



SYNTHESIS, CHARACTERIZATION AND ANTI-BACTERIAL ACTIVITY OF SILVER NANOPARTICLES FROM LEAF EXTRACT OF *PHYLLANTHUS URINARIA*.L.

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Article Received on 01/06/2017

Article Revised on 21/06/2017

Article Accepted on 12/07/2017

ABSTRACT

In present study silver nanoparticles were synthesized from aqueous leaf extract of *Indigo era hirsute* L. plant leaf extracts will be collected were used and compared for their extracellular of metallic silver nanoparticles. Stable silver nanoparticles formed to be treated aqueous by solution of AgNO_3 with the plant leaf extracts as reducing agent Ag^0 to Ag^+ . UV spectroscopy is used to monitor the quantitative formation of silver nanoparticles. The synthesized silver nanoparticle is characterized with FT-IR, XRD, SEM and EDAX Characterization by the above said instrument analysis confirmed the presence, size and stability of the silver nanoparticles. After characterization, the silver nanoparticles were tested at various concentrations to check their bactericidal activity against clinical isolates of five bacterial pathogens. The silver nanoparticles exhibited good bactericidal activity at all concentration against all the tested organisms. Maximum zone of inhibition was of observed against *Vibrio cholera* (18mm at 400 μg) and minimum level of antibacterial activity was observed against *Proteus mirabilis* (8mm at 100 μg). This result suggested the potential use of silver nanoparticles against other clinical pathogens.

KEYWORDS: *Phyllanthus urinaria*, Silver nanoparticles, poultry pathogens.

INTRODUCTION

In recent year nanotechnology filed very important in the world. In 1974, Prof. Norio Taniguchi, was first to introduce the multidisciplinary It is used for all research area discipline covering research and technology from physics, chemistry and biology commonly nanotechnology. The synthesis of nanoparticles has introduced nanotechnology during the last two decades that produced novel compounds applied in various fields. The term "nanoparticles" used to describe a particle with size in the range of 1nm100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally the

nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases.

Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs. [Xu Z P et

al., 2006]. Silver nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical and magnetic properties) which can be incorporated into antimicrobial, applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. This chapter presents an overview of silver nanoparticle. Noble metal nanoparticles (NPs) have attracted a significant interest in materials and biological sciences owing to their potential applications in catalysis, biosensors, biomedical, photonics and heat transfer (Md Jani et al 2013).

Silver (Ag) nanoparticles have high therapeutic potential and exhibit good antimicrobial activity. Ag nanoparticles have a wide range of antimicrobial activities and exhibit high performance even at a very low concentration. Ag nanoparticles have been identified to possess good potential for the treatment of cancer (Govindan et al 2012). Nanoparticles play an important role in pharmaceutical, industrial and biotechnological applications. In particular, the silver nanoparticles are proved to have potential antibacterial, antifungal and antiparasitic and larvicidal properties.

Phyllanthus urinaria Linn. Commonly known as chamber bitter or stone-breaker, belongs to the genus *Phyllanthus* of family Euphorbiaceae. This is a widely used medicinal herb in jaundice, liver and urinary disorders and is often confused with other herbaceous species of *Phyllanthus*. The plant is used for several conditions such as blennorrhagia (gonorrhea), diabetes, dysentery, flu, tumors, jaundice (the yellow color of the skin and whites of the eyes caused by excess bilirubin in the blood), vaginitis (swelling, itching, burning or infection in the vagina), against headache, fever, conjunctivitis (pinkeye or bloodshot eyes), menstrual disorders and dyspepsia (pain or an uncomfortable feeling in the upper middle part of the stomach). Meniran has proven to be ant hepatotoxic, antiviral, antibacterial and hypoglycemic; also used for the elimination of kidney- and gallstones. The genus *Phyllanthus urinaria* Linn. (Euphorbiaceae) has between 550 to 750 species

