



Biosynthesis of Silver Nanoparticles using *Mangifera Indica* (Mango Leaves) and Their Antimicrobial and Antioxidant Studies

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Abstract : The field of nanotechnology is the most active area of research in modern material science. Though there are many chemical as well as physical methods, green synthesis of nanomaterials is the most emerging method of synthesis. In the present work AgNPS were synthesised using the plant leaf extract (Mango Leaves) and their Antimicrobial and antioxidant properties are studied. The synthesized nanocrystals were characterized using UV-VISIBLE SPECTROSCOPY, XRD, FTIR SPECTROSCOPY and SEM. This environmental friendly method provides simple, easy and cost effective faster synthesis of nanoparticles and can be used in medicines.

Key words: Nanoparticles, Antimicrobial, Antioxidant

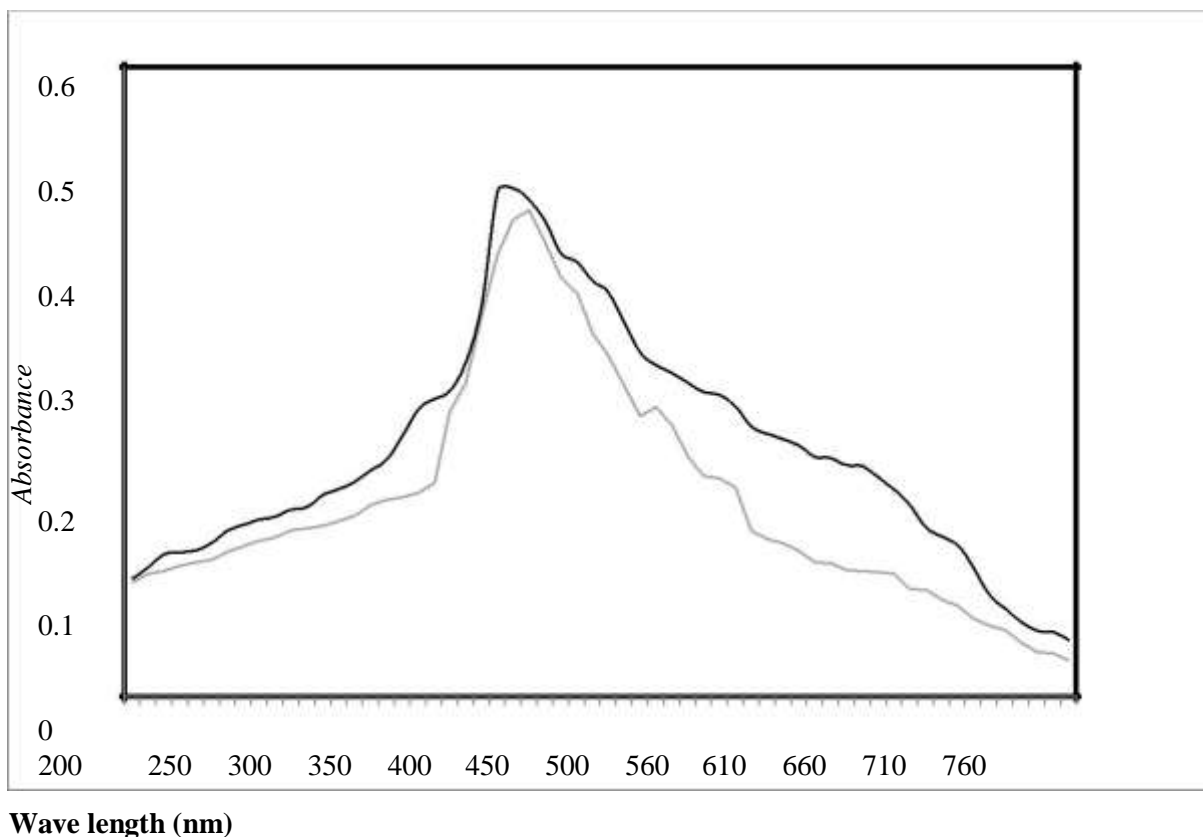
Introduction

Nanotechnology refers a wide range of scientific or technological phenomena that focus on the properties of the nanometer scale (around 0.1-100nm). The basis of this technology is to change the inherent properties of material by reducing its size without changing its chemical composition. This highly potent technology is allowing us to arrange atoms and molecules in most of the ways permitted by physical law. It will give us ability to completely control the structure of materials and developing complex objects with molecular accuracy. [1 to 16]

Results and Discussion

UV- Visible spectroscopic analysis

UV-Vis spectroscopy measurements (Shimadzu, Japan) were carried out at room temperature, operated at a resolution of 1 nm. The reduction of silver ions was monitored by measuring the absorbance of the reaction mixture in a range of wavelength from 200 to 800 nm to find the absorbance peak.



