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Green Synthesis of Silver Nanoparticles from *Alternanthera pungens* Kunth. Leaves Extract and its Antimicrobial Activity

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Abstract*: Nanoparticles are eco-friendly approach for green synthesis of nanoparticles using natural plant extract is gaining a notable importance now a days. The present study deals with the synthesis of silver nanoparticles using *Alternanthera pungens* Aubl petal extract. The complete reduction of silver ions was observed after 48hrs of reaction at 30°C under shaker condition. The formulation of silver nanoparticles was confirmed by UV-Visible spectroscopy, XRD and SEM analysis. The antifungal activity evaluate against *Aspergillus niger* and *Rhizopus stolonifer*. The antibacterial activity evaluated against *E.coli* and *B.subtilis.

Keywords*: Green synthesis, Silver nanoparticles, *C.guianesis.

1. Introduction

Nanoparticles are often referred to as particles with a maximum size of 100nm. Nanoparticles exhibit unique properties, which are quite different than those of large particles (Mohsen *et al.,* 2011). An important area of research in nanotechnology deals with the synthesis of nanoparticles of different chemical compositions, dimension and controlled monodispersity (Swapna *et al.,* 2012). Nano-meter sized metallic particles show unique and considerably changed physical, chemical and biological properties compared to their macro-scaled counterparts, due to their high surface to volume ratio (El-Nour *et al*., 2010).

Fig. 1. Habit of *Alternanthera pungens*

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction (Swapna *et al.,* 2012). A*lternanthera pungens* belongs to the family Amaranthaceae, it is herb 3-8cm tall, annual, stems prostrate, pubescent, branched, brownish to reddish, leaves green, ovate to obtuse, apex obtuse to rounded, glabrous or pubescent, membranaceous sessile. It is used as pain killer and used for treat stomach troubles, diarrhoea and dysentery. A decoction is used internally to treat gonorrhoea.

2. Materials and methods

A. Preparation of flower petal broth

Collect the healthy leaves from the selected plant, weigh 20g of leaves and washed thoroughly with distilled water, then it cut into small pieces, the finely cut into pieces were boiled in 100ml of distilled water for 15 to 20 minutes, then cool the solution and filtered using whatmann no 1 filter paper, collect the solution and used for further purposes (Tahira *et al.,* 2018).

B. Synthesis of silver nanoparticles

Silver nitrate was used as a precursor in synthesis of silver nanoparticles, 5ml of leaf extract was added to 100ml of 1mm AgNO3 (99.99%) aqueous solution in a conical flask at room temperature. The flask were put in shaker at 30°C and reaction was carried out for a period of 48 hrs (Swapna *et. al.,* 2012).

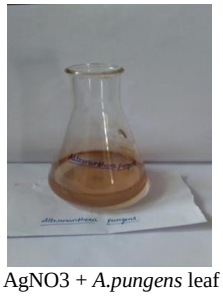


Fig. 2. AgNO3 + *A.pungens* leaf extract

C. UV- visible Spectral analysis

The gradual change in the colour of a sample from light green to dark brown colour was observed and the bio-reduction of silver ions in the solvent extracts was monitored by periodic evaluation of the suspension (2ml) after incubation of 48hrs under dark condition, the aliquotes were subsequently measured for the UV-visible spectral by scanning in the region from 200-800nm.

D. SEM analysis

SEM analysis was undertaken to know the size and shape of the silver nanoparticles biosynthesis using the plant. The analysis was done using Noran system 7, S-3400N model. Thin films if the samples were prepared on tungsten filament by dropping a very small amount of the samples on the gird, extra solution was removed using a blotting paper. Thin film on the SEM gird was allowed to dry and the images of nanoparticles were taken.

E. XRD analysis

XRD analysis the samples was drop-coated onto copper plate by just dropping a small amount of sample on the plate frequently allowed to dry and finally thick coat of sample was prepared the XRD measurements was performed in a Rigako model with step size 0.02 and an angle of 60°-70°.

The particles size of the prepared samples were determined by using Scherrer's equation as follows,

$$D = \frac{k\lambda}{\beta\cos\theta}$$

Where D is the crystal size, **λ** is the wave length of x-ray, θ is the Bragg's angle in radians and β is the full width at half maximum of the peak in radians, k is constant.

