Asian Journal of Innovative Research 2(2) 01-11 (2017)

ISSN 2455 7285

ASIAN JOURNAL OF INNOVATIVE RESEARCH
Available online at http://www.asianjir.com

Research Article Botany

Received 18th Mar.2017;
Accepted 15 Apri. 2017
Online April 2017

CHARACTERZATION AND APPLICATION OF SILVER NANOPARTICLES SYNTHESIZED FROM *Ipomea aquvatica* L.

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ABSTRACT

In this present study to explore that the novel approaches for the biosynthesis of silver nanoparticle using plant. The plant, *Ipomea aquatica* leaves were used in this study, where the dried leaf extract was mixed with silver nitrate in order to synthesis of silver nanoparticles. The synthesized nanoparticle size range from 40 – 80 nm was confirmed in SEM analysis. Silver nanoparticles might be useful for the development of newer and more potent antimicrobial agents. The data represented in our study suggested that leaf extract has controlled nanoparticle synthesized and possess potential biological activity including antimicrobial activity.

Keywords: Silver nanoparticle, SEM, FTIR, UV Vis spectroscopy, *Ipomea aquatica* leaf, Phytochemistry

***Citation*:** G. Subasri, S. Ahmed John, R.Muthumani (2017). Characterzation and application of silver nanoparticles synthesized from *Ipomea aquvatica* L. Asian Journal of Innovative Research 2(2) 01-11 (2017).

INTRODUCTION

Plant derived medicines have been used in traditional health care systems for the treatment of various ailments and diseases since time immemorial. According to the World Health Organisation (WHO), it has been estimated that 80% of the world's population is still dependant on traditional medicines for maintaining their health and combating various diseases. Besides, 56% of world's populations in the rural areas rely chiefly on herbal medicine and supplementation for their primary health care needs. Today bacterial infections, fungal infections, hypertension, diabetes, malaria and cancer are the common health problems in rural communities throughout the world. A huge number of traditionally important medicinal plants have been known to be biologically effective against these diseases. One such potential plant is *Ipomea aquatica* .

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (AgNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects (Jeong *et al.,* 2005). Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Krutyakov *et al.,* 2008). In small concentrations, silver is safe for human cells, but lethal for microorganisms (Sharma *et al.,* 2009). Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology (Prabhu *et al.,* 2010). In this present study to explore that the novel approaches for the biosynthesis of silver nanoparticle using plant. The plant, *Ipomea aquatica* leaves is used in this study, where the dried leaf extracts was mixed with silver nitrate in order to synthesis of silver nanoparticles.

MATERIAL AND METHODS

Collection of plant materials :

The leaf of *Ipomea aquatica* was collected from Thirukattupalli, Thanjavur district, Tamil Nadu, India. The leaves were rinsed with water thrice followed by distilled water to remove the fine dust materials and then the leaves were dried for 1 week to completely remove the moisture.

Preparation of alcoholic extract:

The leaf of *Ipomea aquatica* were first washed well and dust was removed from the leaf. The leaves were coarsely powdered. The powder was extracted with aqueous and 70% methanol for 24 hours. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Synthesis of silver nanoparticles (AgNPs)

Two different conical flasks were taken and add 5 ml of *Ipomea aquatica* leaf extract separately. 45 ml of 1 mM aqueous $AgNO_3$ solution added to the each conical flask. The flask was then incubated in the dark at 4 hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using SEM analysis (Arunachalam *et al.*, 2012).

UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 330-920 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

Spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a FTIR spectrophotometer (Perkin Elmer Spectrophotometer system, USA) followed by previous methods with some modification (Liu *et al*., 2006). A small amount of liquid of silver nanoparticle was respectively placed directly on sample holder of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 400 cm^{-1} and computerized for analyses by using the 21 CFR part 11 software. The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. The peak values of FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using VEGA3 LMU machine. 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 films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Histochemical tests

The powders of *Ipomea aquatica* were treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The *Ipomea aquatica* leaf treated with phloroglucinol and diluted HCl gave red colour indicates lignin, treated with diluted ammonia and H_2SO_4 gave yellow colour indicates flavonoids, and treated with Dragant draft reagent gave brown colour indicates alkaloids, and treated with $Fecl_3$ solution few drops gave dark blue colour indicates Tannin, and treated with diluted Hcl gave dark black colour indicates Crystals, and treated with Lieberman acetic an hydride and H_2SO_4 gave violet colour indicates Steroids, and treated with Toludine blue gave blue colour indicates Polyphenol

and treaded with DNPH gave orange colour indicates Terpenoids , and treated with H_2SO_4 gave Yellow colour indicates Saponin.

