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Research Article

Green Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles (SiNPs)

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Abstract: At present nanotechnology has been flourishing at a remarkable rate in all aspect of Science and Technology. Now, nanobiotechnology is a new branch of nanotechnology and alternating method for chemical and physical methods, and which can be used to green synthesize silver nanoparticles. Here, we present eco-friendly green synthesis method to synthesize silver nanoparticles using tuber extract of *Cyperus rotundus* which can be exploited in antibacterial application. The aqueous silver nitrate solution while exposed to tuber extract was bio reduced and resulted in silver nanoparticles. The green synthesized silver nanoparticles were characterized using UV-Visible spectrophotometer, FTIR Spectroscopy and DLS which were used for support of the biosynthesis and average particles size of silver nanoparticles. Duly characterized silver nanoparticles were used for evaluation of antibacterial activity against *Bacillus thuringiensis* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Pseudomonas* (Gram-negative). As stated by the present literature, this is the first report in green synthesis of silver nanoparticles using tuber extract of *Cyperus rotundus*.
Keywords: Nanoparticles, Silver Nanoparticles (SiNPs), Nanobiotechnology, Characterization techniques and Antibacterial activity.

Introduction

Nanotechnology has started journey from organic chemistry and now it reached to every aspect of Science and Technology. At present it has been drawn a special attention in medical and all allied branches to bring forward its success in the fields of physical, chemical and biological sciences. It is the study of manipulating material at an atomic and molecular scale, and deals with between 1nm to 100 nm size structures in at least one dimension to develop different devices within the size. In 1974, Taniguchi was described nanotechnology mainly consists of the processing of separation, consolidation and deformation of materials by one atom or by one molecule. Nanotechnology term was applied by Eric Drexler (1986) in his book, which proposed the idea of nano-scale and also promoted the technological significance of nano-scale phenomena and devices. Nanotechnology has dynamically developed as significant vicinity of modern research with potential efforts in medicine and electronics (Glomm, 2005, Boisselier and Astruc, 2009).

Nano biotechnology is a new branch of Nanotechnology which combines the biological principles with physical and chemical methods to make nano-size particles with specific functions. It describes an application of biological system and an economic alternative method for green synthesis of functional nanoparticles. Biosynthetic methods can employed either microorganisms or plant extracts for synthesis of various nanoparticles.

In nanotechnology, the development of reliable and eco-friendly method is extremely important for synthesis of metallic nanoparticles. Number of physical and chemical methods is being used to synthesize large quantity of nanoparticles but these are not eco-friendly due to use of organic solvents and toxic reducing agents. Therefore, present is an increasing demand for nanobiotechnology (Singhal *et al.*, 2011) to synthesize different nanoparticles using biological and bio-mimetic approaches. The biosynthesis process is a kind of bottom up approach in synthesis of nanoparticles and microbial or plant phyto-chemicals are usually responsible for reduction of metal compounds into their respective nanoparticles. Both extra-and intra-cellular green synthesis of nanoparticles have been reported till date using microorganisms including bacteria, fungi, and plants (Kowshik *et al.*, 2003; Raut *et al.*, 2010).

Plants are free from toxic chemicals as well as provide natural capping agents and they will provide better platform for synthesis of nanoparticles. The first report of the plant employed in the synthesis of nanoparticles is attributed to *Medicago sativa* (alfalfa) which was capable of synthesizing gold and silver nanoparticles (Gardea-Torresdey *et al.*, 2003). Most of the studies confer the production of nanoparticles by plants that were known to be stable than microbial synthesis (Tripathy *et al.*, 2010; Venkatasubramanian *et al.*, 2010; Lalitha *et al.*, 2013, Raveendran *et al.*, 2003). Plants are widely distributed, easily available, safer to handle and act as a source for several phyto-metabolites thus using plant extracts is the most adopted method for green and eco-friendly synthesis of nanoparticles.

