

STUDY OF PHYSICAL AND ANTIMICROBIAL PROPERTIES OF BIOGENIC SILVER NANOPARTICLES SYNTHESIZED VIA GREEN ROUTE

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Abstract

Health is the primary concern of human beings. Apart from the problem of living and food, health issues have always been a concern for humanity. Various types of hereditary and epidemic diseases are the greatest adversaries of human health. The main cause of their generation and spread is different types of germs. Bacteria are one of the main causes of human health problems, and various medications have been used to treat it. Different treatments have been adopted to deal with the bacteria problems. The use of nanomaterial's for therapeutic purposes, as in other areas of life, have been blessings and encouraging in recent times and is a hope for future. The current research work was focused to synthesize silver nanoparticles from lemon leaves extract and explore their antibacterial activity on different types of bacteria. Leaves extract worked as a reducing agent to form Ag⁰ atoms, which further clustered to form silver nanoparticles. These silver nanoparticles were characterized by using X-Ray Diffraction (XRD) analysis, UV-Visible Spectroscopy and Scanning Electron Microscopy (SEM). The UV-Visible absorption spectra of two different prepared silver nanoparticles samples were obtained and it was observed that these samples have prominent absorption response at energetic end of visible light from 380 nm to 410 nm. The crystalline nature of synthesized silver nanoparticles was confirmed by X-Ray diffraction analysis and showed the diffraction peaks at 31.86°, 37.74°, 43.3°, 64.14°, and 77.08°, corresponding to silver element. The antibacterial activity was tested against different pathogenic bacteria and it was observed that the average inhibition zone diameter was 10 – 13 mm which shows the positive control.

The green synthesis method is very economical, eco-friendly, and simple to implement for synthesis of silver nanoparticles.

1. Introduction

The nanomaterial term is commonly referred to the internal or external structure calculated on nanoscale and have additional or different characteristics [1]. Noble metal nanoparticles have got a lot of interest due to their tunable physical properties such as mechanical, magnetic, and chemical properties that are apart from bulk materials [2]. Nanoparticles could be synthesized from the top down or from the bottom-up approaches [3]. Bulk precursor materials are used as the starting material for the chemically aided breakdown of nanoparticles into smaller bits in the top-down technique [4,5]. The bottom-up technique involves using synthetic or natural/biochemicals to synthesize nanoparticles from the precursor's atomic or molecular species, then allowing the precursor particles to agglomerate in size [3]. Techniques to prepare nanoparticles those use naturally occurring reagents as reducing or capping agents include plant extracts and microbes, which got much interest for nanotechnology [6]. These methods are known as biological or green methods, which are ecofriendly, economical and free of contamination to prepare nanoparticles for their use in different fields, such as in medical and industry to overcome different limitations of physical properties of bulk materials [7]. Many interesting biological or green methods are currently being used to prepare metal nanoparticles using various plant extracts such as hibiscus leaf extract, honey mangifera indica and murraya koenigii leaf, cashew leaf sucrose and maltose, black tea leaf extract, Indian gooseberry fruit extract, sun-dried camphor leaves, and aloe vera plant extracts etc. [8-10]. Those nanoparticles which exhibit localized surface plasmon resonance (LSPR) phenomena are capable of microbial and cancer treatment. Among commonly synthesized nanoparticles, the silver nanoparticles (AgNPs) possess the astonishing surface plasmon field. However, the therapeutic abilities of Ag NPs have not been explored and elucidated in detail up till now. Silver metal has been known to have antimicrobial properties since ancient times,

and silver nanoparticles (Ag NPs) are of particular interest due to their unique properties and wide range of applications [11]. Different methods are being used to produce silver nanoparticles such as green synthesis, sol gel, auto combustion and green synthesis etc. [12,13]. But only green synthesis method is environment friendly while all other methods have some disadvantages and have bad effects on environment [14]. The comparative study of silver nanoparticles prepared by using green synthesis techniques shows that by varying the concentration of extract, the inhibition zone diameter will increase. The different inhibition diameter zones are obtained recently by using nanoparticles prepared by green synthesis technique and applied for different pathogenic bacteria [15, 16], such as, preparation by using endophytic fungus, safflower, ziziphora clinopodioides, macroalgae Padina sp., Carissa carandas L., esculentus extract from 9mm to 12mm. The silver nanoparticles of lemon leaves extract show inhibitory zone range lying between 9mm to 13mm, it can be increased by using different concentrations of silver nanoparticle solution. This study showed that lemon leave extract gives comparatively good results but it still requires to explore effects on physical properties of Ag nanoparticles. It could be made better if we use different concentrations of lemon extract to explore antibacterial properties of Ag nanoparticles.

