Green synthesis, Characterization and Antibacterial property of Silver Nanoparticles using *Ocimum tenuiflorum, Azadirachta indica and Plectranthus amboinicus* leaf extracts

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Abstract - Silver nanoparticles (AgNPs) have received considerable attention due to their attractive physical, chemical, antimicrobial, anti-inflammatory and wound healing properties. This paper reports simple, cost effective and eco-friendly methods for the preparation of AgNPs using silver nitrate solution as the metal precursor using leaf extracts of *Ocimum tenuiflorum, Azadirachta indica and Plectranthus amboinicus*. The AgNPs obtained were characterized by XRD to analyze the particle size. The XRD patterns confirmed the purity, phase composition and nature of the synthesized nanoparticle. UV-Vis and IR spectroscopy was used as a tool to characterize AgNPs. The study also revealed that AgNPs synthesised from all the medicinal plant extracts had inhibitory activity on the gram positive and negative human pathogenic bacteriae *Bacillus* and *Klebsiella*. The broad spectrum of bioactivity of AgNPs makes them promising agents not only to fight infections, but in many other biomedical areas.

Key words: Green Synthesis, XRD, Spectral analysis, Antibacterial study

I. INTRODUCTION

Nanotechnology is one of the modern techniques of material science. The small sized nano particles mean they exhibit enhanced or different properties when compared with the bulk material. The most effectively studied nanoparticles today are those made from noble metals, in particular Silver (Ag), Platinum (Pt), Gold (Au) and Palladium (Pd). Especially silver have drawn the attention of scientists because of their extensive application in the development of new technology in the areas of electronics, material science, nanomedicines, nano- biotechnology and nanotoxicology. New applications of nano particles and nano materials are emerging rapidly. Therefore there is still need for economic, commercially visible as well as environmentally clean synthesis route to prepare silver nano particles (Choudhary R.S et al.,2014).

Physical and chemical methods are more popular for nanoparticle synthesis but the use of toxic compounds limits their applications (Hasna Abdul Salam et al., 2012) and also not economically feasible one. An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. (Sathya et al., 2012). The biosynthesis method employing plant extracts have drawn attention as a simple and viable alternative to chemical procedures and physical methods. The main phytochemicals responsible for the synthesis of nanoparticles from plant extracts are terpenoids, flavones, ketones, aldehyde amides etc. The emergence of nanoscience and technology in the last decades presents opportunities for exploring the bactericidal effect of metal nanoparticles also. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact with microbial membranes and is not merely due to the release of metal ions in solution (Seeram. Hariprasad. et al, 2016).

In this paper AgNPs was synthesized using the extract of *Ocimum tenuiflorum, Azadirachta indica and Plectranthus amboinicus.* The bacterial strains used for the study include *Bacillus species and Klebsiella species. Bacillus* is a rod shaped, gram-positive bacteria and *Klebsiella* is a genus of gram negative, rod shaped bacteria with a prominent polysaccharide-based capsule.

The main objectives of the study includes i) Green synthesis of AgNPs using plant extracts from *Plectranthus amboinicus*, *Ocimum tenuiflorum*, and *Azadirachata indica*. ii) Characterization of AgNPs using X-ray Diffraction, UV-Vis Spectroscopy and FTIR spectroscopy iii) Particle size determination of AgNps iv) Antibacterial study of AgNPs against gram positive (Bacillus sp.) and gram negative (Klebsiella sp.) bacterial strains.

II. MATERIAL AND METHODS

Preparation of Silver nanoparticles

The method involves collection of plant samples, preparation of aqueous extract of the leaf samples and synthesis of AgNPs. The leaves selected were *Ocimum tenuiflorum, Azadirachta* *indica and Plectranthus amboinicus*. The collected leaf samples were cleaned to remove adhering impurities, sand particles and dust. Then the sample was soaked in distilled water, washed and dried. 20 grams of dried leaves was boiled with 200 ml distilled water for 30 minutes. Then the resulting crude extract was filtered through Whatman no.40 filter paper. 100 ml of the aqueous solution of plant extract was added to 50 ml of 1 molar silver nitrate solution. The solution was allowed to react at room temperature. AgNPs formed was filtered, washed and dried. The dried samples were characterized using XRD and IR.

Antimicrobial study

1 milli molar silver nitrate solution was used for the study. 90 ml of 1 milli molar silver nitrate solution was mixed with 10 ml of plant extract. Sterilized nutrient agar medium was used to culture bacteria. The method followed for the antibacterial study was Kirby-Baur Disc diffusion method. Tests were repeated four times and the mean of zone of inhibitions produced were determined. Results are expressed as mean value of diameters of zone of inhibition for four replications. Using commercial antibiotic Ampicilin a control was also maintained for comparison.