Determination of antimicrobial activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans*, *Aspergillus flavus* was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (30µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

RESULTS AND DISCUSSION

Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites. Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. (Sofowara, 1993).

Plants synthesize an array of chemical compounds that are not involved in their primary metabolism. These 'secondary compounds' instead serve a variety of ecological functions, ultimately to enhance the plants survival during stress. In addition these compounds may be responsible for the beneficial effects of fruits and vegetables on an array of health related measures. Medicinal plants are assumes greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase of demand in international trade because of very effective, cheaply available, supposedly have no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments (Liu, 2003).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the *Ipomea aquatica* investigated and summarized in Table-1 and fig-2, 3. The phytochemical screening aqueous extract of *Ipomea aquatica* showed that the presence of tannin, steroids, saponins, terepenoids, carbohydrate, glycosides, anthriquinone and Flavonoids, triterpenoids, alkaloids while, phlobatannins, protein and polyphenolics were absent. Methanol extract of *Ipomea aquatica* showed that the presence of, steroids, saponins, triterpenoids, phenolics, carbohydrate, anthriquinone and glycosides, flavonoids, tannin, protein while terepenoids, phlobatannins and alkaloids were absent.

Reported the occurrence of flavonoids, saponins, diterpenes and phorbol estersin in the aqueous and methanol extracts of *Euphorbia heterophylla*. examined the powdered leaf material of different solvent of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* using and ethanol. Different extracts of *Semecarpus anacardium* were analysed by for its phytochemical properties.

Detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated for phytochemical analysis and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins investigated the phytochemical components of four medicinal plants used for the treatment of malaria in Southwestern Nigeria.

Histochemical studies

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues, it is a powerful tool for localization of trace quantities of substances present in biological tissues (Krishnamurthy, 1998). Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as proteins, lipids, starch, phytin and minerals like calcium, potassium and iron (Krishnan *et al*., 2001). The importance of histochemistry in solving critical biosystematic problems is as popular as the use of other

markers. According to botanical literatures, the use of histochemical characters in taxonomic conclusions is now a common practice. Table 2 and figure 4 represents histochemical studies of *Ipomea aquatica* leaf powder. This study further confirmed the presence of phytochemicals in *Ipomea aquatica* leaf.

Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the grain extract were considered responsible for the reduction of silver ions. It is well known that silver nanoparticles exhibit yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Thirumurgan *et al.*, 2010). The appearances of yellowish-brown colour in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Shankar *et al.*, 2004).

Silver nanoparticles are being extensively synthesized using many different biological sources including fungi, bacteria and plants (Shivaji *et al.*, 2011; Shaligram *et al.*, 2009). Among them the plant mediated nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. This method of nanoparticles synthesis involves no toxic chemicals and termed as green chemistry procedure. In this present study, *Ipomea aquatica* extract was used for the synthesis of silver nanoparticles. The aqueous $AgNO_3$ solution turned to brown colour formed after 4 hours with the addition of leaf extract (Plate 1 shows- $AgNO_3$ and AgNPs), indicating the formation of AgNPs in the reaction solution probably as a result of the excitation of Surface Plasmon Resonance (SPR) bands (Mulvaney, 1996). The control tubes ($AgNO_3$) showed no change in colour when incubated in a similar condition (Plate 1.

Ultraviolet/visible (UV/VIS) spectroscopy

The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, and composition and aggregation state of your sample. Your sample may absorb light, scatter light, or both. As a general rule, smaller particles will have a higher percentage of their extinction due to absorption. Scattering from a sample is typically very sensitive to the aggregation state of the sample, with the scattering contribution increasing as the particles aggregate to a greater extent. For example, the optical properties of silver nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighbouring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions.

