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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *ALLAMANDA BLANCHETII* A.DC., LEAVES EXTRACT AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract: *Nanoparticles are fundamental blocks of nanotechnology. An 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 Allamanda blanchetii A.DC. Leaf extract. The complete reduction of silver ions was observed after 48 hours of reaction at 30ºc under shaker condition. The formation of silver nanoparticles was confirmed by Uv – visible spectroscopy, XRD and SEM analysis. The antifungal evaluate against Aspergillus niger and Rhizopus stolonifer. The antibacterial activity evaluated against Escherichia coli and Bacillus subtillis.*

Key words: *Green synthesis, Silver nanoparticles Allamanda blanchetii A.DC.*

I. INTRODUCTION:

Nanotechnology is a branch of nanoscience and is used to create nanoparticles. Or materials less than 100nm. It converts macromolecules into nanoparticles. (Balashan mugam *et al.,* 2013)**.** The term nanoparticles is used to describe a particles with size in the range of 1-100nm. (Yehia and AI-sheikh. 2014). *Allamanda blancetii* A.DC., Commonly called know as purple *Allamanda*, is an ornamental flowering Plant, The plant inflorescences open two flowers per day and the flowers are hermaphrodite and with gamopetalous corolla with five pink purple petals. (Magnolia *et al.,* 2011). The medicinal potential of *Allamanda blanchetii* grow in Brazil. In terms of their cytotoxic activity against leukemia. Since leukemia is associated with increased angiogenesis in the bone marrow. Which is thought to promote the survival of leukemia cells. The anti angiogenic effect of *Allamanda* extracts, by evaluating its cytotoxic effects against endothelial cells.

II. MATERIALS AND METHODS:

A. Preparation of *allamanda blanchetii* A,DC.:

Collect the healthy leaves from the selected plant, weight 20g of leaves and washed thoroughly with distilled water, then it cut into small pieces, The finely cut 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 1mm $AgNO_3$ (99.99%) aqueous solution in conical flask of 250ml content at room temperature. The flask were thereafter put in shaker (150ml) at 30º and reaction was carried out for period of 48 hours. (Swapna *et al*., 2012)

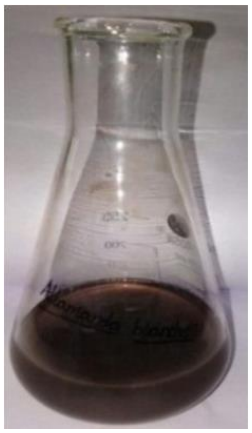


Fig. 2: Plant extract with $AgNO_3$

C. UV- Visible Spectral Analysis:

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

D. SEM Analysis:

SEM analysis was under taken to know the size and shape of the silver nanoparticles biosynthesised using the plant leaf extract of *Alamanda blanchtii* A.DC. The analysis was done using Noran system 7.S-3400 N model. The filme of

the samples were prepared on tungsten filament by dropping a blotting paper. The film on the SEM grid was allowed to dry and the images of nanoparticles were taken.

E. XRD Analysis:

The sample was drop coated onto copper plate by just dropping a small amount of sample on the Plant frequently allowed to dry and finally thick coat of sample was prepared the XRD measurements was performed on a Rigako model with size 0.02 and an angle of 60º –70 º. The particle size of the prepared samples were determined by using Scherrer's equation as follows.

$$\mathbf{D = \frac{K\lambda}{\beta COS\theta}}$$

Where D is the Crystal size, λ is the wave length of x-ray, θ is the Braggs angle in radians and β is the full width at half maximum of the peaks 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 cultures were spread over the plate followed by spread plate technique. Filter paper discs saturated with AgNO3 + plant extract.

Antibiotic (+ve control amphicilin) and filter paper disc with water (-ve control) were Placed onto the plates with the help of forceps. Incubated at 37ºC. Growth zones were read only after 24hours. Zones of Inhibition was calculated.

F. Antifungal Assay:

Antifungal activity was tested by well diffusion method. Aqueous extracts of two $AgNO_3$ samples were tested against. The PDA medium was poured into sterile petriplates and allowed to solidify 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 AgNPs plant extract. Antibiotic (+ve control) distilled water (-ve control) in separated well. The plate was incubated at 25 ºC for 48-72 hours. After incubation zone of Inhibition was calculated.

