RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles using *Zanthoxylum armatum* Seed Extract and Its Characterization, Efficacy on Antioxidant and Antibacterial Properties

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ABSTRACT

Silver nanoparticles are nanosized particle which has antibacterial and antioxidant activity. The present study highlighted a biocompatible and eco-friendly method for the synthesis of nanoparticles through green synthesis using *Zanthoxylum armatum* seed extract. The synthesized nanoparticle was analyzed through analytical techniques. Physically, the presence of AgNPs was confirmed by color change from pale yellow to yellowish-brown color. UV-Visible spectroscopy revealed a peak at 332nm. And the FTIR showed the presence of N-H stretch, O-H stretch, and CN stretch, confirming the phytochemicals reduction and presence of Alkaloids, flavonoids, and Polyphenols. The DLS depicted low PDI values, which indicated the presence of moderate polydispersity. The in vitro antibacterial activity showed the potential of ZAAgNps against bacteria like *Klebsiella pneumoniae, S. aureus, and E. coli,* with zone of inhibition 19mm, 16mm, and 14mm at their highest concentrations. The antioxidant potency of ZAAgNps was estimated by in vitro assays like DPPH and FRAP, which showed their inhibitory and reducing potential at 79.17% and 78.42%. Compiling these data, it revealed that the synthesized AgNPs from *Zanthoxylum armatum* seed extract have capabilities to be them in biomedical application

Keywords: Silver nanoparticles, *Zanthoxylum armatum*, antibacterial activity, green synthesis

INTRODUCTION

Nanotechnology

In this emerging world, one of the rapidly developing technology or we can say biological weapon is the Nanotechnology. They are the wide range of particles that are sized between 1nm and 100 nm. Based on the overall shape they can be in various form,0D,1D,2D and 3D (Tiwari *et al*., 2012). They are important as their size can influence the optical properties of a substance. The technology by which these particles are studied are known as Nanotechnology. And it is known as the field of research since last century. During a famous lecture at California Institute of Technology in 1959 "There's Plenty of Room at the Bottom" Nobel laureate Richard P. Feynman presented "Nanotechnology" and since there happened to be various revolutionary developments in the Nanotechnology field (Feynman, 1960). The Nanoparticles or Nanomaterials aka Nps, are not the simple ones, they are made up of three layers i.e. (a) outer layer which is also known as surface layer which can be functionalized using metal ions, polymers, surfactants and a range of tiny molecules, (b) the shell layer, which differs chemically from the inner or the core layer in every way and the final (c) inner or the core which is the actual Nps itself (Shin *et al*., 2016).
The Royal Society and Royal Academy of Engineering (2004) have recently defined nanoscience as: *"The study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale"* and nanotechnology as: *"the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanometer scale".*With the help of Nanotechnology, new materials and products are developed at nano-scale and this will eventually lead to great impact on the environment in terms of harm and energy saving. According to the Nanotechnology Products Database (NPD) currently, based on Nanotechnology, there are over 11,149 products available in the markets and 3,910 companies are involved in 68 countries. The various domains of these products are Electronics, Medicine, Construction, Cosmetics, Automobile, Textile, Food, Renewable energies, Agriculture etc.

Types of Nanoparticles

Nanoparticles or the Nanomaterials are classified broadly into various types based on their physical and chemical characteristics, size and morphology. Some of them are discussed below:

Carbon based Nanoparticles

Two significant kinds of Carbon-based nanomaterials are fullerenes and carbon nanotubes (CNTs). Allotropic forms of carbon and other nanomaterials composed of globular hollow cages are seen in fullerenes. Their excellent strength, structure, electron affinity, electrical conductivity and adaptability have generated significant commercial interest (Astefanei *et al*., 2015). Pentagonal and Hexagonal carbon units are organized in these materials and each carbon is sp^2hybridised.Carbon nanotubes (CNTs) are elongated tubular structures with diameters ranging from 1 to 2nm (Ibrahim, 2013). Their electrical properties can be classified as either metallic or semiconducting based on their diameter and chirality (Aqel *et al*., 2012). Structurally, they resemble a graphite sheet that has rolled up. These rolled sheets are classified as single-walled (SWNTs), double-walled (DWNTs), or multi-walled carbon nanotubes (MWNTs) based upon their number of layers. They are primarily produced by depositing carbon precursors, particularly atomic carbon, which is vaporized from graphite through laser ablation or electric arc processes onto metal particles. Recently, carbon nanotube s has also been synthesized using chemical vapor deposition (CVD) techniques (Elliott *et al*., 2013).

Metal Nps

Metal nanoparticles (NPs) are entirely composed of metallic precursors. Owing to their well-established localized surface plasmon resonance (LSPR) properties, these NPs exhibit distinct optoelectrical features. NPs made from alkali and noble metals, such as copper (Cu), silver (Ag), and gold (Au), demonstrate a broad absorption spectrum in the visible region of the electromagnetic solar spectrum. The controlled synthesis of metal NPs, in terms of facet, size, and shape, is crucial in today's advanced materials research (Dreaden *et al*., 2012). Thanks to their exceptional optical characteristics, metal NPs are utilized across various research fields. Coating with gold NPs is commonly employed for scanning electron microscopy (SEM) sampling to improve the electronic stream, facilitating the acquisition of high-quality SEM images.

Ceramic Nps

Ceramic nanoparticles (Nps) are solid inorganic materials that lack metallic properties and are produced through heating and subsequent cooling processes. They can exist in various forms including amorphous, polycrystalline, dense, porous, or hollow structures (Sigmund *et al*., 2006). As a result, these Nps are attracting significant attention from researchers due to their potential in numerous applications, such as catalysis, photocatalysis, dye photodegradation and imaging technologies (Thomas *et al*., 2015).

Polymeric Nps

Nps with an organic base are typically referred to as polymeric nanoparticles (PNPs). Most of them have the shape of nanospheres (Mansha *et al*., 2017). At the outer edge of the spherical surface, the other molecules are adsorbed, while the former are matrix particles with an overall mass that is typically solid. The solid mass is entirely contained within the particle in the latter instance (Rao and Geckeler, 2011). Numerous applications of PNPs can be found as they are easily functionalized (Abd Ellah and Abouelmagd, 2016).

Lipid based Nps

As the name itself says, these nanomaterials have lipid moieties and they are used in various biomedical applications. The lipid Nps is typically spherical, with a diameter between 10 and 1000nm. Lipid nanomaterials have a solid lipid core and a matrix of soluble lipophilic molecules, just like polumericNps. The external core of these nanomaterials where stabilized by surfactants or emulsifiers (Rawat *et al*., 2011). (Mashaghi *et al*.'s work on lipid nanotechnology, 2013) is a specialized field that focuses on the design and synthesis of lipid nanoparticles for a range of uses including drug delivery and carriers (Puri *et al*., 2009).

