

CATALYTIC REDUCTION OF P-NITRO PHENOL USING GREEN SYNTHESIZED SILVER  
NANOPARTICLES AND THEIR ANTI-BACTERIAL ACTIVITY

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**ABSTRACT:** In this work, we report a simple and green approach method for the green synthesis of stable silver nano particles (AgNPs) using *Syzygium cumini* leaf extract. Leaf extract acts as both reducing and stabilizing agent. The synthesized nanoparticles were characterized using UV-visible spectroscopy (UV-vis), Fourier transform electron microscopy (FTIR), X-ray powder diffraction (XRD), Scanning electron microscopy (SEM), zeta Potential and Transmission electron microscopy (TEM). The synthesized AgNPs was characterized by a peak of 439-441 nm. The influence of reaction time on the synthesis of nanoparticles were studied. Zeta potential value -14.1 shows a moderate stable of silver nanoparticles with *Syzygium cumini* leaf extract. The TEM image clearly shows the synthesized silver nanoparticles shape is spherical and the average size is 13 nm. Synthesized nanoparticles had significant anti-bacterial activity and it also shows good catalytic activity on the reduction of para nitro phenol (4-NP) to para amino phenol (4-AP).

**Key words** silver nanoparticles, *Syzygium cumini* leaf extract, catalytic activity, anti-bacterial activity.

## INTRODUCTION

Nowadays, nanoparticle synthesis is one of the fast growing field due to its physical, chemical, biological applications in all areas. Noble metals silver, gold, platinum nanoparticles has exhibit different applications like drug delivery, Cancer or virus detection, gene detection, catalysis and antibacterial activity. Among the metals silver nanoparticles show potential applications in various fields such as the bio medicinal, catalysis, optics and electronics. Silver nanoparticles exhibit in chemical synthesis involves toxic solvents and very expensive for lab maintenance. On the contrary the green method is easy, ecofriendly and very cheap (Vinod Kumar et al. 2014) (Reddy et al. 2015) (Bankar et al. 2010). Plant extract provide a good alternative source for the nanoparticle production because of several plants are used in the homeopathy medicine. Plant extracts are also exhibit lot of applications in all areas. The extracts are AgNPs using *Acalyphaindica* and their anti-bacterial activity (Krishnaraj et al. 2010), shape directing role of cetyltrimethyl ammonium bromide in the green synthesis of AgNPs using *Azadiractaindica* (Khan et al. 2012), anti-bacterial activity of *Alternanthera dentata* by using AgNPs (Kumar et al. 2014a), anti-bacterial activity of *Ananacoscopus* using AgNPs (Emeka et al. 2013), anti plasmodial activity of *Neem and ashoka* by using AgNPs (Mishra et al. 2013), seed powder extract of *Artocarpusheterophyllus* by using AgNPs (Jagtap and Bapat 2013), anti plasmodial activity of *catharanthus roseus* using AgNPs (Ponarulselvam et al. 2012), stability of AgNPs using *Laxmitulasi* (Subba Rao et al. 2013), AgNPs using *Delphinium denudatum* and their anti-bacterial and mosquito larvicidal activity (Suresh et al. 2014), Larvicidal activity of AgNPs using *ecliptaprostrata* (Raja kumar and Abdul Rahuman 2011), anti-bacterial activity of *euphorbia hirta* by using AuNPs (Annamalai et al. 2013), anti bacterial activity of *paederia foetida* by using AgNPs (Kumar et al. 2014b), Ag NPs using *gloriosa superba* and their anti-bacterial activity (Ashok kumar et al. 2013), AgNPs using *jatropha curcas* (Bar et al. 2009), enhanced anti-bacterial activity of *Mimosaopselengi* by using AgNPs (Prakash et al. 2013), enhanced mitotic cell division and pollen germination activity of *Terminalia arjuna* by using AuNPs (Gopinath et al. 2013).

AgNPs using glucan extraction from *muhroom* and its anti-bacterial activity (Sen et al. 2013), sodium para hydroxyl benzoate isolated from *vitexnegundo* by using AgNPs (Durai et al. 2014), anti-fungal activity of *Piper bitle* using PdNPs (Mallikarjuna et al. 2013).