III. RESULTS:

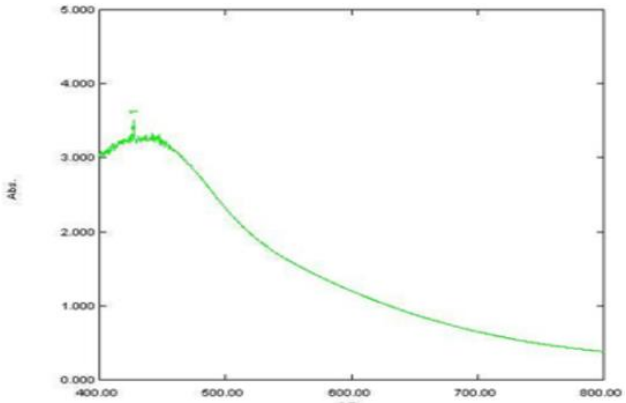
Observation:

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.

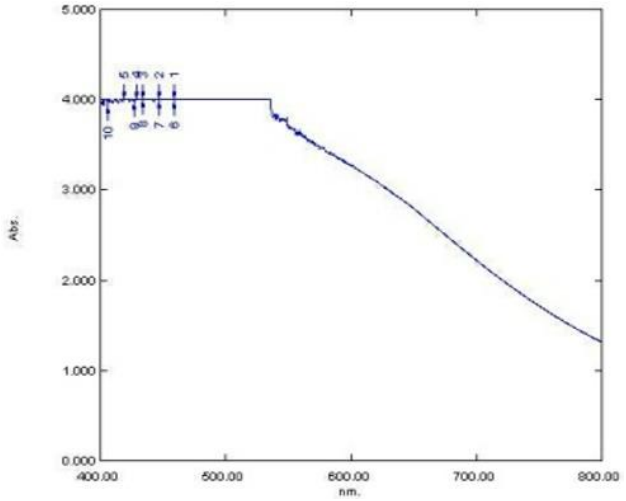
UV-Vis Spectroscopy:

The confirmation, formation and stability of synthesized silver ion nanoparticles was confirmed by UV-Vis spectrum. It was recorded by using AgNPs and bioreduction 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 *Allamanda blanchetii* A.DC. We're observed in before and after the incubation and the UV ranges between 200-800nm.

Before incubation, the synthesized AgNPs Shows peaks at 426 nm of *Allamanda blanchetii* A.DC., respectively. After the incubation period of 48 hrs the synthesized AgNPs showed broad surface Plasmon resonance at 457 nm of *Allamanda blanchetii* A.DC., respectively.



Leaf extract before incubation



Leaf extract after incubation

Graph 1: UV Spectra of *Allamanda blanchetii* A.DC.

Scanning Electron Microscope [SEM]:

SEM analysis of the silver nanoparticles solution were clearly distinguishable owing to their size difference, The SEM images shows the AgNPs synthesized from *Allamanda blanchetii* A.DC., extracts which is further confirms the presence of AgNPs. The shape of AgNPs in

Allamanda extract was spherical and the size of AgNPs is 6.6mm as confirmed by SEM images.

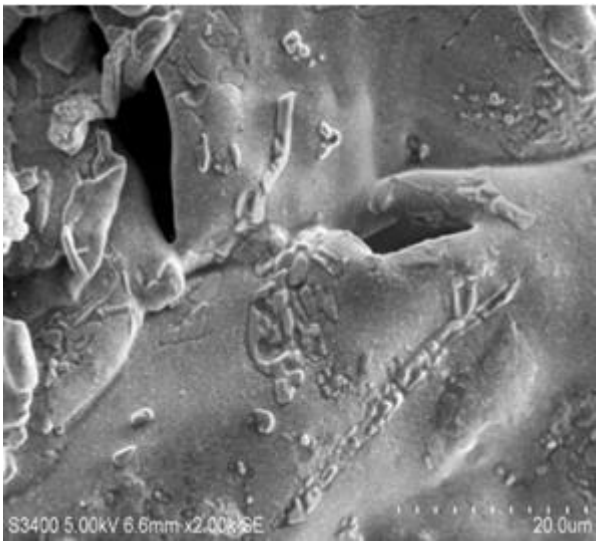


Fig. 3: SEM image of *Allamanda blanchetii*

XRD Analysis:

X ray diffraction study of two samples show different diffraction peaks. *Allamanda blanchetii* plant extract shows 4 peaks at 37.9º, 44.30º, 64.58º, 77.59º, 2θ values and crystalline planes of Ag samples.

