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Biosynthesis and Characterization of Silver Nanoparticles from Tuber Extract of *Cyperus rotundus* And Study of Its Antibacterial Activity.

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ABSTRACT

In current science, nanotechnology has been flourishing at a huge rate in all part of Science and Technology. It deals with between 1 to100 nm sizes of nanoparticles in at least one dimension and involves in creating diverse gadgets. Currently, nanobiotechnology is a commercial alternate process for chemical and physical methods for synthesis of various nanoparticles with specific functions. It is new branch of nanotechnology and joins the natural standards with physical and concoction strategies. Silver nanoparticles were biosynthesized utilizing tuber concentrate of Cyperus rotundus and the biosynthesized nanoparticles was seen inside 30 min. The outcomes were recorded from UV-Visible spectrophotometer, FTIR Spectroscopy, DLS and Zeta potential for help of the biosynthesis, normal particles size and portrayal of silver nanoparticles. The antimicrobial movement of biosynthesized silver nanoparticles was assessed utilizing Whatman No.1 channel paper circles utilizing the insignificant inhibitory focus (MIC) against *Bacillus thuringiensis* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Pseudomonas* (Gram-negative).

Keywords: Nanobiotechnology, Silver Nanoparticles (AgNPs), Green synthesis, Antimicrobial activity.

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INTRODUCTION

Nanotechnology is the investigation of controlling material on a nuclear and atomic scale, and it manages between 1 to 100 nm measure structures in no less than one measurement and includes in creating diverse materials/gadgets inside that size. Nanotechnology primarily comprises of the preparing of partition, union and disfigurement of materials by one atom or by one molecule, was portrayed by Tokyo Science University Professor Norio Taniguchi in 1974. Nanotechnology and nanoscience began in mid 1980's with two noteworthy developments (1) the introduction of group science and the creation of the examining burrowing magnifying instrument and the amalgamation (2) and properties of semiconductor nanocrystals was considered and this prompted a quick expanding number of metal and metal oxide nanoparticles and quantum specks. In 1980, Dr. K. Eric Drexler was advanced the mechanical noteworthiness of nanoscale wonders and gadgets by means of talks and books Engines of Creation: The coming Era of Nanotechnology. (3-6)

Synthesis of nanoparticles

As of now, various physical, synthetic, natural and crossover techniques are accessible for blend of various kind of nanoparticles however physical and concoction methods (7-9) are well known for amalgamation of nanoparticles, utilization of poisonous mixes limits their application. The advancement of eco-accommodating for biogenetic generation is presently of more enthusiasm because of straightforwardness of methodology and flexibility. The accompanying distinctive techniques have been accounted for the blend of metallic nanoparticles.

Importance of silver in biosynthesis of siver nanoprticle preparation:

Nanocrystalline silver particles have been discovered huge applications in the fields of high affectability biomolecular location, diagnostics, antibacterials, therapeutics, catalysis and small scale gadgets. In any case, there is still requirement for financial industrially reasonable and in addition ecologically clean amalgamation course to incorporate the silver nanoparticles. Silver is notable for having an inhibitory impact toward numerous bacterial strains and microorganisms generally introduce in therapeutic and modern procedures (Jiang H, M.S., et al., 2004).

Silver nanoparticles have imperative applications in the field of science, for example, antibacterial operators and DNA sequencing. Silver has been known to show solid poisonous quality to an extensive variety of microorganisms (12-14) (antibacterial applications). The antibacterial property of silver nanoparticles against Staphyloccocus aureus, Pseudomoanas fluorescence aeruginosa and Escherichia coli has been researched (Rai et al., 2009). Silver nanoparticles were observed to be cytotoxic to E. coli it was demonstrated that the antibacterial movement of silver nanoparticles was measure subordinate.

Advantage of biosynthesis method:

The three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the choice of an environmentally benign reducing agent and the choice of a non toxic material for the stabilization (15-17) of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used.