and several of them produce useful secondary metabolites which have been extracted from whole plants (Unander 1996). *Phyllanthus urinaria* Linn. Commonly known as chamber-bitter or stone-breaker. The stems, infusion of leaves and roots of *Phyllanthus* Spp are used in folk medicine for treating intestinal infections, diabetes, the hepatitis B virus and disturbances of the kidney and urinary bladder (Calixto et al., 1998). Several compounds such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated and identified in various species of *Phyllanthus* and have shown ant nociceptive action in mice and other therapeutic activities (Cechinel Filho et al., 1996). *Phyllanthus urinaria*, one of the species belonging to the genus *Phyllanthus* (Euphorbiaceae), is used as a traditional folk medicine for the treatment of several diseases including hepatitis B (Wang et al., 1995) nephrolithiasis (Dias et al., 1995) and some painful disorders (Calixto et al., 1998). *Phyllanthus urinaria* is a herbaceous Euphorbiaceae of the subgenus *Phyllanthus* (Webster, 1957) used in folk medicine for treating intestinal infections, diabetes, the hepatitis B virus and disturbances of the kidney and urinary bladder (Unander, 1998). Diabetes mellitus is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrates, lipids and protein metabolism (Pickup J.C. and Williams G., 2003) Studies have shown that good metabolic control is beneficial in slowing the progression of these complications in diabetes (Floretto et al., 1998). As in many species of *Phyllanthus*, *P. urinaria* is said to possess medicinal properties (Ridley, 1897, 1906; Burkill and Haniff, 1930). For example, Burkill (1935) claimed that a little juice of *P. urinaria* on a bit of cloth may be used to clean a child's tongue, or the juice of a few leaves in coconut milk may be given to stimulate a child's appetite. Further, extracts taken internally are good at stimulating the kidneys. Ridley (1897) reported that the Malays used *P. urinaria* and *P. urinaria* vicariously, internally for diarrhoea, kidney ailments, gonorrhoea, and syphilis; as an emmenagogue, and after miscarriage and childbirth, or young leaves for coughs, especially in children. Recent studies by Nor Azizah (2002) demonstrated an inhibitory effect of extracts of *Phyllanthus* spp., including *P. urinaria*, on the formation and subsequent aggregation of oxalate crystals in kidneys, perhaps supporting the earlier belief among Malays that *P. urinaria* and *P. urinaria* can be used against kidney ailments. Perhaps the most promising use of *P. urinaria* is its medicinal values, as shown by several detailed studies focusing primarily on biochemical extracts and their pharmacological properties. *P. urinaria* was found to have anti-viral qualities (Yang et al., 1987; Unander and Blumberg, 1991; Bagchi et al., 1992; Cruz et al., 1994; Mi. et al., 1995; Prakash et al., 1995; Suthienkul et al., 1995; Liu et al., 1999; Kim et al., 1999; Jikai et al., 2000) and to be effective against bacteria and other pathogens (Cruz et al., 1994; Direkbusarakom et al., 1997; Lin et al., 1999). Extracts were found that could be used to treat liver and kidney ailments (Prakash et al.,