The bio-reduction behavior of different plant leaf extracts such as *Helianthus annuus* (*Asteraceae*), *Basella alba* (*Basellaceae*), *Oryza sativa*, *Saccharum officinarum*, *Sorghum bicolour* and *Zea mays* (*Poaceae*) in the synthesis of silver nanoparticles was investigated and reported by Leela and Vivekanandan (2008). In 2008, Shankar *et al.*, reported the rapid reduction of silver ions to the formation of stable and crystalline silver nanoparticles using *Geranium* leaf extract by treating silver nitrate solution. This study also represents an important advance in the use of plants over microorganisms in the biosynthesis of metal nanoparticles. An eco-friendly method for formation of stable silver nanoparticles has been reported using *Jatropha curcas* seed extract by Bar *et al.*, (2009). Rapid synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its effective inhibitory antibacterial activity against water borne pathogens like *Escherichia coli* and *Vibrio cholera* was reported by Krishnaraj *et al.*, (2010). The extracellular synthesis of stable and crystalline silver nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre and their inhibitory effective against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomoanas fluorescens aeruginosa* and *Klebsiella pneumonia* was studied by Raut *et al.*, (2010).

In 2010, Tripathy *et al.*, reported the biomimetic synthesis, morphology and size of silver nanoparticles using *Azadirachta indica* (Neem) leaves extract. Effective biological route for synthesis of silver nanoparticles by rapid reduction of silver ions (Ag^+ to Ag^o) using hot water *Cyperus rotundus* grass extracts (CRGE) and their morphological features reported in 2014 by Siva *et al.* The integration of nanomaterials with biology has led to the development of antimicrobial activities because of their increase chemical activity. Silver nanoparticles have important in antibacterial agent against *Staphyloccocus aureus*, *Pseudomonas fluorescens aeruginosa* and *Escherichia coli* bas been investigated (Rai *et al.*, 2009).

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The antibacterial properties of the biosynthesized silver nanoparticles when incorporated on textile fabric were investigated by Kong and Jang (2008). The silver nanoparticles were also used for impregnation of polymeric medical devices to increase their antibacterial activity. *Cyperus rotundus* is a medicinal plant and perennial shrub in a height up to 40 cm and found in all types soils (drained and dry soils) or climates. The plant is native to Africa, Southern and Central Europe and Southern Asia, and now found throughout India. It has a dark green thin stem, long and sharp with a width of 1/6 to 1/3 inch of leaves, triangular cross-section of flower stem, 2 to 8 inch length of flowers, and rhizome forms of root system which is grow horizontally and form dark reddish brown tubers or chains of tuber. This plant consists of various chemical compounds viz. alkaloids, vitamins, minerals, epoxides, monoterpene, aliphatic alcohols, luteolin, auresidin, cyperen-1&2, muskatone, kobusone, isokobusone and aromatic & stable oil compounds. It is used as an insect repellent, for perfuming clothing (Venkatasubramanian *et al.,* 2010). Therefore the plant has great medicinal values and is used for medicinal purpose, which is used in both, internally as well as externally. The root extract oil instilled into eyes in conjunctivitis reduces the pain, redness and ocular discharges. Application of its paste on the breasts purifies the breast milk. In obesity, the massage with its dry powder (udvartana) is extremely beneficial for reducing the subcutaneous fat deposition. Internally *C. rotundus* is used in vast range of diseases. It is useful in digestive disorders and also one of the most effective menstrual regulators (Shamkuwar *et al.,* 2012).

In this present study, we performed green synthesis of Silver Nanoparticles (SiNPs) using aqueous solution of tuber extract of *Cyperus rotundus*, their characterization through modern analytical techniques and also antibacterial applications. In fact this is the first and only research study available *Cyperus rotundus* tuber till to the date will demonstrate on study of inhibitory bacterial activity using SiNPs.

Materials and Methods

Preparation of Plant Extract

The medicinal plant, *Cyperus rotundus* was collected from Tirumala hills, Andhra Pradesh, India on the basis of cost effectiveness, ease of availability and medicinal property. Fresh and healthy tubers were collected, thoroughly rinsed with tap water and then distilled water to remove dust and unwanted particles from the tubers. Now the tubers were cut into small pieces and dried at room temperature for 7 days. About 25 gm of incised tuber was weighed and powdered using mixer. About 4 g. of *Cyperus rotundus* tuber powder was weighed and mixed well in 40 ml distilled water at 40^0C temperature for 15 min. The obtained extract was filtered through Whatman No.1 filter paper and then the filtrate was centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and stored at 4^0C for further use.