In the present work we have prepared Ag nanoparticles by using different concentrations of lemon leaves' extract and silver nitrate for Ag⁺ ion source. We studied their physical properties and report on the structural, optical, and antibacterial activities of prepared silver nanoparticles. The antibacterial activities are compared with an antibiotic drug.

1.1 Antibacterial activity mechanism

Though basic reason of antibacterial activity of silver nanoparticles is unknown, various antibacterial effects have been described [16-17]. Nanoparticles can continuously discharge silver ions, which could be a microbe-killing mechanism [18]. Figure 1 represents some

mechanism of silver nanoparticles (Ag NPs), in which due to their small size Ag NPs can penetrate to the cell membrane and plasma membrane [19-21]. Organelle rupture and possibly cell lysis can result from denaturation of the cytoplasmic membrane [22]. Ag NPs can cause cellular toxicity and oxidative stress by producing reactive oxygen species (ROS) [23]. Reactive oxygen species are a major cause of cell

membrane instability and deoxyribonucleic acid (DNA) alteration. Because sulphur and phosphorus are key parts of DNA, the connection of silver ions with the sulphur and phosphorus of DNA in microbial's cells and can influence the DNA replication, cell reproduction, as well as the death of microorganisms [24, 25].

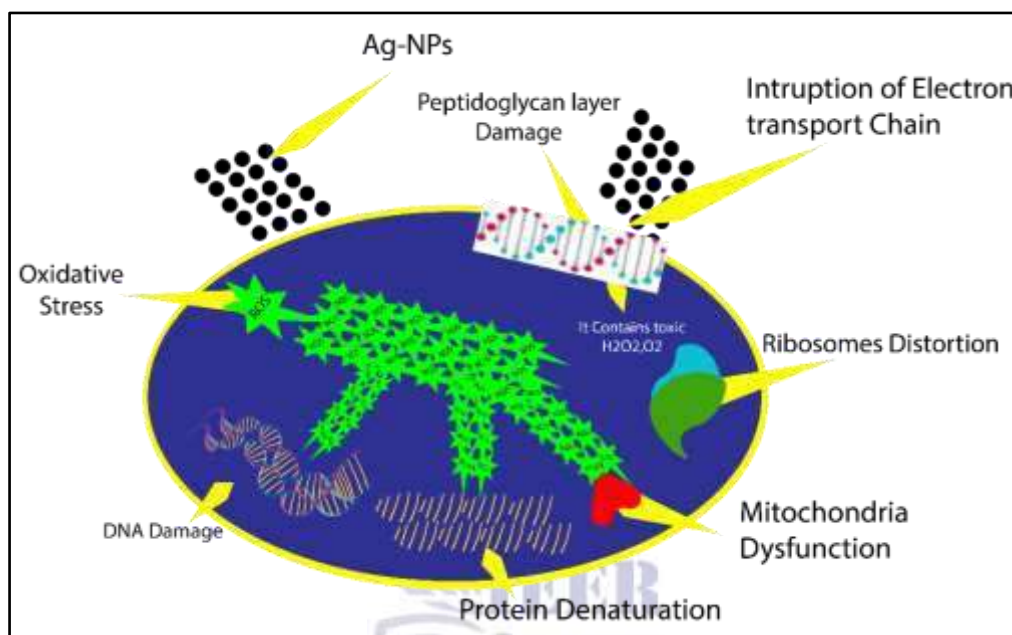


Figure 1: Antibacterial activity mechanism of AgNPs

As the silver nanoparticles reduce from Ag^+ to Ag^0 ions so that when silver nanoparticles react with microbial species, then Ag^0 can accumulate at the microbial's membrane [26-39]. In this condition silver nanoparticles stick to the microbial's membrane and are responsible for the death of cellular cells present at the microbial's [27,29]. The adhesion of Ag NPs is dependent on their positive surface charge [30]. When Ag NPs enter into the cell wall and bind to the cell membrane then they can also stick to the protein and are responsible for decomposition of adenosine triphosphate (ATP) [31,32]. Possible mechanism of antibacterial action of Ag NPs may include; (i) DNA replication and distraction of ATP caused by coalescence of Ag species; (ii) direct demolition of cell wall and membrane on interaction with Ag NPs; (iii) generation of reactive oxygen species (ROS) by absorbing energy of charges existing during LSPR. Ag NPs