A peaks at 435nm(Young leaves) and 424nm(Mature leaves) were obtained due to surface plasmon resonance of silver nanoparticles. This indicates the shift towards shorter wavelength because of the particle size reduction .

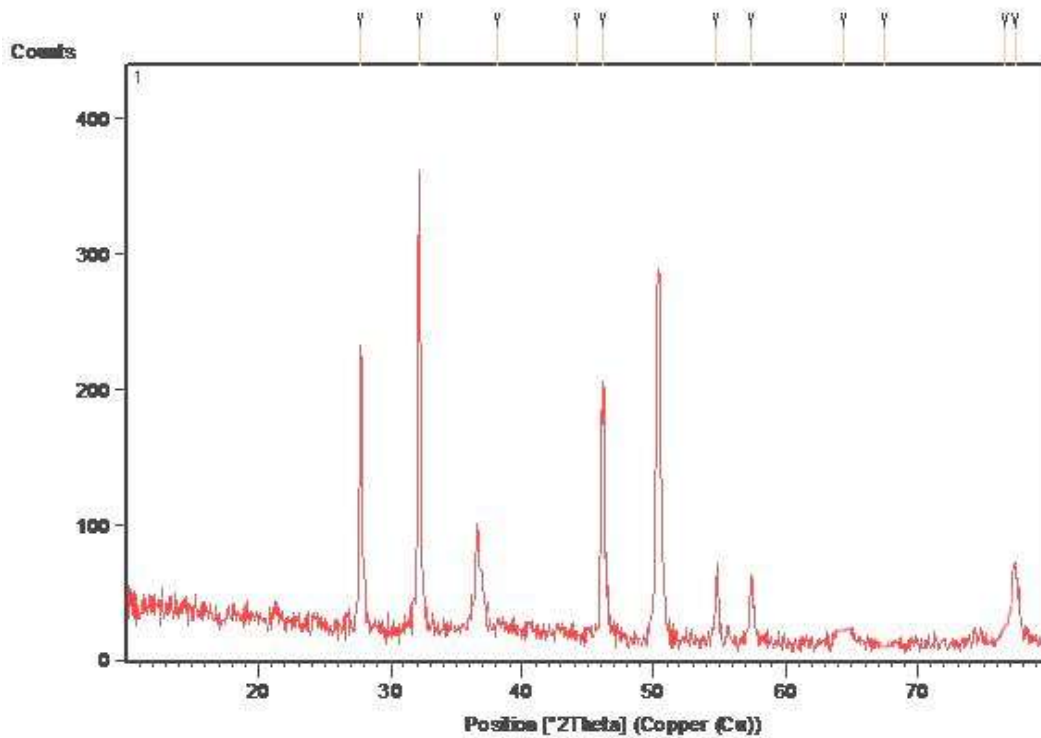
X-ray powder Diffraction analysis

For determination of crystalline size, Scherrer analysis of XRD is used. This technique relies on the broadening of diffraction peaks due to the finite number of diffracting planes. Other factors, such as strain, can broaden XRD peaks, Scherrer analysis generally provides a lower limit on mean crystalline size.

XRD measurements are performed using a Philips diffractometer of „X“ pert company with mono chromatized Cu K α ($\lambda=1.54060\text{\AA}$) radiation. Particle size is determined from the width of XRD peaks using Scherrer's formula,

$$D (\text{nm}) = 0.9\lambda/\beta\cos\theta$$

Where d is the average particle size in nm, λ is the wave length of the X-ray, θ is the bragg diffraction angle in the degrees and β is the full of the peak at half height in radius.



The results obtained from X-ray diffraction study using young mango leaves .