Green synthesis of Silver Nanoparticles (SiNPs)

Aqueous solution (0.01N) of silver nitrate ($AgNO_3$) was prepared and used for green synthesis of Silver Nanoparticles (SiNPs) from tuber extract of *Cyperus rotundus*. 10 ml of *Cyperus rotundus* tuber extract solution was added to 5 ml of 0.01 N of $AgNO_3$ solutions. Within 30 minutes a light yellowish brown color change was observed, indicating that the green synthesis of SiNPs (Figure 1). The bio-reduction of silver nitrate to silver ions was confirmed by color changes from light yellow brown color to dark brown color. Then the solution was allowed for 48 hrs to yield a deep brown color. Now, the bio-reduced solution was centrifuged at 3,000 rpm for 15 minutes to eliminate large aggregates and excess of *Cyperus* extract from solution. Subsequently, the supernatant was collected and kept as final silver nanoparticle product. Accordingly the SiNPs were characterized using UV Visible spectrophotometer, Fourier Transform Infra-Red Spectroscopy and Dynamic Light Scattering (DLS).

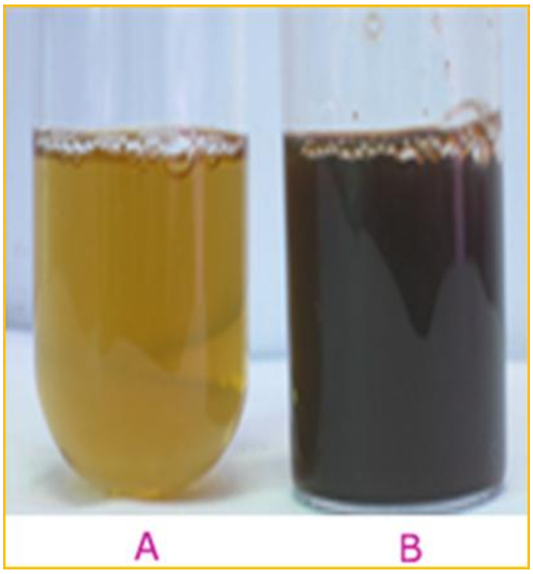

Figure 1. *Cyperus rotundus* tuber extracts (A) and green synthesized SiNPs (B).

Characterization techniques

The green synthesized SiNPs were characterized using the following characterization techniques.

UV-Visible Spectrophotometer

Periodically the samples were collected from aqueous solution and observed the complete bioreduction of silver ions (Ag^+) using UV Visible Spectrophotometer at a wavelength range of 200 nm to 800 nm. The synthesized SiNPs has shown the absorbance due to Surface Plasmon Resonance of silver nanoparticles, and the absorption strongly dependent on particle size, dielectric constant and surface adsorbed species.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrophotometer, with in the range of 4000–500 cm^{-1} was used for analysis of green synthesized silver nanoparticles.

Measurement of particle size and Zeta potential

The average particles size and zeta potential of bioreduced SiNPs were determined by dynamic light scattering (DLS) which is based on LASER diffraction method with multiple scattering techniques. The bio-reduced solution was centrifuged at 25^0 C with 5000 rpm for 15 minutes and then the supernatant was collected. Now, the collected supernatant was diluted for 4 to 5 times and used for mean size of particles and zeta-potential using dynamic light scattering (DLS).

Antibacterial activity

Antibacterial activity of green synthesized silver nanoparticles was evaluated with Whatman No.1 filter paper discs (3/6 mm in diameter) using standard agar disc diffusion method against *Bacillus thuringiensis* (Gram-negative), *Staphylococcus*

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aureus (Gram-positive) and *Pseudomonas fluorescens* (Gram-negative) (Becerro *et al.*, 1994; Mayr-Harting *et al.*, 1972). The green synthesized silver nanoparticles were mixed in 1 ml distilled water and applied to sterile paper discs. The cultured microorganisms on nutrient agar plates were incubated at 27^0 C for 24 hrs and the zones of inhibition were determined that the inhibited visible growth of microorganism.

