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Green Synthesis and Characterization of Silver Nanoparticles from *Justicia Betonica L* Leaves Extract and their Antimicrobial Activities

S. B. Supriya[1], H. T. Navyashree[2]
[1]*Student, Department of Botany, Bharathi College PG and Research Center, Bharathi Nagar, India*
[2]*Assistant Professor, Department of Botany, Bharathi College PG and Research Center, Bharathi Nagar, India*

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 30°c 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.

***Keywords*:** *Green synthesis, Silver nanoparticles Justicia betonica* L.

1. Introduction

Nanotechnology is a branch of nanoscience and is used to create nanoparticles. Or materials less than 100 nm. It converts macromolecules into nanoparticles. (Balashan mugam *et al.*, 2013). The term nanoparticles are used to describe a particle with size in the range of 1-100nm. (Yehia and AI-sheikh. 2014). Nanoparticles have been widely applied in the field of investigation and regulation of cellular stage, drug delivary, artificial implants, etc. Green synthesis of silver nanoparticles is an ecofriendly method for avoiding harmful effects in medicinal applications of silver nanoparticles synthesized by physical and chemical method. (Reenal *et al*., 2015). Nowadays there is a wide application of nanoparticles in diverse fields including catalysis, Energy and medicine (Yehia and AI-sheikh 2014) Nanotechnology is being applied in various fields like biology and medicine. Further earlier indicate that silver nanoparticles are not harmful to humans but acts as effective agent against different bacteria, fungi, and yeast (Raju nalvothula 2014). *Justicia betonica L* includes about 250 genera and 2500 species. Widely distributed in the tropics. (See willis 1966) *Justicia betonica* L is a diffuselly branched under shurb. More or less decumbent and aften rooting at the lower nodes. The stem is cylindrical with distinct swollen nodes. The spikes are terminal as well as axillary. The plant starts flowering in the month of December and continues to flower till the end of May and June. The sepals are approximately half the size of bracteoles. deeply 5 partite and persist. The anther lobes are dorsifixed separated by a broad connective. The gynoecium is bicarpellary, syncarpous with a superior ovary placed on a circular disc. The fruit is a capsule with persistent style. (Bhatnagar and sunitha puri 1970). *Justicia betonica* L.is regarded as a valuable medicinal plant extensively used in folklore medicine. The plant is used in diarrhoea, paralysis, headache, bruises, to lower the cholesterol, to reduce stomach gas and pain. To increase urination and as a cough remedy. (Jollykutty Eapen *et. al*., 2019).

Fig. 1. Habit of *Justicia betonica*

2. Materials and methods

A. *Preparation of the extract*

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 AgNO3(99.99%) aqueous solution in conical flask of 250ml content at room temperature. The flask was thereafter put in shaker (150ml) at 30° and reaction was carried out for period of 48 hours. (Swapna et. al., 2012).

Fig. 2. Plant extract with AgNPs

C. *UV-visible spectral analysis*

Spectroscopy analysis of biosynthesized silver nanoparticles was carried out using uv-visible spectrophotometer. 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 biosynthesized using the plant leaf extract of *Justicia betonica L*, 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 was 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 Braggs angle in radians and β is the full width at half maximum of the peaks i radians. K is constant.

F. *Antibacterial activity study*

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 37 ºC. Growth zones were read only after 24hours. Zones of Inhibition was calculated.

G. *Antifungal assay*

Antifungal activity was tested by well diffusion method. Aqueous extracts of two AgNO3 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.

3. Results

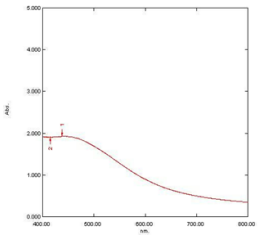
A. *Observation*

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.

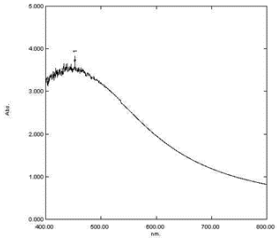
B. *UV-Vis spectroscopy*

The confirmation, formation and stability of synthesized silver ion nanoparticles was confirmed by UV-Vis spectrum. It was recorded by using AgNPs and 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 *Justicia betonica L*, 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 *Justicia bet*onica L, respectively. After the incubation period of 48 hrs the synthesized AgNPs showed broad surface Plasmon resonance at 4 nm of *Justicia betonica L*. respectively.



Graph-1: UV–Spectra of *Justicia betonica* UV spectra of *Justicia betonica* leaves before incubation



Leaves extraction after incubation

C. *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 *Justicia betonica L*, extracts which is further confirms the presence of AgNPs. The shape of AgNPs in *Justicia* extract was spherical and the size of AgNPs is 6.8mm as confirmed by SEM images.

