International Journal of Pharmaceutical Archive-3(5), 2014, 391-399
Available online through www.ijpaonline.info ISSN 2319-7226

RESEARCH ARTICLE

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES AND THEIR POTENTIAL ANTI-MICROBIAL ACTIVITY IN *Artanema fimbriatum*

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(Received on: 27-04-14; Revised & Accepted on: 05-05-14)

ABSTRACT

The present study reports the synthesis of silver nanoparticle using Artanema fimbriatum leaf extract were used as reducing agent for reduction of silver nitrate solution. The synthesis of silver nanoparticles was analyzed by TECAN. The FTIR analysis has shown that size of silver nanoparticles synthesized from leaves extract of Artanema fimbriatum was 200 nm and seems to be spherical in morphology. Morphology of chemically synthesized silver nanoparticles is nearly spherical and of size ranges from 300-500nm. The average particle size analyzed from FTIR analysis eas observed to be 350 nm.This article has discussed the synthesis of silver nanoparticles generated from plant extract, characterization and antibacterial analysis. In this study the antibacterial activity was examined against 8 pathogenic bacteria cultures collected from (Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella, Bacillus, Serattia marcesens, Klebsilla, Psudomonas Aeruginosa). These microorganisms were obtained from the Department of microbiology, central leather research institute, Chennai. These antimicrobial activity of the silver nanoparticles showed maximum zone of inhibition. The silver nanoparticles synthesized using Artanema fimbriatum leaves extract would be a better antimicrobial effective against various bacterial species.

Key words: *nanoparticle, antimicrobial activity, TECAN, FTIR.*

INTRODUCTION

Nanotechnology broadly refers to a field of applied science and technology whose special and unique properties could be attributed to their small sizes and large surface areas. It is the application of science and technology to manipulate the matter at atomic and molecular scale. It has the ability to build micro and macro materials and products with atomic precision. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity. Currently it is employed as a tool to explore the darkest avenues of medical science in several ways like imaging, sensings, targeted drug delivery, gene delivery, and artificial implants. Nanoparticles are being sighted as fundamental building blocks of nanotechnology. In recent years, the research is mainly focused on the metal nanoparticles due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. There is a method known as Green synthesis, which is biosynthesis of nanoparticles using plant extracts, which was exploited to a vast extent because the plants are widely distributed, easily available, safe to handle and with a range of metabolites. Human beings are often infected by microorganisms such as bacteria, molds, yeasts and viruses in the living environment. Research in antibacterial material containing various natural and inorganic substances has been intensive. The use of environmentally bening materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Numbers of plants have been successfully used for the extracellular synthesis of silver nanoparticles including *tarmarind, Helianthus annus, Cinnamomum camphora, Coriander, Capsicum annuum, Avena graveolens.*

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In the present study, we have explored the green synthesis of silver nanoparticles using *Artanema fimbriatum* leaves extract. Synthesized nanoparticles were characterized by TECAN and FTIR. Further, the antimicrobial activity of synthesized silver nanoparticles against 8 pathogenic bacteria cultures collected from (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella, Bacillus, Serattia marcesens, Klebsilla, Psudomonas Aeruginosa*). These microorganisms were obtained from the Department of microbiology, central leather research institute, Chennai.

Artanema fimbriatum

1.1a scientific classification

Kingdom : plantae
Order : Lamiales
Family : Plantaginaceae
Genus : Artanema D. Don.

The genus Artanema is a small group of flowering plant species in the plantain family, plantaginaceae, (but also classified in linderniaceae by some authors).

MATERIALS AND METHODS

3.1 Plant collection and identification

The leaves of *Artanema fimbriatum* were collected from Tirupattur, Tamil nadu in December 2013. The plant samples was identified and confirmed by Dr.Raman, Msc. PhD, Head of the department, Botany, University of Madras.

3.1a Preparation of plant extracts *(P.Rahate-2013)*

The plants leaves were shade dried and grounded using electrical mixer. The powder obtained was used for extraction. Extract was prepared using ethanol and chloroform. 20 g of each of the powdered leaves were placed in a conical flask and 100 ml of 70 % ethanol and chloroform was added and covered using a cotton plug. The flask was left undisturbed for 48 hours at room temperature in dark. After 48 hours, the filtrate was collected by filtration and the crude extract was obtained after the ethanol and chloroform evaporated. This was used for the future processing steps.

$$\%\ \ yield = \frac{Amount\ \ of\ \ substance\ \ obtained(\text{extract})}{Amount\ \ of\ \ substance\ \ taken(\text{gram})} \times 100$$

Preliminary Phytochemical Screening *(Chulet Rahul 2010)*

The phytochemical tests were carried out using the crude extracts obtained. Procedures derived from various literature sources were put into effect.

