	Methylene symmetric vibrational mode
3	2855.07	2854.51	Methylene antisymmetric vibrational mode
4	-	1738.83	Carbonyl stretching
5	1637.15	1640.31	Amide band I
6	-	1459.75	Methylene scissoring vibrations
7	1383.16	1401.16	C=C stretching of aromatic amines
8	1314.12	-	$-\text{NO}_3$ stretching, which comes from silver nitrate
9	1266.80	1278.44	Amide band III
10	1116.49	1117.68	-C-O-C-linkages
11	795.38	-	Corresponds to C-Cl stretching alkyl halides
12	712.02	-	C-H stretching strong aromatic mono-substituted benzene
13	-	653.39	Alpha-glucopyranose rings deformation of carbohydrates
14	618.30	617.91	C=C group/aromatic rings/C=O stretching in carboxyl groups of proteins

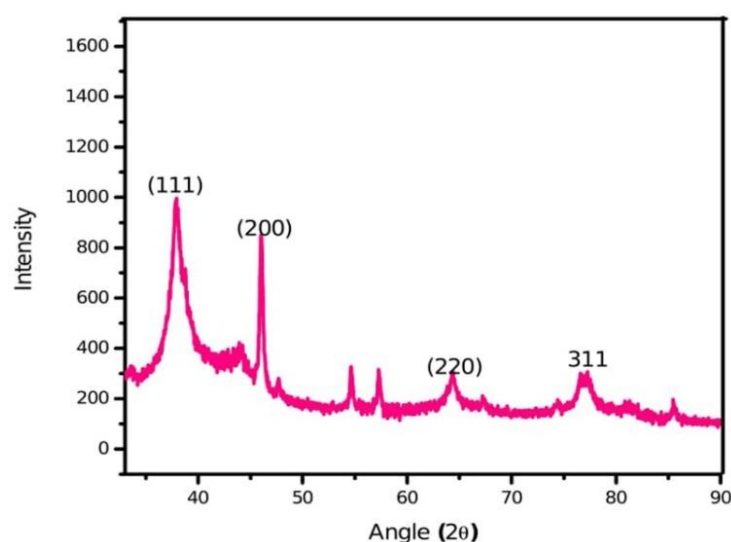


Fig. 7: X-ray diffraction spectrum of silver nanoparticles synthesized by leaf extract of *M. arundinacea*

### X-ray diffraction

It is a very powerful and versatile quantitative technique to elucidate the complete three-dimensional structure of matter at molecular and atomic levels. Elucidation of detail structural features at atomic levels is possible if the specimen is in a crystalline state. The diffraction intensities were recorded from 30° to 90° at 2θ. The XRD pattern of *M. arundinacea* silver nanoparticles synthesized by leaf extract showed four diffraction peaks 37.86°, 44.04°, 64.13°, 76.98° 19° corresponding to (111), (200), (220) and (311) planes respectively (fig. 7). All the peaks in XRD pattern can be readily indexed to a face-centered cubic structure of silver as per database of Joint committee on powder diffraction standards (JCPDS) (file number 893722/04-0783). It confirms the FCC crystalline nature of elemental silver and shape of nanoparticles are spherical and

hexagonal. The Bragg angle which gives the mean size of 8.96 nm of leaf extract of silver nanoparticles synthesized by *M. arundinacea*.

### EDX

The EDX spectrum shows high intense major peak of elemental silver and less intense peak of carbon (C) and oxygen (O) also arises due to the formation of capping layers on the surface of nanoparticles by biomolecules which are present in the leaf extract (fig. 8). The EDX results confirm the reduction of silver ions to elemental silver. Our results are in conformity with other findings [44]. It gives qualitative as well as quantitative status of elements that may be involved in the formation of nanoparticles. The strong signal of elemental peak at approximately 3 keV exist the absorption of metallic silver nanoparticles due to surface plasmon resonance peak [45].

