PHYTOCHEMICAL ANALYSIS, SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL ACTIVITY FROM CYPERUS BREVIFOLIUS L.

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Abstract : Grass extract of *Cyperus brevifolius* was used for the synthesis of silver nanoparticles (Ag NPs) from silver nitrate solution. Ag NPs were characterized by UV–vis spectrophotometer and Fourier transform infrared spectroscopy. The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV–vis spectrophotometer analysis. Fourier transform infrared spectroscopy was performed to analyze the functional group in the process of green synthesis, the role of different functional groups such as hydroxyl; amine and alkyl groups were indicated in the synthetic process. Antibacterial activity of the silver bio-nanoparticles was performed by well diffusion method against *Enterococcus faecalis*, *Bacillus sp*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. The highest antibacterial activity was found against *Bacillus* sp. (18 mm). The synthesized AgNPs has the efficient antibacterial activity against pathogenic bacteria.

IndexTerms - **Grass extract, *Cyperus brevifolius*, silver nanoparticles and antibacterial activity**

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level (Albrecht *et al*., 2006). Recently, biosynthetic methods employing either biological microorganisms such as bacteria and fungus or plants extract (Mostafa *et al*., 2012; Vyom *et al*., 2009) and have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nano materials. Different types of nano materials like copper, zinc, titanium (Retchkiman *et al*., 2011), magnesium, gold , alginate (Ahamad *et al*., 2005) and silver have come up but silver nanoparticles have proved to be most effective as it has good anti microbial efficacy against bacteria, viruses and other eukaryotic microorganisms (Renata *et al*., 2015).

The silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects. AgNPs have potential applications in the bio medical field and has several advantages over physical and chemical methods due to its cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production (Mallikarjuna *et al*., 2010) . The plants can be rich in phenolic compounds, alkaloids, diterpenoid, steroid and other compounds which inhibit the development of various microorganisms. Besides these, phytochemicals in the plant extracts can act a reducing and capping agent in the reduction of metal ions to metal nanoparticles and thus have found widespread use in the biosynthesis of metal nanoparticles which can be used in drug delivery, tissue/tumor imaging, bio sensing, catalysis and surface-enhanced Raman scattering-based sensors **(**Deepshikha *et al***.,** 2013). The *Cyperus brevifolius* belong to the family Cyperaceae. Scientific name *Cyperus brevifolius*. Grasses are used for so many application like antimicrobial, anticancer etc. *Cyperus brevifolius*. belongs to Cyperaceae family (Figure-1). The rhizomes of the grass have been widely used in the paraguayan folk medicine as digestive, diuretic, sedative, tonic, antispasmodic and sudorific. *C. brevifolius* exerts a weak sedative and an interesting anxiolytic-like effect in mice and suggest its potential usefulness for the treatment of anxiety in humans. In this investigation, synthesis of silver nanoparticles was achieved by greener method using fresh leaf extract of *Cyperus brevifolius* and assessment of its characterization and antibacterial activity against pathogenic microorganisms.

Figure.1: ***Cyperus brevifolius***

2. MATERIALS AND METHODS

2.1 Selection and collection of plant material

Cyperus brevifolius were collected from Pattiyagoundanur, Pollachi Taluk, Coimbatore, Tamilnadu and the plant was identified by the PG and Research Department of Botany, NGM College, Pollachi.

2.2. Phytochemical Analysis

Whole plant of *Cyperus brevifolius* was shade dried and Powdered. The methanol extract was prepared according to the method described by Harborne, 1998. The sample was sequentially extracted using a soxhlet apparatus and was subjected to detect the presence of bioactive compounds according to the method described by Kokate, 1994 and Harborne, 1998.

2.3. Preparation of grass extract

Cyperus brevifolius was shade dried and powdered. 25 gm of the powder was extracted with 350 ml of distilled water on boiling for 30-40 minutes. Filter the content with whatman no.1 filter paper and stored it at room temperature.

