Antimicrobial and Catalytic activity of biosynthesized Silver Nanoparticles

A thesis submitted in partial fulfilment of the requirements for the award of the degree of

Master of Science

In

Physics

By

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May 2022

DEPARTMENT OF APPLIED PHYSICS

DELHI TECHNOLOGICAL UNIVERSITY

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CANDIDATE'S DECLARATION

I, Anne Masih (2K20/MSCPHY/05) student of M.Sc. Physics, hereby declare that the project Dissertation titled "**Antimicrobial and Catalytic activity of biosynthesized Silver Nanoparticles**" which is submitted by me to the Department of Applied Physics, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition

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Title of the paper: Catalytic activity of silver nanoparticles synthesized using Crinum asiaticum (Sudarshan) leaf extract

Name of Journal: Materials Today: Proceedings

Author names: Samiksha Shukla, Anne Masih, Aayan, Mohan. S. Mehata

Name of Conference: International conference on design and materials

Date of paper acceptance: December 2021

Date of paper publication**:** April, 2022

Anne Masih (2K20/MSCPHY/05)

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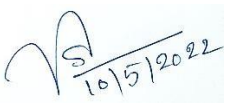
To the best of my knowledge, the above work has not been submitted in part or full for any Degree or Diploma to this university or elsewhere. I, further certify that publication and indexing information given by the students is correct.



Place: Delhi

Date: 10.05.2022

Dr. Mohan. Singh Mehata

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CERTIFICATE

This is to certify that the dissertation titled as "**Antimicrobial and Catalytic activity of biosynthesized Silver Nanoparticles**" submitted to Delhi Technological University ((Formerly Delhi College of Engineering) by **Ms. Anne Masih (2K20/MSCPHY/05)** in the partial fulfilment of the requirements for the award of degree of **Masters of Science in Physics**(Department of Applied Physics, Delhi Technological University) is a bona fide record of candidates' own work carried out under the supervision of **Dr. Mohan. Singh Mehata**. It is further certified that no part of the thesis has been submitted to any university/institute for theaward of any other degree/diploma.

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Signature of Candidate

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ABSTRACT:

A major issue for healthcare medicine in this modern era is the development of a safe, reliable and non-toxic treatment for microbial related diseases. Synthesis methods using biological agents have been gaining attention due to their non-toxic use of precursors. This study focusses on presenting a green route that results in the production of silver nanoparticles (Ag-NPs) using of *Crinum Asiaticum* and *Sphagneticola trilobata* plants. The catalytic activity was investigated by degrading a powerful industrial dye. The degradation of dye will be carried out using biosynthesized Ag-NPs. For investigating the anti-microbial properties of our biosynthesized Ag-NPs, antifungal assay was carried out using Candida isolates which causes life-threatening fungal infections and finding their respective zone of inhibition treated with Ag-NPs, $AgNO_3$ and using fluconazole as a control.

Acknowledgement

I would like to express my indebtedness and deep sense of gratitude to my project supervisor Dr. Mohan. S. Mehata, Assistant Professor, laser-spectroscopy lab, DTU, New Delhi for his valuable discussions, guidance, encouragement and support. My supervisor has been a great inspiration, whose expertise was invaluable in formulating the research and methodology. His work ethics would continue to inspire me in all my future academics and research career.

I would like to extend my thanks to the PhD scholars in the laser-spectroscopy lab, DTU, Mr. Aaryan for his valuable guidance and technical support in UV/Vis, throughout my studies and providing me with tools needed to choose the right direction in completion of my dissertation. I would also like to thank Miss. Aneesha, Miss Namrata and Mr. Mritunjoy for their constant support and guidance.

I would also like to thank my lab partner Miss. Samiksha Shukla for her corporation throughout especially in Chapter 3 and my lovely classmates Ankita, Vidhi and Vaibhav for constantly creating a motivating and enjoyable environment.

A special thanks to my family for their encouragement and great love during my project work.

I would also like to thank Lord God Almighty, for his unfailing love, unmerited favours and blessings that he has showered upon my life.

Anne Masih

2K20/MSCPHY/05

Table of contents:

List of figures:

List of tables

CHAPTER 1:

INTRODUCTION

1.1 Background:

There has been an increasing attention towards the use of Nobel metal nanoparticles and their spectrum range of versatile applications in the direction of medical as well as industrial applications. Their uniquely defined size (1-100 nm) and appreciable surface to volume ratio attributes to various features like surface plasmonic nature, high electrical and thermal conductivity and their photocatalytic behaviour. Silver-nanoparticles were also used to observe strong antimicrobial effect against various bacterial, yeast as well as fungal species.

The green method of synthesis of silver-nanoparticles is emerging rapidly because of their low toxicity levels, low cost of production and its biocompatible nature. The phytochemicals like flavonoids that are components of plant are accountable for the fabrication of silver-nanoparticles of various shape and sizes. [1]

Researchers has been constantly trying to tackle the problem of reducing the effects of harmful dyes that enter water bodies through industrial waste and then come in direct contact of human beings. Such harmful dyes are also a threat to the aquatic life which in turn directly affect human kind.

The ability of small-sized silver-nanoparticles and the phytochemicals that are present in the plant to penetrate the cell membrane and change the basic build of the plant cell makes them responsible for the antimicrobial studies.[2]. Therefore, by opting for the biofriendly green route of synthesizing silver-nanoparticles we can aim to explore a spectrum of uses.

1.2 : Silver-Nanoparticles:

Silver, one of the basic elements in nature and it exists as a native element and in the form of minerals. Silver may exist in one of the four different oxidation state that are Ag^0, Ag^{1+}, Ag^{2+} and Ag^{3+}. Silver has thermal and electric properties due to its appreciable conduction of heat and electricity.

Over a long period of time, silver is known for its medicinal properties and is widely used in surgical prosthesis and splints, curing wounds, fungicides and on burns. Silver is also used in the treatment of various illness like, gonorrhoea, epilepsy and gastroenteritis.

1.3 : Physical parameters

Silver (Ag) is a reflective, soft, white, lustrous transition metal with high electrical conductivity, thermal conductivity and reflectivity.