Peak list for young leaves

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	Left d-spacing [Å]	Rel. Int. [%]
26.427(4)	141(10)	0.28(2)	1.5141	46.21
31.223(2)	361(10)	0.25(2)	2.1115	89.26
35.136(4)	91(6)	0.28(2)	2.1315	57.71
45.943()	199(7)	0.23(2)	1.5161	43.09
50.99(2)	276(6)	0.29(4)	1.6172	24.98
55.13(2)	63(2)	0.22(5)	1.6529	11.98
58.96(2)	52(3)	0.27(4)	1.1547	11.96
78.11(2)	45(4)	0.43(2)	1.2921	15.11

The average crystallite size of silver nanoparticles can be found from XRD data and by using

Debye-Scherer's formula:

$$D = K\lambda / \beta \cos\theta$$

Where **D** is the crystal size of silver nanoparticles, λ is the wavelength of X-ray source used (1.541 Å), β is the full width at half maximum of the diffraction peak (FWHM), **K** is the constant of Debye-Scherer equation with value from 0.9 to 1 and θ is the Bragg angle

XRD peak position

The calculations are shown below.

$$\begin{aligned} \text{(i)} \quad D &= 0.9 \cdot 1.54 / 0.28 \cdot \cos 13.213 = 1.386 / 0.2725 \\ &= 5.086 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(ii)} \quad D &= 0.9 \cdot 1.54 / 0.25 \cdot \cos 15.611 \\ &= 1.386 / 0.24077 \\ &= 5.7582 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(iii)} \quad D &= 0.9 \cdot 1.54 / 0.28 \cdot \cos 17.568 = 1.386 / 0.2669 \\ &= 5.1929 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(iv)} \quad D &= 0.9 \cdot 1.54 / 0.23 \cdot \cos 22.97 \\ &= 1.386 / 0.2117 \\ &= 6.5470 \text{ nm} \end{aligned}$$

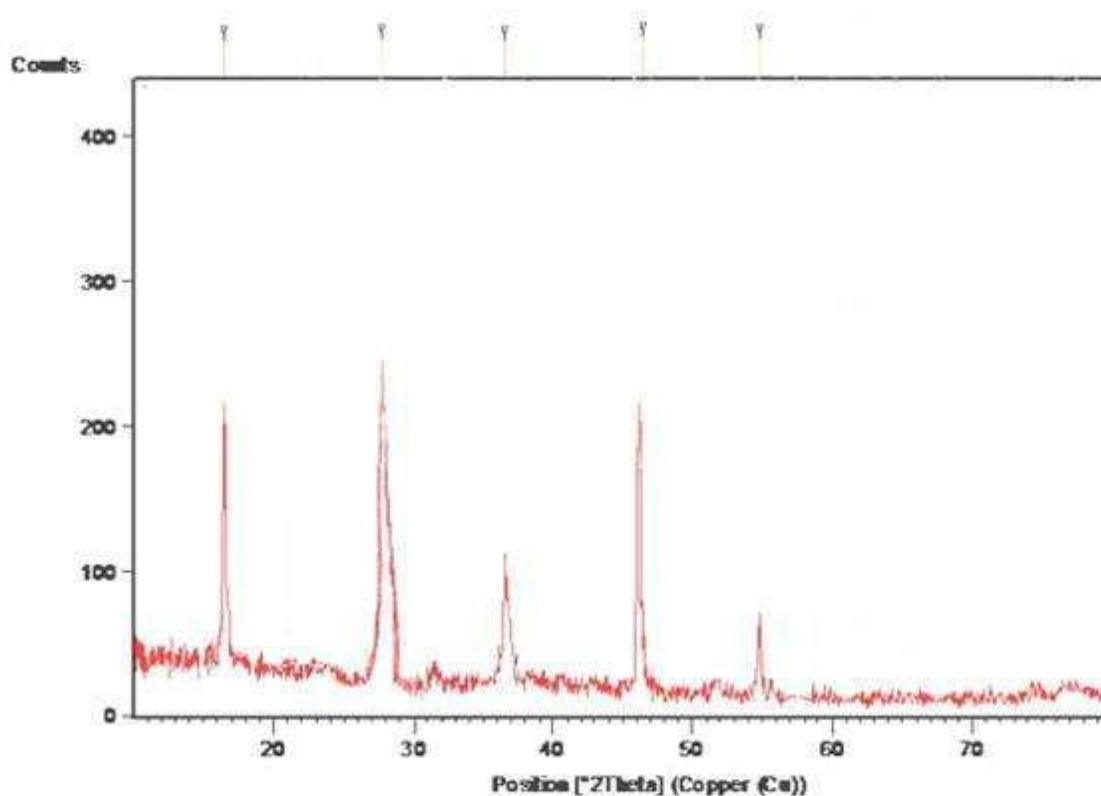
$$\begin{aligned} \text{(v)} \quad D &= 0.9 \cdot 1.54 / 0.29 \cdot \cos 25.495 \\ &= 1.386 / 0.26390 \\ &= 5.251 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(vi)} \quad D &= 0.9 \cdot 1.54 / 0.22 \cdot \cos 27.565 \\ &= 1.386 / 0.1950 \\ &= 7.1076 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(vii)} \quad D &= 0.9 \cdot 1.54 / 0.27 \cdot \cos 29.48 \\ &= 1.386 / 0.2350 \\ &= 5.8978 \text{ nm} \end{aligned}$$

$$\begin{aligned} \text{(viii)} \quad D &= 0.9 \cdot 1.54 / 0.43 \cdot \cos 39.055 \\ &= 1.386 / 0.3339 \\ &= 4.1509 \text{ nm} \end{aligned}$$