2.4 Synthesis of silver nanoparticles

1.5 g of the leaves from *Cyperus brevifolius* was boiled in 100 ml of de-ionized water. 2.5 ml of ammonium solution was added to 5 ml of 1 mM $AgNO_3$ solution, followed by addition of grass extract 1–10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water. The solution turned from pale yellow to bright yellow and to dark brown indicates the formation of silver nanoparticles. After that the solution was incubated at 37◦ C under agitation (200 rpm) for 24–48 h (Kasthuri *et al*., 2009).

2.5. Characterization of Silver Nanoparticles

The bioreduction of $AgNO_3$ was confirmed by sampling the reaction mixture at regular intervals and the absorption maximum was scanned by UV–vis spectra, at the wavelength of 300–700 nm in Labman spectrophotometer. Further, the bioreduced reaction mixture was subjected to centrifugation at 8000 rpm for 30 minutes and the pellet was dissolved in de-ionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing AgNPs was used for FTIR and SEM studies (Xi-Feng *et al*., 2016).

2.6. Antibacterial activity (Well Diffusion Method)

The silver nanoparticles (AgNPs) synthesized from *Cyperus brevifolius* were tested for their antibacterial activity by well diffusion method (Azoro, 2002) against pathogenic bacteria such as *Enterococcus faecalis*, *E.coli*, *Klebsiella pneumonia*, *Bacillus sp* and *Pseudomonas aeruginosa*. The pure cultures were sub cultured on nutrient broth at 35°C on rotary shaker at 200 rpm. Wells of size 6mm have been made on nutrient agar plates using gel puncture. Using micropipette, 50 µl and 100 µl of the sample of nanoparticles solution was poured into wells on all plates. After incubation at 35°C for 18 hr, the different levels of zone of inhibition were measured.

3. RESULT AND DISCUSSION

3.1 Phytochemical screening

Preliminary phytochemical screening of methanolic extract of *Cyperus brevifolius* showed the presence of tannins, terpenoids, saponins, flavonoids and phenol. Similar result was observed in *Cyperus esculentus* and *Butyrospermum paradoxum* seeds, which contain alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, sterols, tannin, terpenoids and reducing sugars (Ibironke *et al*., 2015). The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. Similar result was also observed by Varalakshmi *et al*. (2014) in the aqueous extract of wheatgrass for the presence of bioactive compounds. The results showed the presence of alkaloids, flavonoids, saponin, tannins, amino acids and protein, carbohydrates, coumarins, phenols, alkaloids, terpenoids and cardiac glycosides. But, sterol, steroids and quinone were absent in aqueous extract. Megha *et al*. (2016) in wheat grass leaf extracts (*Triticum aestivum* Linn) also showed similar results. Similarly, Amina *et al*., (2013) obtained oil from camel grass (*Cymbopogon schoenanthus Spreng*.) showed phytochemicals like saponins, saponin glycosides and tannins were also found in *Cyperus brevifolius.*

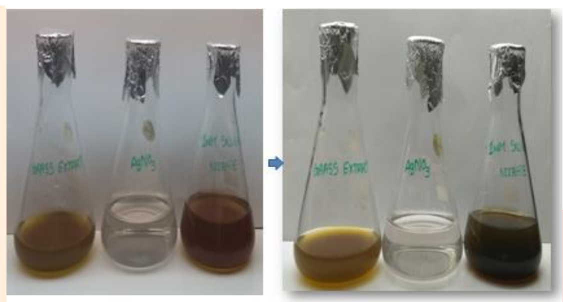

Figure-2 1mM AgNo3solution turned from yellow to bright yellow and then to dark brown