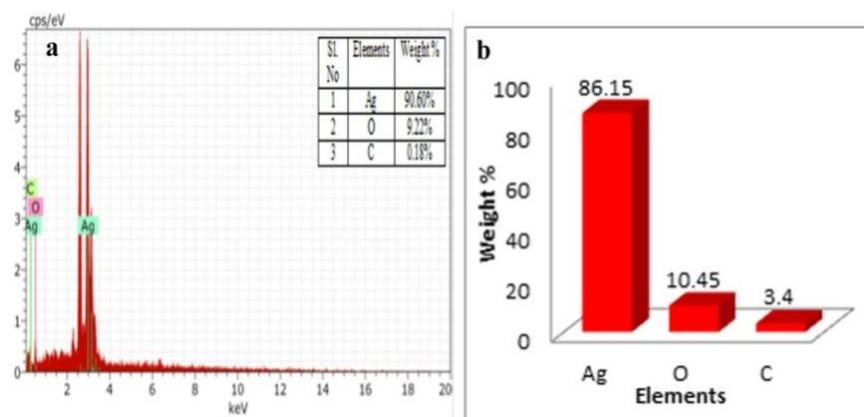


Fig. 8: a) EDX spectrum b) quantitative analysis of biosynthesized silver nanoparticles by using leaf extract of *M. arundinacea*

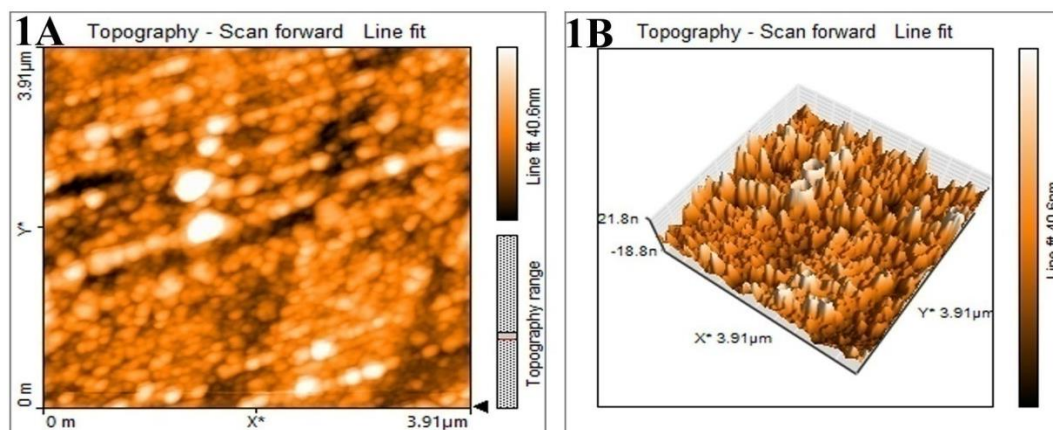


Fig. 9: AFM images of silver nanoparticles synthesized by leaf extract of *M. arundinacea* (1A) Two-dimensional image (1B)-Three-dimensional images

### AFM

The surface morphology of the nanoparticles was visualized by atomic force microscope to analyze the particles shape and size. The two-and three-dimension images of silver nanoparticles synthesized by *M. arundinacea* leaf extract reveals that the particles are monodispersed and spherical in shape with size ranging from 30 to 90 nm (fig. 9). The line graph shows that distribution of the silver nanoparticle and distance between them.

### HR-TEM

The silver nanoparticles synthesized by leaf extract of *M. arundinacea* was analyzed by HR-TEM with different magnification as depicted in fig. 10. The nanoparticles are spherical and no aggregation was evident in fig. 10, size ranges between 5 and 30 nm.