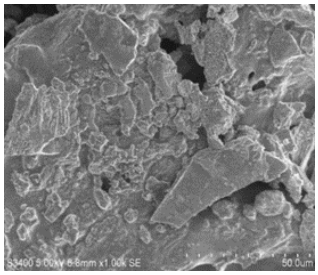


Fig. 3. SEM image of *Justicia betonica* L

D. *XRD Analysis*

X ray diffraction study of two samples show different diffraction peaks. *Justicia betonica* 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 $D = \frac{K\lambda}{\beta \text{Cos } \theta}$ and it is estimated that average size of *Justicia betonica* L shows the XRD pattern of the silver nanoparticle formed in our experiment.

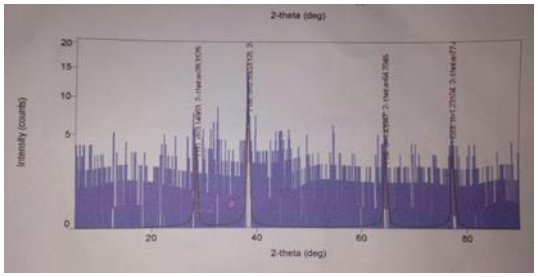


Fig. 4. XRD Analysis of *Justicia betonica L*

Table 1
XRD Analysis of *Justicia betonica L* silver nanoparticles

d-spacing	2-theta	HKL	Average size
3.14	28.31	010	173.93
2.35	38.26	010	178.51
1.43	64.70	010	199.64
1.23	77.47	010	216.21

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 *Justicia betonica* L 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*,

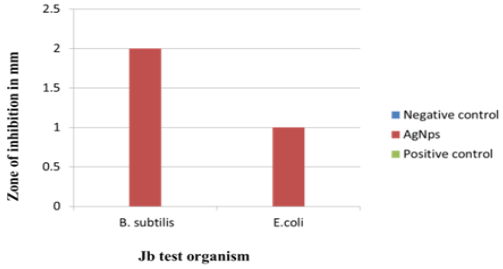
The AgNPs synthesized from leaf extract of *Justicia betonica* L. shows antimicrobial activity using bacteria *B.subtilis* shows inhibition zone of AgNPs (2mm) *E,coli* AgNPs (1.0mm) Negative and positive control of both bacteria shows no results. and *A.niger* shows inhibition zone of AgNPs and negative control (0.1mm) and positive control shows no results. And *R.stolonifer* shows inhibition zone of AgNPs shows (1.0mm) and negative control shows (0.1mm) result. and positive control shows (0.2mm) respectively.

Fig. 4. *B.subtilis, E.coli*

Antibacterial activity of AgNPs of *Justicia betonica* L against two selected bacterial culture by disc diffusion method.
Positive control (Ampicilin),
A-Negative control (Water)
B-AgNPs solution of *Justicia betonica L.*
C-AgNPs solution of *Justicia betonica*

Table 2
Antibacterial activity of *Justicia betonica* L. against two selected bacterial culture

Plant used	Bacterial culture	Diameter of inhibition zone of bacteria in mm		
		Negative control	AgNPs	Positive control
Justicia betonica (L)	*Bacillus subtilis*	-	2.0 ± 0.1	-
	Escherichia coli	-	1.0 ± 0.1	-



Graph-2: Graphical representation of antibacterial activity against AgNPs of *Justicia betonica* L

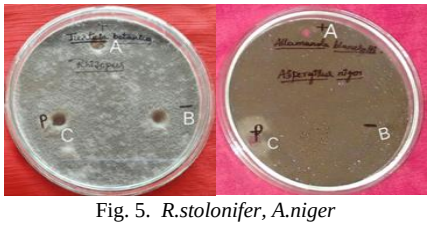


Fig. 5. *R.stolonifer, A.niger*

Antifungal activity of AgNPs of *Allamanda Blanchetti* against two selected fungal culture by well method

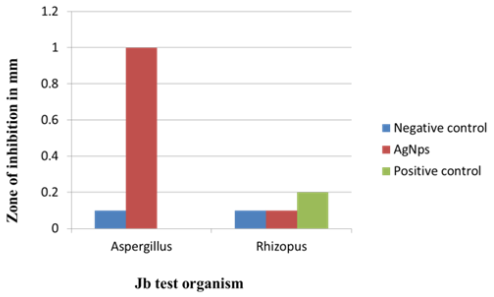
A-Positive control (Ampoxin)

B-Negative control (Water)

C-AgNPs solution of *Allamanda blanchetii* A, DC.

Table 3
Antifungal activity of AgNPs of *Justicia betonica* L. against two selected fungal culture

Plant used	Fungal culture	Diameter of inhibition zone of fungal in mm		
		Negative control	AgNPs	Positive control
Justicia betonica (L)	*Aspergillus niger*	0.1 ± 0.1	0.1 ± 0.1	-
	Rhizopus stolonifer	0.1 ± 0.1	1.0 ± 0.1	0.2 ± 0.1



Graph-2: Graphical representation of antifungal activity against AgNPs of *Justicia betonica* L.

4. Conclusion

The current investigation demonstration that the aqueous extract of *Justicia betonica* L 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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