3.2. UV- visible spectroscopy

Reduction of silver ions into silver nanoparticles using extracts of *Cyperus brevifolius* was evidenced by the visual change of colour for yellow to dark brown (Fig-2) due to excitation of surface Plasmon vibrations in silver nanoparticles. After 24hrs and 48hrs, no significant colour change was observed and concentrations of silver nitrate resulted in a brown solution of nano silver indicating the completion of reaction. Similar results were observed in the grass of *Cyperus esculentus* (Ibironke *et al*., 2015) and *Cyperus rotundus* (Jayasheela *et al*., 2011). The UV-vis spectrophotometer would be used to observe size and shape controlled nanoparticles in aqueous solution. The UV-vis spectra of the silver nanoparticles showed a well defined surface plasmon band at around 380 nm, 460 nm, and 520 nm at different time interval as shown in Figure 2. This is the characteristic of silver nanoparticles and clearly indicates the formation of nanoparticles in solution. Spectra showed (Fig-3) absorption band at 380 nm, 460 nm and 520 nm which correspond to the absorbance of silver nanoparticles. Our results were accordance with Choudhary *et al*. (2014).

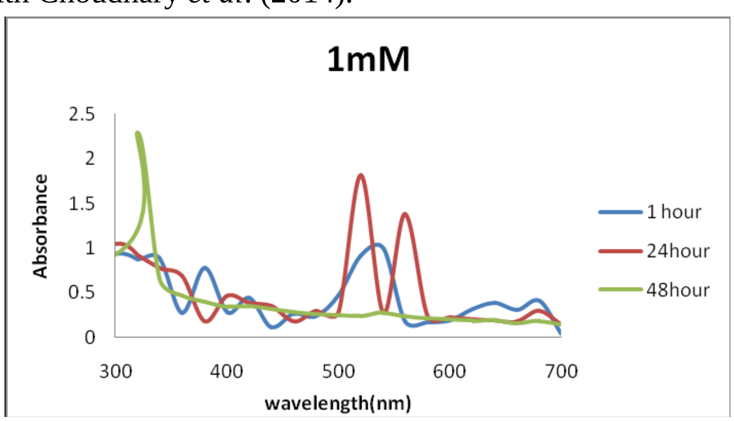

Figure- 3 UV–Vis spectra of SNPs synthesized using the grass extract of *Cyperus brevifolius*

The *Mollugo nudicaulis* plant UV- visible spectra showed a strong Plasmon resonance which was centered approximately at 520 nm (Sridhar *et al.*, 2012). A similar result was observed in *Cyperus brevifolius*. The result of the UV-Vis spectra recorded from the reaction medium absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 460 nm, broadening of peak indicated that the particles are polydispersed (Dhanalakshmi *et al*., 2012) The plant extract *Solanum torvum* formation of silver nanoparticles was monitored by UV-vis spectroscopy. Formation of $AgNO_3$ ions shows a distinct absorption at around 434 nm which corresponds to SPR of silver nanoparticles established peak at 420 nm (Govindaraju *et al*., 2010). The *Cocculus hirsutus* plant UV-Vis absorption spectra of silver nanoparticles in the reaction media has absorbance peak at 360 nm- 380 nm and the broadening of peaks indicated that the particles (Thirupathi *et al*., 2013). Similar results were observed in this study.

FTIR spectroscopy

The FTIR spectra of *Cyperus brevifolius* showed the presence of different functional group line 1285 cm^{-1} (co stretching) OH (3444 cm^{-1}) and –CO (1639 cm^{-1}), and –COV or C≡C stretching AgNP (2088 cm^{-1}) (Fig-4). The appearance of peaks in amide I and amide II peaks regions characteristics of protein or enzymes that have been found to be responsible for reduction of metal ions when using plant extracts of *C.brevifolius* nanoparticles. Noticeable changes were observed in the absorption bands for the spectra of nanoparticles and prominent peak of 2343 which was observed in the spectra suggesting the absorption band for AgNPs. The O-H absorption frequency band was also lowered in the nanoparticles and this consequently led to line broadening. Studies have shown that the presence of hydrogen bonding leads to broad absorption bands.