The average size of the AgNps formed in bioreduction process is determined by using $\boldsymbol{D = \frac{K\lambda}{\beta \text{Cos}\, \theta}}$ and it is estimated that average size of *Allamanda blanchetii* 877.48, 895.85, 981.55, 1064.6, shows the XRD pattern of the silver nanoparticle formed in our experiment.

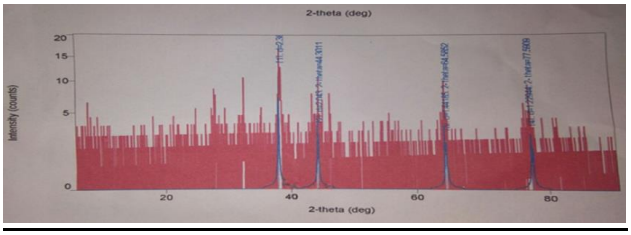


Table 1: XRD Analysis of Allamanda blanchetii A.DC.

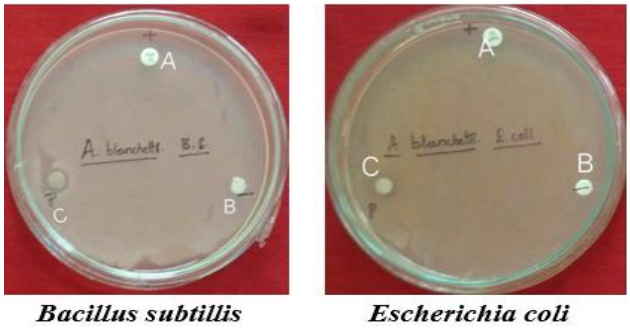
d-spacing	2-theta	HKL	Average size
2.36	37.97	101	877.48
2.043	44.3	101	895.85
1.441	64.58	101	981.55
1.229	77.59	101	1064.6

A Table Shows XRD Analysis of *Allamanda blanchetii* A.DC. silver nanoparticles.

Antimicrobial Analysis:

Toxicity studies on pathogen opens a door for Nanotechnology applications in medicine. Biological synthesis of metal Ag 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 *Allamanda blanchetti* A.DC, 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 *Rhizopus*.

The AgNPs synthesized from leaf extract of *Allamanda blanchetii* A.DC. Shows antibacterial activity using bacteria *B.subtilis* shows inhibition zone of AgNPs (2.0mm) *E,coli* shows inhibition zone of AgNPs (1.0mm) and both the bacteria of positive and negative control shows no results and *Aspergillus niger* shows inhibition zone of AgNPs (0.5mm) positive control [0.1mm] and negative control shows no result. *R.stolonifer* shows AgNPs inhibition zone and positive and negative control shows (0.1mm)



Bacillus subtillis ***Escherichia coli***

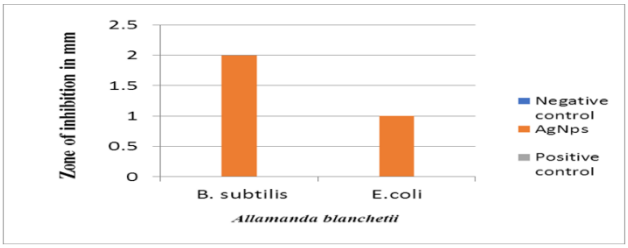
Fig. 4:

Antibacterial activity of AgNPs of *Allamanda Blanchetti* A.DC., against two selected bacterial culture by disc diffusion method.

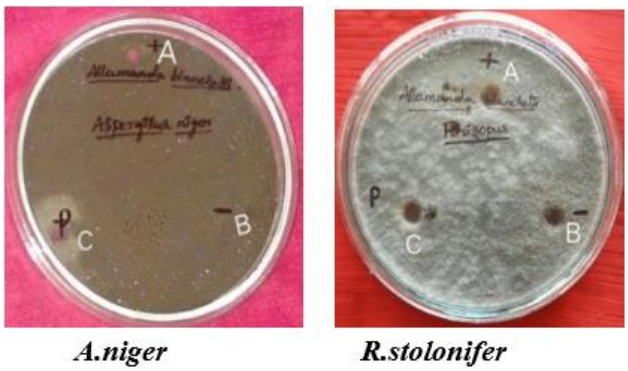
A- Positive control (Ampicillin)
B- Negative control (Water)
C- AgNPs solution of *Allamanda Blanchetii* A,DC.