QUANTITATIVE ANALYSIS

Estimation of tannins *(P.Rahate 2013)*

Determination of total phenolic composition *(Asha kale 2010)*

Estimation of total flavonoids content Aluminium chloride colorimetric Method (Kalita Pallab 2013)

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Activity *(Kalita Pallab 2013)*

Antimicrobial screening for plant leaf extract (*Artanema fimbriatum*)

The antimicrobial activities of the leaf extract from the plant: *Artanema fimbriatum* was determined using 8 pathogenic bacteria (Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella, Bacillus, Serattia marcesens, Klebsilla, Psudomonas Aeruginosa). These microorganisms were obtained from the Department of microbiology, central leather research institute, Chennai. All the microbes were maintained in slant of nutrient Agar. They were all sub culture in to stock culture of nutrient Broth.

Each of the extract weighed 1g were dissolved in 100 ml of 70 % ethanol. Their solvent of extraction to obtained concentration of 10 mg/ml. This concentration was used as the initial concentration to check the Antimicrobial activity of the plant against the tested microbes. Nutrient agar was the medium used as the growth medium for the microbes, and the medium was prepared according to the manufacture's instruction. The medium was weighed and dissolved in measured distilled water in a conical flask, capped with cotton wool and heated to dissolved using Bunsen burner.

It was then sterilized at121 ^{o} C for 15 minutes using autoclave. The medium was allowed to Cool 45^{o} C, and 20 ml of medium was poured to sterile petridishes and allowed to solidify. This screening of the antimicrobial activity of the extracts was carried out using the well diffusion technique. The medium was seeded with the test microbes; the inoculums of each microbe were spread evenly over the surface of the prepared plates with the aid of sterile swab. All the seeded plates were allowed to dry and a stranded cork borer of 5mm in diameter was used to produce the wells on the surface of prepared plates. The extracts were introduced in to the wells. The inoculated plates were all incubated at 37^{o} C for 24 hours, after which the plates were observed for the zone of inhibition of the growth and the zones were measured using a transparent ruler which was recorded in millimeters.

SILVER NANO PARTICLES

EXPERIMENTAL PROCEDURES *(Rajesh Kotcherlakota 29th Jan 2014)*

Preparation of *Artanema fimbriatum* leaf extract in ethanol

300 grams of fresh *Artanema fimbriatum* leaves were thoroughly washed with distilled water (3 times) in a 500 ml beaker. The cleaned leaves were allowed to dry for 2 weeks and then 20 grams of dried leaf powder in 100 ml of 70% ethanol were placed in conical flask. Then filter the Ethanolic extract and dried. Finally 2.45g of *Artanema fimbriatum* leaf crude powder was obtained. 10mg of this crude extract powder was dissolved in 1ml of 70 % ethanol.

125 grams of fresh *Artanema fimbriatum* leaves were thoroughly washed with distilled water (3 times) and grained with500 ml of distilled water by using mortor and pestel. This fresh plant extract has been used for the synthesis & characterizations of AgNPs and other experiments.

Biosynthesis of silver nanoparticles (b- AgNPs) *(Rajesh Kotcherlakota 29th Jan 2014)*

In this present article, we have used *Artanema fimbriatum* leaf extract as reducing as well as stabilizing / capping agent for the formation of silver nanoparticles. The color, shape, size and stability of silver nanoparticles depend on the concentration/ volume of the reducing agent (here *Artanema fimbriatum* extract). Therefore, in order to get optimize $AgNO_3$, we have carried out a series of reactions using the various concentrations of *Artanema fimbriatum* extract (100-500 µl) (table 3) keeping constant concentration of $AgNO_3$ (600µL of 10 $^{-2}$ M). This green chemistry approach for the synthesis of AgNPs is simple, efficient, economically cheap and environmentally friendly method that does not need any special conditions such as sophisticated instrument, vacuum condition, catalyst, template etc., Finally, the reaction was carried out under ambient conditions in water (universally accepted solvent).