Table 1:1 Physical parameters

Parameter	Value
Atomic Number	47
Atomic Mass	107.87 a.m.u
Electronic Configuration	[Kr] $4d^{10}$ $5s^{1}$
Melting Point	1235 K
Density	10.5g/cm^3

1.4 : Electronic band structure and SPR

The size confinements effects greatly influence the conduction of electrons, their excitation. Surface plasmon resonance (SPR) may be defined as collective oscillations of electrons which determine the optical properties of metallic nanoparticles. The broadening of SPR band which occurs due to the confinement effects, gives information about physical parameters given by Mei Theory. The size of nanoparticles decreases with increasing width of SPR band. Hence, the broadening of SPR happens due to two factors, radiation damping of electrons as well as the scattering of electron at the nanoparticle's surface. The shift of SPR band is also explained as well as predicted by Mei Theory. [3]

The number of plasmonic peaks is correlated to the polarization of nanostructures. Therefore, more the symmetric nature of nanoparticles, lesser will be the plasmonic peaks. A spherical nanostructure will have only one peak it can be polarized in only one possible way because of its symmetrical nature. Whereas nanoparticles with cubic nature can be polarized in two ways (dipole and quadrupole modes) and hence would have two peaks.

The symmetry of nanoparticles not only effect the number of plasmonic peaks but as well the intensity of extinction spectra. If mirror symmetry is one of the directions of polarization, the overall cumulation of electrons will cause number of plasmonic peak to increase which in turn increases the extinction spectra intensity.

The wavelength of plasmonic peak depends on the size of nanoparticles because, suppose the surface particles are very far from their respective equilibrium position and hence the restoring force exerted by the ions reduce. [4]

Manikandan and Hoonacker have derived the following relation which gives the dependence of diameter of metallic nanostructures to their λ_{SPR}

$$R = \frac{v_F \lambda_{SPR}^2}{2 \pi c \Delta\lambda} \beta$$

Where: R is the radius of the nanoparticle, $\Delta\lambda$ is the full width at half maxima of UV-Visible extinction spectra, c is velocity of light in vacuum, β is the constant of proportionality and v_F is the fermi velocity of the electron gas. [5]

1.5 Applications:

Due to distinctive nature of silver nano-particles, they are exploited in various applications listed below:

1.5.1 Dye degradation: Industrial waste like pollutants and harmful dyes are released into water bodies which is harmful for the aquatic as well as human health. Degrading harmful dyes is one of the major problems in treating water for usage. Photodegradation from metal nanoparticles is one of the most promising alternatives for treating harmful dyes as they are environment friendly as well as pocket friendly.

Methylene blue is used as a straining agent in the field of medicine as well as used in the industrial field.

1.5.2 Anti-Platelet: Platelets are discoidal shaped nuclear cells. These are formed from the stem cells in bone marrow. Platelets causes the stopping of blood from abrasion as well as coagulation factors. Silver nanoparticles have been found to diminish the mitochondrial activity in the profound murine cells.[6]

1.5.3 Anti-thrombotic: The layer-on-layer fabrication method is exploited to cause the immobilization of biomolecules onto different substrate.

1.5.4 Anti-microbial: The usage of metal nanoparticles is a promising agent for resisting microbes because of the large surface which gives them enough range of invasion. [7]

CHAPTER 2:

Experimental techniques and characterization

2.1 Experimental techniques to synthesize nanoparticles

2.1.1 Top-down approach:

This method uses macroscopic structures in the initial process. These are controlled during the process of fabrication. Top-down approach is mainly depended on grinding of materials. Commonly used top-down approaches are pulsed laser ablation, spray pyrolysis, lithography etc. Thus, these processes are subtractive in nature. [8]

2.1.2 Bottom-up approach:

Bottom-down synthesis of nanomaterials involves miniaturization of different structures to nano scale, resulting in the origination of nanostructures. Common bottom-down approaches include green methods, pyrolysis, solvothermal, coprecipitation etc. Bottom-up approach is based on the principle of molecular recognition or self-assembly. [8]

2.2 Characterization Techniques

To investigate structure, surface morphology, topography, chemical composition, absorbance, emission of synthesized nanomaterials, the following techniques were used:

2.2.1 Structural and Morphological Characterization

2.2.1.1 X-Ray Diffraction (XRD)

Crystal mounted on a sample holder is rotated while being exposed to X-Rays produces a diffraction pattern. The atomic planes present in the atoms causes the incident X-Ray to interfere with each other. [9]

2.2.1.2 Tunnelling Electron Microscope (TEM)

An extremely thin sample is prepared from which electron beam is passed through contributing its interaction with the sample as a result of which, image is produced. This image is further magnified and focussed. [10]

Fig 2.1: A transmission electron microscope

2.2.1.3 Zeta – DLS

Dynamic light scattering DLS works on the principle of scattering of incident light when it strikes nanoparticles. DLS uses Mie theory to estimate the particle size. This technique is also used to find electrostatic and dynamic behaviour of charged particles in a solution, one such property is Zeta potential.[11]

2.2.2 Optical Characterization

The Lambda 750/Vis/NIR is a double beam spectrophotometer with an accuracy of 6 absorbance unit. It utilises tungsten halogen and twin deuterium source lamps for low stray-light performance and for stability.