The **average crystallite size** of silver nanoparticles was found to be **5.6239nm** and this confirms that the synthesized Ag nanoparticles were nanometric in size.



The results obtained from X-ray diffraction study using mature mango leaves

Peak list for mature leaf

Pos. [°2Th.]	Height [cts]	FWHM [°2Th.]	Left d-spacing [Å]	Rel. Int. [%]
16.721(4)	214(8)	0.22(2)	1.2211	39.78
27.124(4)	260(8)	0.41(2)	2.7321	86.34
35.234(4)	101(3)	0.14(2)	1.5951	23.99
45.326(2)	214(4)	0.32(4)	1.1267	23.21
54.421(2)	65(2)	0.24(4)	1.2243	10.12

The average crystallite size of silver nanoparticles can be found from XRD data and by using

Debye-Scherrer's formula:

$$D = K\lambda / \beta \cos\theta$$

Where **D** is the crystal size of silver nanoparticles, λ is the wavelength of X-ray source used (1.541 Å), β is the full width at half maximum of the diffraction peak (FWHM), **K** is the constant of Debye-Scherrer equation with value from 0.9 to 1 and θ is the Bragg angle

XRD peak position Calculation are done as given below

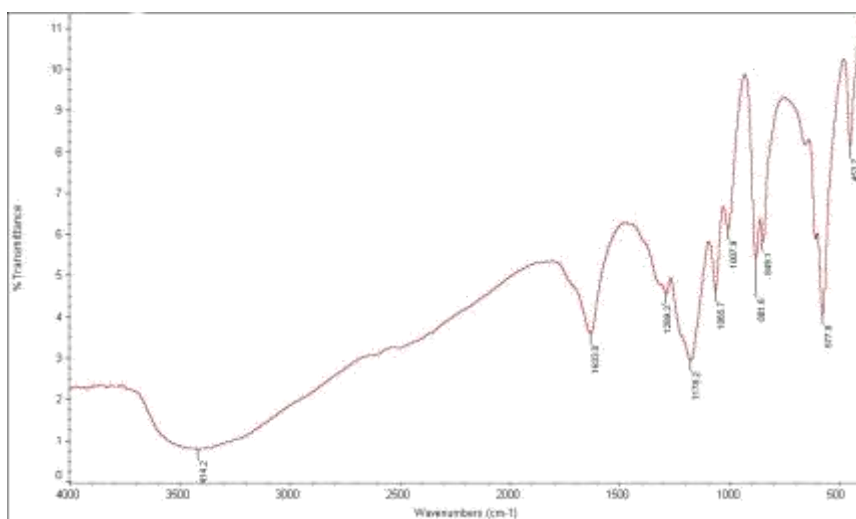
$$(i) \quad D = 0.9 * 1.54 / 0.22 * \cos 8.360$$

$$= 1.386 / 0.2176 = 6.369 \text{ nm}$$

The **average crystallite size** of silver nanoparticles was found to be **6.2853nm** and this confirms that the synthesized Ag nanoparticles were nanometric in size.