also disrupt the ribosome, cause mitochondrial malfunction, and reside in the electron transport chain [33]. The continuous bombardment of Ag NPs will make more Ag^+ ions to penetrate into and out of the cell membrane and bind to the protein, enzymes, nucleoid and plasmid, etc. [34, 35]. Where Ag^+ ions constantly attack these parameters and are responsible for the cellular death. By interfering with cell division and the respiratory chain, Ag NPs cause death of microbial's cells [36, 37]. In this study the bactericidal impact of Ag nanoparticles was investigated against different pathogenic microorganisms [38].

2. Experimental

2.1 Preparation of leaves extract

Healthy leaves from healthy lemon plants were collected and dried under shade, and then grinded in the powder form. The leaves extract was prepared by mixing 50g of leaves' powder

with 500 ml distilled water in a conical flask and boiled for 30 min. Then filtered the extract by using whatman filter paper. Now leaves extract is ready for further synthesis of nanoparticles.

2.2 Synthesis of silver nanoparticles

To prepare Ag NPs we used two different concentrations of lemon leaves extract to optimize their physical and antibacterial properties. First sample was prepared by making a 0.02 M solution of silver nitrate and adding 100 ml of leaves extract in a titration flask and then heated at 80°C for 90 min with constant and continuous stirring. The color transformation of solution confirms the preparation of Ag NPs. Another sample is prepared by adding 250 ml of leaves extract with 250 ml distilled water for 60 min with constant stirring, the color change confirms the particles. After preparation of samples, these were centrifuged for 10 min at 8000 rpm. Then washed the centrifugation tubes with distilled water such that pure samples would leave behind in the tubes. Maintaining the pH, 0.1 M Sulphuric acid and 0.1 M hydroxide solutions were all used. The color shift in the solution indicated the formation of nanoparticles. The impact of variables such as temperature, pH, and the amount of reducing agent on the synthesis of silver nanoparticles was investigated and optimized using response surface methodology. The possible chemical reaction during this process is given below;

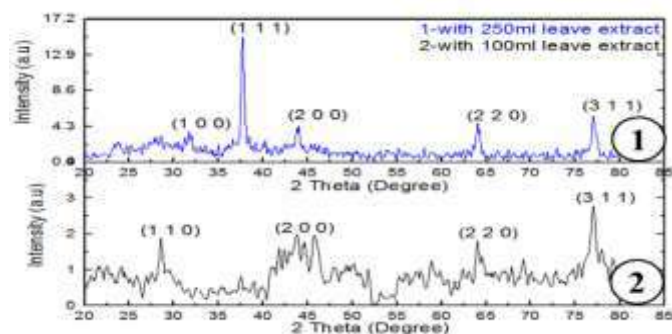
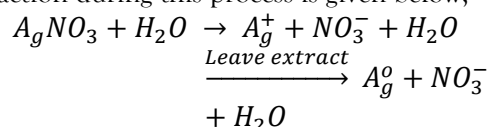


Figure 2: XRD spectra (1) 250ml leaf extract, (2) 100ml leaf extract

Figure 2 (2) shows diffraction pattern of synthesized silver nanoparticle by using 100ml

2.3 Characterization

A simple but reliable characterization for confirmation of Ag NPs formation is by simple visual observation of solution color transformation from yellowish to reddish brown because of the reduction process of Ag^+ to Ag^0 atoms and further formation of Ag NPs confirm the formation of Ag NPs. X-ray diffraction spectra of Ag NPs powder were obtained for 2θ diffraction angle range 20° to 80° using $Cu-K\alpha$ radiations ($\lambda=1.5406\text{\AA}$). UV-Vis spectra is a consistent technique for confirmation of metal nanoparticles by collecting absorption data, we done the absorption for incident light having wavelength range 250-900 nm.