MATERIALS AND METHODS

Cyperus Rotundus

Description

Cyperus rotundus grows all over India up to 2000 meters altitude, especially on the banks of streams and rivers. *C. rotundus* belongs to family Cyperaceae (Table-1). Nut grass is a perennial shrub (19-22) that attains a height of up to 40 cm (Figure-1). It has a dark green thin stem and the leaves are long and sharp with a width of 1/6 to 1/3 inch, divided, alternate spiral, sessile, liner, margin entire, apex acute, base clasping and parallel-veined. The names nut grass and nut sedge are derived from its tubers, that somewhat resemble nuts, although botanically they have nothing to do with nuts. Leaves sprout in ranks of three from the base of the plant. While the flower stem has a triangular cross-section, the flower is 2 to 8 inch in length, has three-stamina and a three-stigma carpel. It is also bisexual. The plant bears flowers in summer and fruits in winter and the fruit is a three-angled achene. The root system of a young plant initially forms white, fleshy rhizomes. Some rhizomes grow upward in the soil, and then form a bulb-like structure from which new shoots and roots grow, and from the new roots, new rhizomes grow (Figure-2). Other rhizomes grow horizontally or downward, and form dark reddish-brown tubers or chains of tubers.

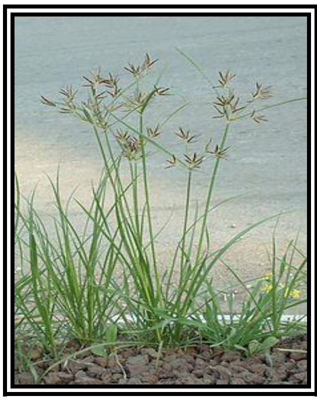


Figure 1: Perennial plant of *Cyperus rotundus;* Figure 2: Tuber of *Cyperus rotundus*

Table 1: Classification and other information of *Cyperus rotundus*

Botanical name	:	*Cyperus rotundus*
Family name	:	*Cyperaceae*
Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Poales
Genus	:	*Cyperus*
Species	:	*Rotundus*
Common name(s)/ Popular name (s)	:	Common Nut Sedge, coco grass, nutgrass and purple nutsedge
Indian name	:	Mustak, nagarmotha, motha and mustaka
Telugu name	:	bhadra-tunga-muste and bhadramuste
Sanskrit name	:	abhrabheda, ambhodhara and ambuda
Habitat	:	Weed found all over India
Product offered	:	Rhizomes and Oil
Parts used	:	Rhizomes/Roots

Plant material and Preparation of extract

The tuber powder of *Cyperus rotundus* was obtained from Karunakaran flour mill, K.T.Road, Tirupati, Andhra Pradesh, India. About 4 g. of *Cyperus rotundus* powder was weighed and transferred into 100 ml beaker containing 40 ml distilled water, mixed well and heated at 40^{0}C temperature for 15 min. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected in a 100 ml Erlenmeyer flask and stored in refrigerator for further use.

Synthesis of Silver nanoparticles

Aqueous solution (0.01N) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of Silver Nanoparticles (AgNPs) from tuber extract of *Cyperus rotundus*. 10 ml quantity of *Cyperus rotundus* extract solution was added to 5 ml of 0.01 N of $AgNO_3$. Within 30 min, a light yellowish brown color change was observed, indicating the biosynthesis of Silver Nanoparticles (AgNPs) (Figure). The reduction of silver nitrate to silver ions was confirmed by the color changes from the light brown to dark brown color. The solution was allowed for 12 hr to yield a deep brown color. The AgNPs dispersion was centrifuged at 3,000 rpm for 15 min to remove any large aggregates and excess free extract of *Cyperus* from the solution. The supernatant was collected and kept as the final silver nanoparticle product. The formation of silver nanoparticles (AgNPs) was confirmed by UV-Visible spectrophotometric analysis.

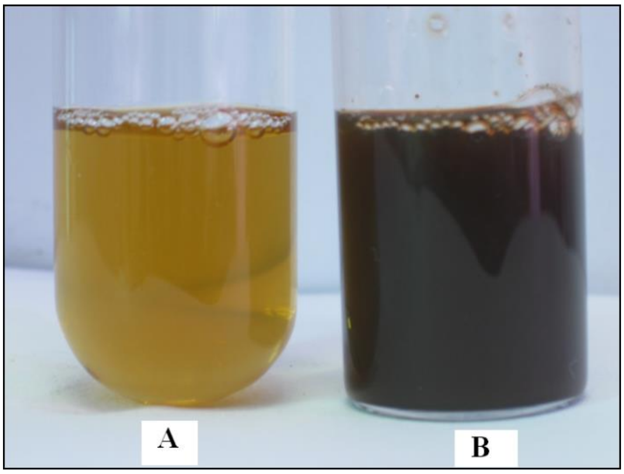

Figure-3: *Cyperus rotundus* tuber extracts (A) and Silver Nanoparticles (AgNPs) (B).