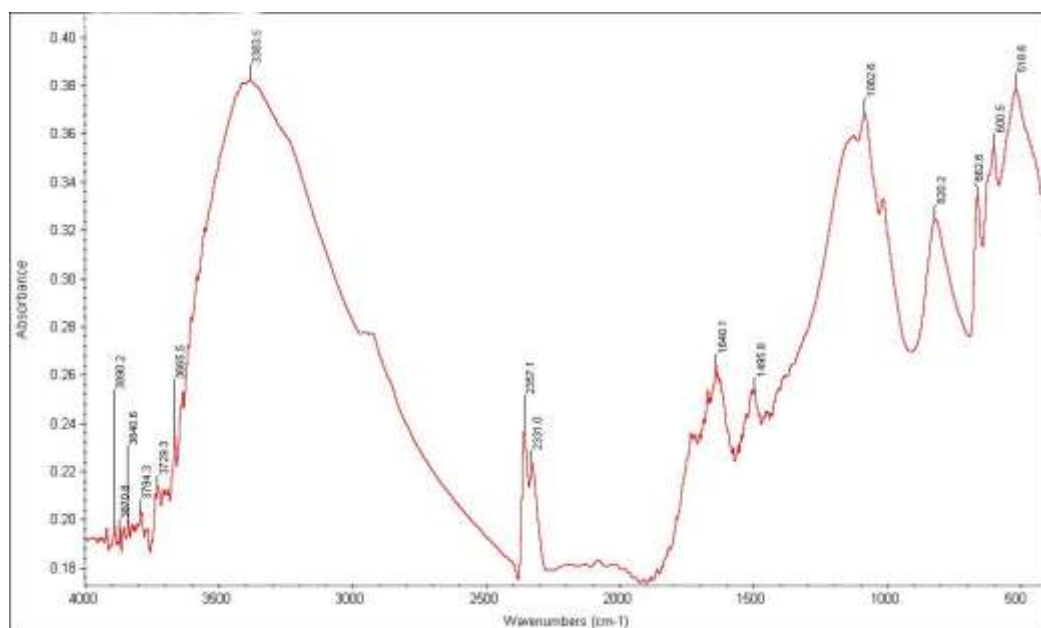
Fourier transform infra-red spectroscopic analysis

FTIR analysis was used for the characterization of the extract and the resulting nanoparticle. The FTIR-spectra of mature leaf shows a band at 3414 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The assignment at 1633 cm^{-1} corresponds to N-H bend primary amines. The peak at 1289 cm^{-1} corresponds to C-N stretching of aromatic amine group and the bands observed at 1178 , 1065 cm^{-1} corresponds to carboxylic acids and esters. The peak at 881 cm^{-1} corresponds to C-H(s) stretch [1]. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, aldehydes and carboxylic acids.



FTIR for mature leaf

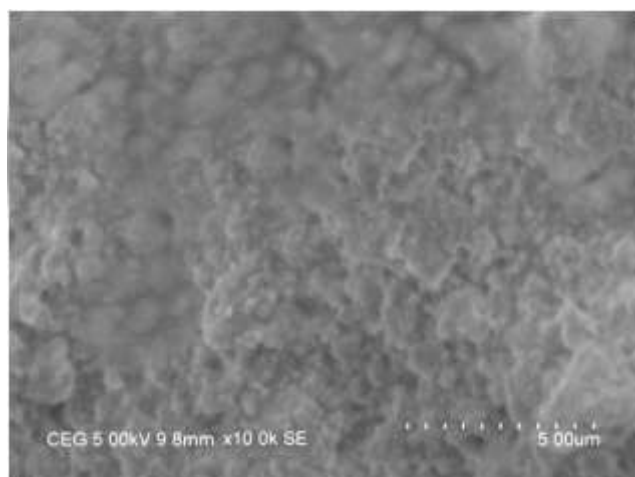
The FTIR spectrum of the mango young leaf extract shows peaks as a band at 3383 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The assignment at 1640 cm^{-1} corresponds to N-H bend primary amines. The peak at 1082 cm^{-1} corresponds to esters. The peak at 820 cm^{-1} corresponds to C-H(s) stretching vibration.



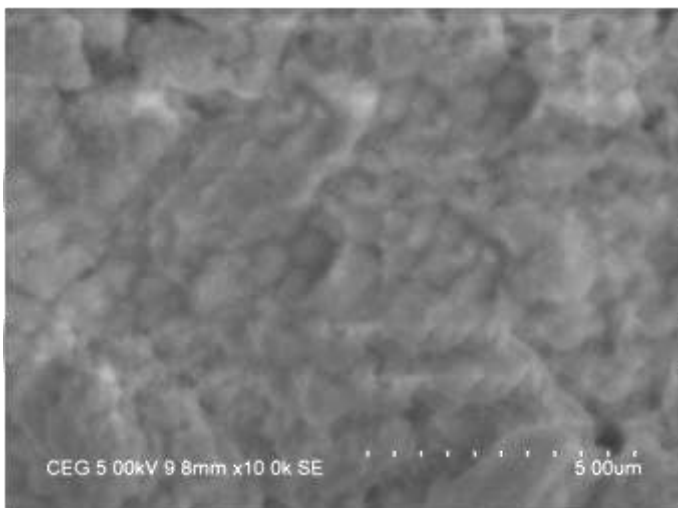
FTIR for young leaf

Scanning Electron Microscope(SEM)

SEM provided further insight into the morphology and size details of silver nanoparticles . From the images it is evident that the morphology of silver nanoparticles from young leaves are oval, spherical and sometime irregular shaped having size ranging **from 19.5nm to 42.55nm** with an average particle size with**23.84nm**.