F. Antibacterial assay

Antibacterial assay was studied using disc diffusion method. Nutrient agar media was prepared, and pour into sterile petriplate and allowed to solidify, using sterile cotton swabe fresh bacterial culture were spread over the plate followed by spread plate technique, filter paper discs saturated with AgNO3+plant extract. Antibiotics (+ve control) and filter paper diac with water (-ve control) were placed on to the plates with the help of forceps. Incubated at 37°C. Growth zones were read only after 24hrs, zone of inhibition was calculated.

G. Antifungal assay

Antifungal activity was tested by well diffusion method. Aqueous extract of two AgNO3 sample were tested against. The PDA media was poured into sterile petriplates and allowed to solidify, then using sterile cotton swabe fresh fungal culture were spread over the place by spread plate technique. 5mm well made on PDA media using a sterile disc. Wells were filled with AgNO3 plant extract. Antibiotic (+ve control), distilled water (-ve control) is separate well. The plate was incubated at 25°C for 48-72hrs, after incubation zone of inhibition was calculated.

3. Results

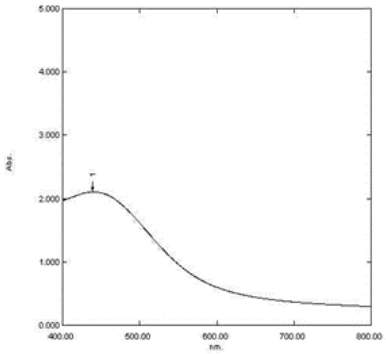
A. Observation

After 48 hrs, distinct change in the colour of experimental sample was observed. The colour of experimental sample turned from light green to dark brown colour. The brown colour confirms that the colour change is due to reduction of silver ions which indicates the formation of Ag nanoparticles.

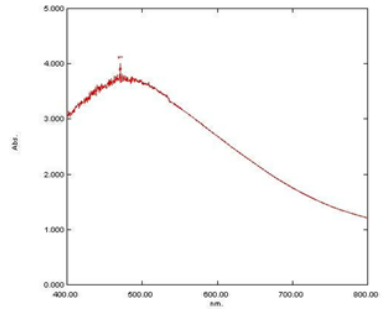
B. UV-Vis spectroscopy

The confirmation, formation and stability of synthesized silver ion nanoparticles was confirmed by UV-Vis spectrum. It was recorded by using AgNPs and bio reduction of Ag+ ion was also monitored in the UV-Vis spectrophotometer. The surface plasmon resonance bands are influenced by size, shape, morphology, composition and dielectric environment of prepared NPs 2ml of synthesized AgNPs solution of *Alternanthera pungens* Kunth. were observed in before and after the incubation and the UV ranges between 200-800nm.

Before incubation, the synthesized AgNPs shows peaks at 230 nm of *Alternanthera pungens* Kunth. Respectively. After the incubation period of 48 hrs the synthesized AgNPs showed broad surface plasmon resonance at 470 nm of *Alternanthera pungens* Kunth. respectively.



Graph-1: UV- spectra of *Alternanthera pungens*



UV- spectra of after incubation

C. Scanning electron microscope analysis

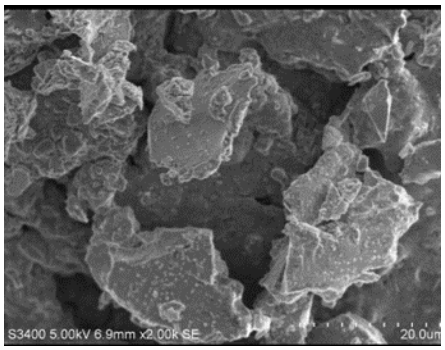


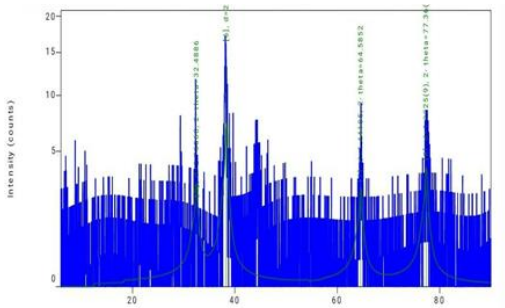
Fig. 3. EM images of *Alternanthera pungens* Kunth. Silver nanoparticle

SEM analysis of the silver nanoparticles solution were clearly distinguishable owning to their size difference. The SEM images shows the AgNPs synthesized from *Alternanthera pungens* Kunth. extract which is further confirm the presence of AgNPs. The shape of the AgNPs in *Alternanthera* extracts was spherical and size of AgNPs is 6.9 mm as confirmed by SEM images.