3. Results & Discussions

3.1 Structural analysis

Figure 2 shows the X-ray diffraction spectra for structural analysis of dry powders of silver nanoparticles. The observed peak broadening and noise are most likely caused by the presence of nano sized particles and various crystalline biological macro molecules in the plant extracts. The synthesized nanoparticle is showing the crystalline nature as shown by the x-ray diffraction analysis. The XRD patterns in figure 2(1) of prepared silver nanoparticles by using 250ml of leaf extract show diffraction spectra at 31.86° , 37.74° , 43.30° , 64.14° , and 77.08° corresponding to the miller indices (1 0 0), (1 1 1), (2 0 0), (2 2 0), and (3 1 1). The green synthesized silver nanoparticles are found to be pure crystalline in nature.

concentration of lemon leaf extract. The X-ray diffraction pattern is obtained at 28.56° ,

43.86°, 64.04°, and 77.02° with miller indices at (1 1 0), (2 0 0), (2 2 0), and (3 1 1). The synthesized silver nanoparticle is less crystalline in nature than is obtained in figure 2 (1). Different diffraction peaks obtained in these spectra revealed that nanoparticles obtained by 250ml leave extract is more crystalline in nature than that obtained by 100ml leave extract. Thus, the crystalline nature of silver nanoparticles is dependent on different concentration of leave extract.

3.2 UV-Visible spectroscopy

The ultraviolet spectroscopy method is widely used to investigate the optical properties of various types of optoelectronic materials. The absorbance properties of nano structure materials are determined by a number of variables such as band distance and surface roughness. The impact of particles on the quantum confinement scale is also revealed by

ultra violet spectroscopy. When particle size increases, the energy of the band gap decreases, and the onset of absorption frequently shifts to a longer Wavelength.

UV-spectrum was utilized to monitor the reduction of silver nitrate to Ag NPs using lemon leaves extract. The UV-visible spectrum was recorded from 250nm to 900nm. Figure 3 shows the ultra violet spectroscopy results obtained for Ag nanoparticles prepared by using 100ml and 250ml leaves extract at pH 9. A characteristic absorption is observed at a wavelength of 385 nm for samples prepared by using 100 ml of leaves extract, while for samples prepared by using 250 ml of leaves extract, absorption is observed at a wavelength of 415 nm. This absorption characteristic is plasmonic resonance, which is the effect of electromagnetic field of incident light with the available conduction electrons present in Ag NPs.

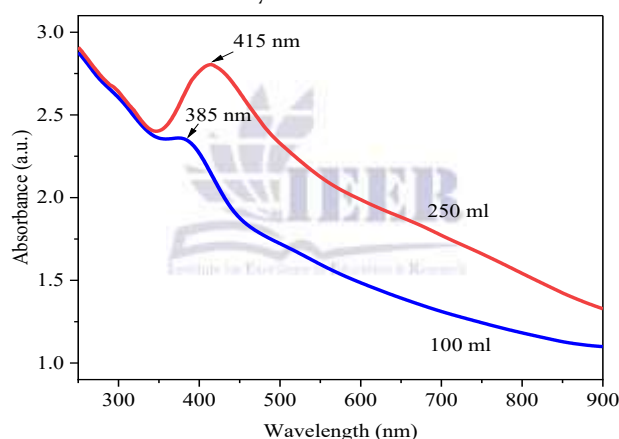


Figure 3: Absorption spectrum of AgNPs prepared by using 100 and 250ml of extract

By comparing with literature [39, 40] it is concluded that the pH level affects the particle size and we can get particles of different nm size.

Recently, several studies on the preparation of silver nanoparticles using different leaves extract shows the absorption peaks lie between 400nm-480nm [41]. If we increase the concentration of leave extract, there is an increase in the peak intensity and wavelength, and it could be better if we increase the concentration of extract [42]. The UV-visible spectra result implies that the quickest bio-reduction is observed using lemon leaf extract.

3.3 Scanning electron microscopy analysis

The size and morphology of prepared silver nanoparticles of lemon leaves extract is described by scanning electron microscopy. The different surface images are taken at different magnifications that completely explore the morphology and size of silver nanoparticles. There are different size and morphology of different materials that are prepared by leaves extract method. The prepared samples of silver nanoparticles have different results at different concentrations because of different parameters are responsible for the morphology and size of silver nanoparticles, such as pH level, temperature and time to make these particles. In fig.4 (a, b, c, d) the different shapes

of prepared silver nanoparticle may be due to change of leaves concentration at different time of preparation. The silver nanoparticles of various shapes, as rod, spherical and cubic, are due to variation of different parameters

mentioned above. The prepared silver nanoparticles are all crystalline in nature having good antibacterial activity against different bacteria.