Semiconductor based Nps

Semiconductor Nps are the nanoscale particles that exhibit tunable optical, magnetic, and electronic properties.These nanocrystals, which typically have sizes between 1 and 100 nm are retrieved from bulk materials which act as a bridge between small molecules and large crystals by exhibiting distinct electronic transitions that resemble isolated atoms and molecules and by making it possible to utilize the beneficial qualities of crystalline materials. The composition-dependent band gap energy, which is the bare minimum of energy needed to excite an electron from the ground state valence energy band into the unoccupied conduction energy band, is a characteristic of bulk semiconductors (Smith AM, Nie S, 2010). They are composed of materials like Silicon, Germanium. Various applications of semiconductor nanoparticles are drug delivery, imaging, and biosensors in biomedical field, also used in LEDs, solar cells, batteries. They also act as water and air purification as part of environment treatment.

Synthesis of Nanoparticles

When it comes to Nanomaterials, to achieve the desired results tremendous significance needs to be given to the synthesis part, as it holds the major stake in controlling the size and shape of the desired nanomaterials. There are basically two broad categories onto which various methods are for the synthesis of Nanostructures. The two classes are: (a) Bottom-up type and (b) Top-down type (Wang and Xia, 2004). In Bottom up type, the nanoparticles are formed from the smaller or simpler substances. And in the Top down method, the bigger materials are broken down into sub-micron level particles, which are ultimately the nanostructures. Theabove-mentioned methods can be broadly put in to three categories which are physical, chemical and biological. Every method has their own advantages and disadvantages and depending upon the availability of the facilities, cost and environmental concerns, the synthesis method is preferred.

Physical Methods

Physical methods for the synthesis of the nanomaterials is the significant approach in the larger field of nanotechnology. It can yield high purity nanomaterials along with minimized contamination. The physical method for nanoparticles synthesis typically followstop-down strategy, where bulk or the large materials are broken down into nanoscale by application of physical forces. There is less chance of contamination because it doesn't call for the use of chemical reagents. The high expense of the laser equipment and the difficulties in expanding the procedure for mass manufacturing, however, continue to be major drawbacks (McNamara *et al*., 2003; Boulos *et al*., 2002). Physical Vapor Deposition (PVD) is another pervasively used physical method for coating and thin firm applications. PVD method of nanomaterials synthesis follows a vacuum environment in which a material is vaporized and then condensed onto a substrate to form nanoparticle. Several sub-techniques, including sputtering, evaporation, and laser ablation, can do this. PVD is perfect for applications requiring high-quality coatings or thin films because it provides exact control over the content and structure of the particles. However, the process necessitates sophisticated equipment and vacuum chambers, which can be costly and challenging to scale up for mass production (Ohring, 2002; Liu *et al*., 2005).

Chemical Method

Similar to physical method of synthesis, chemical methods are also one of the commonly approached synthesis methods in the nanotechnology. By monitoring and adjusting various parameters like temperature, pH, reaction time, and concentration it results in the yield of desired size, shape and composition of the nanoparticles. Methods such as Solvothermal, Hydrothermal, Chemical Vapor Deposition (CVD) and Chemical Reduction, Precipitation, Sol-Gel synthesis, Microemulsion are some of the examples of this category. The Solvothermal and Hydrothermal are one of the most widely used methods for the synthesis Nps. As the name itself says, Solvothermal means they use organic solvent and Hydrothermal means they use water instead solvent for the synthesize of nanomaterials. The key feature of these methods is that they use high-temperature solvents or water to synthesize Nps. Under certain circumstances like proper temperature and pressure, the precursor material is dissolved in the solvent (organic or water) in a sealed container and the reaction is carried out. Under these circumstances, there will be high yield of nanoparticles with uniform size and high crystallinity. These are helpful in the production of inorganic Nps like metal oxides, carbon nanotubes and semiconductors (Feng *et al*., 2007; Zhao *et al*., 2008). Another well-known chemical method is the Chemical Reduction Nps synthesis. In this method, metal salts are reduced to metal nanoparticles with the help of reducing agents, hence the name Chemical reduction. Depending on the system, this extremely adaptable process can be run in either the liquid or gas phase. For instance, Silver nanoparticles are formed from silver nitrate which is reduced in the presence of Sodium citrate. By varying the temperature, reaction time, and reducing agent concentration, the nanoparticles size can be regulated. This method is preferred when there is need to synthesize noble metal nanoparticles like gold, silver, and platinum. Nevertheless, the major disadvantage of this method is potential for the formation of agglomerates and this can reduce the efficacy of the nanomaterials (Xie *et al*., 2015; Daniel and Astruc, 2004). The sol-gel process can be readily adapted for large-scale manufacturing; however, it may necessitate precise control of the reaction parameters to avoid particle aggregation or the development of unwanted phases (Brinker and Scherer, 1990; Aegerter *et al*., 2011).

Biological Methods

The Nanoparticles synthesized through biological methods are often referred to as Green synthesis and it is regarded as the eco-friendly method for the synthesis of Nps. Unlike chemical and physical methods, they are abstained of using harsh and harmful chemicals that can damage the environment. Instead they prefer living organisms like plants, microorganisms, plant extracts to produce nanoparticles. As this method avoids the use of toxic chemicals and harsh chemical reactions, they are cost-effective, sustainable, biocompatible and has high purity. Due to this, they are used in the field of biomedical and environment.

Plant Mediated Synthesis (Green Synthesis)

Plant extracts are one of the most widely used green synthesis method for the synthesis of nanoparticles due to their ease of availability and cost effective. When extract derived from plants are mixed with metal salt solution and kept at room temperature, the nanoparticles are derived within minutes to hours after completing the reaction. Various metals like silver, gold, platinum is derived in this way. For instance, silver nanoparticle derived from plant extracts has exhibited anti-microbial properties which makes them using in the medical devices and wound healing (Siddiqi *et al*., 2018; Kalishwaralal *et al*., 2010). The below figure shows the chemical constituents of plants that are involved in the formation of Nps.

