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Research Article

A RESEARCH STUDY ON SYNTHESIS, CHARACTERIZATION AND ANTI MICROBIAL ACTIVITY OF SLIVER NANO PARTICLES USING ECHINOCHLOA COLONA LEAF EXTRACT

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ABSTRACT

Silver-Nano Particles are nano particles of silver i.e. silver particles of between 1nm and 100nmin size. **Aim:** To develop a facile and eco- friendly method for the preparation of silver nano particles. **Materials:** In the present work, Echinochloa Colona collected from Tirumala hills is used as a biological component in reducing silver ions in silver nitrate solutions to silver nano particles Silver Nitrate was collected from college laboratory for this study. All the glass wares were washed with HNO3 and distilled water and dried in oven. **Methods:** 20gm of dried powdered plant material was weighed and boiled for 15 min in 100ml milli-Q water and then the extracts were filtered through whats man filter paper No.1.The filtered extract was stored in refrigerator at 4 degree centigrade. About 30 ml of 0.01 M aqueous solution of silver nitrate was taken in Erlenmeyer flask; and 2.0, 2.5, 3.0 and 3.5 ml of Echinochloa Colona leaf extract was added separately at room temperature. After 12 hours the solution turned yellow to reddish dark brown indicating the formation of silver nano particles. Bacillus Species and E.Coli bacteria were selected for study of the bactericidal effects of the silver nano particles on organisms with different cell wall structures. **Results and Discusiion:** Bacterial cells were grown in laboratory broth and were spread in a lawn on agar plates. Five cavities of wells were made in a each plate and well no.1, 2 and 3 was filled with 10, 20 and 30µl of 1% of streptomycin (antibiotic) solution. The plates were incubated at 370C overnight, after incubation period zone of inhibition around the wells were measured. In this present work also we observed periodic change colour of the reaction mixture with time. When Echinochloa colona leaf extract was mixed with silver nitrate solutions, after about 12 hours the reaction mixture finally turned into brownish colour and no further change in colour has been observed even after keeping longer period of time. This is the prime indication that silver ions are reduced to fine silver nano particles. Conclusion: The present work describes synthesis and characterization of silver nano particles using Echinochloa colona. Characteristic brown colour indicates the formation of silver nano particles In the reaction mixture. UV-Vis spectroscopy showed absorption maxima at 418 nm which further confirms the formation of silver nano particles.SEM study showed that the average size of silver nano particles is about 75nm.The synthesized AgNPs were proved as excellent antimicrobial agent.

Key Words: Echinochloa colona, Silver Nano Particles, streptomycin, Bacillus Species and E.Coli, SEM, FTIR.

INTRODUCTION

Silver Nano Particles are nano particles of silver i.e. silver particles of between 1nm and 100nmin size. [1,2] while frequently described as being silver some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms [3].

Echinochloa Colona is plant with annual, (Figures 1,2) with fibrous, rather shallow roots. culms stout, usually reddish-purple, erect, ascending or decumbent, often branching from the base, often rooting at the lower nodes,20-60 cm tall, sometimes nodes conspicuously swollen and usually geniculate, compressed [4], lower internodes often exposed. sheath 3-7cm long, compressed, keeled, glabrous, ligule absent, leaf blades light green, sometimes with transverse purple bands,flat,glabrous,elongate,4-10 cm long,3-8mm wide, margins occasionally scabrous,2-4cm

Fig. 1: ***Echionochloa Colona*** **.**

Fig. 2: ***Echionochloa Colona*****.**

Long spreading, ascending, sometimes branched, the lower ones up to 1 cm apart, the upper ones crowded [4].

Taxonomy of *Echionochloa Colona*:

Domain: Eukaryota.
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae.
Class: Monocotyledonae
Order: Cyperales
Family: Poaceae
Genus: Echinochola
Species: *Echinochloa Colona.*

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Plant distribution

India, china, Europe, Indonesia, Cambodia, Pakistan, Philippines, Sri lanka, Thailand, and Vietnam it is distributed throughout tropical Asia and African in fields and road sides; it also invades river banks and shores of lakes and ponds. The species is considered as invasive species in north America where it occurs throughout the continental united states, it is also found in southern Canada from British Columbia east to new found land. it was first spotted in the great lakes region in 1843 [4].

IUPAC name

5,7-dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4H chromen-4-one.

AIM

To develop a facile and eco- friendly method for the preparation of silver nano particles.A biological method offers such versatility and adds further advantage of being a single step. The biologicalcomponents acts as both reducing and stabilizing agents. In the present work, *Echinochloa Colona*is used as a biological component in reducing silver ions in silver nitrate solutions to silver nanoparticles.

MATERIALS AND METHODS

Collection and authentication of plant

The plant *Echinochloa Colona* was collected from Tirumala hills, Chittoor district, Andhra Pradesh and authenticated by Dr.K. Madhavachetty, Assistant professor, Sri Venkateshwara University, A.P.