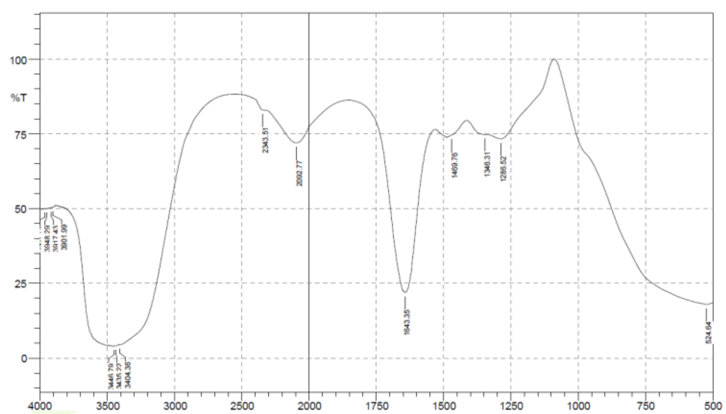

Figure-4 FT-IR spectra of SNPs synthesized using the grass extract of *Cyperus brevifolius*

Siva *et al*. (2014) reported that the FT-IR spectrum of *Cyperus rotundus* Grass Extracts mediated AgNPs is shown in the band at 3428 cm^{-1} corresponds to intermolecular O-H stretching vibrations. In *Cyperus brevifolius* the maximum peak showed at 3435 cm^{-1} and it is nearly close to 3428 cm^{-1}. So it may be corresponds to O-H stretching vibrations. Siva Prasad *et al.* (2016) *Nymphaea rubra* Roxb plant reduced AgNPs and are analyzed by FT-IR spectrum showed broad peaks at 3327 cm^{-1} and 1636 cm^{-1} were assigned for N-H bond of primary amines of proteins and O-H bond of phenols respectively. These results were agreed and similar peaks were showed in *Cyperus brevifolius***.**

Antibacterial activity of synthesized silver nanoparticles

The antibacterial activity of biosynthesized silver nanoparticles, $AgNO_3$, *Cyperus brevifolius* extract was studied against Gram-positive (*Enterococus faecalis* and *Bacillus sp*) and Gram-negative (*Pseudomonas aeruginosa , E. coli, Klebsilla pneumoniae*) bacteria using the agar well diffusion assay. Different concentration (50µl and 100µl) of synthesized silver nanoparticles was showed dose dependent activity of the extract. The results were compared with the standard broad spectrum antibiotic ampicillin, which was used as positive control and the distilled water served as negative control. The results of the zone of inhibition were tabulated (Table-1). The synthesized AgNPs displayed efficient antibacterial activity against both Gram-negative and Gram-positive bacteria. The silver nanoparticles synthesized by *Cyperus brevifolius* leaf extracts showed the maximum zone of inhibition around 18 mm for *Bacillus sp*, which were followed by *Enterococcus faecalis* (17mm) > *E.coli* (16mm) > *P.aeruginosa (15mm)*> *K.pneumonia*(14mm) at 100µl concentration (Fig-5). The maximum zone of inhibition 16 mm was observed in *Basillus sp* followed by the order of *E.coli* (15mm)> *E. faecalis* (14mm) ≥ *P.aeruginosa* (14mm) > *K.pneumonia* (13 mm) at 50µl concentration. On the other hand, the negative control (distilled water) did not exhibit any zone of inhibition. The positive control (ampicillin) displayed antibacterial activity against all tested microorganisms. The maximum zone of inhibition (45 mm) was observed in *E. faecalis* followed by the order of *K.pneumonia* (35 mm), *Bacillus sp* (30 mm), *E.coli* (25 mm) ≥ *P.aeruginosa* (25 mm) at 10µl (50 µg/ml) concentration.