1995; Satyan et al., 1995; Hartini, 2002; Lo, 2002; Zuraihan, 2002) while antinociceptive or analgesic chemicals were also isolated (Dias et al., 1995; Santos et al., 1995; Satyan et al., 1995). Dias et al. (1995) reported that hydro alcoholic extracts caused graded contractions of guinea pig urinary bladders, while Santos et al. (1995) found them to have analgesic effects. Lo (2002) carried out detailed studies showing the effectiveness of extracts of *P. urinaria* and *P. urinaria* to arrest liver damage in mice. Extracts of *Phyllanthus* spp., including *P. urinaria* were also shown to have significant anti-cancer activity (Zurairhan, 2002). Hartini (2002) demonstrated the dose-dependent activities of extracts of *P. urinaria* on the fertility of mice, with 0.1 g/kg of body weight, the orally administered extracts of *P. urinaria* promote embryo production, whereas at 5 g/kg of body weight the extracts function as an anti-fertility agent. Anti-bacterial activity of *P. urinaria* against pathogenic bacteria, such as *Aeromonas hydrophila*, *Streptococcus* sp. and strains of *Vibrio* spp. in fish and shrimp, were reported by Direkbusarakom et al. (1997). In Brazil, Cruz et al. (1994) demonstrated similar activity against a range of common human dietary diseases. The results of this study support the traditional use of this medicinal plant in the treatment of urinary infections. Seeds are transversely ribbed on the back, and sides. It is used against colic, and as an effective remedy to eliminate gall - and kidney stones, urinary tract infection (UTI), bladder inflammation and for other kidney and liver problems in general such as acute - and chronic Hepatitis B. The primary action of shatterstone is on the liver; it acts by the inhibition of DNA polymerase on the hepatitis B virus. The enzyme DNA polymerase is needed for the virus to reproduce. Several studies suggest that *Phyllanthus urinaria* works better than the related species *p. amarus*, *p. debilis* and *p. niruri* in the treatment of hepatitis B. An equally important action is the use against kidney stones (renal calculi), urinary tract- and bladder infections. In preliminary research in animals, extracts of *Phyllanthus* plants have shown promising results in pain relief. The integration of nanomaterials with biology has led to the development of diagnostic device, contrast agent, analytical tools, physical therapy application and drug delivery vehicle. The highly selective approach reduces cost and human suffering, some potentially important application includes cancer treatment with iron nanoparticles or gold particles. The mechanisms involve efflux systems, alternation of solubility and toxicity via reduction or precipitation of metals and lack of specific metal transport systems (Beveridge et al., 1997) To study the synthesis of silver nanoparticles on plant extract and characterization of silver nanoparticles by, UV-Vis spectroscopy, FTIR, XRD, SEM and EDAX analysis and to study the antimicrobial activity of silver nanoparticles by agar well diffusion assay method.

MATERIALS AND METHODS

Plant Collection

Fresh leave *Phyllanthus urinaria*. L plant collected in Bharathidasan University campus at Trichy, Tamil Nadu, and India in the month of December 2015.



Preparation of Plant Extract

The fresh leaves were washed with running tap water in 15 minutes and dry in show at room temperature for one week. The leaves are cut into small pieces and make fine powder, 20g of leaves are weighted and dissolved in 100ml distilled water in a 500 mL Erlenmeyer flask and boiled for 30 min. The extract was filtered with glass cloth again filtered with what men filter paper No.1, were stored in an airtight container and protected from sunlight for further use.

Preparation of Silver Nanoparticles

1Mm of Silver nitrate (AgNO_3) was prepared in 1000ml bottle. The 100ml leave extract were mixed with 900ml silver nitrate solution in (1:9) ratio. The kept in dark condition and colour change of the solution from yellow to brown indicated that the silver nanoparticles were synthesized. Then go for centrifuge in 7000 rpm and 28°C for 15 min. then collect the pellet and kept in hot air oven for pellet is dried. Then further use for characterization studies and antimicrobial activity studies.

Characterization of Silver Nanoparticles

The characterization of silver nanoparticles was carried out by different instrument and technique. It includes visual observation, UV- Vis Spectrophotometer, FTIR, XRD, SEM, and EDAX.

UV-Visible spectroscopy analysis. The UV-visible spectroscopy measurements were performed on an UV - 1800 Shimadzu corporation Japan, spectrophotometer at a resolution of 1 nm from 200 to 800 nm at different time interval up to 24 hours. The UV-visible spectroscopy studies could be considered as the most useful technique for structural characterization of silver nanoparticles. The absorption spectra of nanoparticles increased in intensity as a function of time of reaction. The increase in intensity of colour occurred due to gradually increasing the number of silver nanoparticles synthesized from 200

the reduction of silver ions available in the aqueous solution.

FTIR Spectroscopy Analysis

In Fourier transform infrared (FTIR) analysis, the FTIR spectrum of the dried sample was recorded on a PerkinElmer 1600 instrument in the range 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} . The interaction between protein-AGNPs was analysed by Fourier transform-infrared spectroscopy (FT-IR).