Preparation of silver nanoparticles:

Materials:

Silver Nitrate was collected from college laboratory for this study. All the glassware's were washed with hno3 and distilled water and dried in an oven. *Echinochloa Colona* leaves were collected from Tirumala hills.

Preparation of leaf extract

20gm of dried powdered plant material was weighed and boiled for 15 min in 100ml milli-Q water, and then the extracts were filtered through Whatman filter paper No.1.The filtered extract was stored in a refrigerator at 4^{0C}

Synthesis of silver nanoparticles

About 30 ml of 0.01 M aqueous solution of silver nitrate was taken in Erlenmeyer flask; and 2.0, 2.5, 3.0 and 3.5 ml of *Echinochloa Colona* leaf extract was added separately at room temperature. After 12 hours the solution turned yellow to reddish dark brown indicating the formation of silver nano particles.

Characterization of silver nanoparticles

UV-visible spectra

The reduction of pure silver ions (Ag+ ions) into silver particles (Ag^0) can be identified by measuring UV-VIS spectrum. The sample is prepared by taking a small amount of aliquot from the final reaction mixture (brown colored) and is further diluted with distilled water 1 in 10 dilutions. UV-VIS spectrum for this sample was recorded on UV-VIS spectrophotometer.

Fourier Transform Infrared Spectrum (FTIR)

A sample of FTIR analysis was prepared by centrifuging the final reaction mixture at about 10000 rpm for about 15 min. The sediment was collectedand the dried sample was directly placed on the potassium bromide crystals and the spectrum was recorded in transmittance mode using FTIR (model: spectrum RXI).The spectrumwas recorded in the mid IR region of 400-4000cm^{-1} at resolution of 4cm^{-1} .The spectrum was recorded using attenuated reflectance technique(ATR).

Scanning Electron Microscopy (SEM)

SEM photographs was recorded using software controlled scanned Electron Microscope(MODEL).The energy of electron beam current was continuously adjusted from 1Pa to 1μa to suit the type of examination in progress. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a drop of solution of particles dispersed in distilled water and dried under a mercury lamp for 5mins. Finally a thin film was been formed on the copper grid.

Anti-Microbial Activity of *Echinochloa Colona*

Antibacterial assay

Bacillus Species and *E.Coli* bacteria were selected for study of the bactericidal effects of the silver nano particles on organisms with different cell wall structures.

In the preparation for the well-diffusion assay, bacterial cells were grown in laboratory broth and were spread in a lawn on agar plates. Five cavities of wells were made in a each plate and well no.1, 2 and 3was filled with 10, 20 and 30μl of 1% of streptomycin (antibiotic) solution. The plated were incubated at 37^{0C}overnight, after incubation period zone of inhibition around the wells were measured.

RESULTS AND DISCUSSION

Preparation of silver Nano particles

There are so many ways by which one can confirm the formation of silver Nano particles. The very first instance of Nano particles can be identifiedischange in colour of reaction mixture. The change in colour is very specific and characteristic for each metal nano particles. (Figure3). This kind of observation gives us the preliminary idea about the conversion of metal ions into their particles.

In this present work also we observed periodic change colour of the reaction mixture with time. When *Echinochloa colona* leaf extract was mixed with silver nitrate solutions, after about 12 hours the reaction mixture finally turned into brownish colourand no further change in colour has been observed even after keeping longerperiod of time. (Figure 3)This confirms that the approximate reaction time for the silver ions to reduce to silver particles is about 12 hours. This is the prime indication that silver ions are reduced to fine silver Nanoparticles. A photograph was takenand the same is shown in figure3.This method is environmentally friendly and very facile and easy to obtain silver Nanoparticles in single step. The change in colour may be due to surface Plasmon resonance of deposited silver nanoparticles.

Characterization of silverNanoparticles

UV-Visible spectra analysis

UV-Vis spectroscopy is the basic technique to confirm the formation of mano systems in aqueous media. The UV-VIS spectrum of the sample, silver nano particles, has been shown in (Figure 4) From (Figure 4), It is evident that a strong and intense absorption peak at about 418 nm with high absorbance has been observed. It is confirmedby the literature that every metal nano particles has a characteristic absorption at particularwavelength when exposed to UV-VIS radiation. The absorption peak in between at 410-480nm is characteristic for the presence of silver nano particles (Ingal etal 2008) .This analysis further confirms the formation of silver nano particles when silver ions are reduced with *Echinochloa colona* leaf extracts.