Chemical synthesis silver nanoparticles (c-AgNPs)

Silver nanoparticles were also synthesized by chemical methods in order to compare the biological activity with biosynthesized silver nanoparticles. In a typical experiment, c-AgNPs were prepared by the Reaction of 600 µL of AgNO3 (10 $^{-2}$ M) using 300µl of plant leaf extract (*Artanema fimbriatum*). And kept in the sunlight for 2 hrs. The color will be change. The loose dark ash pellet was collected from chemically synthesized c- AgNPs by centrifugation at 15,000 rpm at 15^{0} C for 15 minutes by using cooling centrifuge ultra 100 and sonication. This chemically synthesized c- AgNPs were used for Antibacterical studies and bacteria culture have been discussed in detail to the Supplementary Material.

Chemical Synthesis of Silver nanoparticles in Acetate Buffer (pH 4.6) with Mannose

Silver nanoparticles were also synthesized by chemical methods in order to compare the biological activity with biosynthesized silver nanoparticles. In the experiment, 5ml of c-AgNPs, 5 ml of 0.5 % of Gelatin solution and stirring at 24 hrs, and Centrifugation at 15000 rpm at 15^{0}C for 15 minutes. The loose dark ash pellet was collected and added 10 ml of acetate buffer (pH 4.6) contains 0.5 % of Mannose and stirrer at 24 hrs and centrifugation. The pellet was collected and used for the antimicrobial studies.

STABILITY TEST

Table: 1 Stability test for silver nanoparticles by using *Artanema fimbriatum*

Exp.No	AgNPs(µl)	Buffer(pH 4.6, 6.4 , 7.4, 8) (ml)	NaCl (ml)	Cystenine (ml)	Histidine (ml)
1	500	1.0	1.0	1.0	1.0
2	500	1.0	1.0	1.0	1.0
3	500	1.0	1.0	1.0	1.0
4	500	1.0	1.0	1.0	1.0
5	500	1.0	1.0	1.0	1.0

Antimicrobial screening for silver nanoparticles (c-AgNPs)

The antimicrobial activities of the silver nanoparticles, silver nanoparticles in acetate buffer (pH4.6) contains Mannose was determined using 8 pathogenic bacteria (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella, Bacillus, Serattia marcesens, Klebsilla, Psudomonas Aeruginosa)* These microorganisms were obtained from the Department of microbiology, central leather research institute, Chennai. All the microbes were maintained in slant of nutrient Agar. They were all sub culture in to stock culture of nutrient Broth. Each of the extract weighed 1g were dissolved in 100 ml of 70% ethanol. Their solvent of extraction to obtained concentration of 10 mg/ml. This concentration was used as the initial concentration to check the Antimicrobial activity of the plant against the tested microbes.

Nutrient agar was the medium used as the growth medium for the microbes, and the medium was prepared according to the manufacture's instruction. The medium was weighed and dissolved in measured distilled water in a conical flask, capped with cotton wool and heated to dissolved using Bunsen burner. It was then sterilized at 121^{o} C for 15 minutes using autoclave. The medium was allowed to Cool 45 ^{o} C, and 20 ml of medium was poured to sterile petridishes and allowed to solidify. This screening of the antimicrobial activity of the extracts was carried out using the well diffusion technique. The medium was seeded with the test microbes; the inoculums of each microbe were spread evenly over the surface of the prepared plates with the aid of sterile swab. All the seeded plates were allowed to dry and a stranded cork borer of 5mm in diameter was used to produce the wells on the surface of prepared plates. The extracts were introduced in to the wells. The inoculated plates were all incubated at 37^{o} C for 24 hours, after which the plates were observed for the zone of inhibition of the growth and the zones were measured using a transparent ruler which was recorded in millimeters.

RESULTS AND DISCUSSION

Result of phytochemical screening and quantitative analysis

The result of the preliminary phytochemical screening give a clear evidence for the presence of Alkaloids, Carbohydrates, Proteins, Amino acids, Glycosides, Steroids, Tannins and Flavonoids. The test also revealed that the absence of saponins. Table 4 showed the results in a qualitative manner. Quantitative analysis for the total Tannins, total Phenolic and Flavonoids of the plant leaf extract showed in table 5

Table: 2 Results of phytochemical screening for the plant leaf extract *Artanema fimbriatum*

Compounds	**Ethanol**	**Chloroform**
Alkaloids	+ +	+
Carbohydrates	+ +	+ +
Proteins	+	+
Amino acids	+	_
Glycosides	+ +	_
Saponins	+ +	+ +
Steroids	+	_
Tannins	+	+
Flavonoids	+ +	+ +

+ Low in abundance, + + High in abundance,-ve Absent.

