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Synthesis of Silver nanoparticles using Periwinkle plant (*Catharanthus roseus* (L.) G. Don and assessment of its Antimicrobial activity

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Abstract

India possesses a great biodiversity of the medicinal plants that were not explored completely yet. The periwinkle species *Catharanthus roseus* (L.) G. Don is a native of Madagascar. Recent nanotechnology and Nano science enhanced the techniques for diagnose, treat and prevent various diseases regarding human health. Silver nanoparticles are one of the most vital and interesting nanomaterial. It plays a significant role in medical and pharmaceutical area. It also has potential to treat the severe disease like cancer. This study is aimed to investigate the antimicrobial properties of this plant species. The antimicrobial properties have been checked against microbial genera like *Bacillus subtilis, Escherichia coli, Salmonella* species, and *Candida albicans*. Yet plant proved to be significant natural substrate for effective diagnostic agent that's why all investigators are has major interest in plants.

Key words : Silver nanoparticles, *Catharanthus roseus,* Antimicrobial Activity.

Traditionally extraction of the silver nanoparticles is carried out by using plant extract. In leaf extract polysaccharides and secondary metabolites are found can reduce Ag+ ions to AgO state and form silver nanoparticles from it. In periwinkle plant large amount of secondary metabolites are present. *Catharanthus roseus* (L.) G. Don is self-compatible species compared to other species in the family due to its medicinal, anti-healing properties it is used in biological field[1]. In *Catharanthus roseus* various essential metabolites and alkaloids are present therefore, it is used for the production of silver nanoparticles. Since from long period periwinkle plant used as wound healer and pain reliever. Sap of the leaves is effective on wound bleeding and protect from other microbial infection[6]. The applications of the crude extract of *Catharanthus* is confirmed and approved by people because it's beneficial effects. Synthesis of silver nanoparticles is carried out by physical, chemical, and biological methods. According to material which is used to produce nanoparticles

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suitable producing method is selected. In physical method silver nanoparticles are produced in specific shape and size without any harmful effect[18]. In contrary in chemical method toxic and harsh chemicals are used due to this contamination are occur sometimes. Hence, physical method is more beneficial than chemical method because chemical methods are complicated and chances of contamination are more in it. Chemical method is complete in two steps *i.e.* "top-down" and "bottom-up" in which grinding of bulk and reduction of chemicals are carried out respectively[4]. The nanoparticles which is made up from using microorganisms are have perfect size and shape. Biological system provides an eco-friendly nature to nanoparticles which is important feature of it.

For the synthesis of silver nanoparticles various methods are used likewise for the characterization of synthesized nanoparticles some techniques are used it includes X-ray Diffraction, Fourier Transform Infra-Red, and Atomic force Microscopy *etc*.[16]. Silver nanoparticles have broad antimicrobial property. This is applied for finding out the types of plant parts are accumulates these active compound within the tissue and promote genetic development for alkaloid production[13]. AgNPs are getting more reactive when they come in contact with antibiotics. Antimicrobial activity is increase in silver nanoparticles according to their size and shape. Silver nanoparticles are non-toxic, eco-friendly and easily available therefore they are used as medicine[20].

Materials :

Fresh leaves of periwinkle plant species *C. roseus*, silver nitrate ($AgNO_3$), Nutrient agar, SS agar and distilled water.

Microorganisms :

Escherichia coli, Bacillus, and Salmonella species where use to investigate the antimicrobial potential of prepared silver nanoparticles (AgNPs). *Candida* species where use to investigate antifungal potential of the prepared silver nanoparticles (AgNPs) is isolated and identify the laboratory of microbiology department.

Preparation of fine powder of Periwinkle leaves:

Operation healthy *Catharanthus roseus* (periwinkle) leaves were collected from college campus and washed properly. Leaves of periwinkle were air dried completely and ground to produce fine powder.

Preparation of leaves extract :

1gm of leaf powder were taken and heated in a 100ml of distilled water in 250ml flask with maintaining temperature at 60°C. After reaching to 60°C temperature leaf extract was collected in separate screw cap tubes after every 15 minutes of interval and four extract was prepared like 15min, 30min, 45min and 60min respectively. These extracts were then filtered with Whatmann's filter paper No. 1 (pore size 25um). These prepared extracts was allowed to cool and used as a stock for further investigation purpose[16].

Preparation of 1mM Silver nitrate ($AgNO_3$) solution :

An aqueous solution of mM of $AgNO_3$

was prepared by referring method of Sulaiman and co-worker using this still water at room temperature[16]. For preparing 100 ml of 1mM silver nitrate solution 0.169 gm. Of silver nitrate was accurately weighed and added to the 100ml of distilled water, mixed properly to get clear solution of $AgNO_3$.