The absorption spectrum of silver nanoparticles changes as the particles transition from a well-dispersed state to an aggregated state following the addition of a concentrated salt solution; as aggregation proceeds, the plasmon peak at 380nm decreases, a secondary peak at 560 emerges, and the baseline elevates due to scattering by aggregates. It is observed that the maximum absorbance of *Ipomea aquatica* silver nanoparticles occurs at 380 nm and silver nanoparticle occurs at 520nm. As the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates) (Tian, 2005).

The rapid and irreversible change in the extinction spectrum clearly demonstrates that the nanoparticles are agglomerating. UV/Visible spectroscopy can be used as a characterization technique that provides information on whether the nanoparticle solution has destabilized over time. In the present investigation the peak was decreased due to destabilization of nanoparticles (Fig 1).

FTIR Spectroscopic analysis of silver nanoparticle

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra are used in the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. FTIR spectrum of *Ipomea aquatica* extract shows bands at 679, 1363, 1630, 2088, 2368, 2719, 2832, 3434 and 3466. The FTIR spectra of the *Ipomea aquatica* is given in the Figure 6 which show the presence of silver nanoparticles, peak at $3464cm^{-1}$ which are assigned as – OH stretching in alcohols and phenolic compounds (Janakiraman *et al*.,2011).The band appeared at about 1640 cm^{-1}can be assigned for aromatic rings. The strong broad band appearing at 3466 cm^{-1}can be associated to the stretching vibrations of alcoholic and phenolic O–H. At $1363cm^{-1}$a peak is observed that could be for plant ascribed to multiplet C=O group (Fig 2).

SEM analysis

SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed spherical Ag-NPs of various sizes. The SEM image showing the high density silver nanoparticles synthesized by the *Ipomea aquatica* extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 40-80nm as well the cubic, face-centred cubic structure of the nanoparticles (**Fig** 3).

Antimicrobial activity

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extract has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Torresdey *et al*., 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The SNPs of shows highest antibacterial activity than *Ipomea aquatica* was observed against *E. coli* and *Staphylococcus aureus* The inhibitory activities in culture media of the Ag nanoparticles reported in table 3 comparable with standard antimicrobiotic viz chloromphenical.

In this study to evaluate the antimicrobial effect of Ag nanoparticles against various bacterial strain such as *E. coli* and *Staphylococcus aureus* here were distinct differences among them. When Ag nanoparticles were tested they effectively inhibited bacterial growth. The results show that Ag nanoparticles having antimicrobial activity against *E. coli* and *Staphylococcus aureus* that was similar to that found by Sondi and Salopek-Sondi (2004).

These results suggest that the antimicrobial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al*., 2011). We think that the lower efficacy of the Ag nanoparticles against *E. coli* and *Staphylococcus aureus*, *Candida albicans* and *Aspergillus flavus* may derive from the difference as a point of membrane structure. To confirm this hypothesis, further comparative study between various gram-negative and gram-positive bacterial species is needed. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent. The AgNPs synthesized from plant species are toxic to multi drug resistant microorganisms. It shows that they have great potential in biomedical applications (Table 3and Fig 4).

Table: 1 Phytochemical screening of *Ipomea aquatica*

S.No	Phytochemical analysis	Aqueous	70% Methanol
1	Tannin	+	++
2	Phlobatannins	-	-
3	Saponin	+	+
4	Flavonoids	+	++
5	Steroids	+	++
6	Terpenoids	+	-
7	Tritrpenoids	+	+
8	Alkaloids	+	-
9	Carbohydrate	+	+
10	Protein	-	+
11	Anthroquinone	+	++
12	Polyphenol	-	+
13	Glycoside	+	+

(+) Presence ; (-) Absence

Table 2 Histochemical studies of *Ipomea aquatica* leaf powder

S.No.	Secondary metabolites	Observation	Result
1	Lignin	Red/Pink	+
2	Flavonoids	Yellow	+
3	Alkaloids	Reddish Brown	+
4	Tannin	Dark Blue to Black	+
5	Crystals	Dark Black	+
6	Starch grain	Blue	---
7	Steroids	Violet to Blue (or) Green	+
8	Poly phenol	Blue green/Red	+
9	Terpenoids	Orange	+
10	Saponin	Yellow	+