*Syzigium cumini* fruits, leaves, bark is very important in medicinal area. *Syzigium cumini* reduces the diabetic levels, improves immunity and bone strength, improves the hemoglobin levels, treats for infections, leaves of the tree are good for digestive system and helps to prevent cancer (Mahpara Safdar et al. 2006).

In the present study, the catalytic and anti-bacterial activity of AgNPs using *Syzigium cumini* leaf extract have been investigated. Preparation of many analgesic and antipyretic drugs, such as paracetamol, phenacetin and so many drugs in market needs 4 amino phenol is an intermediate. It is also used in photography developer, corrosion inhibitor and hair dyeing agent. Thus, being a common precursor material for 4 aminophenol. A newer and cheaper method is always demand for the conversion of 4 nitro phenol to 4 amino phenol. In chemical synthesis the conversion having lot of toxic chemicals used (Wang et al. 2010) (Javaid et al. 2013). Therefore, there is a need for an alternative eco-friendly method for the conversion of para nitro phenol to para nitro amine.

## MATERIALS AND METHODS

### Materials

Silver nitrate,  $\text{NABH}_4$ , Para nitro phenol was purchased from S D Fine Chem Limited Mumbai, India. The *Syzigium cumini* leaves were collected from Osmania university botanical garden Telangana.

### Preparation of leaf extract

The fresh plant leaves were washed several times with running tap water and then distilled water. The leaves were kept shade dried. The dried leaves were made into a fine powder. 10 grams of fine leaf powder was dissolved in a 100 ml double distilled water, heated at  $60^\circ\text{C}$  for 15 minutes and filtered first through sterile muslin cloth and then through what Mann filter paper no.1, the filtrate was used for the preparation of silver nanoparticles.

### Synthesis of silver nanoparticles

30 ml of 3mM aqueous solution of silver nitrate was taken in Erlenmeyer flask and add 2.0 ml of *Syzigium cumini* leaf extract at room temperature and collect the samples every 10min, 20min, 30min, 60min, 120 min, 180min, 1, 2, 3...30 Days.

### Characterization

#### UV-Vis Spectroscopy

In order to confirm the formation of AgNPs, The UV-Vis absorption spectra recorded using a UV-Vis spectrophotometer (UV-3600 SHIMADZU) having a scanning range of 200-800 nm against leaf extract.

#### FTIR

FTIR analysis was carried out in order to determine the possible functional groups of *syzigiumcumini* leaf extract, and prepared AgNPs sample. FTIR spectra recorded the affinity-1 (SHIMADZU). In this we used kbr pellet method in the range of 250-4000  $\text{cm}^{-1}$ .

#### XRD

The crystallinity of the silver nanoparticles was studied by XRD (Rigaku, Miniflex) method using  $\text{CuK}\alpha$  radiation.

### Size Distribution and Zeta Potential

To estimate the size and stability of the synthesized AgNPs by using zeta potential method, the synthesized nanoparticle solution was subjected to data acquisition using zeta sizer Nano instrument and measurement were taken at 532 nm,  $35^\circ\text{C}$  with  $90^\circ$  detection angle.

#### SEM

To determine the morphology of the synthesized AgNPs using leaf extract, the samples were analyzed with zeiss 700 Scanning electron microscope (SEM). EDX was used for elemental analysis in the sample.

#### TEM

The morphology and size of the synthesized AgNPs was distinguished by TEM. The sample grid for TEM measurement was prepared by placing a drop of aqueous AgNPs dispersion on the carbon coated copper grid and subsequently evaporating the water naturally at ambient conditions. The measurement was done on JEOL 2000 FX-II TEM.

### Anti-bacterial property of silver NPs

Anti-bacterial activity of the synthesized AgNPs was carried by disc diffusion method. *Bacillus subtilis* and *Escherichia coli* are gram positive and gram negative were used as a model test strain respectively. Luria –Bertani (LB) agar medium was prepared and transferred in to petri dishes. The medium was make it to solidify and then the petri plates were spread with *Bacillus subtilis* and *Escherichia coli* separately in a Laminar flow hood. Using micro pipette 5micro liter and 10 microliter of silver nanoparticles added to each well to the both plates respectively. The discs were air dried in laminar flow hood and then incubated at 37<sup>0</sup> centigrade for 24h. Then zone of inhibition of bacteria was measured.