RESULTS

UV Visible Spectrophotometer

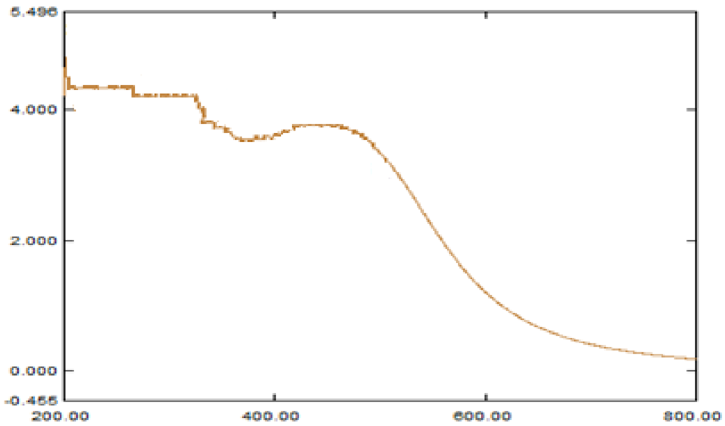

Figure 4: UV-Visible spectra of Silver Nanoparticles (AgNPs) synthesized using tuber extract of *Cyperus rotundus.*

The silver nanoparticles show SPR peak at around 400nm from the different literatures. We found the SPR peak at 400 nm for *Cyperus rotundus*, so we confirmed that *Cyperus rotundus* tuber extract has potential in synthesis of silver nanoparticles specifically the reduction of silver ions into the silver nanoparticles.

Fourier Transform Infrared Spectroscopy

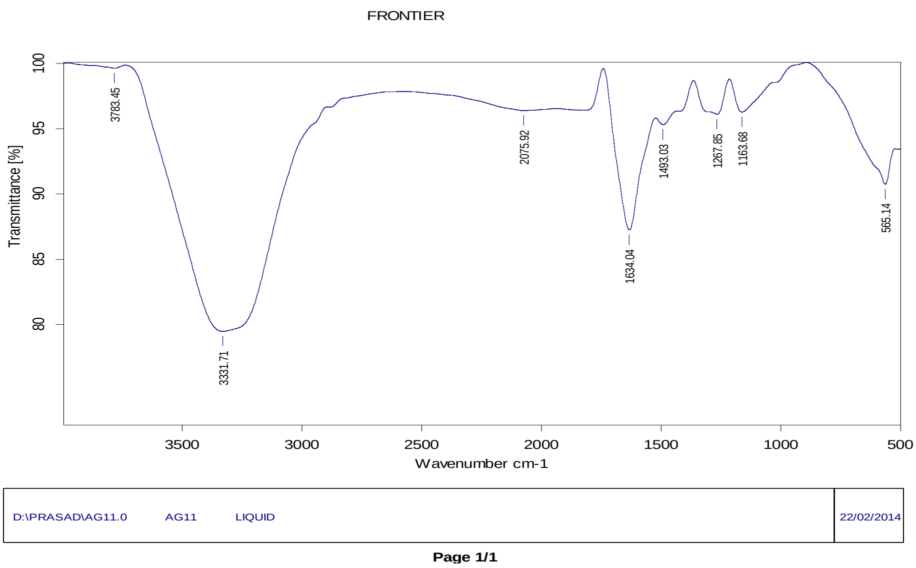

Figure 5: Fourier Transform Infrared (FTIR) spectrum pattern of Silver Nanoparticles (AgNPs) synthesized using tuber extract of *Cyperus rotundus.*

The identification of biomolecules for the capping and stabilization of the biosynthesized nanoparticles were carried out by using the Fourier Transform Infrared Spectroscopy. The FTIR measurements were made by the result of FTIR spectrum of biosynthesized silver nanoparticles and the spectrum was showed between 4000–400 cm^{-1}(Figure-5).

Table 2: Ranging of functional group stretching and it's actual redings.