Fig 2.2: UV-Vis spectrophotometer at DTU

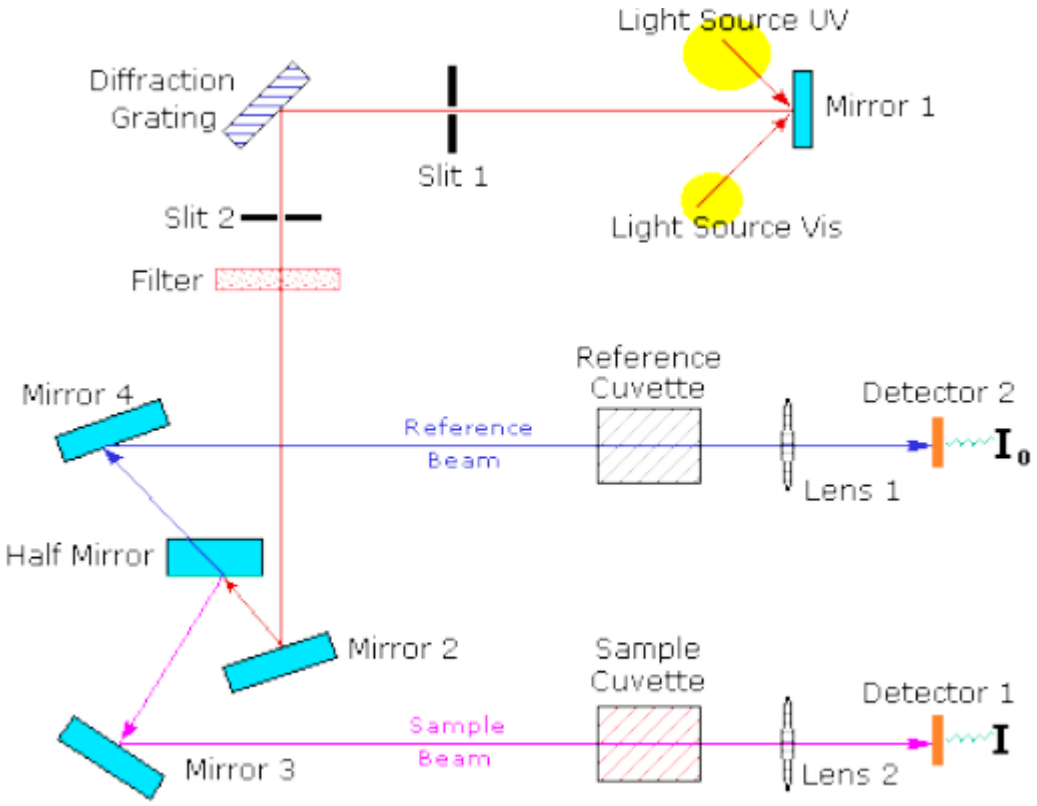


Fig 2.3: Measurement of absorbed radiation in UV-spectrophotometer[12]

CHAPTER 3

Silver nanoparticles and their catalytic study

Abstract:

Usage of biological agents in the production of nanoparticles is gaining lot of attention in the modern era. The less toxic and environment friendly synthesis is a very promising method of synthesis and can be used in various applications. This study focusses on presenting a green route for production of silver nanoparticles (Ag-NPs) using the green leaves extract of *Crinum Asiaticum*. The presence of Ag-NPs can be stated by observing the characteristic absorption peak, which comes out to be around 450 nm exhibiting the surface plasmon resonance. The pH, reaction temperature and plant extract concentration are varied and their effect on the absorbance spectra is observed. Characterization of samples is done using the UV-Vis absorbance spectra, XRD Analysis for crystal structure, Zeta Potential Analysis for stability, DLS for size determination and TEM for morphology. The average particle sizes come out to be 11 nm (from TEM), 14 nm (from XRD) and 54 nm (from DLS). The catalytic activity was investigated by degrading a powerful industrial dye. The degradation of dye will be carried out using biosynthesized Ag-NPs.

3.1 Introduction:

In green synthesis, green agents (plants or microorganisms) are used for capping and reduction of silver [13], [14]. The biomolecules like flavonoids and terpenoids acts as a reducing agent [15] which are accountable for production of Ag-NPs. In case of microorganism mediated green synthesis [16], the antioxidant and reducing properties of microbes govern the reduction process but it requires the need of maintaining elaborate microbe cell cultures and aseptic environments. Hence, green synthesis of Ag-NPs from organic extracts is an efficient bio-friendly one-step alternative to complex physical and chemical routes which often leaves harmful residues. The samples generated from green methods are non-toxic and economical

and hence, can be used in various biological applications. Due to the lack of enough efficient methods for treatment of pollutants like synthetic dyes from industrial wastes, they are often released into water bodies. Thus, high absorbance and catalytic effects on dyes make Ag-NPs desirable to be used as an inexpensive eco-friendly method of degradation of synthetic dyes [17], [18]. Biosynthesized Ag-NPs contain secondary metabolites [19] that are safer alternatives to chemical degrading agents.

3.2 Experimental procedure: The green leaves of C. Asiaticum plant were acquired and cleaned thoroughly, and then oven dried. The dried leaves were crushed into a fine powder. 2g of this powder was added to 30mL water and then boiled and filtered (Fig. 3.1). 1mL of this plant extract was added to 10 mL of $AgNO_3$ (Fig.3.2).

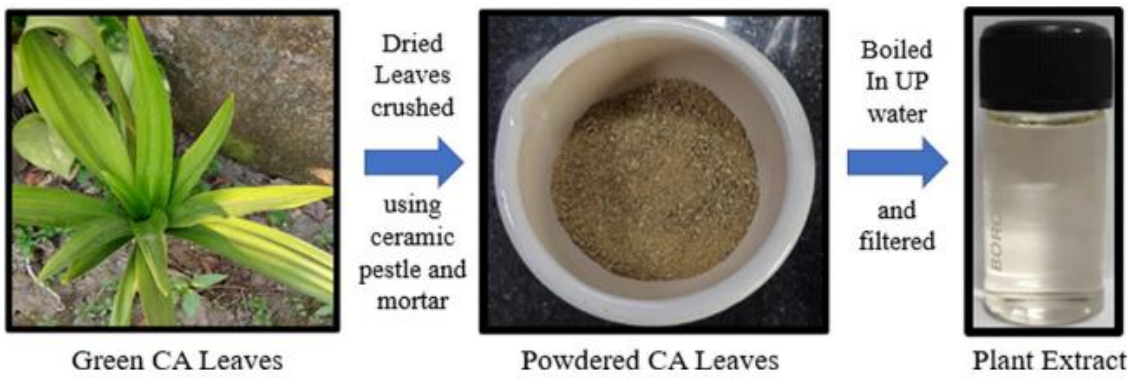


Fig 3.1: Pictorial procedure for producing plant extract

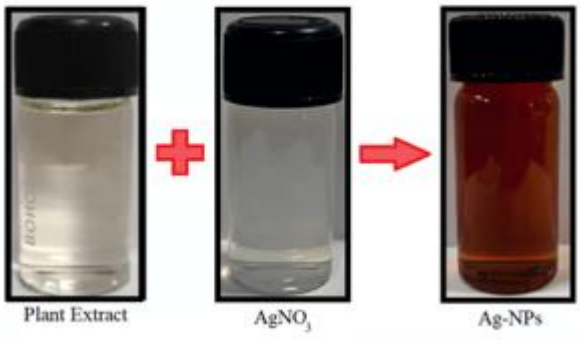


Fig 3.2: Plant extract and $AgNO_3$ when treated together

3.3 Characterization

The spectroscopic characterization was acquired by using a Perkin Elmer LAMBDA 750 UV/Vis/NIR spectrophotometer. The presence of Ag was studied using the XRD patterns obtained by a D-8 Advanced X-Ray Diffractometer by Bruker.(Malvern) Zetasizer Nano-Series ZS analyzer was used for zeta potential analysis. The Morgagni 268D was used to obtain TEM images.