Table -1 Antibacterial activity of silver nanoparticles from *Cyperus brevifolius* grass extract

S.no	Organisms	Zone of inhibition (mm)			
		Synthesis of silver nanoparticles		Ampicillin (10 µl)	Distilled water (50µl)
		50µl	100µl		
1.	*E.coli*	15	16	25	0
2.	*K.pneumonia*	13	14	35	0
3.	*P.aeruginosa*	14	15	25	0
4.	*Basillus sp.*	16	18	30	0
5.	*E. faecalis*	14	17	45	0

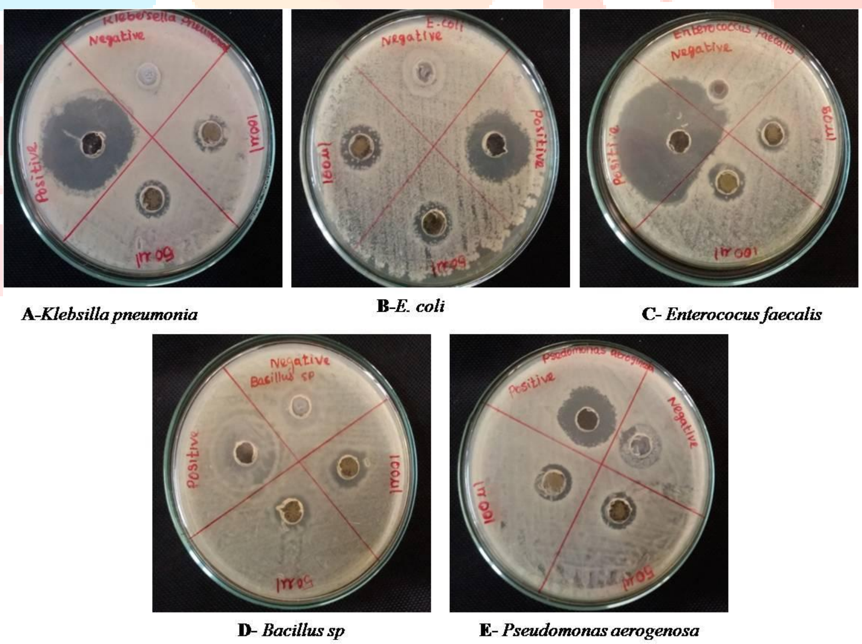

Figure 5- Antibacterial activity of synthesized SNPs against Gram positive and negative bacteria

The synthesized of silver nanoparticles of *Cyperus esculentus* seed extracts showed the antibacterial activity against gram positive and negative bacteria. The zone of inhibition showed in *E.coli* (20 mm), *Bacillus subtilis* (18 mm), *P.aeruginosa* (20) and *K.pneumoniae* (18 mm) at 100 µl concentration (Ibironke *et al*., 2015). In *Cyperus brevifolius,* similar result was observed in *Bacillus sp* and fluctuates in all other tested bacteria. The synthesized silver nanoparticles of *Cymbopogan citratus* (Lemongrass) extracts showed antibacterial activity against multiple drug resistant hospital iso-lates of *E.coli* (10 mm), *S.aureus* (10 mm), *P.mirabilis* (10mm) and *S.typhi* (10 mm), *K.pneumoniae* (10 mm) at 100 µl concentration (Shalaka *et al*., 2011). In this study more antibacterial activity was showed in *E.coli* and *K.pneumoniae*. Antibacterial activity of biogenic silver nanoparticles of *Cymbopogan citratus* extracts was evaluated by zone of inhibition using standard agar disc diffusion method. The nanoparticles showed inhibition zone against all the studied bacteria. Among three different concentrations (25, 50 and 100 µl), 100 µl concentration showed maximum activity against *Klebsiella pneumoniae* (2.2 cm), *Shigella somenesis* (1.2 cm), *P. aeruginosa* (1.5 cm), *E. coli* (1.3 cm), *P. mirabilis* (1.1 cm) and *S. flexaneri* (1.1 cm) (Natesan *et al*., 2014) In this study, similar result was observed in *Pseudomonas aeruginosa* and fluctuates in all other tested bacteria.

The synthesized silver nanoparticles of *Cymbopogon flexuosus* extracts showed strong antibacterial activities against *S. aureus* (22 mm) and *P. aeruginosa* (20mm) while weak activity against *A. bamunn (13 mm)* at 100 µl concentration (Ashish *et al*., 2015). In *C. brevifolius* SNPs showed lower zone of inhibition in *Pseudomonas aeruginosa* while compare to the *Cymbopogon flexuosus* extracts.

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