Results and Discussion

UV Visible Spectrophotometer

The *Cyperus rotundus* tuber extract was added to aqueous silver nitrate solution, the color of the solution was changed from light yellow brown color to dark brown color as a result of surface plasmon resonance. It was confirmed the completion of bioreduction effect between silver nitrate and tuber extract, and indicated that the formation of silver nanoparticles (SiNPs) in aqueous medium (Krishnaraj *et al.*, 2010; Singhal *et al.*, 2011; Namratha *et al.*, 2013; Laitha *et al.*, 2013). The surface Plasmon resonance absorption band was observed by UV Visible Spectrophotometer, the absorption spectrum was formed due to the incidence of electron resonance with the light waves. The maximum absorption spectra of SiNPs were produced in the range of 428 nm (Figure 2). As a result confirmed the *Cyperus rotundus* tuber extract has a potential to green synthesize the silver nanoparticles in aqueous silver nitrate solution.

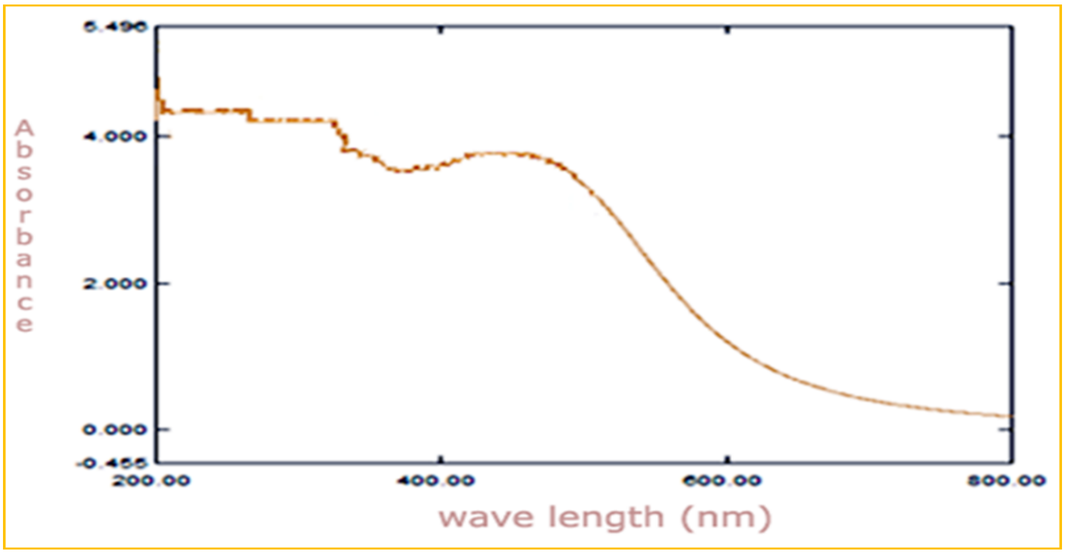

Figure 2. UV-Visible spectra of green synthesized SiNPs using tuber extract of *Cyperus rotundus*

Fourier Transform Infrared Spectroscopy (FTIR)

The identification of biomolecules for capping and stabilization of green synthesized silver nanoparticles were carried out using the Fourier Transform Infrared Spectroscopy (FTIR). The FTIR measurements for green synthesized SiNPs were made by the result of FTIR spectrum was shown between 4000–500 cm^{-1} (Figure 3). It was used to identify the type of biomolecules at different band peaks which were involved in capping and stabilization of SiNPs. Usually, the O-H stretching, H-bonded alcohols and phenols peak bands will give around 3490-3500 cm^{-1}, the alkenes bonds will establish around 2,263 cm^{-1}; the stretch of C-H bond peak will establish around 1500-1550 cm^{-1}, the stretch of N-H peak will establish around 1450-1500 cm^{-1}; the –C-O- groups will establish around 1,320 cm^{-1}; germinal methyls groups will establish around 1,381 cm^{-1} and the stretch of SiNPs will establish around 500-550cm^{-1}.