3.4 Results and Discussion

The characteristic surface plasmon resonance (SPR) band [20] of Ag-NPs came out to be in the range of 420-470 nm in the UV-Vis spectra (shown in fig.3.3) and its sensitivity to variations in pH values, plant extract concentration, and some other reaction parameters.

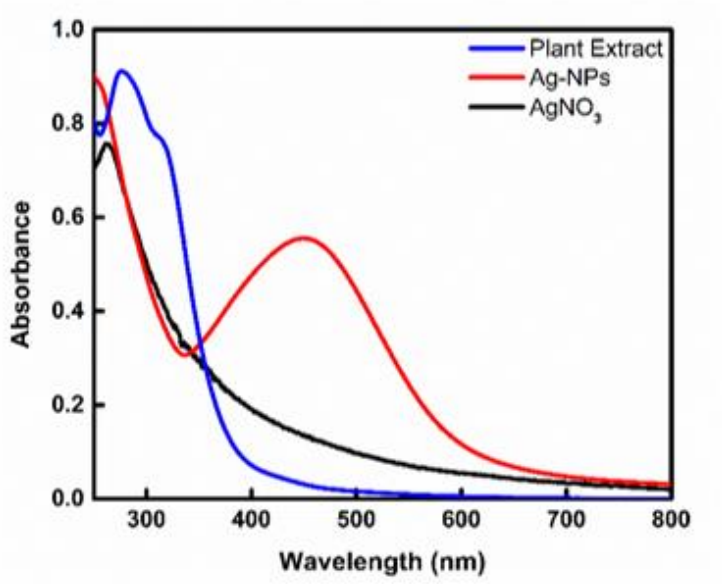


Fig 3.3: The absorption spectra of C. Asiaticum plant extract, $AgNO_3$ and Ag-NPs.

3.4.1 Effect of different concentration of plant extract and reaction time

Three different solutions were prepared with varying ratios of the amount of plant extract to the amount of $AgNO_3$ solution (1:20, 2:20, 3:20). With increasing amount of plant extract, absorption peak intensity of synthesized Ag-NPs sample increases (fig 3.4 (a)).

As soon as we add plant extract to our precursor $AgNO_3$, it changes its colour to slightly yellow from colourless and then it turns completely brown in about 60 minutes (fig 3.5). As time of reaction proceeds, the absorption peak intensity rises (fig. 3.4 (b)).

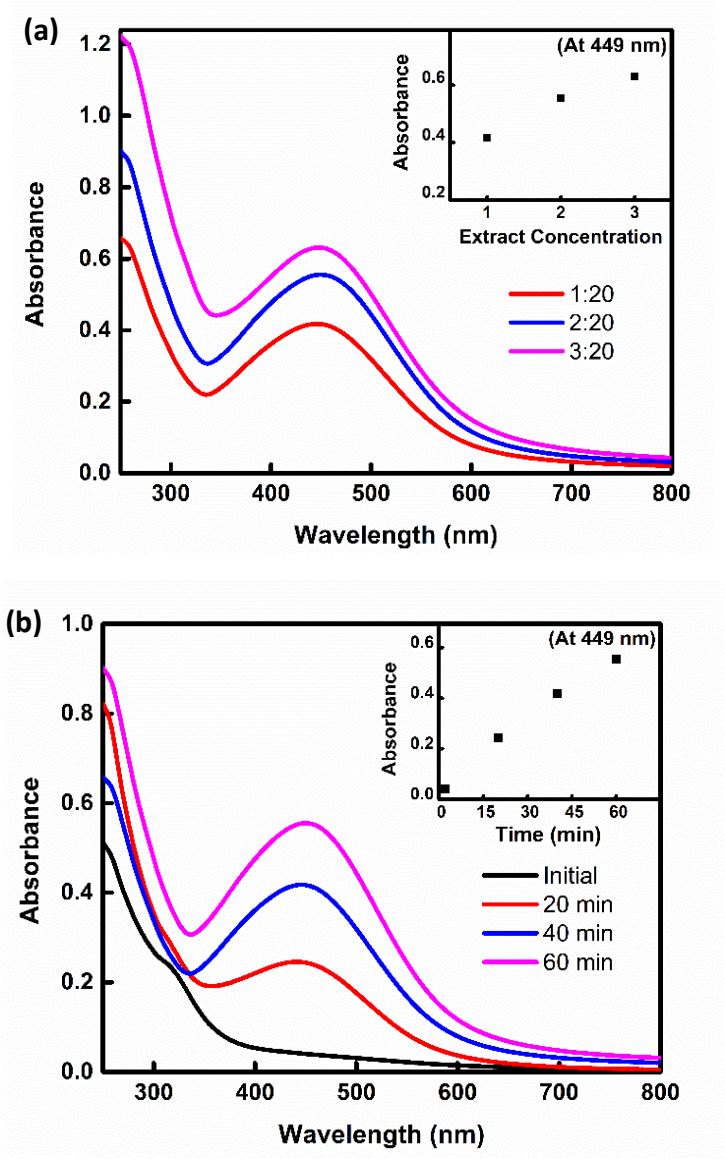


Fig 3.4: The absorption spectra of the synthesized Ag-NPs at (a)different concentrations of plant extract and (b) various intervals of reaction time.