X-Ray diffraction analysis (XRD)

The X-ray diffraction pattern indicated the crystalline structure of silver nanoparticles. The dried fine crystalline powder was used for Xrd analysis. The XRD spectrum confirmed the presence of silver nanoparticles. The diffracted intensities were recorded from 2θ angle.

SEM (Scanning Electron Microscope) analysis

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. The suspension of nanoparticles was dried into powder and about 1mg fine powder was used for the analysis. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDAX (Energy- Dispersive X-ray Spectroscopy) analysis

Energy-dispersive x-ray (EDAX) spectroscopy analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. EDAX is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray spectrum. To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as electrons or protons (see PIXE), or a beam of x ray, is focused into the sample being studied. The number and energy of the x rays emitted from a specimen can be measured by an energy-dispersive spectrometer.

Antimicrobial Activity of Silver Nanoparticles

Antibacterial activity was assayed by well diffusion method. Totally five clinical isolates of bacterial pathogens namely *Escherichia coli*, *Salmonella Typhi*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Pseudomonas mirabilis* were used for antibacterial assay. Nutrient agar medium was used for culturing bacteria. Chloramphenicol antibiotic was used as the positive control and sterile distilled water served as negative control. The assay was done with different

concentrations of silver nanoparticles and aqueous leaf extract of *Phyllanthus urinaria*.

RESULTS AND DISCUSSION

Phyllanthus urinaria extract is used to produce silver nanoparticles in this experiment. Ag^+ ions were reduced to Ag nanoparticles when plant extract is mixed with AgNO_3 solution in 1:9 ratio. Reduction is followed by an immediate change in yellowish to brown color in the aqueous solution of the plant extract due to excitation of surface Plasmon vibration in silver nanoparticle.¹⁸ Further formation of AGNPs in aqueous extract can be monitored by color change. Shows the color changes when the aqueous extract of *Phyllanthus urinaria* plant was mixed with an AgNO_3 solution. The mixture was kept at room temperature for 24 hours. The appearance of a yellowish-brown color in the reaction vessel indicated formation of AGNPs. AGNPs exhibit this yellowish-brown color in aqueous solution due to excitation of surface Plasmon resonance in the AGNPs.



(A) (B) (C) (D)
A- Plant Extract
B- Only Silver Nitrate solution
C- Initial Stage of Silver Nanoparticles Synthesis
D- Synthesis of Silver Nanoparticles

Silver nanoparticles (AGNPs) appear yellowish brown in colour in aqueous medium as a result of surface Plasmon vibrations.¹³ As the different leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint light to yellowish brown to reddish brown and finally to colloidal brown indicating AGNPs formation. Similar changes in colour have also been observed in previous studies and hence confirmed the completion of reaction between leaf extract and AgNO_3 . AGNPs occurred rapidly within the first 15 mins only and the AGNPs in solution remained stable even after 24 h of completion of reaction.

UV-VIS Spectrum analysis

UV-Vis spectra recorded at different time intervals from aqueous solution of silver nitrate with *Ananas comosus* extract. The samples display an optical absorption band peak at about 380 nm, typical of absorption for metallic Ag nanoclusters, due to the Surface Plasmon Resonance (SPR). Effect of the reaction time on AGNPs synthesis was also evaluated with UV-Visible spectra and it is noted that with an increase in time the peak becomes sharper. The increases in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution.

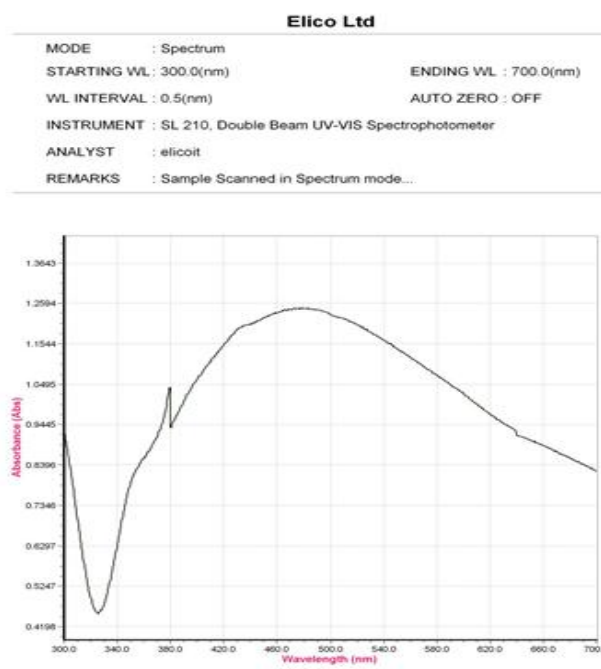


Fig. 1: UV – Spectrum Analysis.