### Antimicrobial activity

The silver nanoparticles showed good antimicrobial activity against both Gram-positive (*S. aureus*, *B. polymyxa*) and Gram negative bacteria (*E. coli*, *S. typhi*). Silver nanoparticles at different concentrations (25 μl, 50 μl, 100 μl and 250 μl) showed different zone of inhibition (ZOI) with respect to different microorganisms (fig. 11 and table 2). In the present investigation, silver nanoparticles synthesized by leaf extracts of *M. arundinacea* show that higher antibacterial activity in Gram negative bacteria than the Gram-positive bacteria. Gram-negative bacteria are more sensitive to silver nanoparticles due to difference in the cell wall structure of bacteria. The cell wall of the Gram-positive bacteria is composed of a thick layer of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides, thus forming more rigid structure leading to

difficult penetration of the silver nanoparticles compared to the Gram-negative bacteria where the cell wall possesses thinner layer of peptidoglycan [46]. Due to species specificity *S. typhi* are more sensitive to silver nanoparticles compared to *E. coli*. The *E. coli* was shown sensitive towards the silver nanoparticles of leaf extract of *M. arundinacea* ( $5.100 \pm 0.078$  mm). The highest zone of inhibition  $7.033 \pm 0.033$  mm was observed in *S. typhi* at  $250 \mu\text{l}^{**}$  and lowest zone of inhibition  $4.100 \pm 0.066$  mm was seen in *B. polymyxa* at  $250 \mu\text{l}^{**}$ . The moderate zone of inhibition  $6.033 \pm 0.033$  mm and  $5.100 \pm 0.078$  mm was observed in *S. aureus* and *E. coli* at  $250 \mu\text{l}^{**}$  (table 2). As per statistically analysis reveals that zone of inhibition is varied with different concentration. Results obtained varied significantly with each treatments. It was observed that ZOI formed by *E. coli*, *S. typhi*, *S.*

*aureus*, and *B. polymyxa*, was significant at the level of  $p < 0.05$ . The mechanism behind the activity of silver nanoparticles on bacteria not yet fully explored; the three most common mechanisms proposed to date are (1) the disruption of ATP production and DNA replication by uptake of free silver ions, (2) Reactive Oxygen Species (ROS) generation by silver and silver nanoparticles and (3) direct damage of silver nanoparticles to the cell membranes [47]. Feng *et al.*, (2008) and Matsumura *et al.*, (2003) proposed that silver nanoparticles release silver ions which interact with thiol groups of many enzymes, thus inactivating most of the respiratory chain enzymes, leading to the formation of reactive oxygen species enzymes, which causes the self-destruction of the bacterial cells and interruption of DNA replication due to damage of the DNA [48-50].

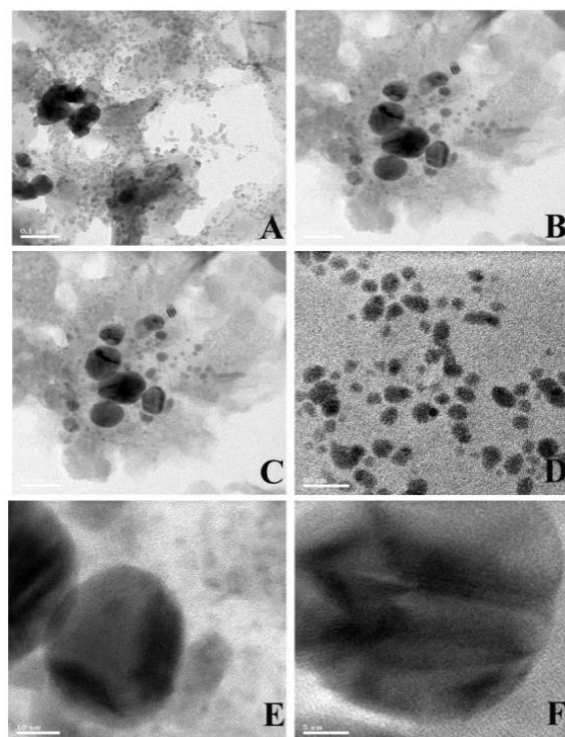


Fig. 10: HR-TEM Images of silver nanoparticles synthesized by leaf extract of *M. arundinacea* (A-0.1  $\mu\text{m}$ , B-100 nm, C-50 nm, D-20 nm, E-10 nm, F-5 nm)