Functional Group	Type of vibration	Peak shows in cm^{-1}
OH	Streching	3490 - 3500 cm^{-1}
CH	Streching	1500 – 1550 cm^{-1}
NH	Streching	1450 – 1500 cm^{-1}
AgNps	Streching	500 – 550 cm^{-1}
OH	Streching	3331.71 cm^{-1}
CH	Streching	1493.03 cm^{-1}
AgNps	Streching	565.14 cm^{-1}

Therefore the biosynthesized silver nanoparticles were surrounded by proteins and metabolites. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles specifically capping of silver nanoparticles to prevent agglomeration and thereby stabilize the medium.

Dynamic light scattering (DLS)

This method measurement depends on the size of the particles core, the size of surface structures, particles concentration and the type of ions present in the medium. The biosynthesized silver nanoparticles (AgNPs) were used for measurement of particle size distribution (Figure-6) which in present in the solution. The DLS pattern reveals that silver nanoparticles synthesized by this method have a Zeta average diameter of 122.3 nm with polydispersity index (PDI) of 0.439.

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201402221118003.nsz

Measurement Results

Date	:	Saturday, February 22, 2014 11:18:26 AM
Measurement Type	:	Particle Size
Sample Name	:	Ag 11
Scattering Angle	:	173
Temperature of the holder	:	25.0 °C
T% before meas.	:	6
Viscosity of the dispersion medium	:	0.894 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	1130 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	149.0 nm	47.4 nm	142.0 nm
2	—	— nm	— nm	— nm
3	—	— nm	— nm	— nm
Total	1.00	149.0 nm	47.4 nm	142.0 nm

Cumulant Operations

Z-Average	:	122.3 nm
PI	:	0.439

Molecular weight measurement

Molecular weight	:	---
Mark-Houwink-Sakurada parameters	:	---

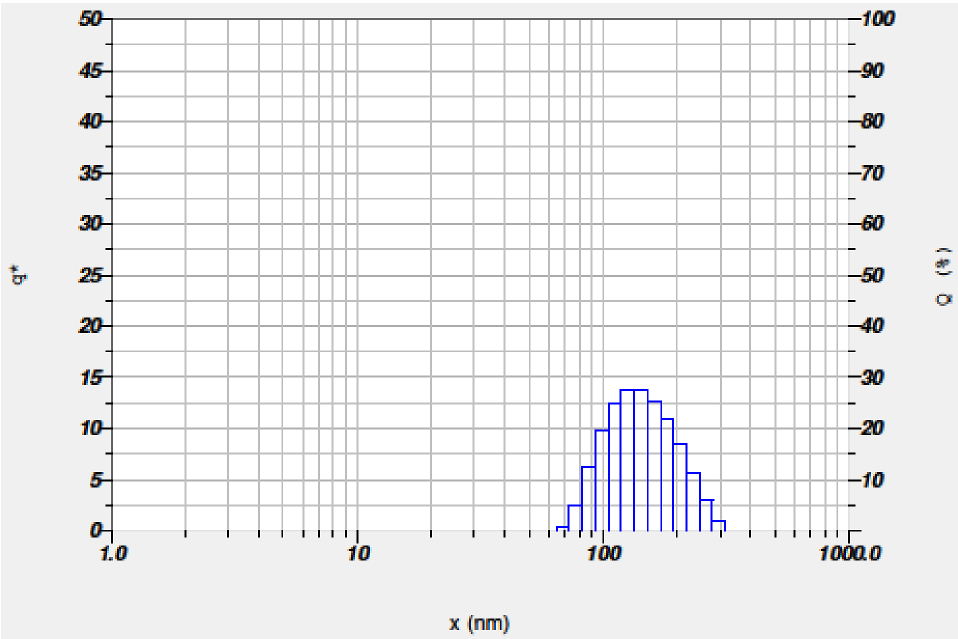

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Figure 6: Particle size distribution of Silver Nanoparticles (AgNPs) produced by tuber extract of *Cyperus rotundus*.

Zeta potential

The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion The Zeta potential measurements of silver nanoparticles synthesized is 34.0 mV (Figure-7). Nanoparticles are very small in size for which they are energetically very unstable so the nanoparticles undergo for agglomeration or aggregation to stabilize them. As a result there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis.