FTIR Analysis

FT-IR measurement was carried out to identify the possible biomolecules in *Phyllanthus urinaria* responsible for capping leading to efficient stabilization of the AGNPs. FT-IR spectroscopy is used to identify the functional groups that play an important role in reducing and stabilizing the AGNPs prepared using *Phyllanthus urinaria* leaf extract. The FT-IR spectra of *Phyllanthus urinaria* leaf extract and AGNPs. The IR spectra of *Phyllanthus urinaria* leaf extract shows prominent bands at 3433, 2921.1, 1631.7, and 1384.4 cm⁻¹. The strong band observed at 3433 cm⁻¹ corresponds to hydroxyl functional groups in polyphenols [Sheny et al., 2011]. The IR band due to N-H bend is observed at 1631.7 cm⁻¹. The band observed at 1384.4 cm⁻¹ is identified as the alkanes due to the carbonyl stretch vibrations in the alkane's linkages of the proteins.

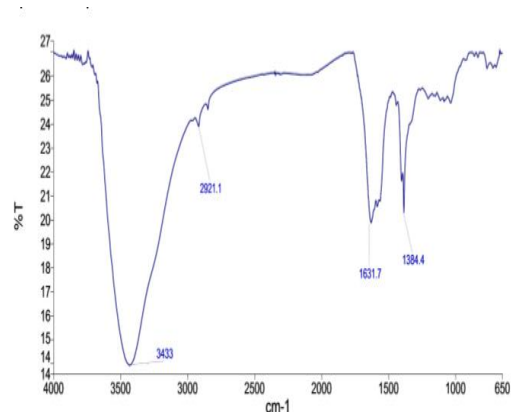


Fig. 2: Ftir Spectrum.

Table 1: FTIR spectrum characteristic features of silver nanoparticle synthesised *Phyllanthus urinaria*.

Frequency.cm-1	Bond	Functional group
3433	O-H Stretch, H-Bonded	Alcohols, phenols
2921.1	C-H Stretch	Alkanes
1631.7	N-H Bend	1° amines
1384.4	C-H Bend	Alkanes

The phytochemical analysis of the dried leaf extract of *Phyllanthus urinaria* has been reported to show the presence of vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans and phenolic. The flavonoids present in the leaf extract are powerful reducing agents which may be responsible for the reduction of Ag⁺ ions. The flavonoids and terpenoids present in *Phyllanthus urinaria* leaf extract can act as surfactant to attach on the surface of AGNPs and it stabilizes AGNPs through electrostatic stabilization. Thus it is found that *Phyllanthus urinaria* leaf extract has the ability to perform dual functions of reduction and stabilization of AGNPs.

X-Ray Diffraction Analysis (XRD)

Further demonstration and confirmation of the presence of silver nanoparticles biosynthesised by using the leaf extract of *Phyllanthus urinaria* in the reaction media was observed by X-Ray Diffraction (XRD) images. The characteristic peaks obtained in the XRD pattern. The XRD Patten showed four intense peaks in the spectrum of 2 theta values of 38.06°, 44.64°, 64.58 and 77.62° corresponds to 111, 200, 220, and 311 planes for silver nanoparticles. The TEM images reveals that the silver nanoparticles obtained by the reduction of Ag⁺ by the *Phyllanthus urinaria* leaf extract were predominantly spherical shaped. The size of the silver nanoparticles ranges between 30nm to 50nm. The selection area electron diffraction (SAED) pattern of the silver nanoparticles representing the face centered cubic (FCC) crystalline structure of silver nanoparticles is show in 3.