In silver nanoparticles solution, prominent bands of FTIR absorbance were observed (Figure 3) at around 3331.71, 2075.62, 1634.04, 1493.03, 1267.85, 1163.68 and 565.14 cm^{-1} (Table 1). The observed peaks denote O-H stretching, H-bonded alcohols & phenols; alkenes bonds; stretch of C-H; stretch of N-H; C-O-; germinal methyls and stretch peak of SiNPs, respectively (Table 1). These bands indicate the stretching vibrational bands which are responsible for terpenoids and flavonoids compounds and are responsible for the capping and stabilization of silver nanoparticles (SiNPs). The FTIR studies also designate the carbonyl groups from amino acid residues and proteins have the stronger ability to bind metal nanoparticles. The proteins were specifically involved in capping of silver nanoparticles to prevent the agglomeration thereby the solution was stabilized. These studies proved that the biological molecules were performed the formation and stabilization of silver nanoparticles in an aqueous solution of *Cyperus rotudus* tuber. Terpenoids play an important role in reduction of metal ions by oxidation of aldehyde groups to carboxylic groups. These carboxylic groups interact on surface of silver nanoparticles and were involved in stabilization and formation of silver nanoparticles (Siddiqui *et al.*, 2000).

Table 1. FTIR frequency and Bond or Stretching of biomolecules

S/N	Frequency (cm^{-1})	Bond or Stretching
1	3331.71	O-H stretching
2	2075.62	H-bonded alcohols & phenols
3	1634.04	Alkenes bonds
4	1493.03	Stretch of C-H
5	1267.85	Stretch of N-H; C-O-
6	1163.68	Germinal methyls
7	565.14	Stretch peak of SiNPs

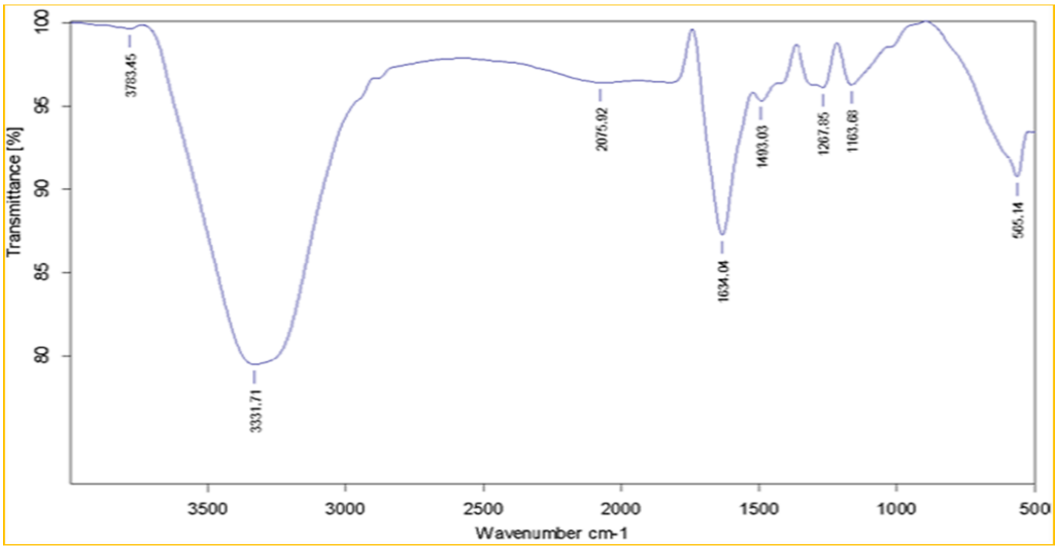

Figure 3. FTIR spectrum pattern of SiNPs

Measurement of particle size

After green synthesis, the diameter and poly-dispersity index of silver nanoparticles in solution was measured using dynamic light scattering. The Z-average (average particle size) was found at 122.3 nm. The particle size analysis showed the presence of silver nanoparticles with polydisperisty index (PDI) of 0.439 (Figure 4) (Sivaraman *et al.*, 2013; Aruna *et al.*, 2014).

