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BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES USING *CALLISTEMON VIMINALIS* (BOTTLE BRUSH) BLOOMS CONCENTRATE AND STUDY OF THEIR ANTIBACTERIAL ACTIVITY

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Abstract

In the present study, the silver nanoparticles (AgNPs) were synthesized through green synthesis using blooms concentrate of Callistemon viminalis. The aqueous Ag^+ ions were reduced into AgNPs when the $AgNo_3$ solution was mixed with the Callistemon viminalis blooms concentrate. The biologically synthesized AgNPs were characterized by UV–vis, FT-IR, XRD and SEM analysis. The phytochemical analysis of the plant Callistemon viminalis blooms concentrate reveals the presence of flavonoids, alkaloids, cardiac glycosides and saponins. The synthesized AgNPs have shown good antibacterial activity against Klebsiella aerogenes, Staphylococus aureus, E-coli and Pseudomonas aerogenes.

Keywords: Callistemon viminalis, biological synthesis, AgNPs, antibacterial activity, XRD.

I. Introduction

Nanotechnology is the science and engineering involved in the synthesis, design, characterization and application of materials of nanometric scale. Nanoparticles can be produced using various approaches such as physical, chemical, and biological approaches. The synthesis of nanoparticles by chemical method is quick and more quantity of nanoparticles can be produced. These methods also require capping agents for size stabilization of the nanoparticles, which are toxic and end up with toxic byproducts. Therefore there is a need for ecofriendly and nontoxic methods for the synthesis of nanoparticles. This lead to the development of biological methods of synthesis of nanoparticles. Many biological methods for nanoparticles synthesis have been reported till date using bacteria [1], fungi [2]-[3], and plants concentrate [4-6].

Plants mediated nanoparticles synthesis is of more advantageous as they are free from toxic chemicals and provide natural capping agents. Plant concentrate can also reduce the isolation and culture of microorganism [7].

Silver nanoparticles are widely used because of their unique properties in chemical sensing, bio-sensing, catalytic, photonics, electronics, and pharmaceuticals [8]. These have a good potential as antibacterial activity [9]. Because of the antimicrobial properties the AgNPs find many applications in household products like food storage containers, textiles, home appliances, and medical devices [10]. Silver is an effective antimicrobial agent and exhibits low toxicity [11]. These are used in tropical ointments to prevent infection against open wounds and burn [12]. Silver nanoparticles are reported to possess anti-inflammatory, anti-platelet, anti-angiogenesis, antiviral, and antifungal activity [13].

For the present study the blooms of *Callistemon viminalis* were selected for biological production of AgNPs. *Callistemon* is a genus of shrubs belongs to the family *Myrtaceae*. *Callistemon* species have commonly referred as bottle brushes because of their cylindrical, brush like flowers resembling a traditional bottle brush. This popular evergreen tree has a dense, low-branching, multi trunked, pendulous growth habit and a moderate growth rate (Fig. 1). These blooms appear in great abundance during March to July. The flowers are followed by persistent woody capsules which are not noticed unless closely observed [14].

Fig.1 Callistemon viminalis

II. Experimental

Collection and Preparation of Blooms Concentrate

Callistemon viminalis blooms were collected from the campus garden of Shridevi Institute of Engineering and Technology, Sira Road, Tumakuru, Karnataka, India. The blooms of *Callistemon viminalis* were washed thoroughly with tap water to remove the dust and dirt particles and then washed with double distilled water. 20 g of chopped blooms were added to 100 ml double distilled water and stirred at 60°c for 30 min on heating mantle. After boiling, the mixture was cooled for 15 min and filtered through Whatman filter paper number-1. The collected blooms concentrate (red color) was used as reducing and capping agents in AgNPs biological synthesis.

Phytochemical Analysis

The blooms concentrate of *Callistemon viminalis* were assessed for the qualitative determination of Secondary metabolites constituents i.e. amino acids, terpenoids, flavonoids, alkaloids, phenols, oxalate, anthraquinones, saponins, tannins and cardiac glycosides by applying standard procedures [15].