Microbial Mediated Synthesis
Another important green synthesis method for the nanoparticles is by use of microorganism. The microorganisms can be bacteria, algae and fungi. The synthesis is similar to the plant extract but one of the major advantages being the availability of whole cell or cell-free extracts. These extracts reduce the metal salt or ions into nanoparticles. Microorganisms like *Aspergillus niger, Escherichia coli, Bacillus subtilis, Streptomyces and Trichoderma harzianum*are capable to synthesize nanoparticles. (Bansal *et al*., 2009; Jha *et al*., 2009). (Bose and Sahu, 2012).
The Green synthesis of metal nanoparticles have various advantages over the traditional chemical and physical methods. The use of non-toxic chemicals, minimizing the reaction time and reducing the adverse effects of chemicals on environment and majorly biocompatibility has shown the intense and vast usage in the biological and medical fields.

Bacterial Mediated Synthesis
It is another kind of green synthesis method which shows great ability for the nanoparticle formation. This is due to their high metabolic activity and the capacity to produce various reducing agents. Bacteria like Escherichia coli, Staphylococcus, Streptomyces, Bacillus subtilis has the ability to reduce metal salt solution to synthesize nanoparticles. The nanoparticles synthesized from bacteria have excellent stability and functionalization potential making them as controlled scalable process which are ideal for the biomedical applications. (Bose and Sahu, 2012).

Biomolecules Mediated Synthesis
In addition to various Microorganisms and plants, biomolecules are also used for the successful synthesis of nanoparticles. Biomolecules such as enzyme, protein and peptides are used as reducing and stabilizing agents for nanoparticles synthesis. For an instance, enzymes can facilitate the reduction of metal ions and stabilization of nanoparticles resulting as efficient route for nanoparticles synthesis. This way of synthesis of nanoparticles is promising for the production of nanoparticles which have complex surface chemistry, and they are used in the applications like drug delivery and bio sensors (Brennan *et al*., 2015).

Significance of Green Synthesis and Silver Nanoparticles
Though the traditional synthesis methods are effective and widely used, they do result in the environment concern as it involves use of toxic chemicals, harmful environmental byproducts, longer reaction time and complex protocols. This is where the Green synthesis method comes where it minimizes the use of toxic chemicals and are bio compatible unlike the conventional methods. Green synthesis is Eco-friendly alternative, cost-effective and environment sustainable as it involves the use of plant extracts, microorganisms to produce nanoparticles. Reducing agents like Sodium borohydride or hydrazine, are toxic to the environment while natural reducing agents like polyphenols, flavonoids are utilized in green synthesis which are generally found in plant extracts and they also operate under ambient conditions and are low cost making it an ideal method for the synthesis of nanoparticles. Along with this, their enhanced bio compatibility due to the presence of natural capping agents like phytochemicals reduces their toxicity, making it safety and ideal for various bio medical applications. And incorporating this method in the synthesis of silver nanoparticles has ample number of benefits to the world. Silver nanoparticles has been studied extensively as they have shown excellent anti- inflammatory, antimicrobial and antioxidant activities (Raj, M., *et al*. (2009). Even though they can be synthesized through the conventional methods, biological or the green synthesis has been effective and safe when it is used for the medical purposes. The Phytochemicals like flavonoids, phenolic acids, tannins, alkaloids present in the plant extract acts as natural capping agents making them safe. Due to their exceptional antimicrobial activity, silver nanoparticles are incorporated in medical devices, wound healing and also in agriculture pest control. (Kumar *et al*., 2019). They also possess antioxidant activity which reduces the oxidative stress by scavenging the free radical. In addition to this, they are also useful in treating conditions like arthritis and wound making them act as anti-inflammatoryagents. (Patel *et al*., 2018). Kumar, R., *et al*. (2019).

OBJECTIVES OF THE STUDY

- To collect the sample and authenticate.
- To perform qualitative and quantitative analyses of the phytochemical present in the extract.
- To synthesize AgNps (Silver Nanoparticles) using the *Zanthoxylum armatum* seed extract.
- To characterize the synthesized nanoparticles.
- To evaluate the biological properties of nanoparticles.

REVIEW OF LITERATURE AI

Green Synthesis of Silver Nanoparticles and its Significance
Plants are known for their immense medicinal benefits. When chemically synthesized drugs were not available in the early days, people used plants as natural medicine. People generally link them to religious and spiritual thing for many healing and cure. Over the past few years, the environmentally friendly synthesis of silver nanoparticles (AgNPs) has gained significant interest based on their diverse applications and decreased environmental footprint. Traditional physical and chemical synthesis routes, although effective, tend to use harmful chemicals and high amounts of energy. Conversely, green synthesis offers a sustainable, cost-effective, and eco-friendly alternative by using biological species such as plant extracts with a mix of phytochemicals that have the ability to convert silver ions (Ag^{+}) into silver nanoparticles (Ag^{0}).

Role of Light Conditions in Nanoparticle Synthesis
Light has been found to have a marked effect on the synthesis and properties of silver nanoparticles. Light can result in photoactivation of silver nitrate, which can be responsible for uncontrolled reduction and nanoparticle agglomeration. Hence, a number of studies have underscored the necessity of carrying out synthesis in the dark to keep the reaction in check and produce uniform nanoparticle properties.

Green Synthesis Using Some plants
In a study by International Journal of Nanomedicine, 2021, ginger *(Zingiber officinale)* extract was utilized for green AgNPs synthesis. The scientists modified the pH to 12 and left the mixture in dark conditions for 24 hours to avoid photoactivation. Dark brown color change indicated nanoparticle formation, which was further confirmed by UV-Vis spectroscopy and other characterization methods. The dark condition enabled a slow and controlled process of reaction, improving the stability and homogeneity of the particles (International Journal of Nanomedicine, 2021). Silver nanoparticles were synthesized in dark conditions using safflower extract according to a rather interesting study published in Heliyon back in 2021. Incubation of reaction mixture in dark conditions prevented unwanted side reactions precipitated by light thereby resulting in significantly improved AgNPs quality. External conditions like light and pH played a pivotal role in nanoparticle synthesis success (Heliyon 2021).

Ferulago macrocarpa extract facilitates green synthesis rather remarkably according to a study published in Journal of Experimental Nanoscience during 2020. They obtained optimal synthesis using 1 mM silver nitrate at pH 11 and keeping mixture in darkness at 80°C for two and half hours. Nanoparticle formation occurred successfully as solution turned brown signifying a rather complex chemical reaction had taken place quietly. Proper reaction conditions especially darkness play a crucial role in green synthesis procedures according to a recent study published in Journal of Experimental Nanoscience in 2020. A study carried on with the green synthesis of silver nanoparticles with *Zanthoxylum armatum* stem extract showed the successful formation of silver nanoparticles having characteristic absorption in UV-Vis Spectroscopy at 385nm (Habib, U., Ahmad khan, A., Rahman, T. U., Zeb, M. A., and Liaqat, W. (2022). The literature also reviewed the further confirmation of nanoparticle by SEM having the size range of 46.66nm to 60.12nm, spherical shape and XRD showed FCC structure. FTIR analysis exhibited the presence of secondary alcohol and phenol groups over the AgNps. The antibacterial activity against the specific bacteria showed zone of inhibition of 21mm for Staphylococcus aureus, 17mm for Pseudomonas aeruginosa, 18mm for Salmonella enteric.