III. RESULT AND DISCUSSION

1. Characterization of silver nanoparticles

XRD analysis

Figure 1a, 1b and 1c shows the XRD of AgNPs obtained from different plant samples. The peaks corresponding to (111), (200), (220) and (311) planes of silver was observed and compared with the standard powder diffraction card of JCPDS (Silver file no.JCP2-03-065-2871). The XRD study confirmed that the resultant particles are (FCC) silver nanoparticles. Table 1 shows the determination of average particle size of AgNPs from four plant extracts.

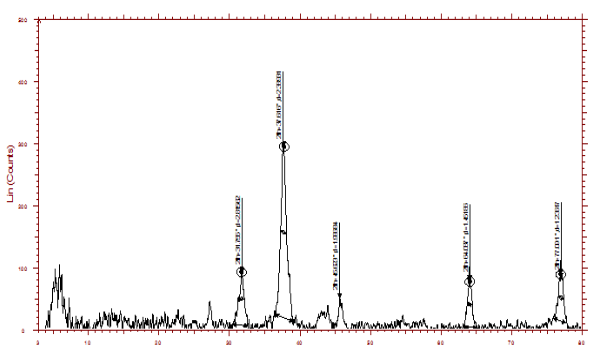

Fig 1a. XRD of Silver nanoparticle prepared from *Azadirachta indica*

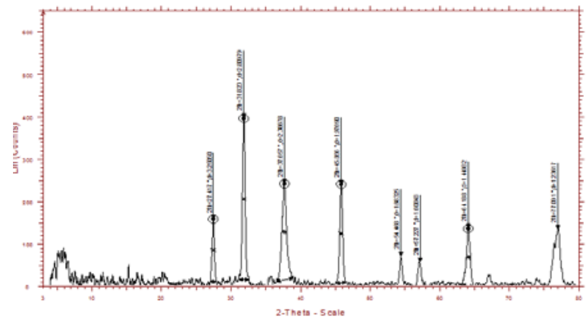

Fig 1b.XRD of Silver nanoparticle prepared from *Plectranthus amboinicus*

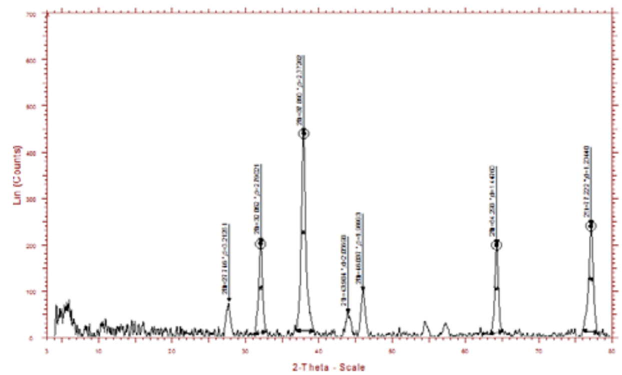

Fig 1c.XRD of Silver nanoparticle prepared from *Ocimum tenuiflorum*

UV-Visible spectroscopy

UV – Visible spectra taken for all the 3 samples showed absorption peaks in the range 400 – 450nm. This is in good agreement with literature (Li T. et al 2007), clearly indicating the formation of AgNPs and is reported to be due to Surface Plasmon resonance. Figure 2, 3 and 4 shows the formation of AgNps prepared from *Plectranthus amboinicus*, *Ocimum tenuiflorum*, and *Azadirachata indica* in comparison with the UV spectrum of the plain plantextract.