Synthesis of Silver Nanoparticles using Blooms Concentrate

10 ml of *Callistemon viminalis* blooms concentrate was added to the 90 ml of $AgNO_3$ solution in a conical flask and kept at room temperature and stirred continuously for 15 min using magnetic stirrer. The mixture was allowed for 24 hours for complete biological reduction process.

III. Characterization

UV-Vis Spectroscopy: The sample was analysed by UV-Vis spectrophotometry (model Shimadzu UV) for its maximum absorbance v/s wavelength to confirm the formation of AgNPs.

Fourier Transform Infra-Red Spectroscopy Analysis

The FTIR measurement sample was recorded in the range of 400-4000cm^{-1} using Nicolet Avatar model. It gives information on the rotations and vibrations modes were identified and purposed to determined the distinct functional groups present.

X-Ray Diffraction Analysis

The reduced AgNPs powder was coated on a glass substrate and the X-ray diffraction measurement were carried out by using a powder X-ray (PAN analytical BV model) instrument operating at a voltage of 40kV and current of 30mA. The output was recorded in the form of a graph with 2θ on x-axis and then intensity on y-axis. The crystallite average size of particle was calculated by using the Debye-Scherrer formula.

$D=k\lambda/\beta\cos\theta$, where λ is wavelength, D is particle diameter size, β is the full width half maximum, k is a constant (value 0.9) and θ is Braggs diffraction angle.

Scanning Electron Microscopy Analysis

The Ag nano particle size and its morphological distribution were assessed with Scanning Electron Microscopy.

Antibacterial Activity

The antibacterial activity of AgNPs produced by *Callistemon viminalis* blooms concentrate were evaluated by the disc diffusion method. *Psedomonus aerogenes, Staphylococus aureus, Klebsiella aerogenes* and *E-coli* bacterial strains were collected from department of microbiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumakuru, Karnataka, India. These bacterial strains were developed in nutrient broth (NB) media for 24 h at 37°c and 1 ml of each broth culture was spread over the nutrient agar media. 5 mm sterilized filter paper discs were dipped in biological synthesized AgNps suspension (10µg/ml), double distilled water (negative control), Taxim (1µg/ml) as standard and blooms concentrate was placed over the agar plates and incubated for 24 h at room temperature.

IV. Results and Discussion

Phytochemical Analysis

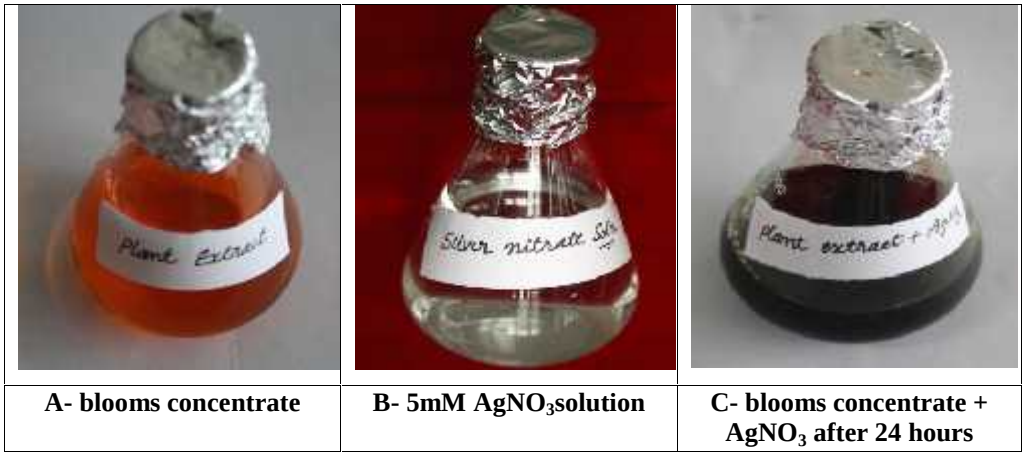
The results of phytochemical analysis of *Callistemon viminalis* are presented in table. 1 and amino acids, terpenoids, flavonoids, alkaloids, phenols and cardiac glycosides are present.