(+) Presence ; (-) Absence

Table 3 Antimicrobial activity of *Ipomea aquatica* AgNPs

Microorganism	AgNPs	Extract	$AgNO_3$	Std.
Escherichia coli	5.10±0.35	1.30±0.09	0.20±0.01	8.20±0.57
Staphylococcus aureus (mm)	4.20±0.29	0.90±0.06	0.10±0.01	8.30±0.58
*Candida albicans (*mm)	4.10±0.28	0.50±0.035	0.20±0.01	7.90±0.55
Aspergillus flavus (mm)	3.50±0.24	0.20±0.15	0.10±0.01	7.70±0.53

Values were expressed as Mean ± SD.
AgNPs = Silver Nanoparticles; $AgNO_3$ = Silver nitrate
Bacterial standard - Chloramphenicol

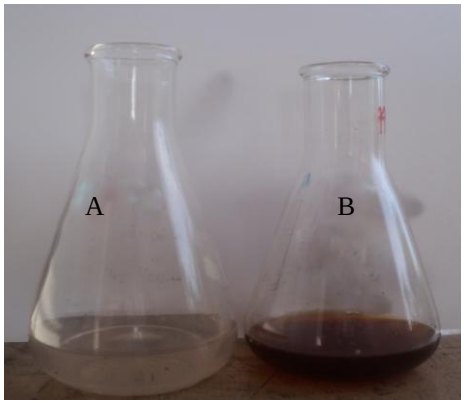


Plate 1. (A) Aqueous solution of 1mM $AgNO_3$ with. (B) Silver nanoparticles

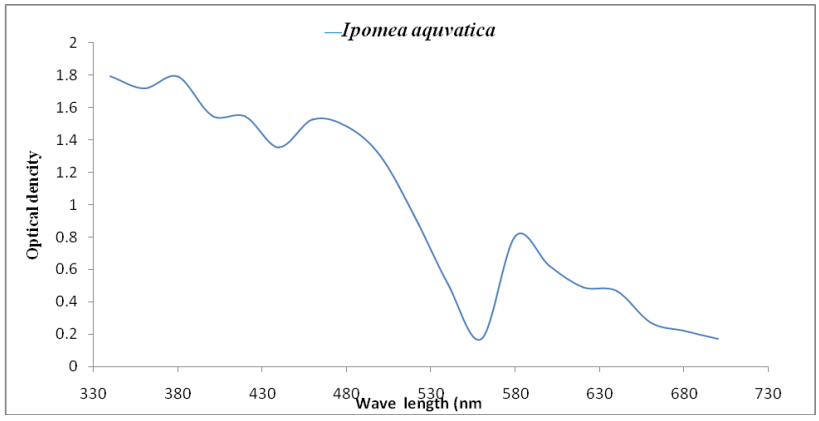
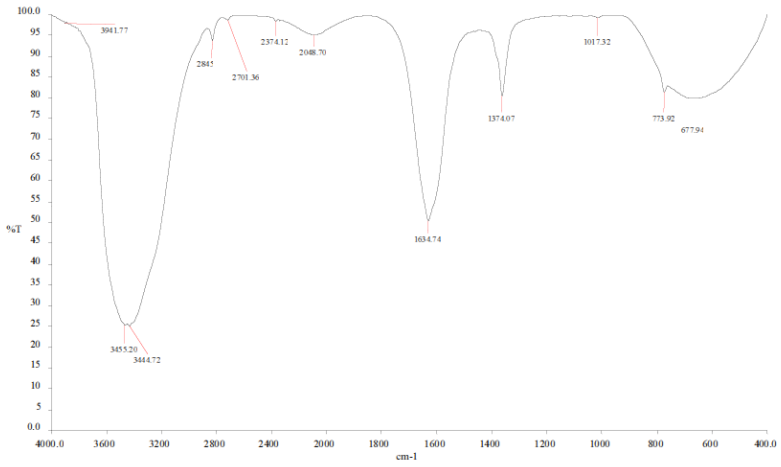