Table 1: The table shows antibacterial activity of AgNPs of *Allamanda Blanchetii* A.DC. Against two selected bacterial culture.

Plant used	Bacterial culture	Diameter of inhibition zone of bacteria in mm		
		Negative control	AgNPs	+control
Allamanda blanchetii A,DC.	*Bacillus subtilis*	-	2.0 ± 0.1	-
	Escherichia coli	-	1.0 ± 0.1	-



Graph 3: Graphical representation of antibacterial activity against AgNPs of Allamanda blanchetii A.DC.



A.niger *R.stolonifer*

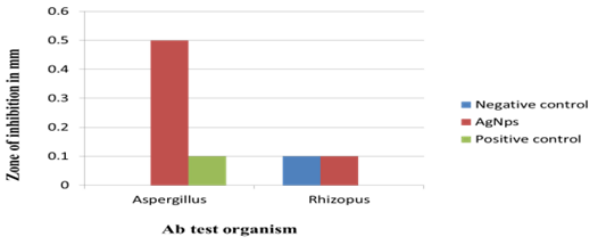
Fig.5:

Antifungal activity of AgNPs of *Allemande blachetti* against two selected fungal culture by well method.

A- Positive control (Ampoxin)
B- Negative control (Water)
C- AgNPs solution of *Allamanda blanchetii* A.DC.

Table 2: Shows Antifungal activity of AgNPs of *Allamnda Blanchetti* A.DC. Against two selected fungal culture.

Plant used	Fungal culture	Diameter of inhibition zone of Fungal in mm		
		yake control	AgNPs	Positive control
Allamanda Blanchetii (A.DC.)	*Aspergillus niger*	-	0.5 ± 0.1	0.1 ± 0.1
	Rhizopus stolonifer	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1



Graph 4: Graphical representation of Antifungal activity against AgNPs of Allamanda Blanchetii A.DC.

IV. CONCLUSION:

The current investigation demonstration that the aqueous extract of *Allamanda blanchetii* A.DC., leaves showed noticeable antimicrobial potential and was also capable of producing $AgNP_3$ extra, cellular, furthermore, the biosynthesized particles had an excellent antibacterial activity against some gram positive and gram negative bacteria. Nanoparticle will definitely pave way towards minimizing the utilization of this multipurpose nanotechnology.

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REFERENCES:

1. Balashan mugan P, Santhaeh S, Mukesh kumar D. J, Anbazharan S, Kalaichelvan P.T, 2013, *Synthesis of Plant mediated Silver nanoparticles using Aerva lanata Leaf Aqueous extract and evalution of its Antibacterial Activities*, Indo American Journal of Pharmacestial Research, Vol 4 (1).
2. Magnolia martins Alves; Manoel Bandeira de Albuquerque; Walter esfranin Pereira, Maria de Fatima de Araujo LUCENA, Jao da silva AZEVEDO, 2017, *Morpho Physiological Analysis of Allamanda Blanchetti A.DC., Seedlings under water deficit*, Biosci J, Uberlandia, Vol 3(5); 1134-1143.
3. Swapna Alex, Preetha Rani M, Soni K.B, Deepa S, Nair and Reghunath B.R, 2012, *Biosynthesis of Silver nanoparticles with Antibacterial Activity using Leaf extract of Michelia champaca.* Jour PI Science Research, 28(1), 121-126.
4. Tahira Akther., Mohdshahanbaj Khan, Hemalatha srinivasan 2018 *Novel Silver nanoparticles synthesized from anthers of Couropita guiansis Abul. Control growth and Biofilm formation in Human Pathogenic Bacteria.* Nano biomed, Eng, Vol 10 (3); 250-257.
5. Yehia R.S, AI– Sheikh H, 2014, *Biosynthesis and Characterization of Silver nanoparticles produced by Pleurotus Ostreatus and their Anticandidal and Anticancer activities.* World Journal Microbial Biotechnology Vol 10(5).