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Measurement Results

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Measurement Results

Date : Tuesday, February 18, 2014 3:40:40 PM
Measurement Type : Zeta Potential
Sample Name : Ag11
Temperature of the holder : 25.0 °C
Viscosity of the dispersion medium : 0.894 mPa·s
Conductivity : 0.480 mS/cm
Electrode Voltage : 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	177.8 mV	0.001378 cm2/Vs
2	-98.6 mV	-0.000764 cm2/Vs
3	-24.9 mV	-0.000193 cm2/Vs

Zeta Potential (Mean) : 34.0 mV
Electrophoretic Mobility mean : 0.000263 cm^2/Vs

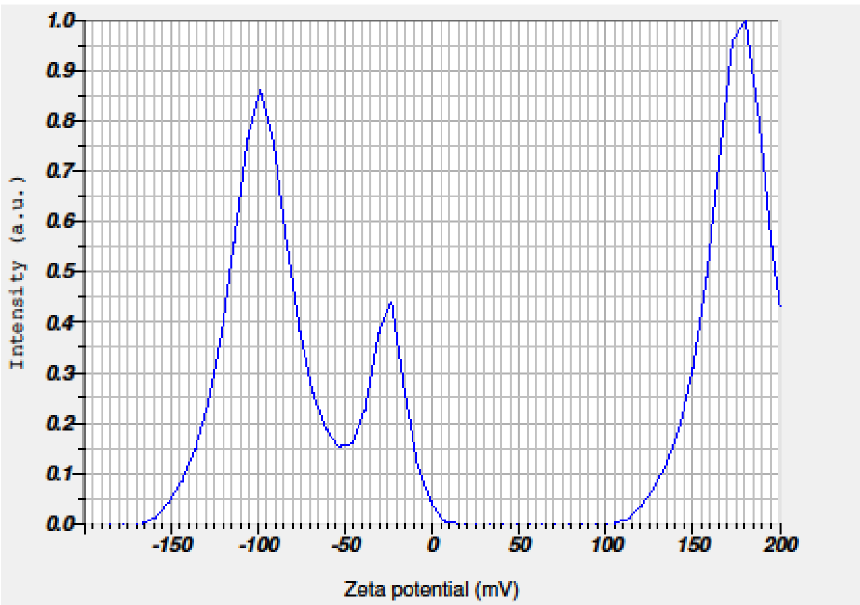

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Figure 7: Zeta potential of Silver Nanoparticles (AgNPs) produced by tuber extract of *Cyperus rotundus*.

Antibacterial activity

The antibacterial activity of the sample was identified by the formation of Zone of Inhibition.

Antibacterial potential of silver is known for many years (W. Raut Rajesh, *et al.,* 2009). In our study, the AgNPs synthesized using *Cyperus rotundus* tuber extract exerted a fairly significant antibacterial action on the tested microorganisms. This is evident by the values of diameter of zone of inhibition obtained during assessment of antibacterial activity. Figure-8,9&10 shows the zones of inhibition of *Bacillus thuringiensis, Staphylococcus aureus* and *Pseudomoanas fluorescens against Cyperus* tuber extract, silver nitrate and silver nanoparticles (AgNPs). For three bacterial strains, no zone of inhibition was observed for silver nitrate solution. Zones of 17mm, 15mm and 16mm were observed for *Bacillus thuringiensis, Staphylococcus aureus* and *Pseudomoanas fluorescens*. Coupling of inherent property of *Cyperus rotundus* tuber extract with that of AgNPs has really proved to be beneficial to minimize the dose that needs to be administered for total microbial reduction.

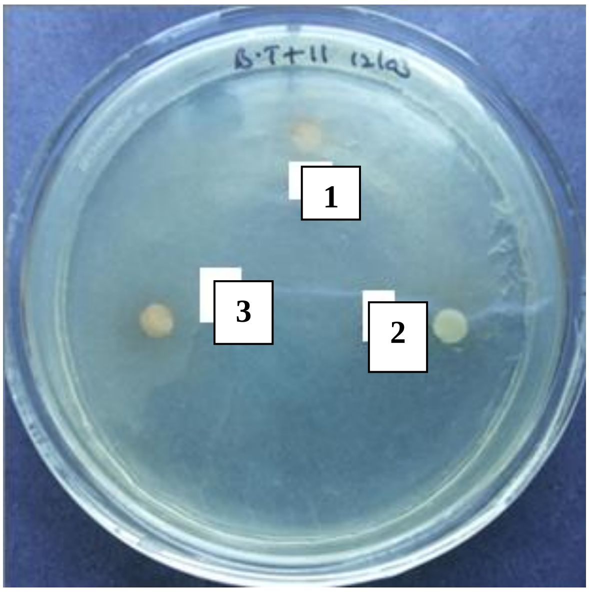

Figure 8: Antibacterial activity against *Bacillus thuringiensis (Gram-negative)* of (1) Silver nitrate solution (2) Extract of *Cyperus rotundus* and (3) Biosyntheiszed Silver Nanoparticles (AgNPs).