Fourier Transforms Infra-Red Spectroscopy (FTIR) Analysis

(Figure5) Represents the FTIR spectrum of synthesized silver nano particles prepared by using *Echinochloa colona* leaf extract. Three absorption peaks at 1635, 2350 and 3433cm^{-1} wave lengthswere observed in the FTIR spectra for dried silver nano particles. The absorption peak at 2350cm^{-1} corresponds to the stretching vibrations of –C-H groups present in leaf extract. The peak at 3433cm^{-1} corresponds to the bounded hydroxyl (-OH) or amine (-

NH) groups of leaf extract. The peak at 1635cm^{-1} corresponds stretching vibrations of carboxyl groups (-C=O).This analysis provides evidence for the presence of proteins as capping agent, which helps in increasing the stability of the synthesized silver nano particles apart from acting as reducing agents in reducing silver ions into particles.

Scanning of Electron Microscopic (SEM) Analysis

(Figure6) Scanning electron microscopy will provide further insight into the morphology and size and shape of the nano particles.SEM micrograph of the synthesized silver nano particles using the *Echinochloa colona* leaf extract is shown in figure 6.The synthesized silver nano particles were dispersed without aggregation and also possessing near spherical shape. The average particle size was found to be less than 75 nm.SEM results confirmed that the morphology of synthesized silver nano particles.

Antimicrobial activity of silver nano particles

(Figure7) Synthesized Ag NPs exhibited effective antimicrobial activity against pathogenic Bacillus and *E. Coli* species. Leaf exract has not shown any inhibition, where as Ag NPs showninhibition towards both Bacillus and *E. Coli* species. The zone of inhibition increased as the concentration of Ag NPs increased from 10 to 30µL.Thisclearly indicates that the antimicrobial activity is due to Ag NPs. The exact mechanism for the anti-microbial activity of Ag NPs is not yet known, but hypothetically, many studies reported that the Ag NPs could bind to the bacterial membrane, invade the cell and cause dissipation of proton motive force which leads to the disruption of bacterial cell by forming pores on the bacterial cell wall.

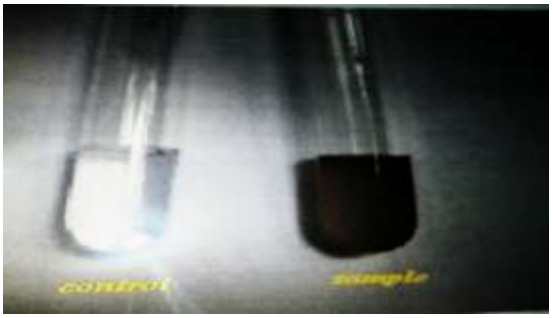

Fig 3: A Visible observation of change in color during silver nano particles formation.

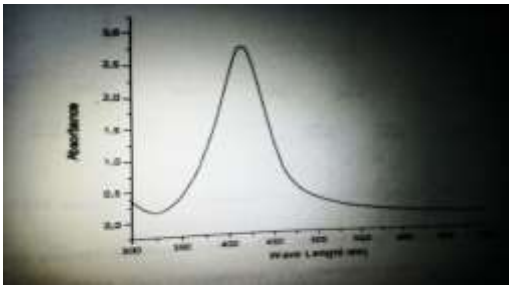

Fig 4: UU-VIS Spectra of silver nano particles with *Echinochloa colona leaf extract.*

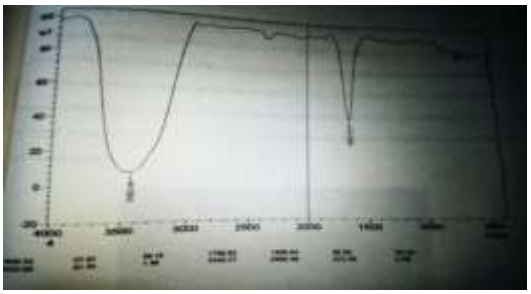

Fig 5: FTIR spectra of silver nano particles synthesized using *Echinochloa Colona* extract.

Fig 6: SEM micrographs of synthesized silver Nano particles using the *Echinochloa colona extract.*

Fig 7: Petri Plates showing anti-microbial activity of silver Nano particles.

CONCLUSION

The present work describes synthesis and characterization of silver nano particles using *Echinochloa colona.* Characteristic brown color indicates the formation of silver nano particles. In the reaction mixture. UV-Vis spectroscopy showed absorption maxima at 418 nm, which further confirms the formation of silver nanoparticles.SEM study showed that the average size of silver nano particles is about 75nm.FTIR analysis indicated the possible role of carboxyl(-C==O),hydroxyl (-OH) and amine (-NH) groups of leaf extract in fabrication of silver nano particles. Use of *Echinochloa colona* extract offers affordable, environment friendly technique for synthesis of large scale silver nano particles. The synthesized Ag NPs were proved as excellent antimicrobial agent.

CONFLICT OF INTREST

As a main author for this study I claims there is no conflict of interests, the present research is free of bias and is conducted in genuine to estimate and create awareness on Breast Cancer among teenagers, there are no manipulations for positive results and is not influenced by others from outside.

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