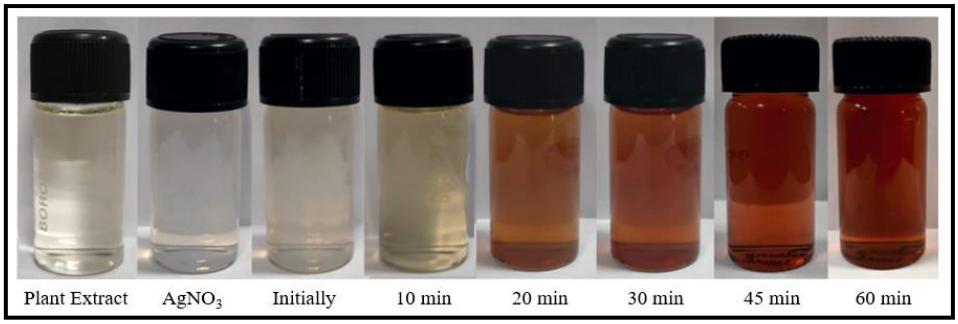


Fig.3.5: Formation of Ag-NPs with time.

3.4.3 Variation of pH and reaction temperature:

The UV-Vis absorption of biosynthesized Ag-NPs using C. Asiaticum leaf extract at various pH values is shown in fig. 3.6 (a). At higher pH values the absorption peak deviates slightly with almost no shift in the wavelength or the intensity of the absorption. This indicates that there is no alteration in the polydispersity [21] index when pH is varied. Fig. 3.6 (b) shows a gradual broadening in the absorption peak as we raise the temperature of Ag-NPs solution. Due to the localization of SPR, [1,2] the phonon-electron scattering rate increases with rising temperature which causes the broadening of the absorption spectra peak [23].

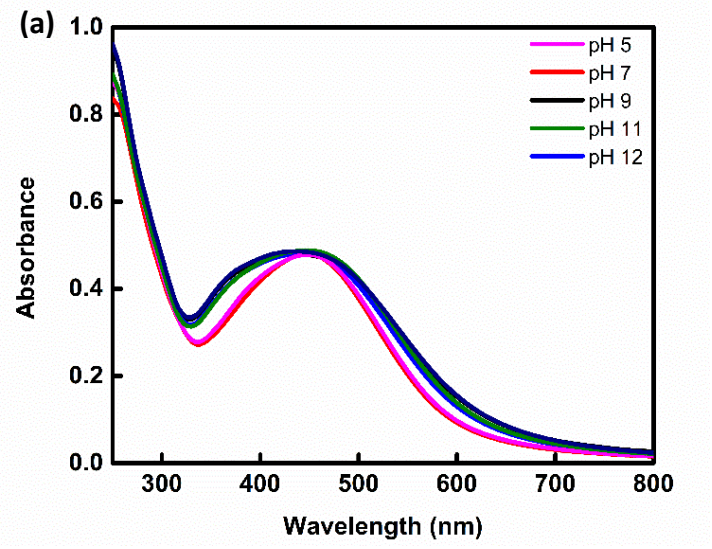


Fig. 3.6 (a): The absorption spectra at different values of pH.

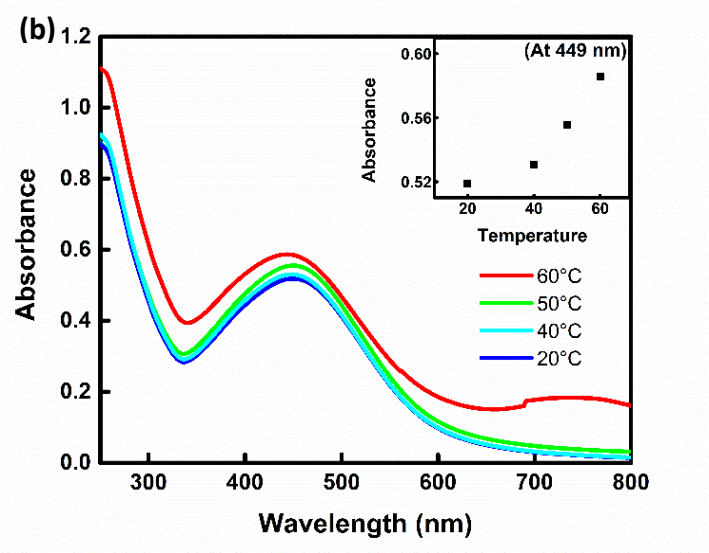


Fig. 3.6 (b): The absorption spectra at various rection temperature.

3.4.4 XRD Pattern

From the XRD pattern (obtained by drop coating method) we can observe that the four key Bragg's reflection intensity peaks occur at 38.24°, 46.32°, 64.72° and 76.96°. (fig. 3.7), indicating that our sample has an FCC (face-centred cubic) lattice [21] structure. Therefore, the obtained XRD pattern verifies the presence of silver in the sample. Debye-Scherrer relation [24] is used to find the average crystalline size of the sample, coming out to be 14nm.

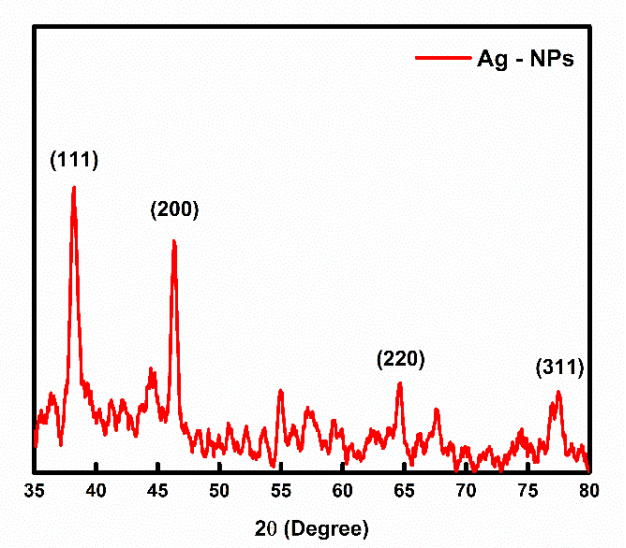


Fig. 3.7: The XRD pattern of biosynthesized Ag-NPs.

3.4.5 Zeta Potential

Zeta potential of the colloidal Ag-NPs was explored using DLS as shown in fig. 3.8. The zeta potential came out to be – 23 mV. Higher value of zeta potential indicates a powerful electrostatic repulsion between suspended negatively charged nanoparticles. These negative charges account for the prevention of agglomeration which controls shape and size of the suspended nanoparticles [25].