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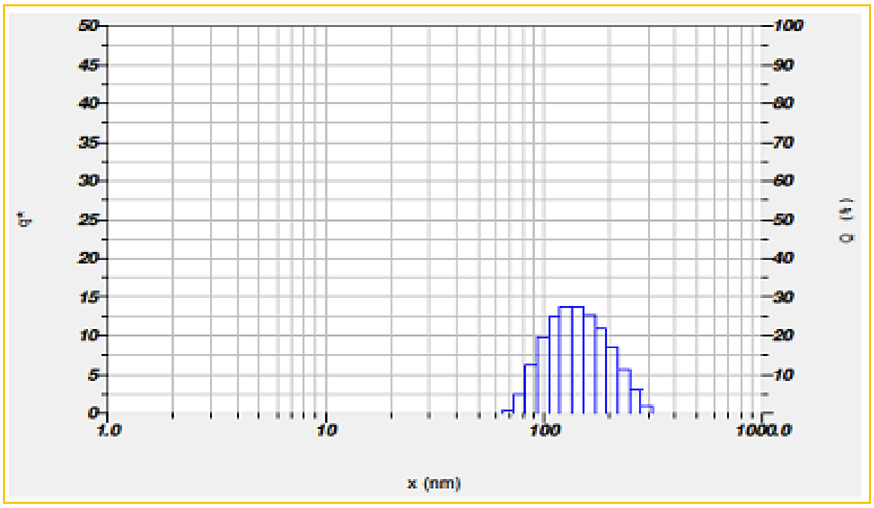

Figure 4. Particle size distribution of SiNPs

Zeta potential

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability i.e. the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. The zeta potential has direct relation with the stability of silver nanoparticles in solution (Table 2) (Raveendra *et al.*, 2003). For green synthesized SiNPs, the zeta values were measured and found at 34.0 mV (Figure 5). These values were provided moderate stabilization of silver nanoparticles.

Table 2. Zeta potential (surface potential) has direct relation with the stability of a form or structure

S/N	Zeta potential (mV)	Stability behavior of the colloid
1	From 0 to ±5	Rapid coagulation or flocculation
2	From ±10 to ±30	Incipient instability
3	From ±30 to ±40	Moderate stability
4	From ±40 to ±60	Good stability
5	More than ± 61	Excellent stability

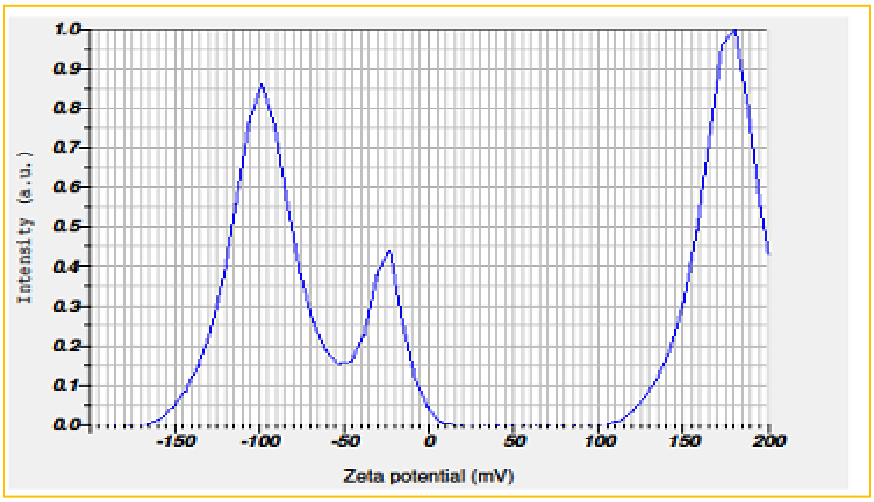

Figure 5. Zeta potential of AgNPs produced by tuber extract of *Cyperus rotundus*