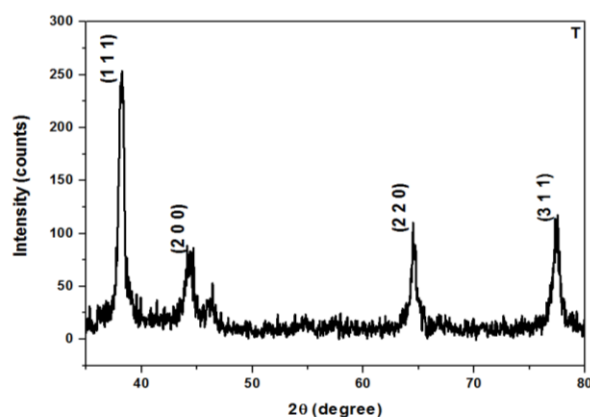


Fig. 3: Xrd Analysis.

SEM Analysis

SEM analysis provided further detailed insight into the morphology and size details of the silver nanoparticles. Our experiments results showed that the size of synthesized nanoparticles in the colloidal solution ranges from 50-83 nm. Particles observed are predominantly spherical in shape and they are quite well distributed without any agglomeration. The synthesized nanoparticles were well stabilized by capping agent (plant phytochemicals) hence they were not in direct contact even within the aggregates as seen in SEM image. Since these phytochemicals are involved in bonding with nanoparticles they provide charge to the nanoparticle.

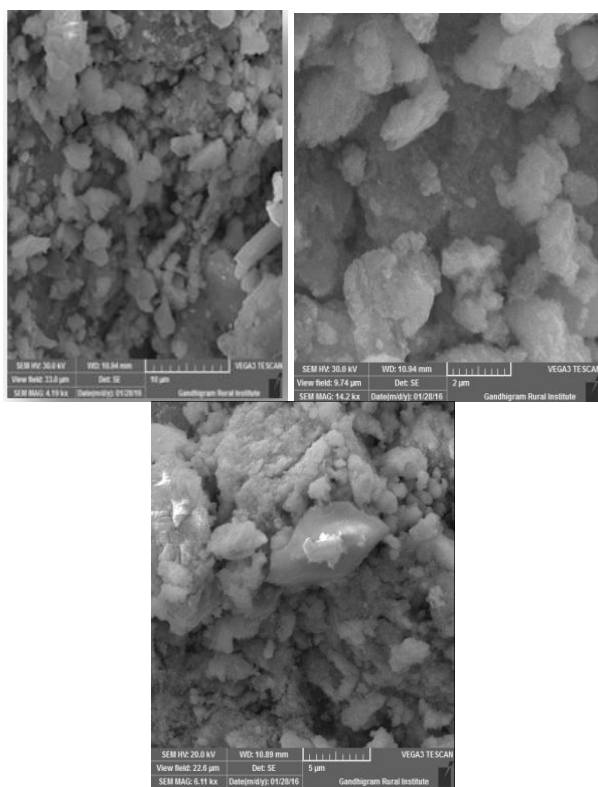
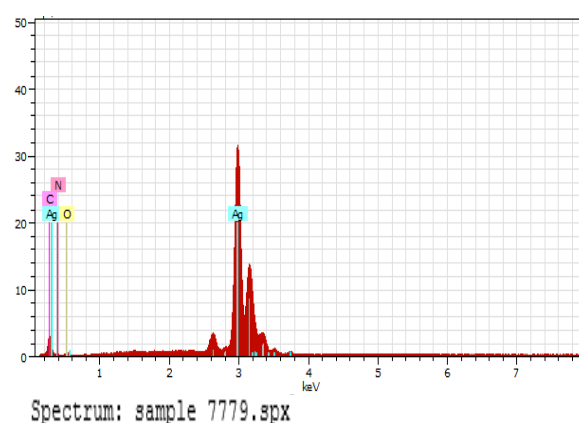


Fig. 4: SEM Analysis.

