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ANTIBACTERIAL ACTVITY OF SILVER NANOPARTICLES SYNTHESIS FROM ACACIA NILOTICA PLANT LEAF

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ABSTRACT

Silver nanoparticles have long been known for their antibacterial, antifungal, anti-viral and anti-inflammatory properties. Recently, researchers have extended their use into chemotherapy as a device for delivering various payloads such as small drug molecules or large bio molecules to specific targets. Silver compounds were shown to be effective against both aerobic and anaerobic bacteria by precipitating bacterial cellular proteins and by blocking the microbial respiratory chain system. The silver nanoparticles show antimicrobial activity which heals the wounds and infectious disease. The synthesis of nanoparticles gets concern in nanotechnology due to the variable size, shapes, chemical composition and controlled disparity and their potential use in the medical science for the better treatment of human benefits. The main mechanism considered for the synthesis of nanoparticles mediated by the plants due to the presence of phytochemicals these responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides. In producing nanoparticles using plant extracts, the extract mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced by this way.

KEYWORDS: Acacia nilotica, Silver nanoparticles, Plant extract, phytochemicals, Microbial bacteria.

INTRODUCTION

Plants are the principal tools of traditional medicinal system. Although in origin, many traditional medical paradigms and their pharmacopeia have evolved in to quite sophisticated system, using thousands of plants and their natural system. The medicinal plants are being looked upon not only as a source of health care but also as a source of income. India has a rich diversity of medicinal plants. Indian Materiel Medico accounts for nearly 35000 species under various crude drugs both of indigenous and exotic origin (Jain, 2010; Bhattacharya, 2008). The nanoparticle research is an intense scientific research due to its wide potential application in biomedical, optical and electronic fields and their narrow bridge in between bulk materials and molecular and atomic structures. Bulk materials have constant physical properties because they have grain structures with random grains individually oriented in space and contacting each other across grain boundaries but nanomaterials are made up of a single grain with all the atoms oriented in a crystalline lattice (Sharma *et al.,* 2009) and they shows different properties such as quantum confinement, Surface Plasma Resonance (SRP), decrease in melting temperature which are directly related to the crystal lattice of the nanomaterials. Due to nano size feature it easily used for chemical imaging drugs agents and versatile function used for the cellular delivery as they are widely available, rich functionality, good biocompatibility, this is also a good carrier of targeted drug delivery and controlled drug release (Xu *et al*., 2006). It's a completely advantageous material for medical science. The silver nanoparticle shows antimicrobial activity which heals the wounds and infectious disease (Ravishankar and Jamuna, 2011). The synthesis of nanoparticle gets concern in nanotechnology due to the variable size, shapes, chemical composition & controlled disparity and their potential use in the medical science for the better treatment of human benefits.

Silver nanoparticles are nanoparticles of silver of between 1 nm to 100 nm in size **(**Graf *et al*., 2003). While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used are spherical silver nanoparticles but diamond, octagonal and thin sheets are also popular nano-silver particles are mostly smaller than 100 nm and consist of about 20-15,000 silver atoms (Jain *et al*., 2009). Silver nanoparticles have long been known for their antibacterial, antifungal, anti-viral and anti-inflammatory properties. Recently, researchers have extended their use into chemotherapy as a device for delivering various payloads such as small drug molecules or large bio molecules to specific targets. Once the AgNPs has sufficient time to reach its target, release of the payload could be triggered by internal or external stimulus and targeting the accumulation of nanoparticles to a designated area ensures high drug concentration at specific sites and thus minimizes side effects (Pickup *et al*., 2008).

Biological properties of silver nanoparticles

Anti-bacterial properties of silver nanoparticles

The utilization of silver as a disinfecting agent is not new, and silver compounds were shown to be effective against both aerobic and anaerobic bacteria by precipitating bacterial cellular proteins and by blocking the microbial respiratory chain system (Thomas *et al*., 2007) Before the advent of silver nanoparticles, silver nitrate was an effective antibacterial agent used clinically (Wyatt *et al*., 1900). However, because of the larger surface area to volume ratio, AgNPs may have much better efficiency.