Fig 1. UV-Visible spectrum of AgNPs synthesized from *Ipomea aquatica* extract

Fig 2. FTIR Spectroscopic analysis of silver nanoparticle



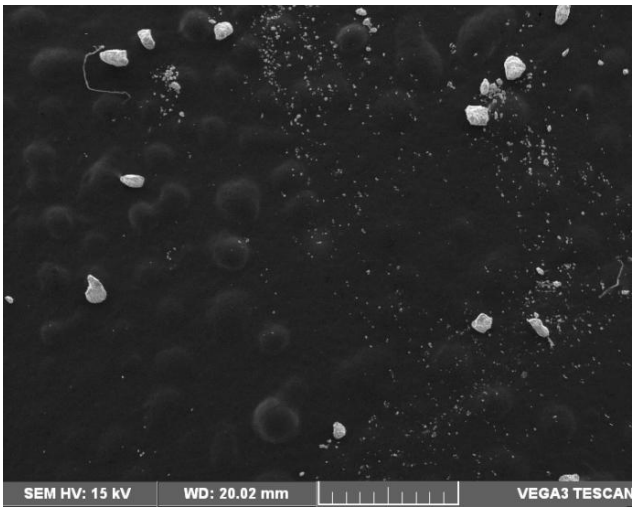
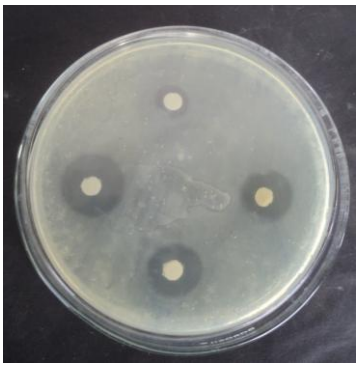
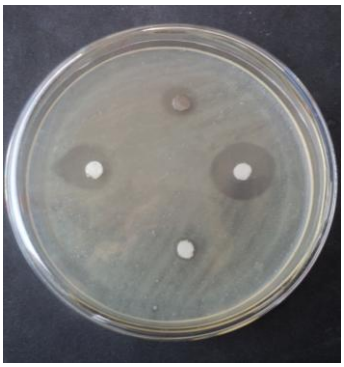


Fig 3. High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed AgNPs ranged between 40–80 nm.

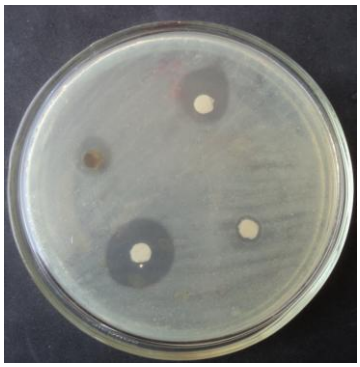
Fig 4: shows the antibacterial activity of *Ipomea aquatica* AgNPs



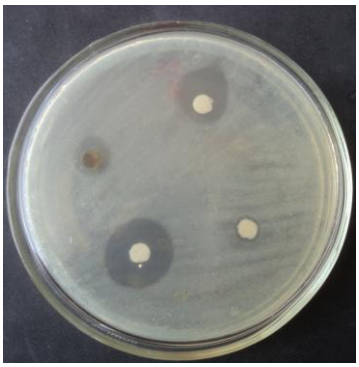
Escherichia coli



Staphylococcus aureus



Candida albicans



Aspergillus flavus

The present study exhibit a simple environmentally benign method of synthesis of silver nanoparticles from a novel primitive plant source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. The synthesized nanoparticle size range from 40 – 80 nm was confirmed in SEM analysis. Silver nanoparticles might be useful for the development of newer and more potent antimicrobial agents. The data represented in our study suggested that leaf extract has controlled nanoparticle synthesized and possess potential biological activity.

Acknowledgement

The authors are grateful to Director, Harman Institute of Science Education and Research (www.harmanresearchcentre.com), Thanjavur, Tamil Nadu for his support and facility.

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Source of support: Nil;
Conflict of interest: None declared