D. XRD analysis

X-ray Diffraction studies of two samples show different diffraction peaks. *Alternanthera* pungens Kunth. plant extract shows four different diffraction peaks at 32.48°, 38.33°, 64.58 and 77.36°. 2 theta values and crystalline planes of Ag sample.

The average size of the AgNPs formed in bio-reduction process is determined by using $D = \frac{k\lambda}{\beta\cos\theta}$ and it is estimated that average size of *A.pungens* Kunth. 119.77, 121.74, 136.03 and 147.3 shows the XRD pattern of the silver nanoparticles formed in our experiment.



Graph-2: XRD result of *Alternanthera pungens* Kunth. silver nanoparticle

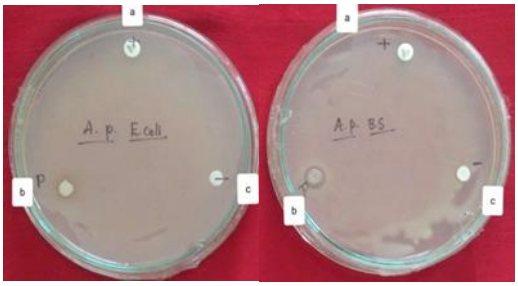
Table 1
XRD result of *Alternanthera pungens* Kunth. silver nanoparticles

d-spacing	2-theta	H	K	L	Average size
2.753	32.48	1	2	2	119.77
2.346	38.33	1	0	2	121.74
1.441	64.58	1	2	2	136.03
1.232	77.36	1	1	2	147.3

E. Antimicrobial analysis

Toxicity studies on pathogen opens a door for Nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of a plant extract as a new awareness for the control of disease, besides being safe and no phyto-toxic effects. The biologically synthesized AgNPs using *Couroupita guianensis* Abul. And *Alternanthera pungens* kunth. were found to be highly toxic against different pathogenic bacteria and fungi of selected species. The AgNPs against followed by *E.coli* (Gram positive), *Bacillus* (Gram negative), Antifungal activity was observed against *A.niger* and *R.stolinifer*.

The use of plant extract is effective against various micro-organisms including plant pathogens. Oligidynamic silver antimicrobial efficiency extends well beyond it's vitrotoxicity. The ionic silver strongly interacts with thiol groups of vital enzymes and in activate the vital activity. Experimental evidence indicate that DNA losses it's replication ability, once the attributed their stability in the medium as a colloid, which modulates the phosphotyrosine profile of the pathogen proteins and arrests it's growth, the growth of micro-organisms was inhibited by the synthesized AgNPs showed variation in the inhibition if growth of micro-organisms may be due to the presence of peptide-glycin, which is a complex structure and after contains teichoic acuds or lilotichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria.



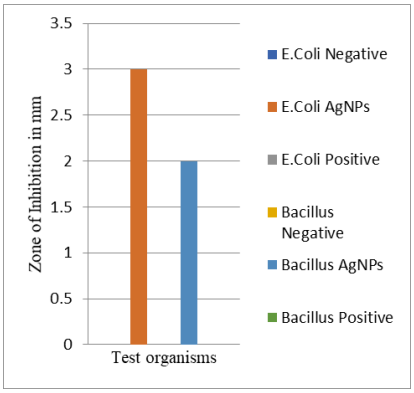
Escherichia coli *Bacillus subtilis*
Fig. 4. Escherichia coli and Bacillus subtilis

Table 2
Antibacterial activity of AgNPs of *Alternanthera pungens* Kunth. against to selected bacterial culture by disc diffusion method

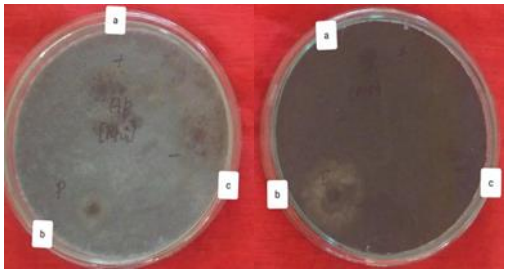
Plants used	Bacterial culture	Diameter of Inhibition zone of bacteria(in mm)		
		-ve control	Ag NPs	+ve control
Alternanthera pungens Kunth	*Escherichia coli*	-	3±0.1	-
	Bacillus subtilis	-	2±1	-

a) Positive control - Ampicillin
b) AgNPs solution - *Alternanthera pungens* Kunth.
c) Negative control- Water

A table shows antibacterial activity of AgNPs of *Alternanthera pungens* Kunth. against selected bacterial culture.