Table 1: Phytochemical analysis (blooms concentrate**)**

S. No	Phytochemicals	*Callistemon viminalis*
1	Flavonoids	+++
2	Alkaloids	++
3	Phenols	+
4	Tannins	—
5	Cardiac glycosides	++
6	Saponins	—
7	Anthraquinones	—
8	Amino acids	+
9	Oxalate	—
10	Terpenoids	++
+: Confirms, —: Absent.		

Synthesis of Silver Nanoparticles using Blooms Concentrate

10 ml of *Callistemon viminalis* blooms concentrate were added to the 90 ml of $AgNO_3$ solution at ambient temperature and stirred continuously for 10 min using magnetic stirrer. After 24 h red color of the mixture was turned into dark brown color which indicates the formation of AgNPs (Fig.2). The AgNPs obtained from the solution was purified by repeated centrifugation at 8,000 rpm for 15 min using Remi Cooling centrifuge C-24. The AgNPs obtained were dried and stored for further analysis.



A- blooms concentrate	**B- 5mM $AgNO_3$solution**	**C- blooms concentrate + $AgNO_3$ after 24 hours**

Fig.2: Formation of AgNPs

UV-Vis-spectroscopy Analysis

UV-vis spectra of AgNPs synthesized by *Callistemon viminalis* blooms concentrate was observed at 433nm which is a broadening peak with an increase in absorbance due to increase in number of AgNPs formed as a result of reduction of Ag^+ ions present in the aqueous $AgNO_3$ solution (Fig. 3).

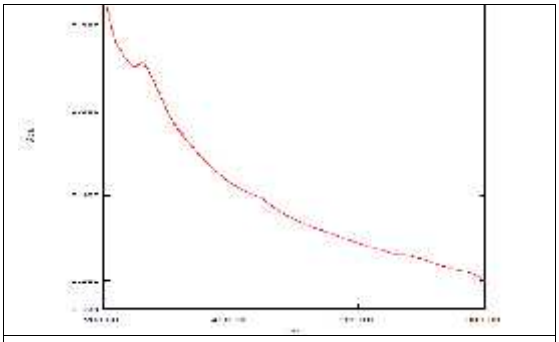


Fig. 3: UV-vis spectrum of AgNPs

FT-IR Analysis

FT-IR spectrum was performed to identified and assigned to determine the different functional groups present in the AgNPs synthesized by *Callistemon viminalis* blooms concentrate (Fig.4). The IR bands were observed at 3309, 2896, 1589, 1380, 1066 and 546cm^{-1}. The strong band which appeared at 3309 cm^{-1} seconadry amine N-H stretch,medium bands which appeared at 2896 cm^{-1} alkane C-H, 1589cm^{-1} Amine N-H, 1380 cm^{-1} alkane C-H, strong band which appeared at 1066 cm^{-1} Primary alcohol C-O and the low band at 546cm^{-1} halo compound C-Br.

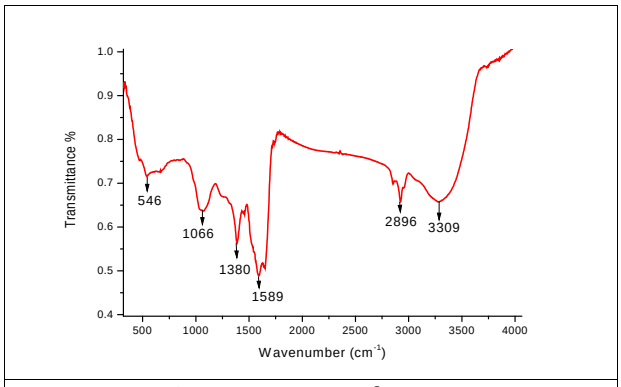


Fig.4: IR spectra of AgNPs

Scanning Electron Microscopy Analysis

The formation of AgNPs in the SEM image (Fig. 5) has shown separate AgNPs as well as particle agglomeration. This indicates, the particle size is irregular and shape of the particles has spherical in morphology with an average size of 43.9 nm ranging from 32 to 56 nm.