The possible mechanisms action are

1. Better contact with the microorganism-nanometer scale silver provides an extremely large surface area for contact with bacteria. The nanoparticles get attached to the cell membrane and also penetrate inside the bacteria (Rai *et al*., 2009).
2. Bacterial membranes contain sulfur-containing proteins and AgNPs, like Ag+, can interact with them as well as with phosphorus-containing compounds like DNA, perhaps to inhibit the function (Liu *et al*., 2010).
3. Silver (nanoparticles or Ag+) can attack the respiratory chain in bacterial mitochondria and lead to cell death (Salopek-Sondi and Sondi 2004).
4. AgNPs can have a sustained release of Ag+ once inside the bacterial cells (an environment with lower pH), which may create free radicals and induce oxidative stress, thus further enhancing their bactericidal activity (Tian *et al*., 2007).

Synthesis of silver nanoparticles from plants

The plant synthesized nanoparticles are spontaneous, economical, eco-friendly protocol, suitable for large scale production and single step technique for the biosynthesis process (Huang *et al.*, 2008). It have a broad variety of metabolites that can aid in the reduction of silver ions, and are quicker than microbes in the synthesis. The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals these responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides (Prabhu and Poulose, 2012). In producing nanoparticles using plant extracts, the extract mixed with a solution of the metal salt at room temperature. The reaction is complete within minutes. Nanoparticles of silver, gold and many other metals have been produced this way (Li *et al*., 2014). The nature of the plant extract, its concentration, the concentration of the metal salt, the pH, temperature and contact time are known to affect the rate of production of the nanoparticles, their quantity and other characteristics (Dwivedi and Gopal, 2010). Synthesis of silver nanoparticles using a leaf extract of *Polyalthia longifolia*. An average particle size of about 58 nm was obtained. Silver and gold ions could be reduced to nanoparticles using a leaf extract of *Cinnamomum camphora* the reduction was ascribed to the phenolics, terpenoids, polysaccharides and flavones compounds present in the extract. These nanoparticles were found to have a peak bactericidal activity at a concentration of 45 µg/mL (Huang *et al*., 2008). They were most active against the yeast *Candida albicans* (Saxena *et al*., 2011).In this study silver nanoparticles were synthesised from plant Acacia nilotica.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$), *A. nilotica*, Culture (9 strains), Nutrient broth and Agar, Magnetic bead, Whats' man no.1 filter paper, Aluminum foil, Conical flasks, Glass funnel, Measuring cylinder, Falcon tubes, Petri dishes, Cotton swab. Double distilled water was used in all experiments.

Bacterial strains

Bacterial strains used for determining antimicrobial activity were *S. marcescens* and *K. pneumonia, B. ariyabattai, B. megaterium, B. cerus, P. putida, B. subtilis* cultures.

Instruments used

Magnetic stirrer (REMI), Centrifuge, Laminar air flow (Lab tech), Shaking incubator (Lab tech), Auto clave.

Preparation of plant extract

The whole plant of A. nilotica collected from local area of University campus was washed thoroughly with distilled water several times to remove dust and dried under shade. The dried plant was cut into small pieces and its aqueous extract was prepared using soxhlet apparatus (Fig.1). Then the extract was reduced under pressure to make a powder.

Acacia nilotica

Fig 1: *Acacia nilotica* plant leaves.