SEM image of young leaves



SEM image of mature leaves

From the images it is evident that the morphology of silver nanoparticles from mature leaves are spherical and irregular shaped having size ranging **from 18.3nm to 37.21nm** with an average particle size with **21.34nm**.

Antibacterial activity

The antibacterial activities of AgNps were carried out by disc diffusion method. Nutrient agar medium plates were prepared by suspending 28 g of nutrient agar powder in 1 litre of distilled water. Boiled to dissolve completely and sterilized at 121°C for 20-25 minutes. Cooled to 50°C and Swirled thoroughly to mix agar and nutrients. Poured 25-35 ml per petri plate. After solidification, bacterial cultures were swabbed on these plates .

Potato Dextrose Agar was used for the cultivation of fungi. Potato Dextrose Agar was composed of dehydrated Potato Infusion and Dextrose that encourage luxuriant fungal growth. Agar was added as the solidifying agent. Suspended 39 g of the medium in one litter of purified water Heated with frequent agitation and boiled for one minute to completely dissolve the medium. Autoclaved at 121°C for 15 minutes

The sterile discs were dipped in silvernanoparticles solution (50µg/ml) and placed in the nutrient agar plate and kept for incubation at 37°C for 24 hours. Zones of inhibition for

1. Negative Control – DMSO
2. Positive control – Streptomycin
3. Plant extract
4. Silver nanoparticles solution synthesized from Young leaves
5. Silver nanoparticles solution synthesized from mature leaves

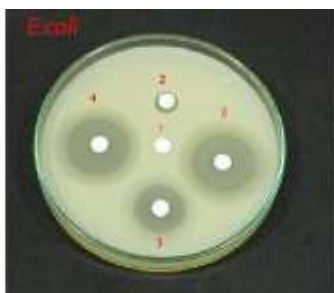
Result of antibacterial activity of young and mature leaves

		Treatment – Zone of Inhibition (in mm)				
					Silver	Silver
		Negative				
			Positive		Plant	nanoparticles
S.No	Microorganism	Control –				
		DMSO	control	–	extract	solution
			Streptomycin		from Young	from Mature
					leaves	leaves

1	<i>Bacillus subtilis</i>	0	2	4	6	8
2	<i>E.coli</i>	0	3	8	10	12
3	<i>Aspergilluslavus</i>	0	4	7	9	10



Zone of inhibition for *Aspergilluslavus*



Zone of inhibition for *E. coli*



Zone of inhibition for *Bacillus subtilis*

The results obtained shows that silver nanoparticles of mature mango leaves show better antimicrobial property in comparison with young leaves. This is in correlation with the size of the silver nanoparticles obtained from XRD analysis.

Antioxidant activity

Interference:

AgNps sample concentrations above 5mg/ml will not give valid results.

Only DMSO or methanol can be used as diluting solvents

Data Acquisition and Calculation:

All values obtained are acquired from colorimeter for assays.

%Antioxidant activity.

$$= \frac{(\text{absorbance at blank}) - (\text{absorbance at test})}{(\text{absorbance at blank})}$$

Result for Antioxidant properties of young and mature leaves

S.NO	SAMPLES		DPPH activity (%)
	Ag Nps	5mg	74.21
1	From Young mango Leaves	3mg	55.78
		1mg	42.76
2	Ag Nps	5mg	84.98
	From mature mango leaves	3mg	65.43

		1mg	51.76
3	Standard-BHT		99.9

Conclusion

AgNPs nanoparticles were prepared from the aqueous leaf extracts of mango leaf. The formation of AgNPs were confirmed by the colour change from transparent green to dark brown and is also shown calculating uv-visible calculating absorption maximum at the wave length region of 435nm for young leaves and 424nm for mature leaves. XRD study reveals that the silver nanoparticles formed as nanometric in size. The synthesized silver nanoparticles shown good antimicrobial activity against aspergillus lavus, E.coli, Bacillus subtilis. In this method there is no need to use high pressure, energy, temperature and toxic chemicals as in case of chemical and physical method. Thus it can be concluded that mango leaf extract can be used as simple low cost and eco friendly biomaterial for synthesis of AgNPs with high antimicrobial and antioxidant activity.

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