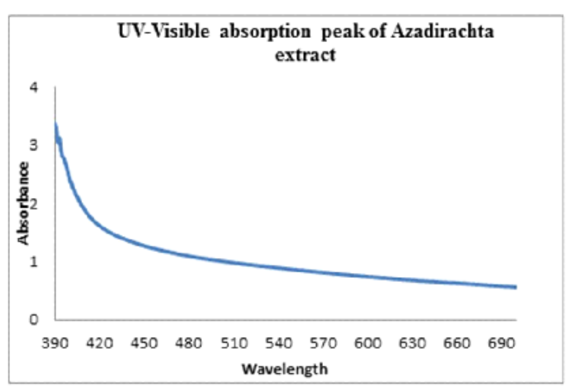

Fig 2a UV visible absorption peak of *Azadirachta* extract

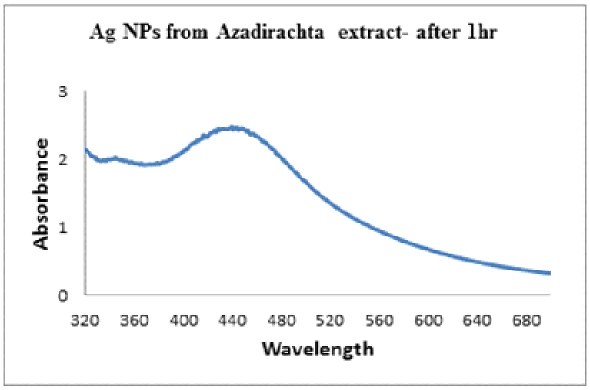

Fig 2b AgNPs from *Azadirachta* extract after 1 hour

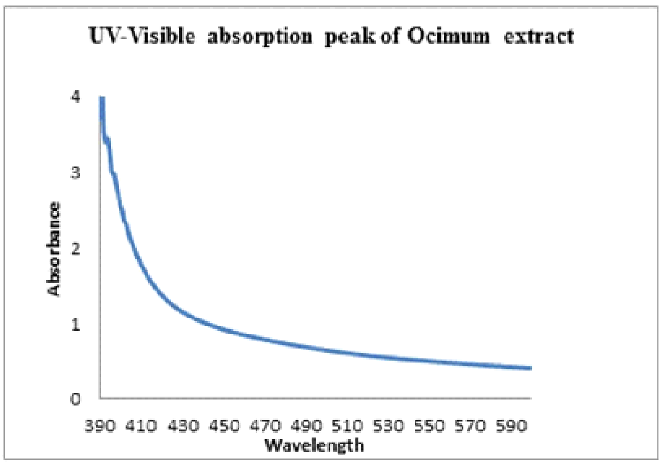

Fig 2c UV visible absorption peak of *Ocimum* extract

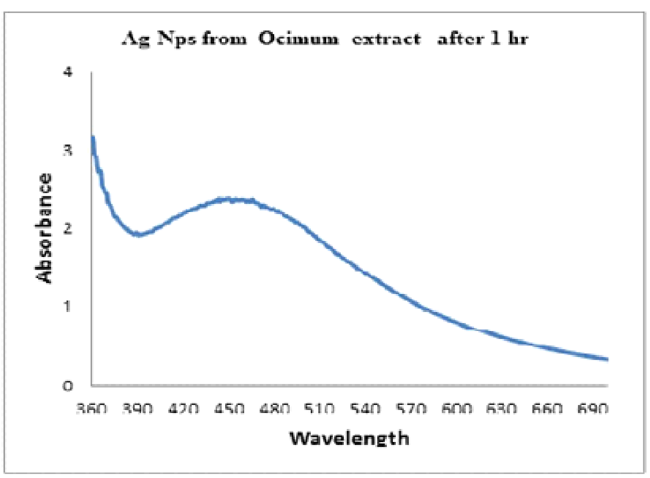

Fig 2d AgNPs from *Ocimum* extract after 1 hour

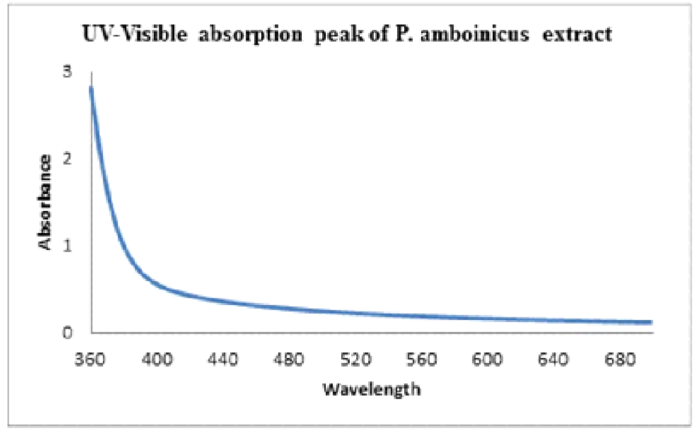

Fig 2e UV visible absorption peak of *P.amboinicus* extract

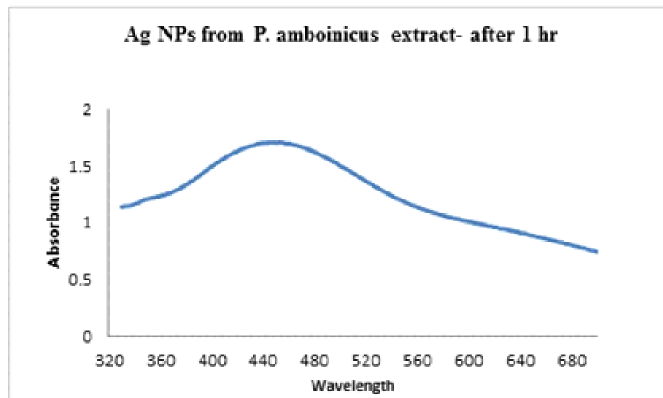

Fig 2f AgNPs from *P.amboinicus* extract after 1 hour

Analysis of FTIR spectrum of AgNPs prepared by *Ocimum tenuiflorum* (tulsi) extract