EDAX (Energy- Dispersive X-ray Spectroscopy) analysis

Further analysis of the silver particles by energy dispersive spectroscopy confirmed the presence of the signal characteristic of elemental silver. The Energy Dispersive Absorption Spectroscopy photographs of derived AGNPs. All the peaks of Ag are observed and are assigned. Peaks for O and C are from the grid used and the peaks for Ag and N correspond to the protein capping over the AGNPs. Silver Nano crystallites display an optical absorption band peak at approximately 3 KEV, which is typical of the absorption of metallic silver Nano crystallites due to surface.



El	AN	Series	unn.	C norm.	C Atom.	C Error (1 Sigma)
			[wt.%]	[wt.%]	[at.%]	[wt.%]
Ag	47	L-series	59.19	83.30	38.34	1.91
C	6	K-series	5.99	8.43	34.85	1.05
O	8	K-series	3.99	5.62	17.42	1.01
N	7	K-series	1.88	2.65	9.39	0.62

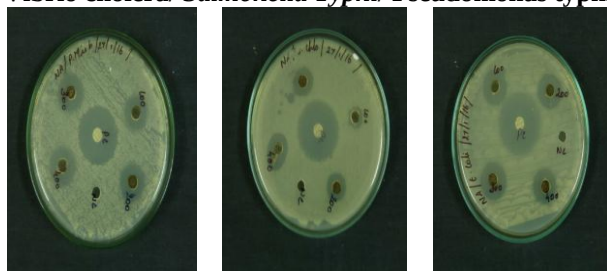
Fig 5: EDAX Analysis.

Antimicrobial Activity of Silver Nanoparticles

Antibacterial activity Silver nanoparticles pretence to have strong bactericidal activity against gram- negative and gram-positive bacteria including multidrug resistant strains. In the present study antibacterial activity of the biosynthesized AGNPs against different poultry pathogens. It is apparent that the AGNPs showed inhibition zone against almost all the test organisms the synthesized AGNPs were found to have higher inhibitory action when compared to the *Phyllanthus urinaria*. Moreover, AGNPs exhibit effective zone of inhibition against gram-negative bacteria (*E. coli* and *P. aeruginosa*) compared to the gram-positive bacteria (*S. aureus* and *B. subtilis*). Antibacterial effects of AGNPs obeyed an action mechanism of antibacterial activity. The potential reason for the antibacterial activity of silver is that AGNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller AGNPs having the large

surface area available for interaction would give more antibacterial effect than the larger AGNPs. It is also possible that AGNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria.

Vibrio cholera/ Salmonella Typhi/ Pseudomonas typhi



E.coli

Klebsiella Sps.

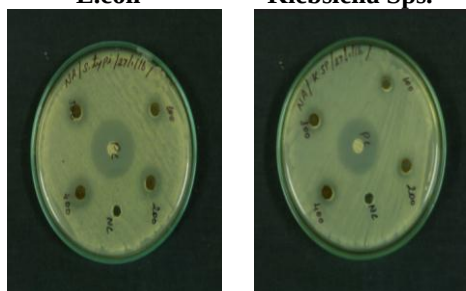


Fig. 6: Inhibitory action of control, *Phyllanthus urinaria* leaf extract of silver nanoparticle synthesis and AGNPs against Poultry disease causing organisms.

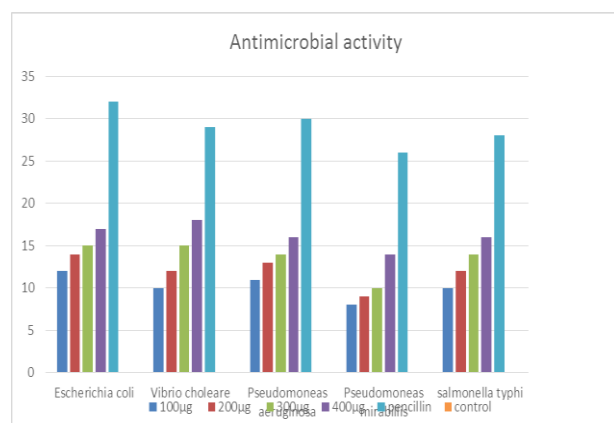


Fig. 7: *Phyllanthus urinaria* synthesised silver nanoparticles zone of inhibition against poultry pathogens.