Though some research have been conducted with different parts of *Z.armatum*, primarily fruits and leaves for nanoparticles synthesis. They have been successfully demonstrated the effectiveness of gold and silver nanoparticles. However, research with seed remain scarce which is highlighted through this study with the hydroalcoholic solvent ratio (70:30). Results from current research on *Zanthoxylum armatum* seed extract in water-ethanol solvent medium which comprises 70% ethanol apply fairly generally. Changes in light exposure and reaction conditions can markedly enhance synthesis yield by drawing cues from experiences garnered through various prior studies.

Zanthoxylum armatum

Zanthoxylum armatum, also known as Timur or tooth ache tree is a traditional medicinal plant which are predominantly grown in Himalayan regions. They are endemic to India, Japan, Korea and China. The different parts of plants are used for various purposes in different parts of region. For an instance, it is used by Bhotiya communities of Uttaranchal for food, medicines and trade. Bhotiyas people of Pithoragarh district of Kumaon region uses fruit while the sticks of *Zanthoxylum armatum*has been collected and traded to the pilgrims of Badrinath, Kedarnath, Yamunotri and Gangotri by peoples of Kamoli district of Garhwal. However, the seeds, barks and leaves are used as condiment in Indian households. Seeds of Z. armatum are rich in essential oils, alkaloids, flavonoids, lignans, and fatty acids, making them suitable for nanoparticle synthesis (Shrestha and Jha, 2010). The plant belongs to Ruttaceae family and it is an aromatic deciduous, spiny shrub which grows to 3.5 meters in height. It is commonly known as Timur in Nepal, Tejphal, Tumru, Trimal in Hindi. Differentvarieties of the tree are found in various parts of India such as Jammu and Kashmir, Manipur, Assam, Nepal, Meghalaya, Nagaland, Orissa, Andhra Pradesh.

Taxonomic Classification

Taxonomical Classification of *Zanthoxylum armatum* (Table:1).

MATERIALS AND METHODS

Preparation of Plant materials

Zanthoxylum armatum seeds were bought from the verified local source and they were rinsed with water to remove the dirt or dusts. The seeds were then shade dried at room temperature for several days so that the moisture present in the seed would be eliminated without the loss of phytochemical constituents. Then they were hand grounded into coarse powder using conventional mortar and pestle and stored in zip lock bag or air tight container. Fig:1. Zanthoxylum armatum seed powdered sample.

Solvent Preparation

Based on the desired components, the solvent was preferred. For this study, water and ethanol (hydroalcoholic) in the ratio 70:30(v/v). Ethanol dissolves non-polar compounds while water extracts polar components providing a broad spectrum of phytochemicals for nanoparticle synthesis.

Phytoextraction

It was carried out in the ratio 1:10 i.e., for 1gm of sample 10ml of solvent was used. Therefore, 10gm of seed powder was dissolved in 100ml of hydroalcoholic solvent in a 200ml beaker and they were covered with aluminium foil to prevent the vaporization of ethanol. They were boiled at 60^0C for 20min and filtered with cheese cloth after cooling. Further, the extraction was kept in the glass beaker sealed with aluminium foil and stored at 4^0C in the refrigerator. The extraction mixture is shown below (Fig: 2. An Extracted Sample).

Nanoparticle Synthesis

Silver nitrate procured from Vels University Central Reagent Lab was used for the synthesis process. To synthesize Silver nanoparticles, 1mM aqueous solution of Silver nitrate was prepared by dissolving approximately 169.7mg of silver nitrate in 1000ml of distilled water. From that, 90ml of the solution was transferred into a 200ml beaker and 10ml of the plant extract was added into it, making it as in the ratio (1:10) and the mixture was continuously stirred at room temperature. They were sealed with aluminium foil and stored at room temperature in dark conditions to prevent photoreduction. The mixture was monitored for any visible color change. The color change from pale yellow to brown color was observed over 24hrs if there's formation of silver nanoparticles. Reaction mixture after the immediate addition of Seed extract (Fig: 3). Reaction mixture after 24hrs of incubation in dark condition (Fig: 4).

Characterization Techniques

After physically confirming the presence of Silver Nanoparticles with the help of color change from pale yellow to dark yellowish-brown, they were further studied for the characterization. The characterization techniques help in the characterization of the Nps synthesized therefore it is the essential step that can confirm the formation of nanoparticles. They also help to understand their morphology, size and stability. The analytical tools for the characterization of nanoparticles are UV-Visible Spectroscopy, Dynamic Light Scattering, Fourier Transform Infrared Spectroscopy.

UV-Visible Spectroscopy

UV-Vis or Ultraviolet-Visible Spectroscopy is the first and foremost analytical technique used for the confirmation of nanoparticles. It measures the light in the ultraviolet and visible region of the spectrum. It is one of the useful techniques used to analyze the amount of substance present in a sample. This technique is based on the principle of Beer Lambert's Law which states that amount of light absorbed is directly proportional to the concentration or amount of substance present in a sample. It is given by $A = \varepsilon bc$ *where,* A is the absorbance of light, b is path length, c is concentration of absorbing compound and ε is the molar absorptivity. The UV-Vis spectrum ranges from 200nm-800nm and each nanoparticle has their own wavelength or to put it scientifically, each metal nanoparticles has a distinct surface plasmon resonance (SPR) band onto which they show peak or spike. Likewise, for AgNps (Silver Nanoparticles) the SPR usually appears between 400nm-450nm due to the continuous oscillation of electrons on the nanoparticles surface when excited by light (Kora and Rastogi, 2013). And peak within this range confirms the presence of AgNps.