Table: 3 Result of percent extract value

Extract	*% Extractive value (w/w)*
Ethanol	12.297%
Chloroform	6.115%

Table: 4 Result of quantitative estimation of total Tannins , Phenolics and Flavonoids

Plant	**Extract**	**Total Tannins (mg)/100 mg**	**Total phenolics (mg)/100 mg**	**Total Flavonoids (mg)/100 mg**
Artanema fimbriatum	Ethanol extract	82.00	1.3632	o.1561

Figure: 5 Standardization of Total tannins content

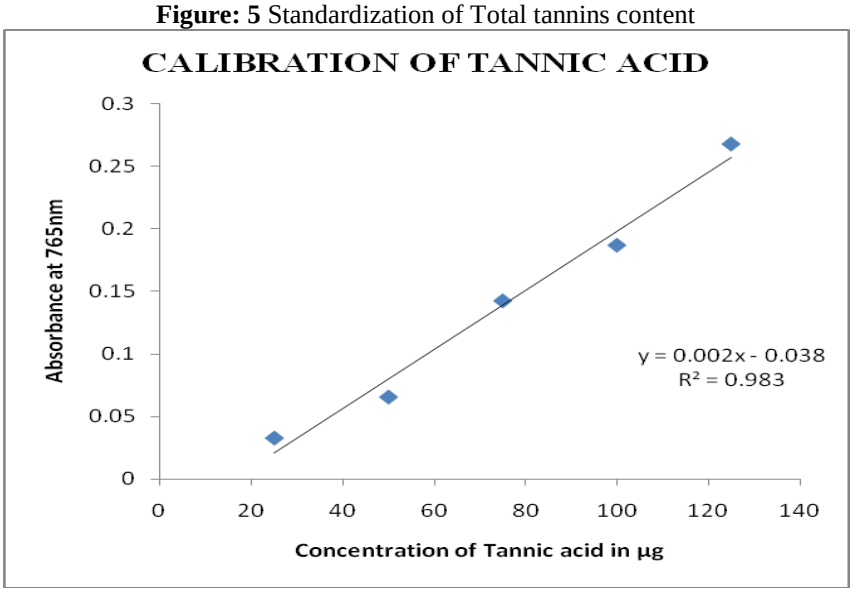


Figure: 6 Standardization of Total phenolic content

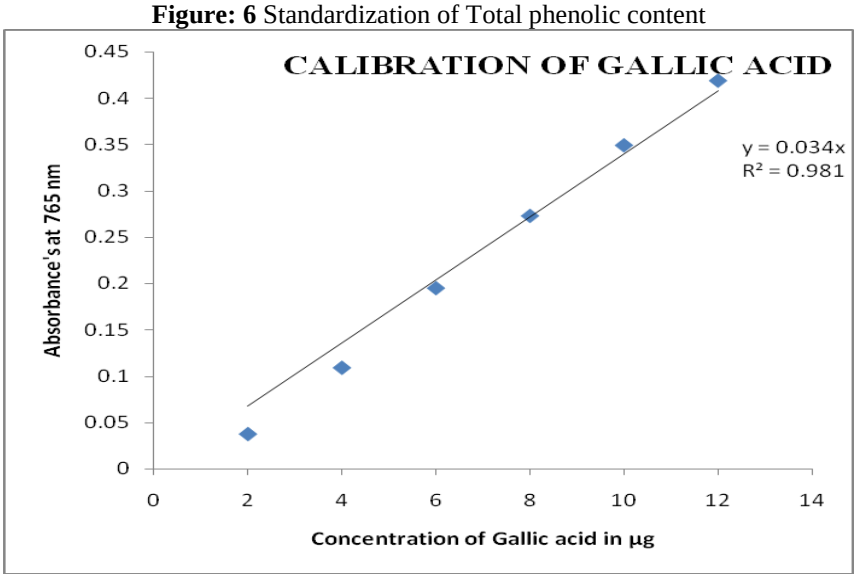
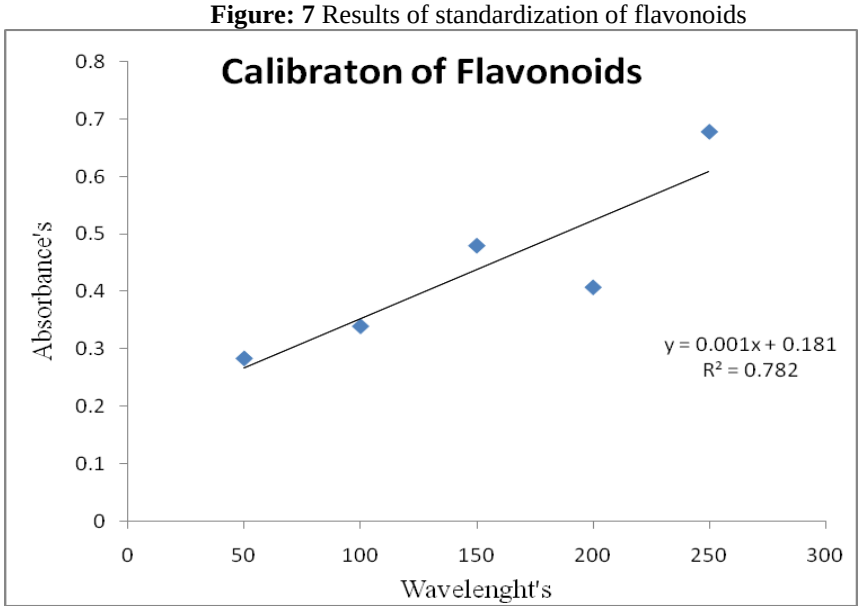