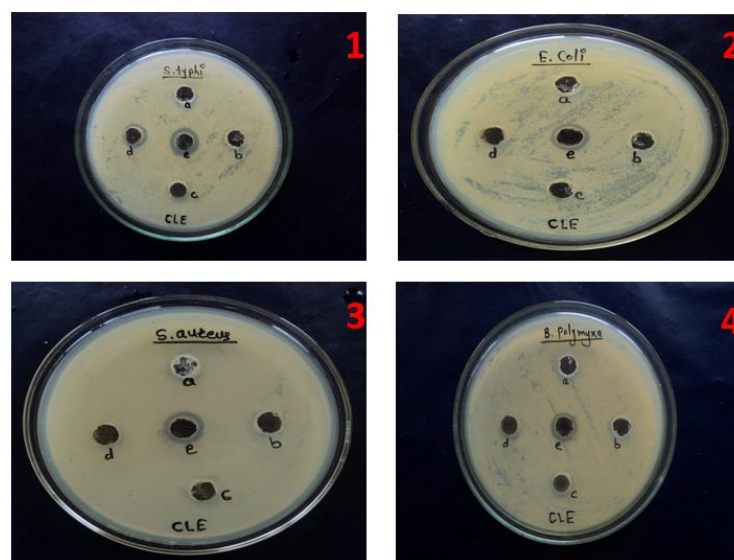


Fig. 11: Antimicrobial activity of concentrations of silver nanoparticles 1) *Salmonella typhi* 2) *Escherichia coli* 3) *Staphylococcus aureus* 4) *Bacillus polymyxa*. (a) Control, (b) 25, (c) 50, (d) 100 and (e)  $250 \mu\text{l}$

Table 2: Antibacterial and antifungal activity of silver nanoparticles by leaf extract of *M. arundinacea*

Different concentration of AgNPs (µl)	Zone of inhibition (mm)			
	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Bacillus polymyxa</i>
Control	-	-	-	-
25	-	-	-	-
50	-	-	-	-
100	-	2.100±0.058 <sup>b</sup>	-	-
250	5.100±0.078 <sup>a</sup>	7.033±0.033 <sup>a</sup>	6.033±0.033 <sup>a</sup>	4.100±0.066 <sup>a</sup>

Note: Each value represents the mean ±SE of triplicates; mean in each column followed by similar letters are not significantly different at 5 % probability level using tukey's test

## CONCLUSION

Plant-mediated biosynthesis offers a rapid, cheap, clean, safe and eco-friendly approach. The spherical shaped silver nanoparticles were formed by using leaf extract as reductant and stabilizer. The silver nanoparticles were tunable by simply changing the pH, reaction temperature, leaf extract concentration and silver nitrate concentration of the reactions. The influences of physico-chemical factors increase reduction of silver precursor due to increased activity of leaf extract constituent. As a result, the number of nucleus and, thus size of the silver nanoparticles decreased with basic pH higher temperature of the reaction. The silver particles became more spherical-like in shape. The synthesized silver nanoparticles showed good antimicrobial activity against multi-drug resistant *Staphylococcus aureus*, *Bacillus polymyxa*, *Escherichia coli*, *Salmonella typhi*. In the present investigation, silver nanoparticles highly toxic to *Salmonella typhi*. The Gram-negative bacteria shows highest antimicrobial activity than Gram-positive bacteria.

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## AUTHORS CONTRIBUTIONS

Research was done by the main author Veena Rokhade under the guidance of Prof. T. C. Taranath.

## CONFLICTS OF INTERESTS

There are no conflicts of interest

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