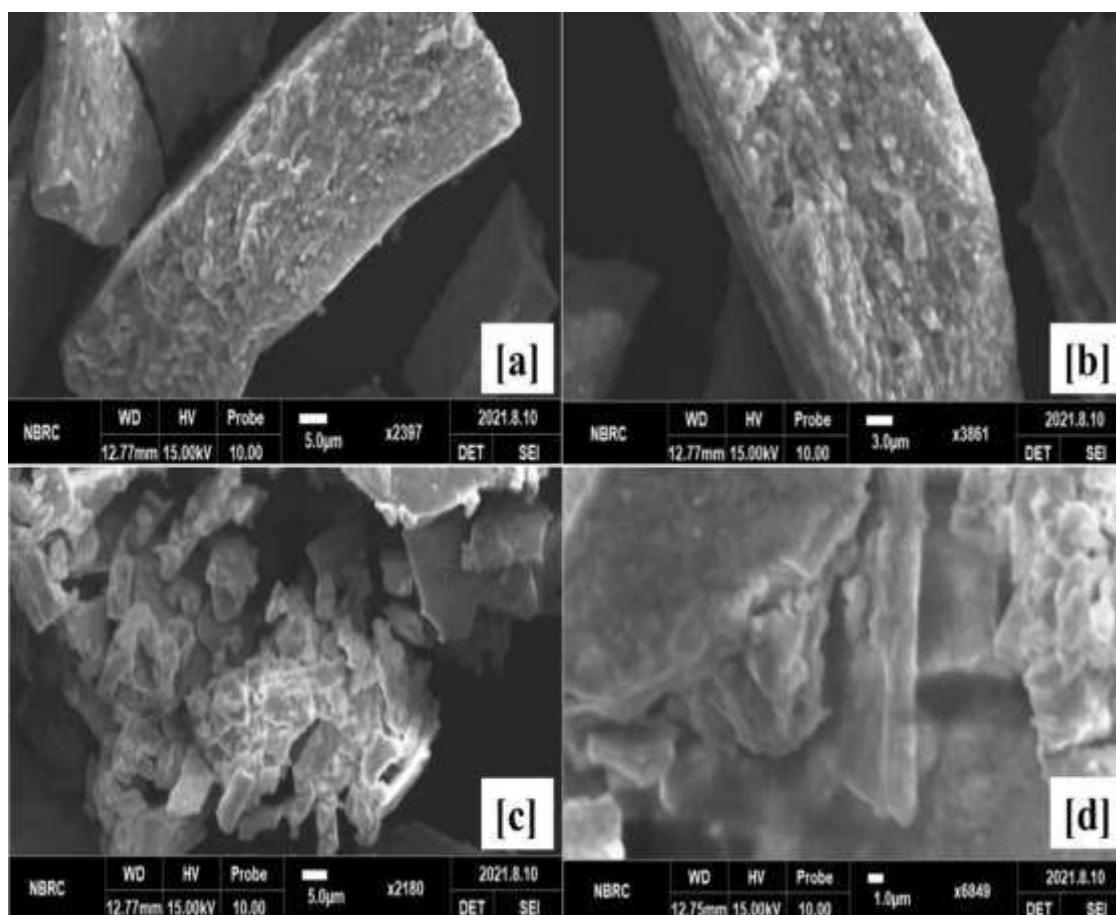


Figure 4: SEM micrograph of prepared AgNPs; [a], [b] prepared by 250 ml of extract, & [c], [d] prepared by 100 ml of extract.

3.4 Antibiotic effect

If an antibiotic, with or without nanoparticles, has no effect on bacteria, the diameter of the inhibitory zone will be smaller. This suggests that micro-organisms are not totally stopped. The inhibition zone is larger if prepared nanoparticle or any antibiotic effect is positive and inhibition zone is smaller if prepared nanoparticle or antibiotic effect is smaller. We can say that growth of such bacteria is stopped to some limit by that medicine or nanoparticle. The antibacterial activity of synthesized Ag NPs is investigated by using agar disc diffusion method on p.aeru and s.aeru (a type of negative and positive gram bacteria respectively). In fig.5 (A & D), results are obtained by using two different concentrations of doses; 10mg and

20mg of NPs prepared by using 100ml leaf extract. Also, B & C results are obtained by using two different concentrations of doses; 10 mg and 20 mg of NPs prepared by using 250ml of leaf extract. Fig.5 (E) shows that diameter is in good size comparison to confirm the antibacterial activity of Ag NPs. The diameter size is moderate which shows that synthesized Ag NPs have moderate effect on bacteria. In fig.6 the bar graph shows the inhibition zone lies between 9 mm to 13 mm in size using 100 ml and 250 ml extracts with different concentration doses when tested against two different pathogenic bacteria. The different antibiotics used against different bacteria result in few mm in inhibition zone. The most benefit thing of green synthesis process is that it is very

economical, ecofriendly and very easy to use. So, nanoparticles prepared by this method are more suitable for medical applications due to the toxic-chemical free nature of synthesized nanoparticle. As well as the size, shape and morphology can be controlled using green synthesis process.