### Catalitic Activity of P-Nitro Phenol

The catalytic activity of 4- NP to 4-AP was done in presence of sodium boro hydride and AgNPs. In this we take 1.9 ml of 4-NP and add 0.2m NABH<sub>4</sub> in a cuvette. At this stage we observed immediate color change from light yellow to deep yellow color. then we add 50micro liter 3mM prepared silver nanoparticles we added to the above solution. The reaction was spectro photo metrically monitored in the range of wavelength range 200-700 nm range with different time intervals.

## RESULTS AND DISCUSSION

### UV-Visible spectroscopy

The UV-vis spectroscopy is the most important technique for determining the formation and stability of nanoparticles in aqueous solution. Formation of AgNPs was primarily observed by UV-vis spectroscopy. The UV-vis spectra were recorded AgNPs at different time intervals. The absorption peaks are 10min, 20min, 30 min, 60min, 120 min, 180min, 1, 2, 3...30 Days respectively (fig.1). It is observed from the spectra that the AgNPs SPR peak at 439-441nm. If the reaction time increases the intensity of SPR peak increased indicating the silver ions converted into AgNPs. However the UV-vis spectra recorded after 3 days showed that there was no increase in the absorption which confirmed the reaction completed. The complete reaction mixture was monitored 30 days for stability. Even after 30 days AgNPs shows same absorption intensity with same wavelength without aggregation. Hence the colloidal mixture was stable for 30days, which was supportive and convenient for the synthesis of AgNPs.

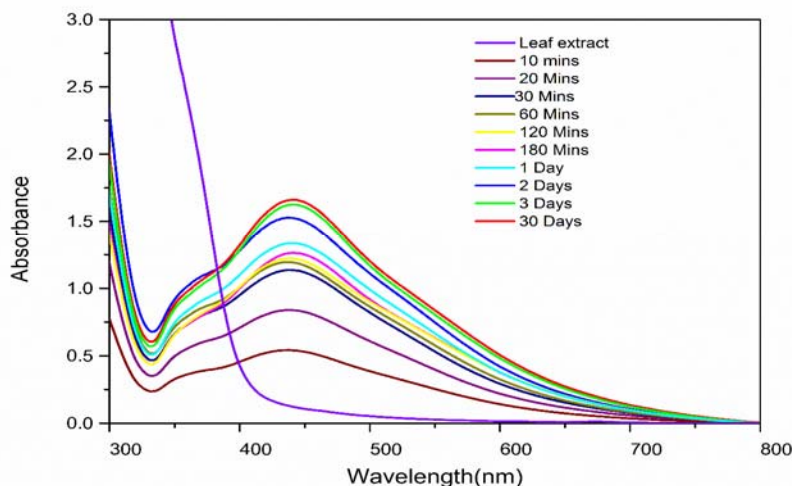


Fig-1: The UV-VIS Spectra of Ag nanoparticles

### FTIR Spectra

Figure 2a and b indicates *Syzygium cumini* leaf extract and AgNPs stabilized *Syzygium cumini* leaf extract. The major stretching frequencies in the spectrum of *Syzygium cumini* leaf extract are observed at 3404, 1739, 1618, 1452, 1350, 1192, 1041 (curve a in figure 2). While AgNPs stabilized *Syzygium cumini* leaf extract shows characteristic peaks at 3419, 1718, 1623, 1452, 1184, 1035 (curve b in figure 2). The bands observed at 3400-3412 cm<sup>-1</sup> indicates poly phenolic -OH group. The peaks 1710-1740 indicates ester group. The absorbance peaks at 1359-1448 cm<sup>-1</sup> corresponds to the amide group respectively. The peaks at 1080-1360 indicates C-N stretching. A shift in the peaks of the FTIR spectrum was observed 1618 to 1623 and the remaining peaks unchanged suggesting the protein possibility will bind AgNPs (Kumar et al. 2010).