Figure: 7 Results of standardization of flavonoids



Result of antioxidant activity

The result of antioxidant activity of the plant extract was exerted as the inhibitory effects of these extracts against DPPH stable free radical. The IC_{50} inhibitory concentration of Ethanolic extract of the plant as well as quercetin, ascorbic acid and butaylated hydroxy anisole (positive control), are expressed in the table 6. Inhibitory effects of plant extract at 200 µg / 200 µl.

- The percentage inhibition of Artanema fimbriatum was found to be 60.5858 %.

Table: 5 Result of DPPH inhibition by the plant extract as well as positive control (the result expressed IC_{50} in µg of extract / µl)

Plant and control	**IC_{50} of DPPH inhibition in µg /µl (Ethanolic extract)**
Artanema fimbriatum	3.2566 ± 0.0412
Quercetin	0.0954 ± 0.0133

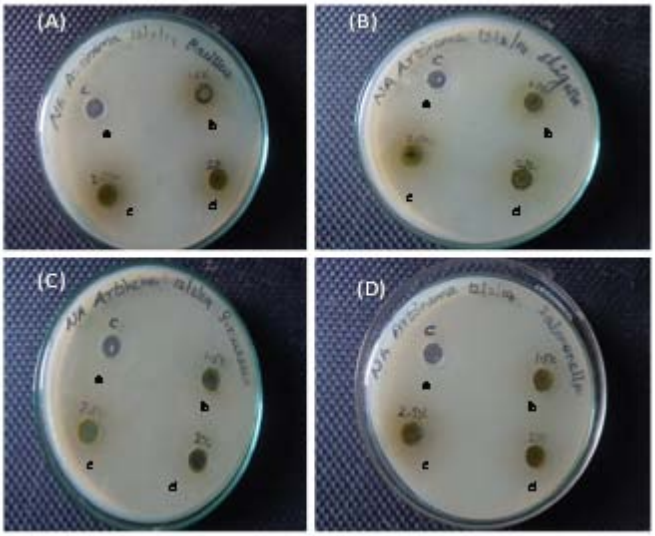
Result of Antimicrobial Activity in plant extract *Artanema fimbriatum*

Ethanol successive extract is test for this antibacterial activity. The doses of 1 mg/1ml of extrac were made by dissolving appropriate quantity of extracts. The solution of test compounds (1 %, 2 %, 3 %) are added in the cups by using micropipettes and these plates are incubated at 37^0 C for 24 hrs. The zone of inhibition is measured in mm for each organism. Controls did not show any activity. The crude extract shows positive antimicrobial activity.