The comparative study of silver nanoparticles prepared by using green synthesis techniques shows that by varying the concentration of prepared extract inhibition zone diameter will also increase. The different inhibition diameter zones are obtained recently by using green synthesis technique on different pathogenic bacteria [43, 44-48] reported using endophytic

fungus, safflower, ziziphora clinopodioides, macroalgae *Padina* sp. , *Carissa carandas* L., *esculentus* extract from 9mm to 12mm. The silver nanoparticles obtained using lemon leaves extract showed inhibitory zone range lies between 9mm to 13mm, it could be increased by using different doses concentration of silver nanoparticle solution. This study showed greater concentration of leaf extract gives more good results than lower concentration, and it could be made better if we use more or greater concentration of extract to explore more antibacterial properties.

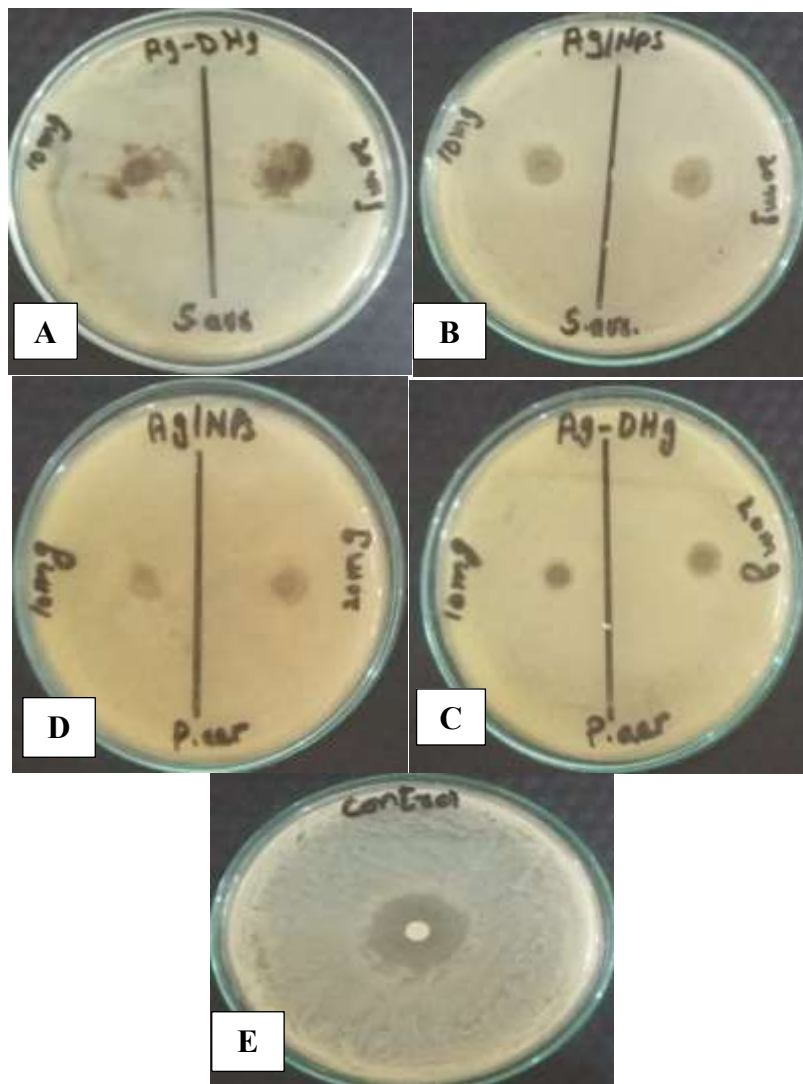


Figure 5: AgNPs activity against different bacteria

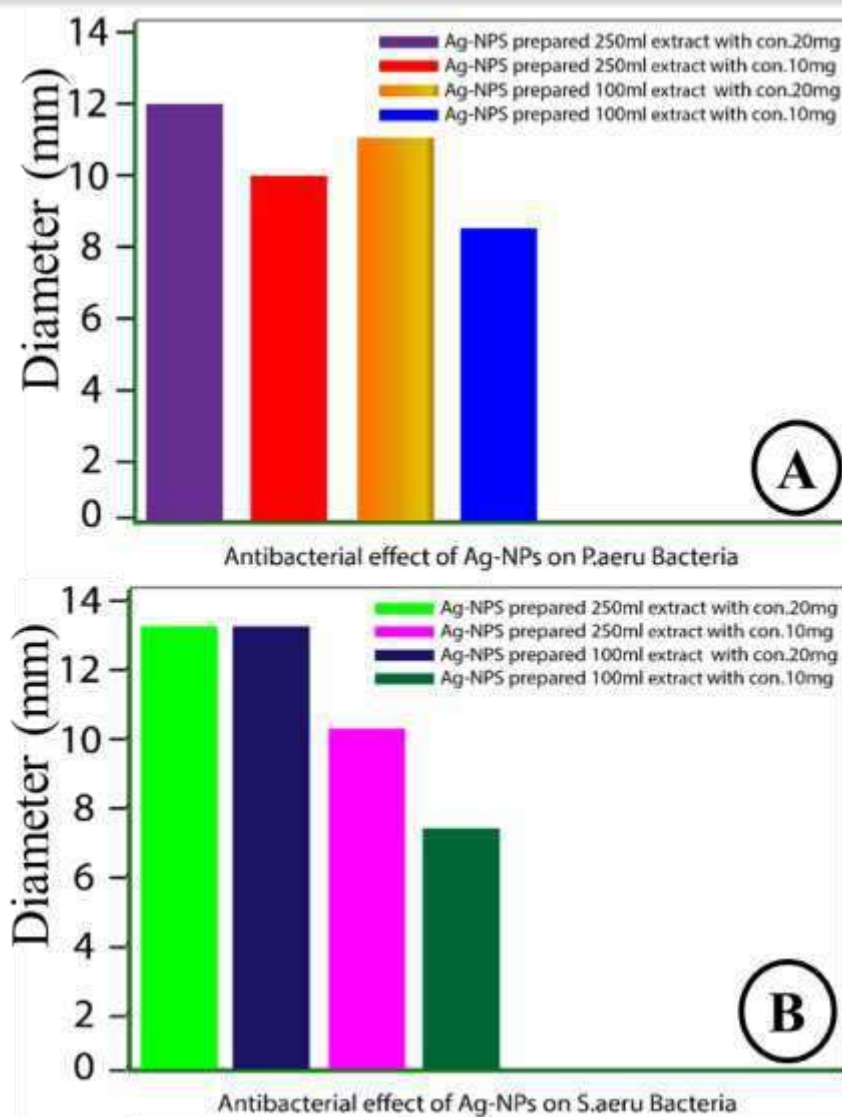


Figure 6: Observed diameter of control AgNPs on bacteria

Conclusions

In this study, the silver nanoparticles were prepared by green synthesis using lemon leaves extract. The XRD peaks were obtained at angles of 28.56° , 43.86° , 64.04° , and 77.02° . Two different concentrations of leaves extract were used to produce silver nanoparticles and the silver nanoparticle obtained using 250ml leaf extract was more crystalline in nature than prepared using 100ml leaf extract. The observed silver nanoparticle's crystallinity is dependent on the concentration of leaves extract. The greater quantity of leaves extract produces greater crystals and the smaller quantity produces smaller crystals. The Ultra Violet spectroscopy results showed that the particle obtained having a wavelength in the range from 380nm-410nm and confirms that

the nanoparticles were yielded. The prepared silver nanoparticles were then applied to different types of bacteria species that produced the inhibition zone having different diameters in between 7mm-13mm which showed that prepared nanoparticle has a good effect on bacteria and more diameter of inhibition zone can be achieved by increasing concentration of leaves extract.

The most advantageous feature of the green synthesis process is that it is cost-effective, non-toxic, and simple to implement. As a result of the harmful chemical-free nature of the nanoparticles created using this process, they are more acceptable for medicinal purposes. The size, shape, and morphology of the organism can all be regulated utilizing the green synthesis process.

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Conflicts of Interest

The authors declare no conflict of interest.

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