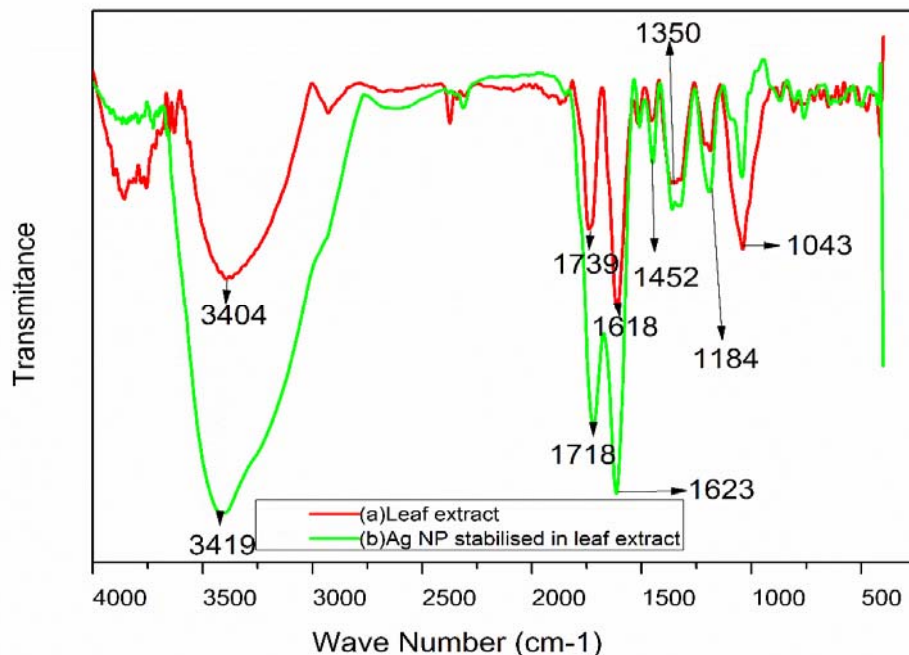


Fig-2: FTIR spectrum of *Syzigium cumini* leaf extract, Ag NP stabilised leaf extract.

### XRD

XRD Spectrum of synthesized AgNPs (fig.3) shows the peaks at  $2\theta$  degree of 38.2, 44.4, 64.6, 77.5 can be attributed to the (111) (200) (220) and (311) crystalline planes of face centered cubic crystalline structure of metallic nanoparticles respectively. The average crystallite sizes are calculated by using Debye-Scherrer formula,

$$D = \frac{K\lambda}{\beta \cos \theta}$$

In this equation where D is the crystalline size of catalyst, K is the Scherrer constant with value from 0.9 to 1,  $\lambda$  the wavelength of the X-ray,  $\beta$  full width at half maximum and the Bragg angle in radians, the average crystallite size of silver nanoparticles was found to be about 12.4 nm.

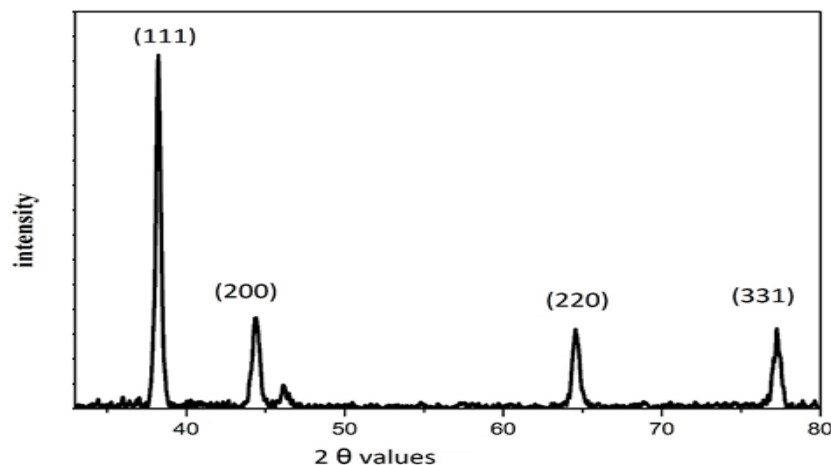
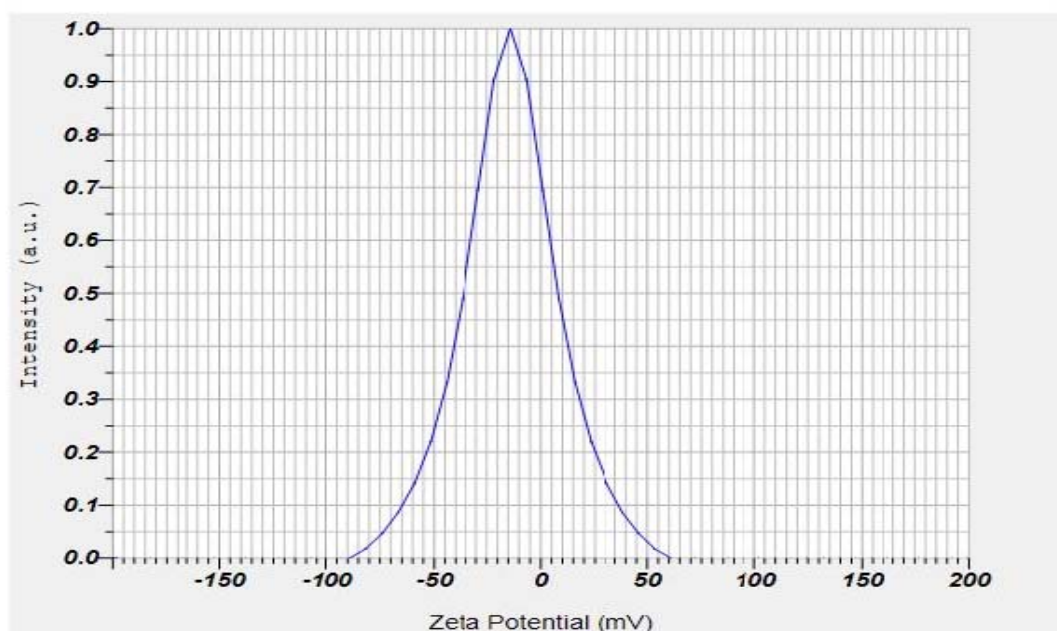