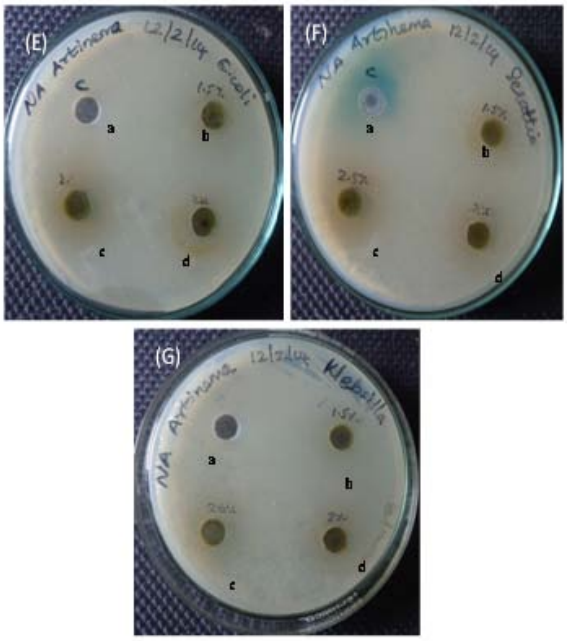
Table: 6 *Zone of inhibition for Artanema fimbriatum in*

Organism	**1.5% of extract(mm)**	**2% of extract (mm)**	**2.5 % of extract (mm)**
E.coli	11	12	21
Salmonella	10	16	23
Shigella	11	16	21
Klebsiella	12	19	23
S.aureus	08	17	22
Bacillus	10	15	22
Serratia	09	15	19

Figure: 8



A. Inhibition zone against *Bacillus*
B. Inhibition zone against *Shigella*
C. Inhibition zone against *Staphylococcus aureus*
D. Inhibition zone against *Salmonellatyphi*



E. Inhibition zone against *Escherichia coli*
F. Inhibition zone against *Serattia marcesen*
G.Inhibition zone against *Klebsilla*

RESULTS OF SILVER NANOPARTICLE

Biosynthesized silver nanoparticles (b-AgNPs) have been synthesized using Artanema fimbriatum leaf extract, which acts as reducing as well as stabilizing capping agent. The one step synthesis reaction was carried out at ambient conditions (room temperature and atmospheric pressure) in water solvent, a universally accepted solvent. Our biosynthesis method for b-AgNPs follows almost all principles of green chemistry approach In order to get optimized and stable b-AgNPs, We have carried out a series of reactions using the various concentrations of *Artanema fimbriatum* leaf extract (Table1). According to experimental conditions described in Table 3 and characterization data (discussed later), the experiment number #3 is the optimized reaction conditions for the biosynthesis of nanoparticles (b-AgNPs-500) base on color, stability, size and charge of nanoparticles. therefore the optimized silver nanoparticles (b-AgNPs-500) have been utilized for detailed characterization and further *in-vitro* assays for several biomedical applications of antibacterial studies

Result of Antimicrobial Activity in silver nanoparticles, silver

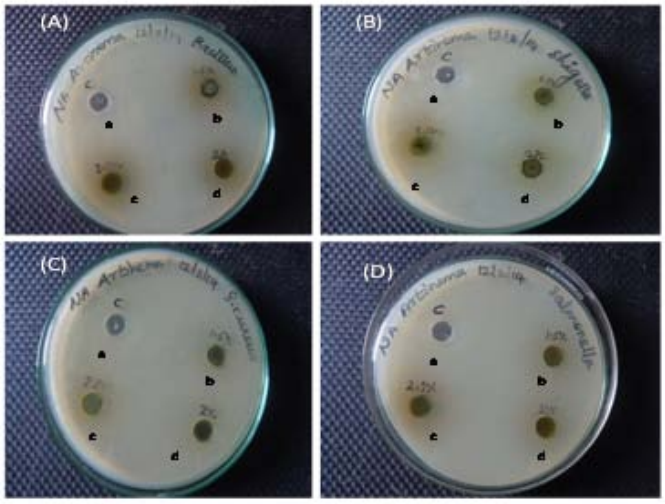
nanoparticles in acetate buffer (pH 4.6) contains mannose

Silver nanoparticles, silver nanoparticles in acetate buffer (pH 4.6) contains mannose is test for this antibacterial activity. The solution of test compounds (20µl, 40 µl, 60 µl) are added in the cups by using micropipettes and these plates are incubated at 37^0 C for 24 hrs. The zone of inhibition is measured in mm for each organism. Controls did not show any activity. The Silver nanoparticles, silver nanoparticles in acetate buffer (pH 4.6) contains mannose shows positive antimicrobial activity.