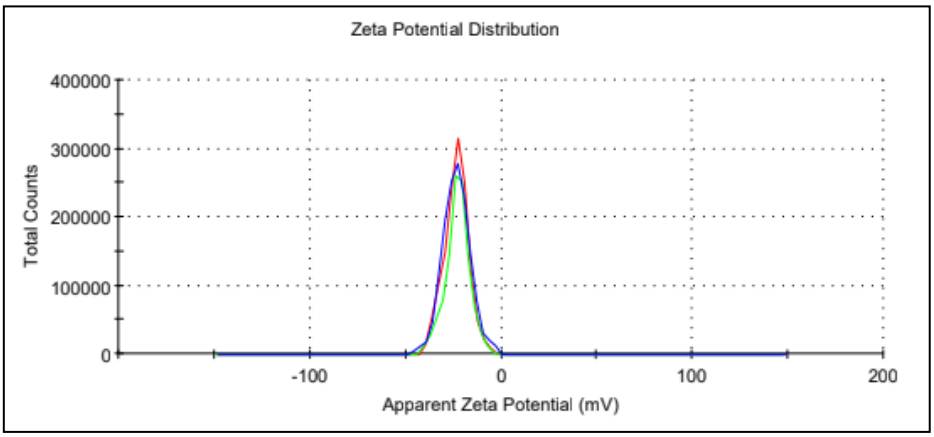


Fig. 3.8: Zeta potential of Ag-NPs.

3.4.6 Morphology

TEM images were used for morphology. A copper mesh grid was taken and a drop of Ag-NPs was coated which was then used for TEM. The uniform distribution of particles in TEM (fig. 3.9 (a)) indicates that formed Ag-NPs are stable. The particle size distribution histogram in fig 3.9 (b) gives an average size of 11nm.

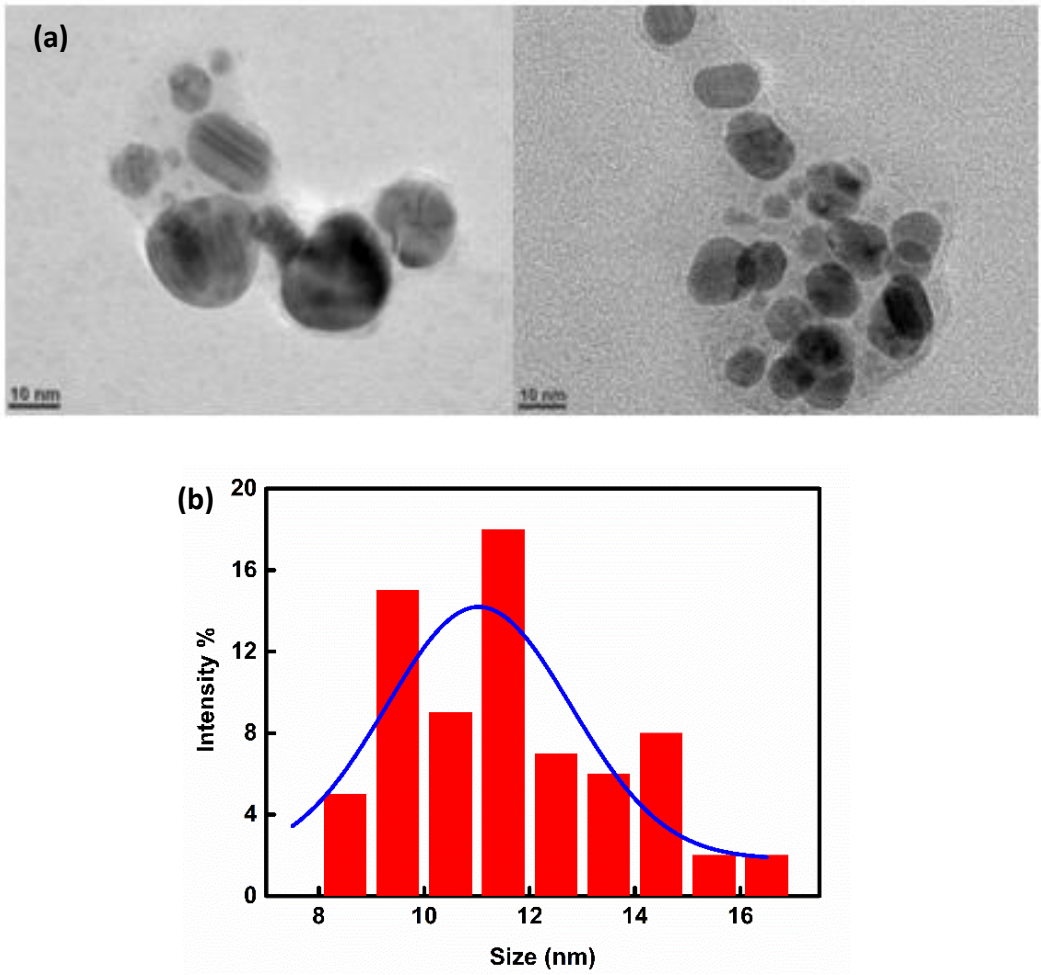


Fig. 3.9: (a)TEM images and (b) Histogram of Particle size distribution of biosynthesized Ag-NPs from plant extract of *C. asiaticum.*

3.5 Methylene Blue Dye degradation

The catalytic activity of biosynthesized Ag-NPs was explored using Methylene Blue dye by degrading 10 µM solution of M. blue dye by Ag-NPs, $NaBH_4$ and Ag-NPs + $NaBH_4$.

Firstly, 100 mL of the dye divided into 3 equal sections, the first section of dye was degraded using biosynthesized Ag-NPs, the other section using only $NaBH_4$ and the last section using both $NaBH_4$ and Ag-NPs together. We will firstly study the effects dye treated with of $NaBH_4$ and Ag-NPs.

The absorption curves were taken and the maxima is observed around 664 nm for which n-π* transitions [26] are accountable. After 20 minutes, decrease of absorption peaks were observed with increasing time. Colour of the dye was changed from deep blue to pale brown was observed in about 20 minutes, indicating a degradation of the dye as shown in fig 3.10. A similar behaviour is observed for just $NaBH_4$ and just Ag-NPs. We observed a much slower degradation process when dye was treated with only $NaBH_4$ and Ag-NPs individually, rather than together. Ag-NPs acts as a catalyst by triggering a reduction reaction and $NaBH_4$ acts a reducing agent [27].