The band at 3444.27 cm^{-1} corresponds to either OH stretching of hydrogen bonded alcohol and phenols. The peak at 1592.7 cm^{-1} corresponds to N-H bend primary amine. The band at 1340.38 cm^{-1} corresponds to C-N stretching of aromatic amine and 1155.84 cm^{-1}, 1002.98 cm^{-1} corresponds to CO stretch of alcohol, ethers, acid, anhydride. The peak at 1495.14 cm^{-1} and 1434.64 cm^{-1} is due to OH in plane of bending. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional group of alcohols, ketones, aldehydes and carboxylic acid. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues has the stronger ability to bind metal indicating that the proteins could possibly form a coating on the metal nanoparticles to prevent agglomeration and thereby stabilizes them. This suggests that the biological molecules could possibly perform dual function of formation and stabilization of AgNPs in the aqueous medium [K.Mallikarjuna et al 2011]. Figure 5 shows the FTIR spectrum of AgNPs prepared by tulasi extract.

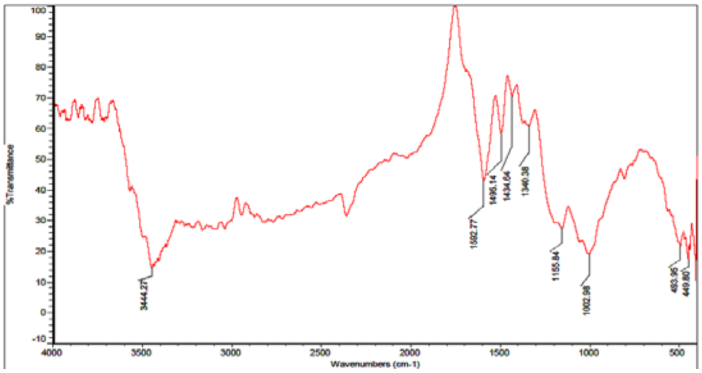

Fig 3. FTIR Spectrum of silver nanoparticles obtained from green synthesis

iv) Antimicrobial Property of AgNPs

AgNPs displayed antimicrobial activity against studied pathogenic microorganisms, with varying degrees, as suggested by the diameter of inhibition zone. Against treatment with bacillus species, commercial antibiotic ampicillin produced an average inhibition zone of 17.4, *ocimum* showed only 7.6, *Azadirichta* showed 9.8 and *Plectranthus* 8.6 mm. Gram negative bacteria *Klebsiella* was more susceptible to AgNPs as indicated by the zones of inhibition produced. Against the treatment with *Klebsiella* ampicillin produced inhibition zone with diameter 14.8 mm where as AgNPs produced from *ocimum* produced very large inhibition zone measuring 18 mm in diameter. *Azadirichta* produced 10 mm and *plectranthus* 9.8 mm of ZOI. Figure 6 &7 shows the Zones of inhibition produced by *Bacillus* and *Klebsiella respectively*.