Synthesis of silver nanoparticles (AgNPs):

From the four extract of periwinkle leaves 1ml of leaves extract were separated in screw cap tubes and labelled as 15min, 13min, 45min and 60min extract then 9ml of $AgNO_3$ aqueous solution were added in it respectively. The tubes were kept in dark for avoid the photo-reactivation and incubated at room temperature. After half an hour colour change was observed. The colour of solution in tubes is changed into pale yellow to dark brown. The results were recorded and the synthesized colored solution was used for next experimental analysis[2].

Characterization of silver nanoparticles :

UV- spectral analysis :

Primary characterization, monitor the stability and synthesis of silver nanoparticles (AgNPs) are performed using UV- 1800 Shimadzu visible spectrophotometer[19]. The solution of silver nanoparticles was taken in the quartz cuvette from each respective screw cap tube and spectra analysis is carried out by scanning the solution in the range of 300 to 700 nm to get lambda max value for confirmation of synthesis of silver nanoparticles and the spectra obtained was studied[7].

Detection of stability of synthesized Silver Nanoparticles (AgNPs) :

Stability of silver nanoparticles was confirmed by using UV- spectral analysis. The UV spectrum of silver nanoparticles solution was taken in 15 minute of time interval 15min, 30min, 45min and 60min respectively in scanning range of 300- 700nm.

Antimicrobial assessment of silver nanoparticles :

The silver nanoparticles produced from using *Catharanthus reosus* (periwinkle) was tested against different active cultures of Gram positive and Gram negative microorganisms. For confirming its presence and antimicrobial activity standard agar disc diffusion method is used. The seeded agar was prepared by using active culture of *Escherichia coli, Bacillus subtilis, Salmonella* species and *Candida albicans* species in 50% nutrient agar[3].

Antibacterial bioassay :

Seeded agar platting was done and allowed to solidify. After solidification sterile filter paper discs dipped in each respective tube and placed on the seeded agar plates of *Escherichia coli, Bacillus subtilis, Salmonella* species[8]. Each concentration of extract with respect to time interval up 1 hour is placed on the field plate of microorganisms that is 15 minute, 30 minute, 45 minutes and 60 minutes, then all the plates was incubated at 37^0C for 24 hour. After incubation zone of inhibition was observed, measured and recorded[17].

Antifungal bioassay :

The seeded agar of fungi *Candida albicans* was poured in a petri plate and allowed to solidify[5]. Then filter paper discs was dipped into respective solution and placed on seeded agar plate and incubated at 37^0C for 24 hour. After incubation zone of inhibition was observed, measured and recorded[9,11].

Synthesis of Silver Nanoparticles (AgNPs):

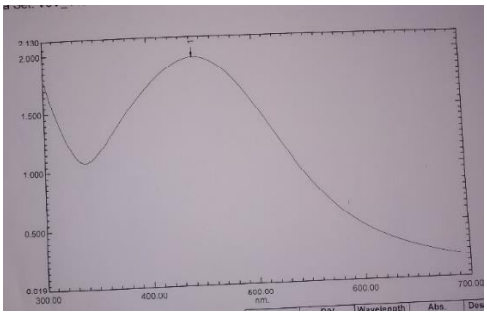
Synthesis of silver colloids from the reduction of aqueous solution of silver nitrate is common and mostly used method. According to the study, the production of AgNPs from *C. roseus* (periwinkle) detected and investigated. The emergence of the pale yellow colour turned into dark reddish colour is suggested the formation of AgNPs in screw cap tube. The Fig. 1 shows the colour change in the screw cap tubes containing 9ml of $AgNO_3$ and 1ml of plant extract before and after heating for 5 minutes. The colour change is compared with each other according to the extraction of plant extract respect to the 15 min of time interval up to one hour. (*i.e* 15 min, 30 min, 45 min and 60 min).

UV-Visible spectral analysis of AgNPs :

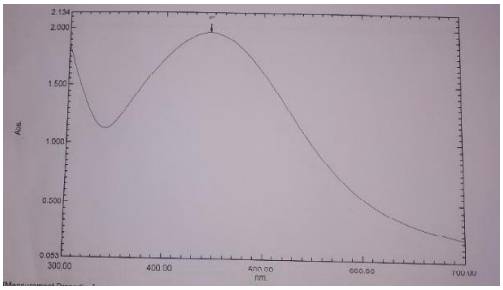
The changes obtained in pale yellow colour of leaves extract into dark reddish brown indicate the formation of silver nanoparticles. This sample shows reduction of silver nitrate and presence of silver ions in spectrophotometric analysis. The reduction of silver ions and absorbance peak was monitored by analyzing the absorbance of solution mixture in a range of wavelength from 300 to 700 nm using spectrophotometer[19]. The incubated four samples *i.e* 15 min, 30 min, 45 min and 60 min of extract showed well defined SPR peak at 438.50 nm, 445 nm, 442 nm and 443.20 nm respectively due to reduction of silver ions and formation of AgNPs. Fig. 2 and Fig. 3 showing the SPR peaks of the AgNPs formation.

(A) **(B)**

Fig. 1. Photograph showing colour changing **(A)** 4 pale yellow colour of aqueous leaf extract of *C. roseus* with control
(B) changing colour from pale yellow to dark reddish brown after adding 9ml of $AgNO_3$ and heating for 5 min.