Silver nanoparticles exhibited brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles (Shankar, et al, 2004). As the *Phyllanthus urinaria* leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to brown due to reduction of silver ion; which denoted formation of silver nanoparticles. UV-Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [Shankar et al, 2006]. Absorption spectra of silver nanoparticles formed in the reaction

media had absorbance peak at 380 nm and the broadening of peak indicated that the particles were polydispersity.

FTIR analysis was used further for the characterization of the resulting nanoparticles. FTIR absorption spectra of silver nanoparticles showed absorbance bands (before bioreduction) in the region of 3433, 2921, 1631.7, 1384.4. In particular, the 1226 cm⁻¹ band arose most probably from the C–O group of polyols such as hydroxyflavones and catechins. The total disappearance of this band after the bioreduction might be due to the fact that the polyols were mainly responsible for the reduction of silver ions, whereby they themselves got oxidized to unsaturated carbonyl groups leading to a broad peak at 1226cm⁻¹ for reduction of silver.

The biosynthesised silver nanostructure by employing *Phyllanthus urinaria* plant leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and the structural view under the scanning electron microscope. The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging from 40 to 80. Average size of the particles synthesized was 15nm with size range 40 to 80nm with cubic and orthorhombic crystals.

The SEM images showing the high density silver nanoparticles synthesized by *Phyllanthus urinaria* plant leaf further confirmed the development of silver nanostructures.

The typical XRD pattern revealed that the sample contained a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 15 nm derived from the FWHM (full width at half maximum) of peak corresponding to 111 plane.

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Phyllanthus urinaria* plant leaf extract as observed by the UV-Vis spectroscopy revealed the presence of silver nanoparticles. The XRD, SEM, TEM analyses showed the particle size between 25-50nm as well the cubic structure of the nanoparticles. FTIR analysis confirmed that the bio reduction of silver ions to silver nanoparticles was due to the reduction by capping material of plant extract. The present study, thus showed a simple green route for rapid and economical synthesis of silver nanoparticles.

In the analysis by Energy Dispersive Spectroscopy of the AgNPs, the presence of elemental metal signals was confirmed. EDAX analysis gives quantitative and qualitative status of elements that may be involved in formation of nanoparticles synthesized nanoparticle using *Phyllanthus urinaria* plant aqueous leaf extracts and confirms the formation of silver nanoparticles. Spectrum also indicates some unidentified peaks in image which

may be due to copper grid used for EDAX analysis. EDAX results also show higher counts at 5 keV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance. The anti-bacterial activity was done on poultry pathogenic *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella Typhi* by the standard disc diffusion method.

CONCLUSION

Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields.

The field of nanotechnology is new and upcoming research area in the Indian scenario. Much has already been studied in synthesis of nanomaterials through physical and chemical processes. But synthesis of nanomaterials by biological agents offer several advantages like establishment of green chemistry lead to a pollution free production of nanomaterials in contrast to energy dependent, pollution based physical and chemical methods.

Nanotechnology has dynamically as an important field of modern research with potential effects in electronic and medicine. The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Among the various known synthesis methods, the use of plants for synthesis of AGNPs are rapid low cost, eco-friendly, safe for human therapeutic uses and a single step methods for biosynthesis process.

The silver nanoparticles synthesized by *Phyllanthus urinaria* plant leaf extract were characterized by UV-VIS spectrophotometer, FTIR, SEM, EDAX and XRD analysis.

EDAX results also show higher counts at 5 KEV due to silver nanoparticles. Generally metallic silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance.

We have found that the silver nanoparticles synthesized in our study effectively resistant to the growth and multiplication of pathogenic bacteria like *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Pseudomonas mirabilis*, *Salmonella Typhi*.

ACKNOWLEDGEMENT

We thankful to Dr. D. Dhanasekaran, Department of Microbiology, Bharathidasan University for providing Lab facilities and culture to carry out the research project.

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