Domain : Eukaryota

Kingdom : Plantae

Phylum : Spermatophytasu

Sub phylum : Angiospermae

Class : Dicotyledonae

Order : Fabales

Family : Fabaceae

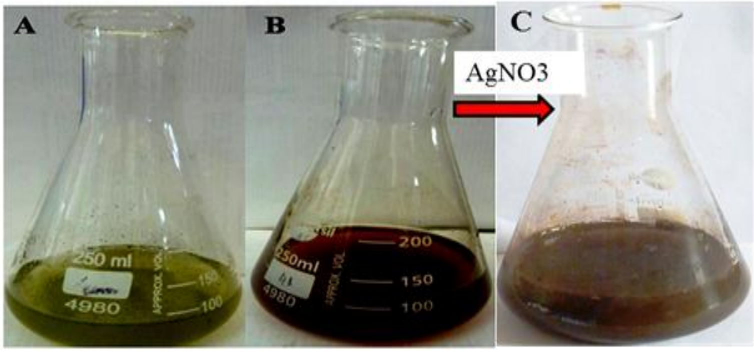
Sub family : Mimosoideae

Genus : *Acacia*

Species : *nilotica*

Synthesis of silver nano particles

Aqueous extract of A. nilotica powder (5g) was dissolved in 100 ml of distilled water and then, 10 ml of the above extract was added to 90 ml of 0.1 M $AgNO_3$ solution and then it was transferred into a round bottom flask and heated to 70˚C with continuous stirring 15 min, and the solution allowed to incubated for 24 h the solution turned to brown color indicating the formation of AgNPs. The solution was centrifuged and the mixture was collected after discarding the supernatant and the pellet used for further characterizations (Fig.2).



Plant extract Before After

Characterization of silver nanoparticle

Antimicrobial activity assay and UV-Vis absorption spectra of the synthesized AgNPs were recorded on spectrophotometer (max in nm) equipped with 1-cm quartz cell and FT-IR, XRD, SEM, EDAX pattern were recorded.

Antibacterial determination by well diffusion method

Agar well diffusion assay

The antibacterial activity of synthesized AgNP of A. nilotica plant extract were tested against human bacterial pathogens viz., Staphylococcus aureus, Escherichia coli, Bacillus megaterium, Bacillus ariyabatai, Bacillus putida, Klebsilla pneumonia, The silver nano particles ($AgNO_3$) prepared were tested to evaluate the Minimum Inhibitory Concentration (MIC) required to inhibit the growth of the test pathogens selected in the study. For all the plates were incubated at 35˚C for 24 h. After the stipulated time period, the zone of inhibition formed at the minimum dilution for each pathogen was recorded. Based on the above MIC replicates were maintained for each pathogen and the mean diameter value was expressed in millimeters.

UV- Visible analysis

The resulting solution was then diluted with a small aliquot of 100 μL of the sample with 1 mL de-ionized water and assayed in UV Visible spectroscopy. UV Visible spectral analysis has been done to know the surface plasmon resonances band by using Shinadzu UV visible absorption spectrophotometer with the resolution of 1 nm between 200 and 800 nm, possessing a scanning speed of 300 nm/minutes (Fig.3). The reduction of pure Ag+ ions to form AgNPs using *A. nilotica* plant leave extract was characterized by UV-Visible spectrum of the reaction medium.

Fourier-Transform Infrared Spectroscopy (FT-IR Spectroscopy)

Fourier-Transform infrared spectroscopy is a technique that provides information about the chemical bonding or molecular structure of materials whether organic or inorganic. The bonds and group of bonds vibrate at characteristic frequencies. A molecule that is exposed to infrared rays absorbs infrared energy at frequencies which are characteristics to that molecule. During FT-IR analysis a spot on specimen is subjected to a modulated IR beam. The specimen's transmittance and reflectance of infrared rays at different frequencies is translated to an IR absorption plot consisting of reverse peaks. The resulting FT-IR spectral pattern is then analyzed and matched with known signatures of identified materials. The parameters used in FT- IR analysis were: spectral range 4000-200cm-^{1}, resolution 4cm.-^{1} The 10% plant extracted sample was subjected to FT-IR spectroscopic analysis (Thermo Nicolet, Avatar 370), equipped with KBr beam splitter with DTGS (Deuterated triglycine sulphate) detector (7800-350cm-^{1}), at Periyar university, Department of Physics, Salem, Tamil Nadu, India.