Fourier Transform Infrared Spectroscopy

FTIR or Fourier Transform Infrared Spectroscopy is another powerful analytical technique for the characterization of nanoparticles. It helps in the identification of organic and inorganic molecules of the synthesized nanoparticles which are on the surface of nanoparticles after interacting with the plant extract. The principle of FTIR is a beam of infrared radiation is passed through a sample to produce an infrared spectrum, and the transmission percentage of the radiation is recorded as a function of transmitted radiation wave number. A certain portion of infrared radiation is transmitted through the sample and other remaining portion are absorbed. The radiation of infrared absorbed and transmitted by sample produces molecular fingerprint from the spectrum. Two regions can be distinguished in an infrared spectrum. One of them is fingerprint region which is between 1000 and 400 cm^{-1} while another is functional group region where majority of the functional groups exhibit absorption bands in the 4000-1000 cm^{-1} area. These two regions usually detect the molecular vibrations corresponding to various bonds such as O-H, C=O, and N-H where these bonds represents the phytochemicals present in the sample (e.g., alkaloids, phenols, flavonoids). These phytochemicals get participated in the capping and reduction of nanoparticles (Mittal *et al.*, 2013).

Dynamic Light Scattering

Another important analytical technique for the characterization of nanoparticles. Dynamic Light Scattering (DLS) also known as Quasi-elastic light scattering. It helps in the study of size of small particles which ranges from nanometer to sub-micrometer, suspended in a liquid. The fundamentals of DLS is that it is based upon the Brownian movement of particles in a fluid. It determines the hydrodynamic diameter of nanoparticles in suspension by measuring the fluctuations in light intensity scattered by particles undergoing Brownian motion, which are then correlated with particle size. The speed of this motion is inversely proportionally to particle size, i.e., smaller the particle faster they move and inverse. A laser beam is directed at the particle suspension from monochromatic light source. When the laser hits particle in suspension, the light gets scattered in various directions. Overtime, due to the particle movement, the intensity of the scattered light gets fluctuated. And these fluctuations are recorded at fixed angle by a detector. The hydrodynamic diameter is calculated using Stokes-Einstein equation

$$D = k_B T / 3\pi n d_H$$

where,
D= diffusion of coefficient
k_B=Boltzmann constant
T= absolute temperature
n= viscosity of the solvent
d_H= hydrodynamic diameter of the particle
It also provides data on the PDI (polydispersity index) indicating the uniformity of particle size in sample.

Antibacterial Activity
The Antibacterial activity was performed in Avigen Biotech Lab, Chrompet, and Armats Biotech Lab, Guindy, Chennai. The Antibacterial activity which is usually done against the bacterial pathogens was carried out by agar well diffusion method (Mosachristas, 2018). The pure culture of *Klebsiella pneumoniae* was obtained and maintained in Nutrient Agar (NA) slant. The bacterial strain of *Klebsiella pneumoniae* were grown overnight in NA liquid medium or broth on a shaking incubator at 37^0C at 100rpm. The Mueller-Hinton Agar was prepared and sterilized by autoclaving. The inoculum containing a microbial load of $1x10^5$ CFU/ml was then applied to respective agar plates. A 6mm diameter of wells were punched aseptically with sterile well cutter. Then they were loaded with different concentrations of sample (ZAAgNps) (25, 50, 75, 100ug/ml) respectively. Further, 20ul of gentamycin (positive control) into another well which acted as control, 10ug). The plates were incubated at 37^0C for 24hrs. Further, zone of inhibition (mm) was measured which are appeared around the wells. For *Staphylococcus aureus (25923)* and *Escherichia coli (25922)*bacteria, Muller-Hinton Agar was prepared and sterilized by autoclaving. The control used was antibiotic tetracycline (50µg/mL). The *E. coli* and *S. aureus* strains were inoculated in the nutrient broth and incubated overnight at 37^0C. Then on the sterile MHA plates, *S. aureus* and *E. coli* bacterial culture were uniformly spread on their respective plates and using well cutterfour wells (C, 250uL, 500uL, 1000uL) were made in both the plates. Using sterile micropipette, 100uL of sample (ZAAgNps) was added into the wells. The plates were incubated at 37^0C for overnight. The next day zone of inhibitions were measured.

Antioxidant Assay
This assay was carried out in Avigen Biotech Lab, Chrompet, Chennai and Armats Biotech Lab, Guindy, Chennai. Antioxidant activity exhibits the potential of sample to scavenge free radicals. To assess the antioxidant activity, DPPH scavenging activity were performed, by using 1,1-diphenyl-2-picrylhydrazyl (Manzocco *et al*, 1998) and Ferric Reducing Power Assay (FRAP).

DPPH Assay
0.4mM solution of DPPH were prepared using methanol and 2ml of this sample was added to different concentration of the sample ranging from 20ul, 40ul, 60ul, 80ul, and 100ul. And then they were kept at room temperature for 15min. Then, the absorbance was read at 517nm against blank samples. Lower the absorbance of reaction mixture, higher the free radical scavenging activity. The percentage of DPPH radical scavenging is calculated by below equation:

Radical scavenging activity/ Inhibition (%) = (Abs Control-Abs sample/ Abs control) x 100

FRAP Assay
FRAP or Ferric Reducing Antioxidant Power Assay is a method that uses antioxidant as reductants in a redox-linked colorimetric reaction. Different concentrations of the extracts (20 – 120 µg/mL) were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 minutes. Then, 1 mL of 10% trichloroacetic acid was added to the mixture. Further, 1 mL of 0.1% of freshly prepared ferric

chloride was added and the absorbance of the resultant solution was measured at 700 nm. The percentage of reduction was calculated by the below formula:

% of reduction = Sample - Control × 100/ Sample

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

The UV-Visible spectroscopy was carried out through Systronics (India) Limited, UV-VIS Spectrophotometer-119. The sample are analyzed in the UV-Visible region or spectrum which ranges from 200nm -800nm. It is the primary technique used to confirm the formation of nanoparticles or any mixture. After the presence of silver nanoparticles (AgNps) by physical confirmation, this technique is specialized to provides insight into the behavior of nanoparticles through surface plasmon resonance (SPR). In the current research carried out with seed extract of *Zanthoxylum armatum,* in the hydroalcoholic solvent ratio 70:30 displayed absorbance at 332nm. The extraction was carried out under 60^0C for 20 min and further the extract was mixed with 1mM silver nitrate solution for the synthesis of nanoparticles. However, the solvent used in this research is water and ethanol depicted the peak at 332nm which is not corresponding to the characteristics of silver nanoparticles but the literature [9] carried out with the same plant but different part revealed peak at 385nm and confirming the presence of Silver Nanoparticles. Though, it is not corresponding to the characteristics of AgNp, it may have less stability or could have low concentration of silver nanoparticles in the obtained mixture. The lack of SPR band indicates that either the phytochemical concentration in the extract was insufficient to facilitate reduction or it may also be due to reaction conditions. The below figure. 5 describes the SPR of the synthesized silver nanoparticles.