Fig-3: XRD spectra of silver nanoparticles stabilized in *Syzigium cumini* leaf extract.

### Zeta size and zeta potential analysis

From fig.4 The AgNPs size distribution with Z-average value 65 nm and zeta potential value -14.1 shows negatively charged groups in AgNPs solution. The above negative zeta potential value clearly indicates a moderate stable of silver Nano particles with *Syzigium cumini* leaf extract.





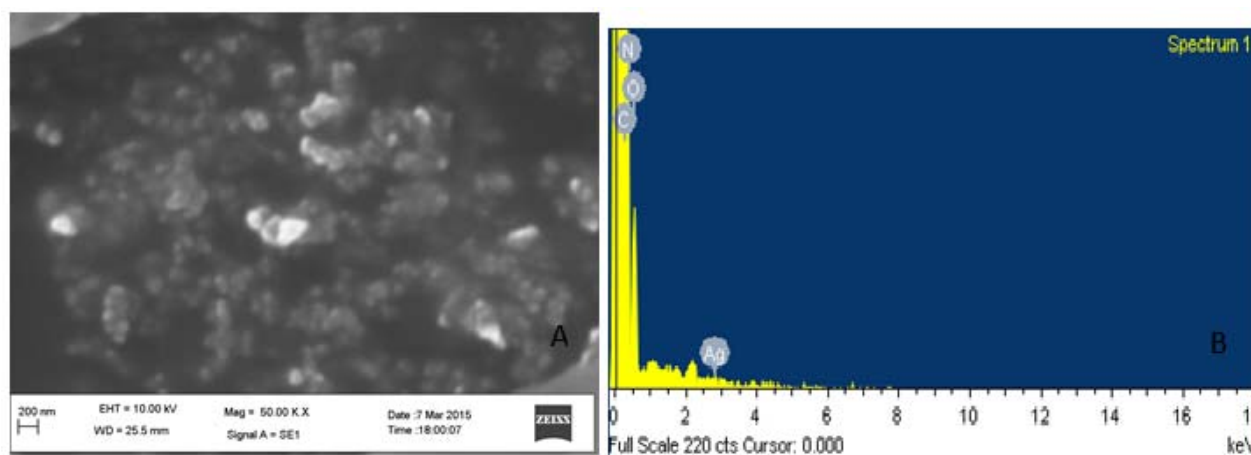
**Fig-4: Zeta potential of synthesized silver nanoparticles**

### SEM Analysis

SEM technique visualized the size and morphology of the AgNPs. The formation of AgNPs clearly shows spherical (fig 5a). Energy dispersive X-ray analysis (EDX) spectra recorded from the silver Nano particles were shown in fig (5b). It is clear that silver signal along with oxygen, nitrogen, carbon peak which may originate from the molecules that are bound to the surface of the AgNPs. There are no silver compound peaks were observed. This data clearly explains silver compound has been reduced completely to AgNPs as determined by the spectrum.