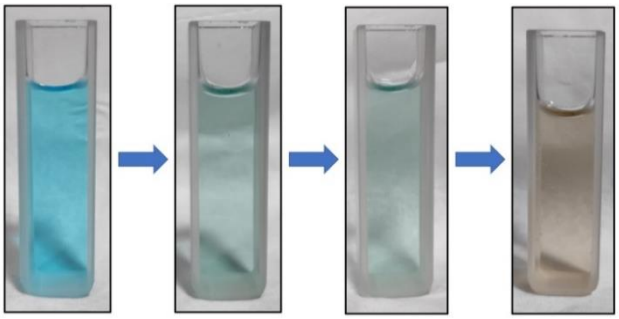
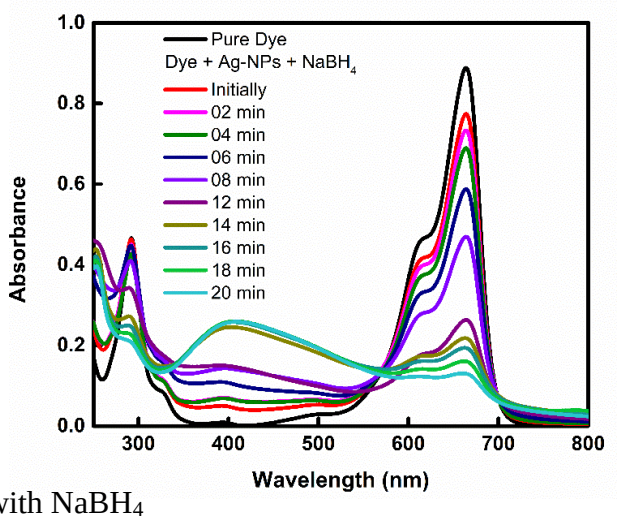
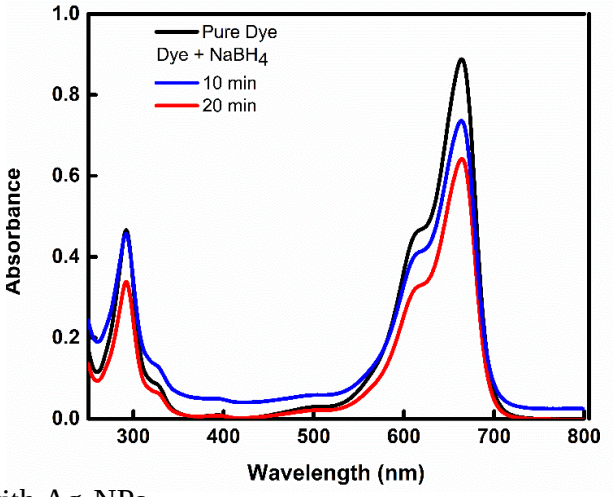


Fig.3.10: Dye treated with both $NaBH_4$ and Ag-NPs.

(a) degradation with $NaBH_4$ and Ag-NPs



(b) degradation with $NaBH_4$



(c) degradation with Ag-NPs

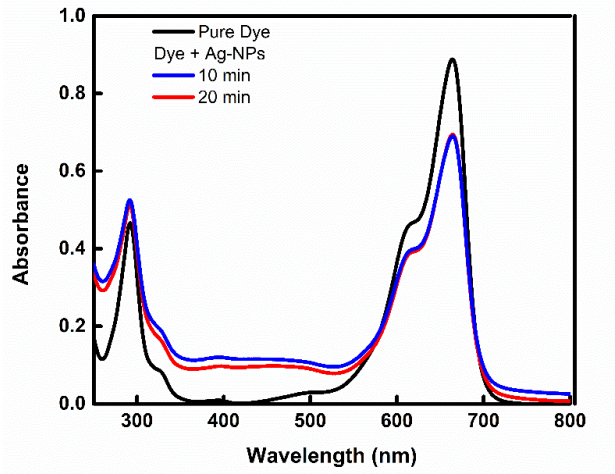


Fig. 3.11: Absorption spectra of M. Blue dye degradation treatment.

CHAPTER 4

Silver nanoparticles and their antimicrobial study

Abstract:

Evolution of effective and safe therapeutic treatment of fungal infections is a huge challenge for medicine. Colonization of fungal infections has been a major contribution to the rising mortality rate among the low immunised group of people. The study focusses on presenting a green route for the production of silver nanoparticles (Ag-NPs) using the extract of green leaves of *Sphagneticola trilobata*. The existence of Ag-NPs in our samples was given by observing the absorption spectra, the characteristic absorbance peak of Ag-NPs comes out to be around 450 nm exhibiting the surface plasmon resonance. The average particle sizes come out to be 12 nm (from TEM). For investigating the anti-microbial properties of our biosynthesized Ag-NPs, antifungal assay was carried out using different Candida strains and finding their respective zone of inhibition treated with Ag-NPs, $AgNO_3$ and fluconazole.

4.1 Introduction:

Diseases caused by fungal infections have accorded abundantly to the rising morbidity and mortality rates within patients with low immunity. These types of fungal infections can spread while implementing hospital related devices such as catheters, contributing significantly to the colonization of fungal invasion by Candida species. [28] Candida is a type of yeast infection, which commensals in a person. If a low immunity system pertains, Candida species invade the human body and causes various infectious diseases. [29]

Usually, the Candida species is related to the medical care implementation, where the it enters the bloodstream and produces more persistent systemic infections. Candida related infections are very common invasive infection related with the hospital atmosphere.

Candida species are Amphotericin, Caspofungin, Fluconazole, Micafungin and Voriconazole resistant and many multidrug companies use these elements in their anti-fungal medicines. The resistant components are alarming, because these are usually used against invasive mycosis, less susceptible. These antifungals have been described as a result of membrane permeability reduction [30], [31]. Therefore, we can adopt novel alternatives to control these infections such as biosynthesized Ag-NPs which exhibit low toxicity and low sensitivity as compared to conventional antifungals.