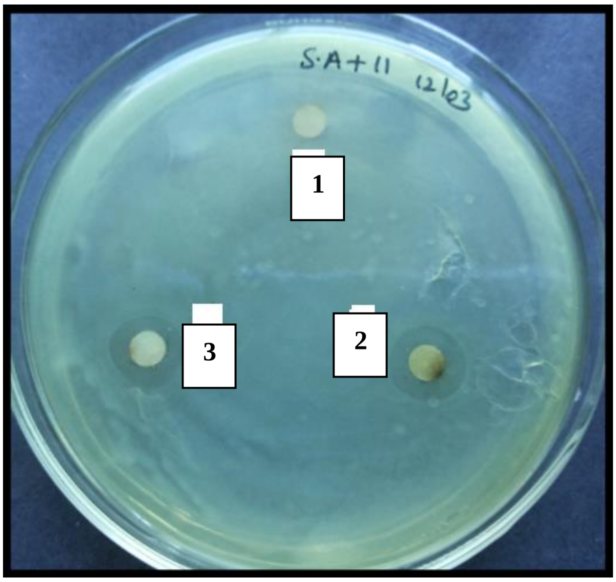

Figure 9: Antibacterial activity against *Staphylococcus aureus (Gram-positive)* of (1) Silver nitrate solution (2) Extract of *Cyperus rotundus* and (3) Biosyntheiszed Silver Nanoparticles (AgNPs)

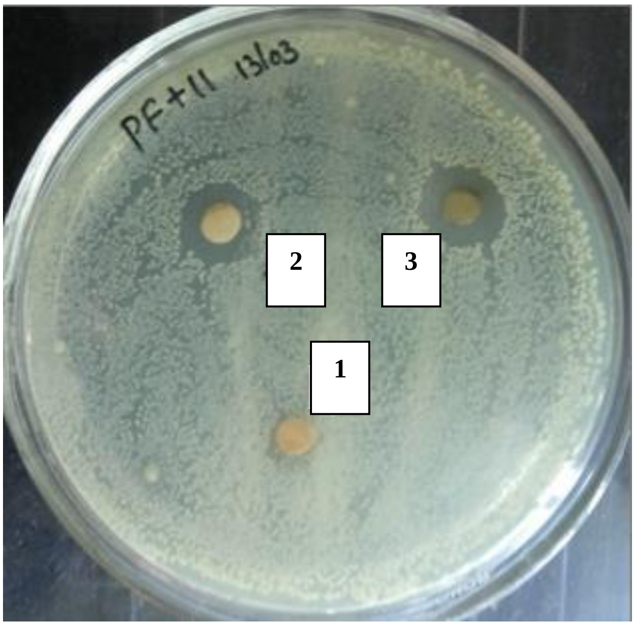

Figure 10: Antibacterial activity against *Pseudomoanas fluorescens* (*Gram-negative)* of (1) Silver nitrate solution (2) Extract of *Cyperus rotundus* and (3) Biosyntheiszed Silver Nanoparticles (AgNPs).

CONCLUSION

The bio-reduction of Ag^+ ions by the tuber extract of *Cyperus rotundus* plant has been demonstrated. This green synthesis technique has many advantages towards the synthesis of AgNPs. UV-Visible spectroscopy results states that the SPR peak found at 400 nm so, the *Cyperus rotundus* tuber extract has potential in synthesis of silver nanoparticles. From the measurement of FTIR peaks we can conclude that the AgNPs were surrounded by terpenoids, flavanoids and eugenol having functional groups of alcohols, phenols, amines, carboxylic acids, ethers and esters. The DLS pattern reveals that silver nanoparticles synthesized by this method have a Zeta average diameter of 122.3 nm with polydispersity index (PDI) of 0.439. The Zeta potential measurements of silver nanoparticles synthesized is 34.0 mV (Figure-7). The antibacterial activity of silver nanoparticles was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that silver is a very effective antibacterial agent.

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