**Table 1: The composition of silver nanoparticles synthesized from *Syzigium cumini* leaf extract**

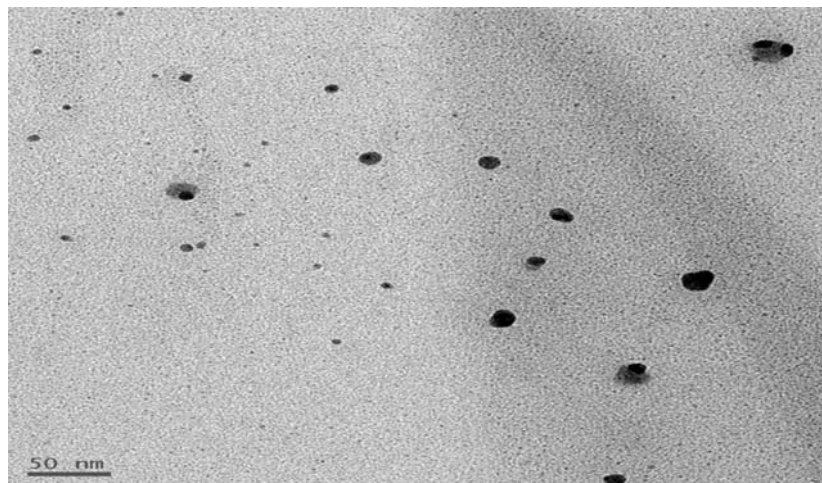
ELEMENT	WEIGHT %	ATOMIC %
C K	53.5	58.45
N K	30.86	28.91
O K	15.38	12.61
Ag L	0.26	0.03
<b>Total</b>	<b>100</b>	



**Fig.5 a and b SEM image EDS spectra of silver nanoparticles stabilized in *Syzigium cumini* leaf extract.**

**TEM**

The size, morphology and shape of synthesized silver AgNPs were elucidated by with the help of TEM. Figure (6) Shows the synthesized AgNPs were mainly spherical shape and some irregular shaped particles was observed. The average particle size obtained from these micrographs was about  $13\pm 2$  nm.



**Fig-6: TEM images of silver nanoparticles**

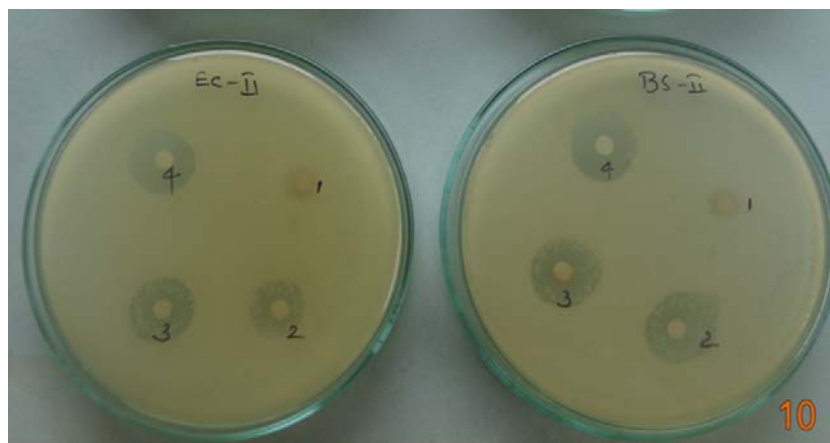
**Antibacterial Activity of Silver Nanoparticles**

The silver nanoparticles exhibited antibacterial activity against *Bacillus subtilis* and *Escherichia coli* are gram positive and gram negative bacteria at different concentration of nanoparticles progressively inhibit the growth (fig 7) as shown. Ampicillin was used positive control for both gram positive and negative discs. *Syzygium cumini* leaf extract itself did not form zone of inhibition. So, it does not exhibit anti-bacterial activity alone. However, silver nanoparticles using leaf extract shows significant anti-bacterial activity.

In the present study, a higher inhibition zone was observed for the gram positive *Bacillus subtilis* compare with gram negative *Escherichia coli* bacteria. It was previously reported that the anti-bacterial activity is directly proportional to the zeta potential. The zeta potential value of the AgNPs was found -14.1. The interaction between gram positive bacteria and AgNPs was stronger than gram negative bacteria. The cell wall of gram negative bacteria provides an outer membrane, which acts as a barrier and protects against antibacterial agents. But the cell wall of gram positive bacteria does not consist of a cell membrane (Kora et al. 2010).