Graph-3: Graphical representation of antibacterial activity against AgNPs of *Alternanthera pungens* Kunth.



A. niger *R.stolonifer*

Fig. 5. Niger and Stolonifer

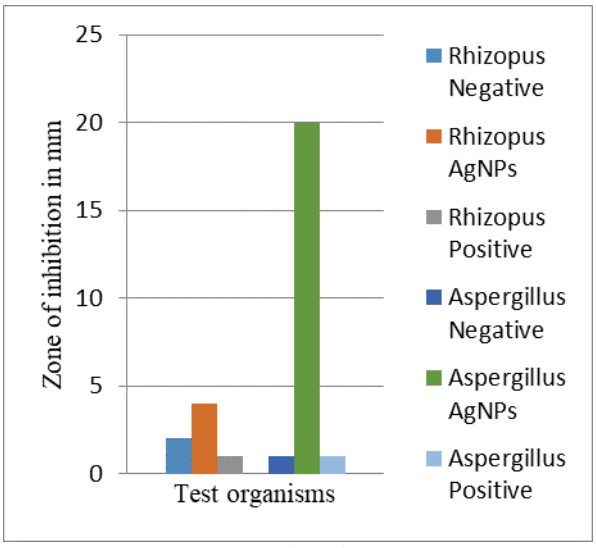
Antifungal activity of AgNPs of *Alternanthera pungens* Kunth. against to selected fungal culture by well method.

a) Positive control - Ampoxin
b) AgNPs solution - *Alternanthera pungens* Kunth.
c) Negative control - Water

Table 3
Antifungal activity of AgNPs of *Alternanthera pungence* Kunth. Against selected fungal culture.

Plants used	Fungal culture	Diameter of Inhibition zone of Fungal (in mm)		
		-ve control	AgNPs	+ve control
Couroupita guianensis Aubl	*Rhizopus stolonifer*	2±0.1	4±0.2	1±0.1
	Aspergillus niger	1±0.1	20±0.3	1±0.1

A table shows Antifungal activity of AgNPs of *Alternanthera pungence* Kunth. against selected fungal culture.



Graph-4: Graphical representation of Antifungal activity against AgNPs of *Alternanthera pungence* Kunth

4. Conclusion

The current investigation demonstrated that the aqueous extract of *Alternanthera pungens* leaf showed noticeable antibacterial potential and was also capable of producing AgNPs extract, cellularly, furthermore the biosynthesized practicles had on excellent antibacterial activity against some gram +ve and gram -ve bacteria. Nanoparticle well definitely pave way towards minimizing the utilization of this multipurpose nanotechnology.

Acknowledgement

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References

[1] El-Nour About K.M.M, Al-Warthan A and Ammar R.A.A., 2010. Synthesis and applications if silver nanoparticles. Arabian Journal of Chemistry, vol. 3 (iss 3); 135-140.
[2] Mohsen Zargar, Azizah Abdul Hamid, Fatima Abu Bakar, Mariana Nor Shamusudin, Kamyar Shameli., Fatemeh Jahanshiri and Farah Farahani., 2011. Green synthesis and antibacterial effect of silver nanoparticles using Vitex negundo L. Molecules, vol. (26); 6667-6676.
[3] Swapna Alex, Preetha Rani M. R, Soni K. B, Deepa S.Nair and B.R. Reghunath., 2012. Biosynthesis of silver nanoparticles with antibacterial activity using leaf extract of Michelia champaca. Jour Pl Sci Res, vol 28(iss.1); 121-126.
[4] Tahira Akther, Mohd Shahanbaj Khan, Hemalatha Srinivasan., 2018. Novel silver nanoparticles synthesized from anthers of Couroupita guianensis Abul. Control growth and biofilm formation in human pathogenic bacteria. Nano Biomed. Eng, vol. 10 (Iss. 3); 250-257.