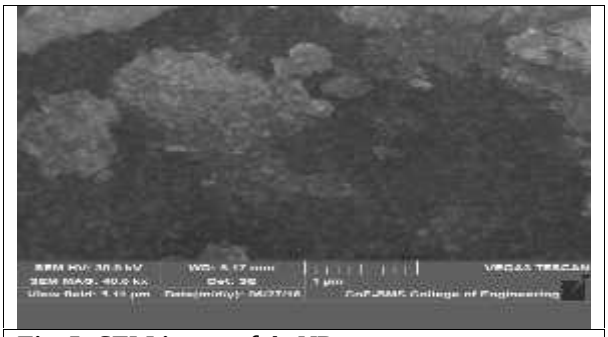


Fig. 5: SEM image of AgNPs.

X-ray Diffraction

X-ray diffraction pattern (XRD) was recorded for the synthesized AgNPs (Fig. 6). The diffraction peaks at 2θ = 38.26°, 44.29°, 64.35° and 77.23° were indexed with the planes (111), (200), (220) and (311) for the fcc lattice of obtained silver as per the JCPDS card No. 04-783 was matched with database. The average size (D) of synthesized AgNPs was found to be 43.76 nm as calculated by using Debye-Scherer formula.

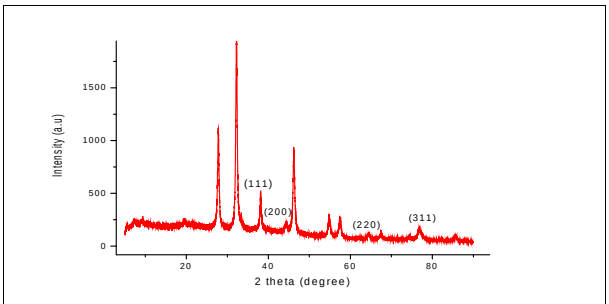


Fig. 6: XRD pattern of AgNPs

Antibacterial Assay

The biologically synthesized AgNPs by the blooms concentrate of *Callistemon viminalis* have shown significant antibacterial activity against *Staphylococus aureus* followed by *E-coli, Psedomonus aerogenes* and *Klebsiella aerogenes* (Fig.7; Table 2).

E-coli	Pseudomonas aeruginosa	Klebsiella aerogenes	Staphylococus aureus

Fig. 7: Antibacterial activity of AgNPs

Table 2: Antibacterial zone formation

Zone of Inhibition (mm)					
S. No	**Strains**	**Control**	**Standard**	**AgNPs**	**Blooms concentrate**
1	E-coli	_	9	13	_
2	Pseudomonasaerogenes	_	6	8	_
3	Klebsiellaaerogenes	_	8	12	_
4	Staphylococus aureus	_	8	15	_

1. Control - sterile distilled water, 2.Standard -Taxim, 3.AgNPs - Silver Nanoparticles, 4.blooms concentrate of *Callistemon viminalis*.

V. Conclusion

Biological synthesis of AgNPs is achieved by using a simple eco-friendly approach from *Callistemon viminalis* blooms concentrate at room temperature. The phytochemical screening of *Callistemon viminalis* blooms concentrate has shown the presence of amino acids, terpenoids, flavonoids, alkaloids, phenols and cardiac glycosides. The formation of AgNPs was identified by the change of color of *Callistemon viminalis* blooms concentrate and the synthesized AgNPs were characterized by UV-Visible spectroscopy, XRD, FT-IR and SEM, which confirms the formation of AgNPs. The synthesized AgNPs which shown significant anti-bacterial activity against four tested bacterial strains.

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Conflict of Interest

Conflict of interest declared none.

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