Microorganism	Concentration of AgNPs used	Zone of inhibition(mm)
<i>Bacillus subtilis</i>	5 $\mu$ l	12
	10 $\mu$ l	15
<i>Escherichia coli</i>	5 $\mu$ l	10
	10 $\mu$ l	12

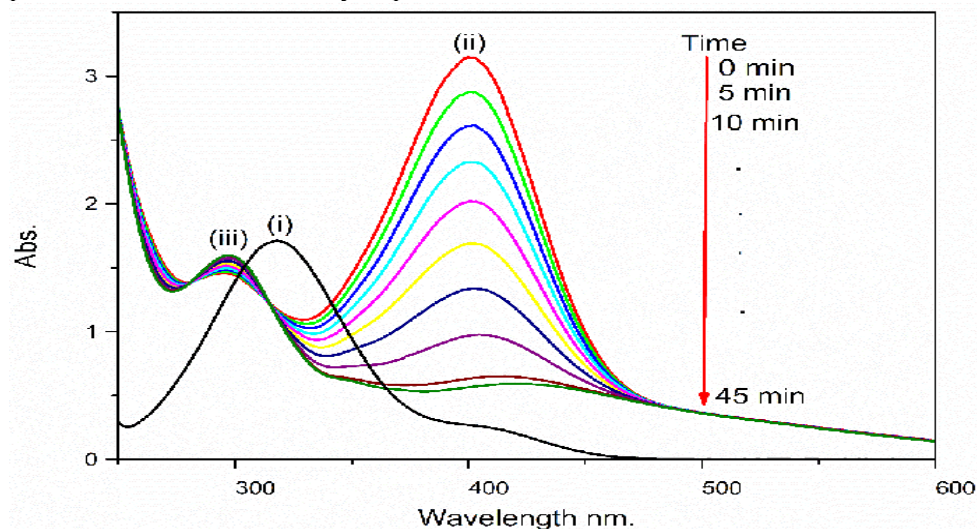
Based on the results, the synthesized AgNPs shows significant anti-bacterial activity.



**Fig-7: Antibacterial activity of AgNPs against B.Subtilis and E.coli after 24 h. inhibition 1) 5µ L *Syzigium cumini* leaf extract.2)5µ L of AgNPs.3)10 µ L AgNPs.4)5µ L ampicillin**

### Catalytic Activity

Catalytic activity of silver nanoparticles explained by 4-NP to 4-AP by using sodium borohydride. In this experiment 1.9ml of 0.2mM 4-NP was taken in a quartz cuvette. 4-NP shows its characteristic absorption peak at 320nm. After the addition of  $\text{NaBH}_4$  the solution immediately changes light yellow to intense yellow and the absorption peak shows at 400 nm, indicating the formation of para nitro phenolate ion (fig.8). In the absence of AgNPs the phenolate ion peak unchanged. When we adding the AgNPs to phenolate ion and placed in a UV-Vis spectrophotometer. The phenolate ion peak was slowly decreased. The reaction was observed every five minutes in the range of 200-600nm. A decrease in the intensity was observed and a new peak observed at 296nm. It clearly shows the 4 amino phenol is formed without any by products formation.



**Fig-8: UV-VIS spectra recorded during the reduction of 4-NP with  $\text{NaBH}_4$  catalysed by AgNPs. I) 4- NP .II) reduction of nitrophenolate ion with time interval of 5 Min's. III) 4 -AP**

### CONCLUSION

*Syzigium cumini* is an efficient source for the synthesis of AgNPs. *Syzigium cumini* acts as both reductant and stabilizer. The synthesized AgNPs were characterized by various techniques. UV-vis spectroscopy clearly shows the formation of silver nanoparticles without agglomeration for 30 days. zeta potential value -14.1 indicate the silver nanoparticles are moderate stable. The XRD pattern showed the synthesized AgNPs shows face centered cubic (FCC) crystalline nature. The TEM images clearly shows the size of the nanoparticles 13 nm and spherical shape. The synthesized AgNPs showed significant antibacterial activity in both gram positive and gram negative bacteria respectively. The catalytic activity of green synthesized silver nanoparticles was determined by the 4-NP to 4-AP reaction.

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