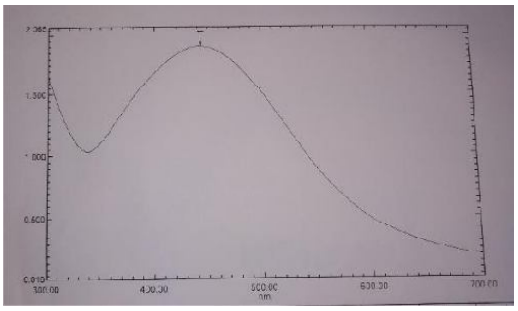


(A)

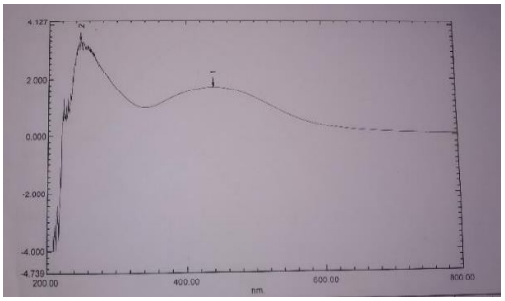


(B)

Fig. 2. (A) Showing UV absorption spectra peak of reduction of silver ions to AgNPs of 15min sample and (B) 30min sample.



(A)



(B)

Fig. 3. (A) showing UV absorption spectra peak of reduction of silver ions to AgNPs of 45min sample and (B) 60min sample.

Table-1. Effect of time interval in sample extraction on stability of AgNPs.

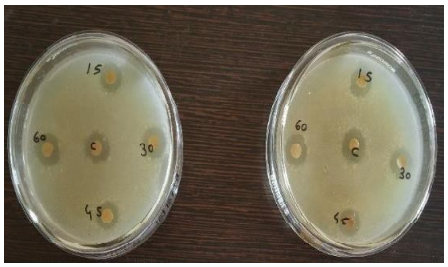
Serial No.	Extracted sample in minutes	Peak (nm)	Optical density
1	15min	438.50	1.708
2	30min	445.00	1.960
3	45min	442.00	1.954
4	60min	443.20	1.895

Antimicrobial assay of AgNPS :

Synthesized silver nanoparticles exhibited antimicrobial activity against *Escherichia coli, Bacillus subtilis, Salmonella* species and *Candida albicans*. As it showed a clear zone of inhibition in Fig. 4. The inhibition is tested according to the time intervals of sample extraction *Escherichia coli* from periwinkle species i.e. *C. roseus*. The interaction of silver nanoparticles with surrounding microorganisms on agar plate resulting into disturbing the permeability of the cell membrane and respiratory function. Due to that growth is inhibited and produce the clear zone of inhibition as showed in a figure.

(A) *Escherichia coli*

(B) *Bacillus subtilis*



(C) *Salmonella* species

(D) *Candida albicans*

Fig. 4. Shows the antimicrobial activity assay of AgNPS against different pathogens by disc diffusion method.
(A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Salmonella* species and (D) *Candida albicans*. The extract was placed according to time and the $AgNO_3$ placed as a control in a centre of the plate.

The illustrated zone of inhibitions in each two plates of pathogens were measured and mentioned as plate A and plate B in Table-2.

Table-2 Represents the zone of inhibition obtained on agar plates in mm.

Serial number	Time in minutes	*Escherichia coli*	*Bacillus subtilis*	*Salmonella* species	*Candida albicans*
1	15min	A 0.3 B 0.2	A 0.3 B 0.2	A 0.3 B 0.5	A 0. 5B 0.6
2	30min	A 0.4 B 0.6	A 0.3 B 0.3	A 0.2 B 0.4	A 0.4 B 0.4
3	45min	A 0.5 B 0.6	A 0.2 B 0.2	A 0.4 B 0.3	A 0.5 B 0.4
4	60min	A 0.6 B 0.5	A 0.3 B 0.2	A 0.4 B 0.4	A 0.6 B 0.5
5	Control $AgNO_3$	A 0.6 B 0.8	A 0.4 B 0.3	A 0.2 B 0.3	A 0.3 B 0.4

Using aqueous extract of periwinkle species *Catharanthus roseus* the silver nanoparticles were synthesized and explored under different conditions. The silver nanoparticles were characterized and analyzed by UV- visible spectroscopy to confirm its presence. The method of green synthesis investigation is less time consuming and alternative to chemical and physical method, as it is low cost, eco-friendly and pollution free.

In present research it is investigated and confirmed that, the aqueous extract of silver nanoparticles played an important role in reducing silver ion and produce the silver nanoparticles. It is also confirmed that this synthesized silver nanoparticles capable to disturbing metabolic activity of the micro-organisms due to that they showed antimicrobial activity against both gram positive and gram negative pathogens.

According to this study further use and application of the silver nanoparticles are to be investigated for future benefits in pharmaceutical and medical areas.

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