Fourier Transform Infrared Spectroscopy

FTIR was performed in Vels University Central Instrumentation Laboratory with Spectrum Two FT-IR/Sp10 software model, PERKIN ELMER, USA. Fourier Transform Infrared Spectroscopy is another analytical technique which are used to identify the functional groups in a mixture. They are carried out by the measurement of infrared absorption of molecules present in the compound. It provides important information of the phytochemicals that are present in the plant. During the interaction with metal ions, the phytochemicals of the plants react with them and reduces to form nanoscale structures. The functional groups that are involved in the reducing of metal ions to nanoparticle are analyzed and detected by the infrared spectrum and are portrayed as in the form of peaks with %T (Transmission). In the study conducted with dried seed extract of *Zanthoxylum armatum,* in the hydroalcoholic solvent ratio 70:30 displayed two peaks when analysis was carried out with FTIR in the infrared range 4000 to 400cm^{-1}. The peaks were formed at 3341.89cm^{-1}, 51.66%T and 1636.16cm^{-1}, 72.31%T. These peaks were corresponding to the N-H stretch, C-N stretch, O-H stretch, C=O stretch and these are corresponding to the Alkaloids, Flavonoids and Polyphenols. So, FTIR peaks displayed in the Fig 6, showed the presence of phytochemicals like polyphenols, alkaloids and flavonoids. This shows the confirmation of phytochemicals of the silver nanoparticles of *Zanthoxylum armatum*seed extract.

Dynamic Light Scattering

DLS was performed in Vels University Central Instrumentation Laboratory with Nanotrac Wave II model, Microtrac, Inc. USA.Dynamic Light Scattering is a powerful analytical tool in the characterization of nanoparticles. They analyze the size distribution and stability of nanoparticles suspended in liquid media. It is based upon the Brownian motion of particles suspended in liquid medium. When a beam of light usually laser is made pass through the solution mixture, these get hit by the particles in the solutions due to which fluctuations happen. It measures the fluctuations in the intensity of scattered light caused by Brownian motion of particles in suspension and these fluctuations are used to determine the hydrodynamic diameter of the particle. This gives insights of their distribution, agglomeration and their average size. DLS result of ZAAgNps (Fig:7). The study carried out with *Zanthoxylum armatum* seed extract in the hydroalcoholic solvent ratio 70:30 showed three peaks. This may be due to the solvent preference or the reaction conditions that there's size variability. These were identified using the PDI (Polydispersity index) which tells how uniform the sample in the mixture are. The PDI value obtained is 0.24 which indicates there's moderate polydispersity. The lower the PDI values better the quality of particles, i.e., uniformly sized and inverse for the higher PDI values.

Antibacterial Activity

The antibacterial activity was carried out by Agar well diffusion method. The pure culture of bacterial strain *Klebsiella pneumonia* was obtained and maintained in nutrient agar slant for 37^{0}C. Further, the bacterial strain of *Klebsiella pneumoniae* were grown overnight in NA liquid medium or broth on a shaking incubator at 37^{0}C at 100rpm. The inoculum containing a microbial load of 1x10^5 CFU/ml was then applied to respective agar plates. A 6mm diameter of wells were punched aseptically with sterile cork borer. Then they were loaded with different concentrations of sample (AgNps) (25, 50, 75, 100µL) respectively. Further, 20ul of gentamycin (positive control) into another well which acted as control, 10ug). The plates were incubated at 37^{0}C for 24hrs. Further, zone of inhibition (mm) was measured which are appeared around the wells. After 24hrs of incubation at 37^{0}C, the study carried out with the silver nanoparticles synthesized from *Zanthoxylum armatum* seed extract showed prominent effect or the inhibition was best in the concentration 100ug/ml showing 19mm of zone of inhibition. The positive control used was gentamycin which has the zone of inhibition 20mm against the bacteria *Klebsiella pneumoniae.* And the AgNps of *Zanthoxylum armatum* seed extract had the zone of inhibition in its highest concentration 100ug/ml of 19mm. Higher the concentration, better the zone of inhibition. The test results showed following zone of inhibitions (mm) 0, 14, 17, and 19 in the respective concentrations (µL) 25, 50, 75, and 100 against the pathogen *Klebsiella pneumoniae.* The zone of inhibitions against the bacteria *Klebsiella pneumoniae* are showed in the below. The figure 8 shows the activity of AgNps from *Zanthoxylum armatum* seed extract against the bacteria. For the *Staphylococcus aureus (25923)* and *Escherichia coli (25922),* the positive control used was tetracycline (50µg/mL) which has the standard zone of inhibition value of 35mm and 23mm. For these two bacteria the concentrations used were 250µL, 500µL, and 1000µL respectively. After the incubation at 37^{0}C, there were significant formation of zone of inhibitions. The Zone of Inhibitions for *E. coli* are 11mm, 11mm, 14mm and that for *S. aureus* is 15mm, 16mm, 16mm respectively. Therefore, the highest (mm) of zone of inhibitions in both the bacteria is corresponding to the concentration 1000µL indicating that, higher the concentration higher the amount of zone of inhibitions. The Zone of inhibitions of *E. coli and S. aureus* are shown in the figure 9 and figure 10. *S. aureus* had better zone of inhibition when compared to*E. coli.* Table: 2. Result of ZAAgNps against *Klebsiella pnemoniae.* Fig: 8. ZAAgNps (Silver Nanoparticles) activity against *Klebsiella pneumonia.* Table:3. Result of ZAAgNps against *E. coli and S. aureus.*

Antioxidant Assay

DPPH Assay

The antioxidant assay was performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity.0.4mM solution of DPPH were prepared using methanol and 2ml of this sample was added to different concentration of the sample ranging from 20ul, 40ul, 60ul, 80ul, and 100ul. And then they were kept at room temperature for 15min. Then, the absorbance was read at 517nm against blank samples. Lower the absorbance of reaction mixture, higher the free radical scavenging activity. The study carried out with AgNps of *Zanthoxylum armatum* seed extract, it showed potential effect in the concentration 100ul which had the absorbance of 0.201. The control had the 0% inhibition at absorbance 0.965 but the concentrations (uL) from 20, 40,60, 80 and 100 showed the inhibitions 4.45, 9.32, 20.93, 45.80, 79.17 with their corresponding absorbance 0.922, 0.875, 0.763, 0.523, 0.201 respectively. The higher the concentration of sample higher the scavenging potential.

The inhibitions were calculated with the formula

Radical scavenging activity/ Inhibition (%) = (Abs Control-Abs sample/ Abs control) x 100

ZAAgNpsResults of DPPH antioxidant assay (Table: 4). Chart depicting % of inhibitory activity of ZAAgNps (Chart: 2). DPPH Antioxidant Assay of ZAAgNps (Fig:11).