Antibacterial activity

The formation of zone of inhibition was used to identify the antibacterial activity of a compound and the test microorganism was susceptible to that compound. The range of inhibition zone area was used to measure the compound's effectiveness. In this study, *Cyperus* tuber extract, aqueous silver nitrate solution and green synthesized SiNPs were used to investigate the antibacterial property by growing selected microorganisms viz., *Bacillus thuringiensis*, *Staphylococcus aureus* and *Pseudomonas fluorescens* on nutrient agar plates (Figures 6, 7 & 8). The controls were separately maintained for all microorganisms. The zones of inhibition and values of diameter of zones of inhibition were obtained in assessment of antibacterial activity. The obtained zones of inhibition indicate the maximum antibacterial activity of the sample. The bacterial inhibition zones by SiNPs were prepared from *Cyperus rotundus* tuber extract show maximum inhibition for *Bacillus thuringiensis* (17 mm) (Gram-negative), *Staphylococcus aureus* (15 mm) (Gram-positive) and *Pseudomonas fluorescens* (16 mm) (Gram-negative). Also, in comparison to silver nitrate solution and SiNPs, there was no such prominent antibacterial activity in case of the plant tuber extract when used as crude form. No zone of inhibition was observed in case of all controls. These studies proved that the SiNPs to be beneficial to minimize the dose that needs to be administered for bacterial inhibition (Morones *et al.*, 2005; Gogoi *et al.*, 2006; Shahverdi *el al.*, 2007).

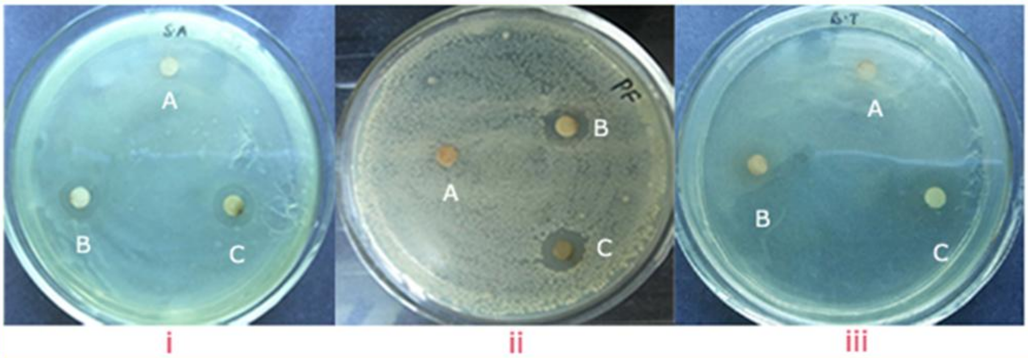

Figure 6, 7, & 8. Antibacterial activity against (i) *Staphylococcus aureus* (Gram-positive) (ii) *Pseudomonas fluorescens* (Gram-negative) (iii) *Bacillus thuringiensis* (Gram-negative) of (A) Silver nitrate solution (B) Tuber extract of *Cyperus rotundus* (C) green synthesized Silver Nanoparticles (SiNPs)

Conclusion

The bio-reduction of silver ions (Ag^+) through tuber extract of *Cyperus rotundus* leading to formation of silver nanoparticles (SiNPs) has been demonstrated. The green synthesized SiNPs were characterized using UV-Visible spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and DLS. From the results of characterization techniques we analyzed the Surface Plasmon resonance of SiNPs, identified the capping agents of SiNPs such as terpenoids, flavanoids and eugenol having functional groups of alcohols, phenols, amines, carboxylic acids, ethers and esters and measured the average diameter of SiNPs. The green synthesized SiNPs were applied for antibacterial activity and confirmed the zone of inhibition area. Applications of such eco-friendly green synthesized SiNPs in bactericidal, wound healing and other medical and electronic applications makes this green synthesis method potentially exciting for large scale production of silver nanoparticles (SiNPs) using *Cyperus rotundus*.

Conflicts of interest: There is no conflict of interest of any kind.

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