X-ray diffraction (XRD)

X- ray diffraction (XRD) pattern analysis was done to know the face centre cubic crystalline nature of the nanoparticles. The biosynthesized AgNPs using 10% plant extract was centrifuged and the pellet powder was collected. The powdered or dried AgNPs were coated on XRD grid and the spectra was recorded by using Rich seifert p 300 instrument operated at a voltage of 40 KV and a current of 30 mA with Cu Kα radiation.

SEM analysis

Scanning electron microscopic (SEM) analysis was performed using the Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by simply dropping a very small amount of the sample on the grid, with excess solution being removed using blotting paper. The film on the SEM grid was then allowed to dry by putting the grids under a mercury lamp for 5 min.

EDAX analysis

The particles were isolated by centrifuging 20ml of suspension in deionised water containing Ag nanoparticles for 20min at 10,000 rpm. The pellets were collected and were dried in the oven at 50◦C to remove any excess water. The sample was collected in the powder form and was used for EDX analysis. In order to carry out EDX analysis, the leaf extract-reduced

AgNPs were dried and drop coated on to carbon film. EDX analysis was then performed using the Hitachi S-3400N SEM instrument equipped with a Thermo EDX attachment.

DISCUSSION AND CONCLUSION

Silver based topical dressing has been widely used as a treatment for infections in burns, open wounds and chronic ulcers, silver nanoparticles and Ag^+ carriers can be beneficial in delayed diabetic wound healing as diabetic wounds are affected by many secondary infections and it can help the diabetic patients in early wound healing with minimal scars (Mishra *et al.,* 2008). Silver nitrate (Wright *et al.,* 1999) is still a common antimicrobial used in the treatment of chronic. Another area where silver nanoparticles have proven to be effective is in controlling and suppressing bacterial growth and it have been developed several applications which use the bactericidal effect of silver nanoparticles. The antibacterial properties of silver are documented since 1000 B.C., when silver vessels were used to preserve water. The first scientific papers describing the medical use of silver report the prevention of eye infection in neonates in 1881 and internal antisepsis in 1901. After this, silver nitrate and silver sulfadiazine have been widely used for the treatment of superficial and deep dermal burns of wounds and for the removal of warts (Rai *et al.,* 2009). The silver's mode of action is presumed to be dependent on Ag^+ ions, which strongly inhibit bacterial growth through suppression of respiratory enzymes and electron transport components and through interference with DNA functions (Li *et al.* 2006).

The study reveals the environment friendly cheap and simple method for the synthesis of silver nanoparticles using a *Acacia nilotica* plant leaf extracts of which itself played a dual role of stabilizing and reducing agent. The UV-spectra reveals the silver nanoparticles of A. *nilotica* synthesized at 416 nm and antimicrobial activity of plant extract shows more antimicrobial activity against *Escherichia coli* (25mm), and less activity against *Staphylococcus aureus* (12mm). FT-IR measurements were carried out to identify the bimolecular for capping and efficient stabilization of the metal nanoparticles synthesized. The FT-IR spectrum of *A. nilotica* plant leaf extract of silver nanoparticles showed the band between 850–2528 cm-1 corresponds to the different functional groups. The XRD peak corresponding to the 2θ=77.46, 64.62, 58.46, 53.98, 35.56, 21.84. The present method does not require the addition of any external reducing agents, The sizes of the AgNPs were it is observed from the SEM images that spherical AgNPs are polydispersed in nature with the average size of spherical NPs of 93.33-106.61nm and EDAX silver nanoparticles of *Acacia* *nilotica* and mostly showed strong signal energy peaks for silver atoms in the range 3.3–4.8 keV. These results could provide a possible mechanism for the synergistic or enhanced effects of antibiotics and AgNPs. These results suggest that AgNPs could be used as an adjuvant for the treatment of various infectious diseases caused by Gram-negative and Gram positive bacteria. Thus, our findings support the claim that AgNPs have considerable effective antibacterial activity, which can be used to enhance the action of existing antibiotics against Gram-negative and Gram-positive bacteria.

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