FRAP Assay

Ferric Reducing Antioxidant Power **Assay** is a method that uses antioxidant as reductants in a redox-linked colorimetric reaction. Different concentrations were used to assess the reducing potential of ZAAgNps. The study carried out with AgNps of *Zanthoxylum armatum* seed extract, it showed potential effect in the concentration 120ul which had the absorbance of 1.400. The control had the 0% inhibition at absorbance 0.302 but the concentrations (µg/mL) from 20, 40,60, 80, 100 and 120 showed the inhibitions (%)14.44, 55.97, 69.70, 75.84, 76.38 and 78.42 with their corresponding absorbance 0.353, 0.686, 0.997, 1.250, 1.279 and 1.400 respectively. The higher the concentration of sample higher the percentage of inhibitions. The % of inhibitions were calculated with the formula:

% of reduction = Sample - Control × 100/ Sample

ZAAgNpsResults of FRAP antioxidant assay (Table: 5). Chart depicting % of reducing activity of ZAAgNps (Chart: 2). FRAP Antioxidant Assay of ZAAgNps (Fig:12)

CONCLUSION

The study green synthesis of silver nanoparticles using *Zanthoxylum armatum* seed extract revealed the preliminary indications of reaction like color change, peaks formation in UV-Vis spectroscopy, presence of Alkaloids, flavonoids and polyphenols which are all corresponding to the silver nanoparticles. Though the color change to yellowish-brown suggested some reactions between the seed extract and silver nanoparticles, the characteristic surface plasmon resonance was absent due to which the formed AgNps may have less stability with the solvent preferred with 70:30 hydroalcoholic. suboptimal reagents, or the need for certain conditions like temperature, pH etc. Despite of the lesser stability of ZAAgNps, it showed prominent activity against *Klebiella pneumoniae* and *S. aureus*with 19mm of zone of inhibition and 16mm in 100ug/ml and 1000µLconcentration. And the DPPH assay resulted their inhibitions of 79.17% at 100uLof concentration and 78.42% of reduction at 120µg/mL in FRAP Assay.

SUMMARY

The present study was undertaken to explore the green synthesis of silver nanoparticles using Zanthoxylum armatum seed extract in the solvent ratio 70:30 hydroalcoholic. The Plant based method offers a biocompatible and environment friendly route compared to traditional methods, making it easily approachable for nanoparticle production. During the study, the reaction mixture showed a visible color change from pale yellow to yellowish-brown color which typically indicates the presence of silver nanoparticles in the initial stage. Further, confirming with the UV-Vis spectroscopy revealed peak at 332nm confirming the presence of silver nanoparticle with reference to the literature [9]. The characterization using FTIR spectroscopy indicated the presence of functional groups hydroxyl and carbonyl with two peaks at 3341.89cm^{-1}, 51.66%T and 1636.16cm^{-1}, 72.31%T which played the role in stabilization and reduction process. The DLS results were not conclusively supportive but the PDI values indicated the presence of moderate polydispersity. The antibacterial activity against *E. coli*, *S. aureus* and *Klebsiella pneumoniae*, and antioxidant activity were also promising with the isolated silver nanoparticles using green synthesis of *Zanthoxylum armatum* seed extract.

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Table: 1: Taxonomical Classification of *Zanthoxylum armatum*

Botanical name	***Zanthoxylum armatum***
Family	Rutaceae
Kingdom	Plantae
Sub kingdom	Viridaeplantae
Domain	Eukaryote
Phylum	Tracheophyta
Sub phylum	Euphyllophytina
Infra phylum	Radiatopses
Class	Magnoliopsida
Sub class	Rosidae
Super order	Rutanae
Order	Spindale
Sub order	Rutineae
Genus	*Zanthoxylum*

Table.2: Result of ZAAgNps against *Klebsiella pnemoniae*

Pathogens	**Zone of Inhibition (mm)**				
	Positive Control (C)	**25 µL**	**50 µL**	**75 µL**	**100 µL**
Klebsiella pnemoniae	20	0	14	17	19

Table. 3: Result of ZAAgNps against *E. coli and S. aureus*

Pathogens	**Zone of Inhibition (mm)**			
	Positive Control (C)	**250 µL**	**500 µL**	**1000 µL**
E. coli	23	11	11	14
S. aureus	35	15	16	16

Table. 4: ZAAgNps Results of DPPH antioxidant assay

Concentration (µL)	**Absorbance**	**Inhibition (%)**
Control	**0.965**	**0**
20	**0.922**	**4.455958549**
40	**0.875**	**9.32642487**
60	**0.763**	**20.93264249**
80	**0.523**	**45.80310881**
100	**0.201**	**79.17098446**

Table. 5: ZAAgNps Results of FRAP antioxidant assay

S. No.	**Conc. (µg/mL)**	**Absorbance @700nm**	**% of Reduction**
1.	Control	0.302	-
2.	20	0.353	14.44
3.	40	0.686	55.97
4.	60	0.997	69.70
5.	80	1.250	75.84
6.	100	1.279	76.38
7.	120	1.400	78.42

	
Fig: 1. *Zanthoxylum armatum* seed powdered sample	**Fig: 2. An Extracted Sample**
	
Fig: 3. Reaction mixture after the immediate addition of Seed extract	**Fig: 4. Reaction mixture after 24hrs of incubation in dark condition**

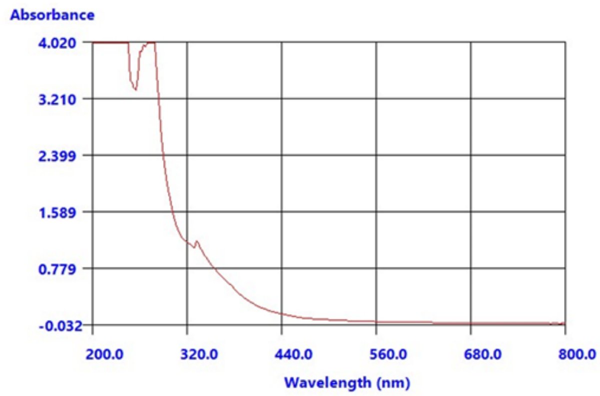

Fig: 5. UV-Vis Spectroscopy of ZAAgNps (Silver nanoparticles) showing peak at 322nm.