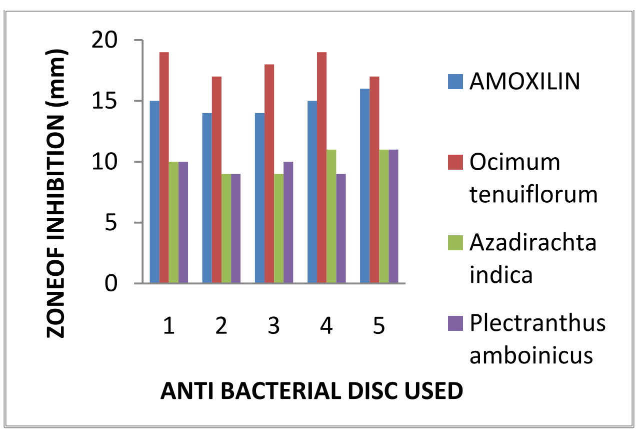

Fig 4. shows the zone of inhibition produced by *Bacillus Sp.*

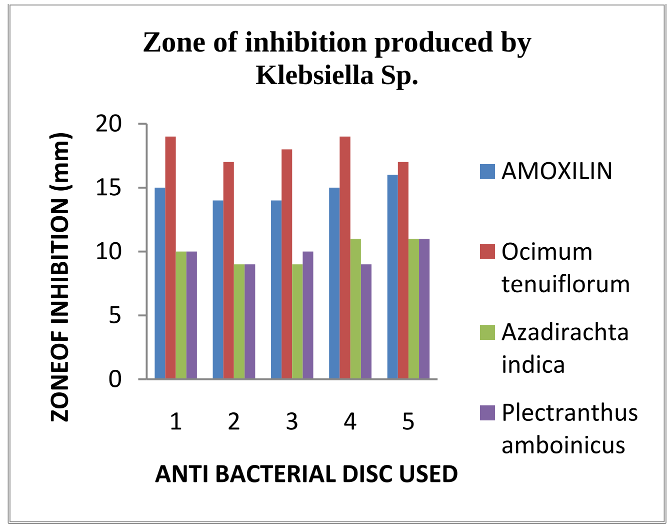

Fig 5. shows the zone of inhibition produced by *Klebsiella Sp.*

Gram positive bacteria bacillus showed smaller inhibition zones against AgNPs when compared with the antibiotic ampicilin. Whereas the gram negative bacteria *Klebsiella* developed larger inhibition zone with AgNPs synthesized from *ocimum*. It should be noted that morphology of the synthesized nanoparticles plays a very important role in the antibacterial property (Pham Van Dong et al., 2012). The Gram negative bacteria *Klebsiella* showed larger zones of inhibition, compared with the Gram positive bacteria Bacillus, which may due to the variation in cell wall composition. The cell wall of Gram positive bacteria composed of a thick peptidoglycan layer, consisting of linear polysaccharide chains cross linked by short peptides, thus forming more rigid structure leading to difficult penetration of the AgNPs, while in Gram negative bacteria the cell wall possesses thinner peptidoglycan layer (Rai et al., 2014).

AgNPs can induce cell death in bacterial cells inducing structural and morphological changes. When they come in contact with bacterial cells, AgNPs will adhere to cell membranes and go into the cell through the membrane. Within the bacterial cell they interacts with phosphate-containing compounds like DNA and RNA, while another portion adheres to the sulphur-containing proteins on the membrane. These interactions will cause the formation of pits and pores on the cell membrane resulting in the release of cellular components to the extracellular fluid due to the osmotic difference. Within the cell DNA replication is also prevented by AgNPs causing the death of the cell.
Further increasing their effect, when silver comes in contact with fluids, it tends to ionize, which increases the nanoparticles bactericidal activity. This has been correlated to the suppression of enzymes and inhibited expression of proteins that relate to the cell's ability to produce ATP. It has been seen that in general, AgNPs with an average size of 10 nm or less show electronic effects that greatly increase their bactericidal activity. This could also be partly due to the fact that as particle size decreases, reactivity increases due to the increase in the surface area to volume ratio (Kaur and Sharma, 2016). Table 2 and 3 shows the statistical analysis of the significance of the data obtained from

zone of inhibition produced by Bacillus and Klebsiella respectively.

IV. CONCLUSION

The present study concluded that the plants *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), *Plectranthus amboinicus* (Panikkurkka) can be used as an excellent source for preparing AgNPs. In our study we used leaf as a source which is easily available, cost effective and environment friendly. The primary confirmatory for the AgNPs was colour changes, XRD and UV-vis absorption spectra. The particle size of AgNPs formed was approximately between 10-15 nm in size. FTIR spectrum of AgNps explains the role of biomolecules in performing the dual function of formation and stabilization of AgNPs in the aqueous medium. Antibacterial study revealed that AgNPs synthesized from all the medicinal plant extracts had inhibitory activity on the gram positive and negative human pathogenic bacteria *Bacillus and Klebsiella*. The inhibition zones were clearly visible on 24 hour incubation at 37^0C. Significant difference were obtained in the inhibition zone diameters (IZDs) for AgNPs obtained from different plant extracts. This shows the difference in antibacterial potency of these plant extracts. The response of the test microbe with these extracts varies greatly. The Gram negative bacteria *Klebsiella* showed larger zones of inhibition, compared with the Gram positive bacteria *Bacillus*, which may due to the variation in cell wall composition. The broad spectrum of bioactivity of AgNPs makes them promising agents not only to fight infections, but in many other biomedical areas.

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