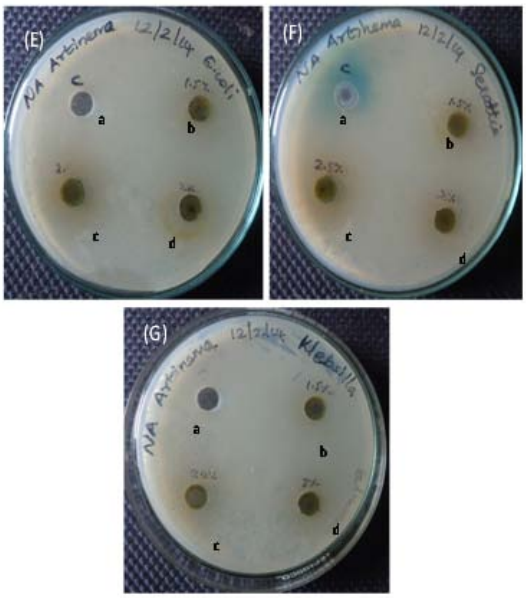
Table: 7 *Zone of inhibition for Artanema fimbriatum*

Organism	1.5% of extract(mm)	2% of extract (mm)	2.5 % of extract (mm)
E.coli	13	14	23
Salmonella	12	18	26
Shigella	13	19	24
Klebsiella	14	21	25
S.aureus	10	19	22
Bacillus	12	17	21
Serratia	11	18	23

Figure: 13



A. Inhibition zone against *Bacillus*
B. Inhibition zone against *Shigella*
C. Inhibition zone against *Staphylococcus aureus*
D. Inhibition zone against *Salmonellatyphi*



E. Inhibition zone against *Escherichia coli*
F. Inhibition zone against *Serattia marcesen*
G. Inhibition zone against *Klebsilla*

CONCLUSION

The Artanema *fimbriatum* plant leaf contains alkaloids, carbohydrates, amino acids, steroids, flavonoids, tannins and phenolic compounds, which posses antioxidant property. Hence the further investigation and proper isolation of more active principles might help in the findings of new lead compounds which will be effective against free radical mediated diseases. we have developed a simple green chemistry approach for the synthesis of silver nanoparticles (b-AgNPs) by *Artanema fimbriatum* leaf extract that demonstrate the multifunctional activities of bio-synthesized AgNPs. Surprisingly, we have observed the biocompatible nature of b-AgNPs. Bio-synthesized silver nanoparticles (b-AgNPs) shows enhanced antibacterial activity compared to chemically synthesize silver nanoparticles (b-AgNPs). All results together demonstrate the multifunctional activities of bio-synthesized AgNPs towards biomedical applications that could be applied as anti-bacterial. A plausible mechanistic approach has been investigated for anti-bacterial of b-AgNPs. Formation of reactive oxygen species induced by b-AgNPs is one of the plausible mechanisms for anti-bacterial activity. We strongly believe that bio-synthesized AgNPs will open a new direction towards various biomedical applications in near future.

REFERENCES

1. Uma Maheswari. R "Plant mediated synthesis of silver nanoparticles by using leaf extracts *Dioscorea Oppositifolia* L. and their antimicrobial activity".
2. Sarika Sharma. (26th Oct 2013) "synthesis, characterization and antibacterial potential of silver nanoparticles by *Morus nigra* leaf extract".
3. Rajesh Kotcherlakota.(16th Dec 2013) "Potential Theranostics Application Of Bio-synthesized Silver Nanoparticles".
4. Preetha Devaraj.(8th July 2013) "Synthesis and characterization of silver nanoparticles using cannon and their cytotoxic activity against MCF-7 cell line".
5. Amany O Abdeen.(April 2013) "Green synthesis of silver nanoparticles using *Carob* leaf extract and its anti-bacterial activity".
6. Mohamed S. Abdel Aziz.(6th Oct 2013) "Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract".
7. Yamini Sudhalakshmi.G(2011) "Green synthesis of silver nanoparticles from *Cleome viscose*: synthesis and antimicrobial activity".
8. Bhaskar Mitra.(2012) "Green-synthesis and characterization of silver nanoparticles by aqueous leaf extracts of *Cardiospermum helicacabum* leaves".
9. Balashanmugam. P(2014) "Synthesis of plant mediated silver nanoparticles using *Aerva lanata* leaf aqueous extract and evaluation of its anti-bacterial activities".
10. Linga rao.M (December 2013) "Green synthesis of silver nanoparticles by *Allamanda cathartica* L. leaf extract and evaluation for antimicrobial activity".

Source of support: Nil, Conflict of interest: None Declared