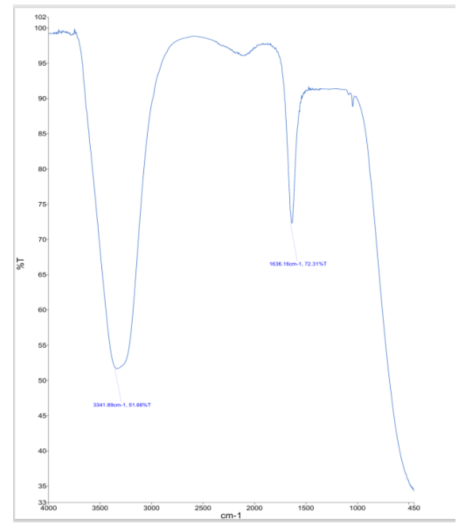

Fig: 6. FTIR peaks of silver nanoparticles of *Zanthoxylum armatum* seed extract (AgNps)

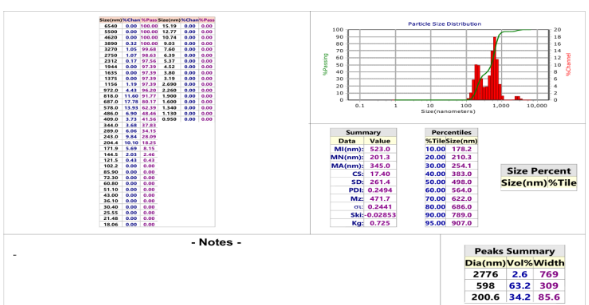

Fig: 7. DLS result of ZAAgNps

Fig: 8. ZAAgNps (Silver Nanoparticles) activity against *Klebsiella pneumoniae*

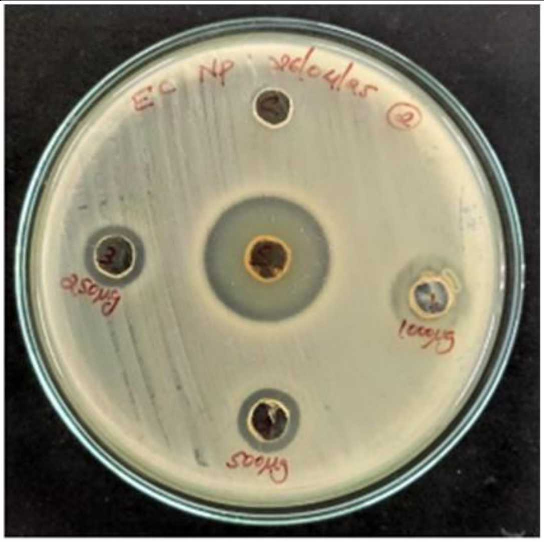

Fig: 9. ZAAgNpsactivity against*E. coli*

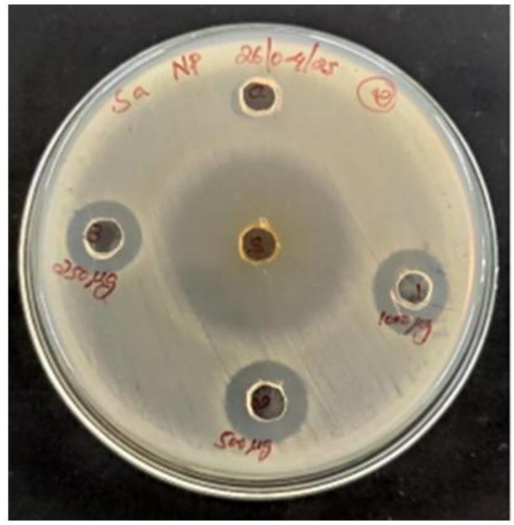

Fig: 10. ZAAgNps activity against *S. aureus*

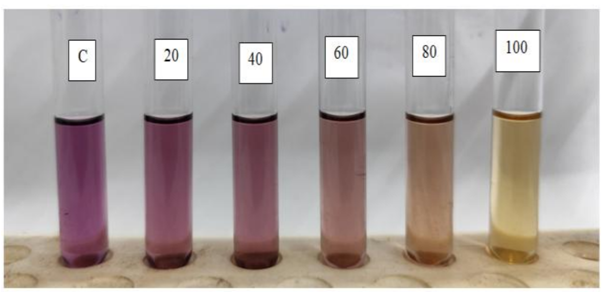

Fig: 11.DPPH Antioxidant Assay of ZAAgNps

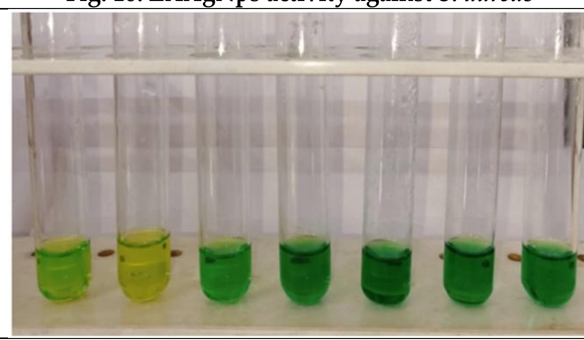

Fig: 12. FRAP Antioxidant Assay of ZAAgNps

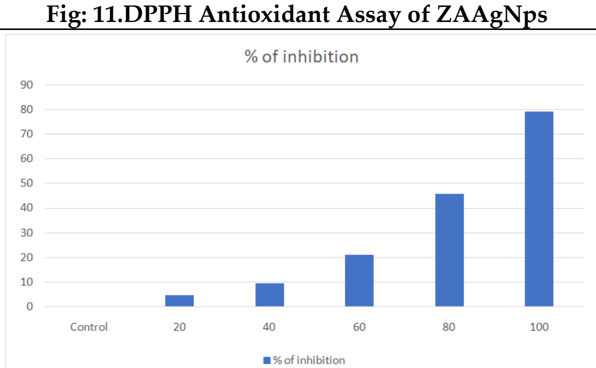

Chart: 1. Chart depicting % of inhibitory activity of ZAAgNps

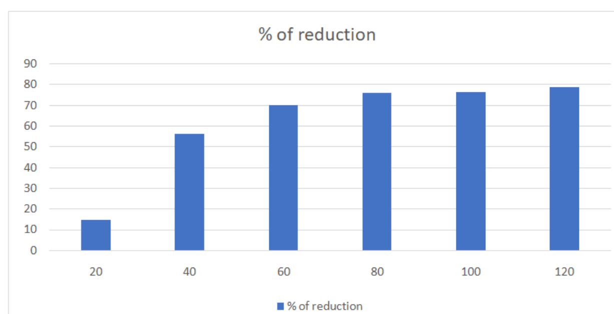

Chart: 2. Chart depicting % of reducing activity of ZAAgNps