4.2 Experimental procedure:

The green leaves of S.T plant were taken and cleaned, and then dried in the oven at 90 C. The dried leaves were crushed into a fine powder. 2g of this powder was added to 30mL water and then boiled and filtered (Fig. 4.1). 1mL of this solution was added to 10 mL of $AgNO_3$ (Fig.4.2).

Fig 4.1: Schematic procedure for producing plant extract.

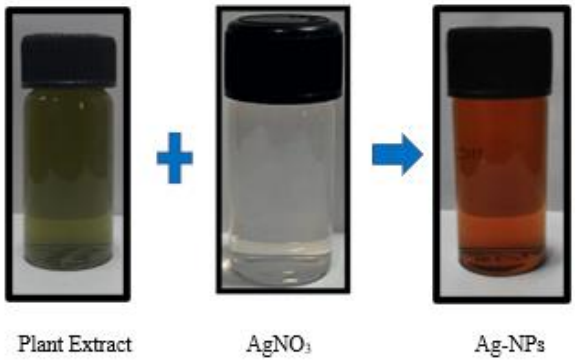


Fig. 4.2: Plant extract and $AgNO_3$ when treated together.

4.3 Characterization

The spectroscopic characterization was done using a Perkin Elmer LAMBDA 750 UV/Vis/NIR spectrophotometer. Zetasizer Nano-Series ZS analyzer was used for zeta potential analysis. The Morgagni 268D was used to obtain TEM images.

4.4 Results and Discussion

The characteristic SPR band [20] of Ag-NPs came out to be in wavelength band of 420-470 nm, as observed from the UV-visible absorption spectra (fig.4.3.)

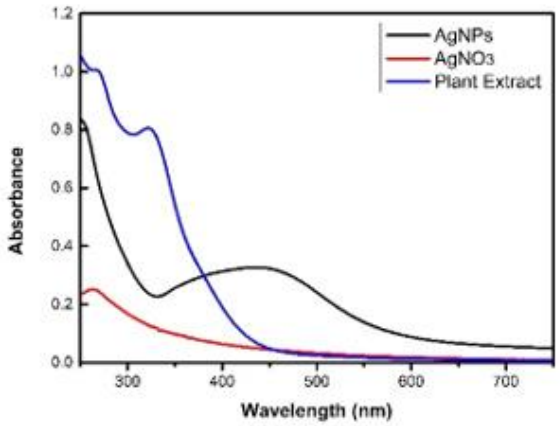


Fig.4.3: The absorption spectra of S.T plant extract, $AgNO_3$ and Ag-NPs.

4.4.1 Zeta Potential

Stability analysis of biosynthesized Ag-NPs was done by DLS, shown in fig 4.4. The zeta potential value came out to be – 21.7 mV. The value that is considered most stable for zeta potential is about of ± 30 mV [32] for nanosuspensions. Higher value of zeta potential indicates

a powerful electrostatic repulsion between suspended negatively charged nanoparticles. These negative charges account for the prevention of agglomeration which controls shape and size of the suspended nanoparticles [25]. Large negative value of zeta potential also infers that the stabilizing catalyst is anionic.

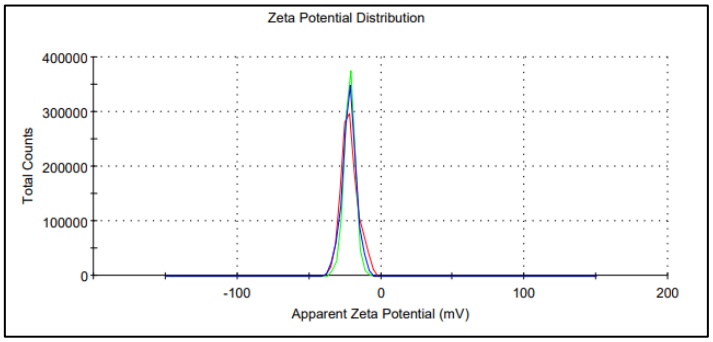


Fig. 4.4: Zeta potential of Ag-NPs from S.T leaves.

4.4.2 Morphology

TEM images were obtained for morphology. The Ag-NPs sample solution was sonicated for about 15 min before coating it on copper mesh grid, used for obtaining TEM images Uniform distribution of particles, as shown in fig. 4.4 gives information regarding stability (a). Particle size distribution curve was plotted from the TEM images, given by fig. 4.4 (b). The average particle size calculated from this size distribution curve comes out to be 12 nm.

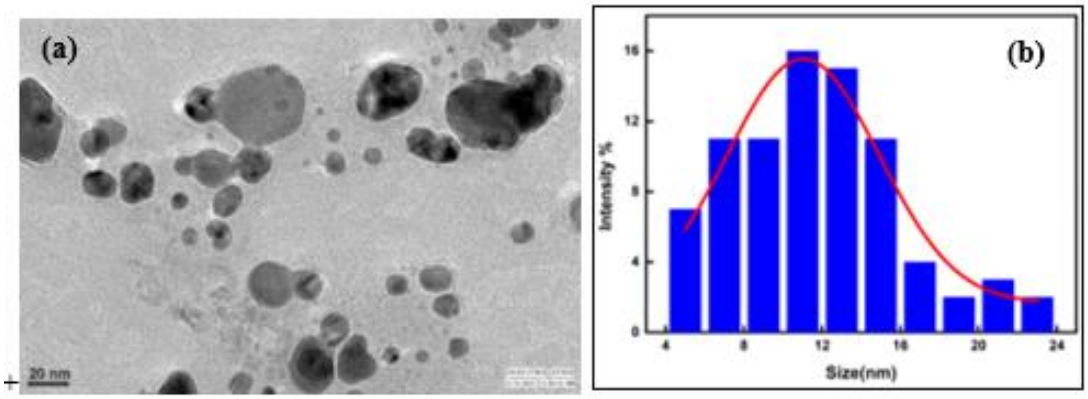


Fig.4.4 (a) TEM image (b) Histogram of Particle size distribution of biosynthesized Ag-NPs from plant extract of S.T.

4.5 Antifungal Activity

4.5.1 Disk Diffusion: All organisms are subcultured onto Sabouraud dextrose agar (SDA). Suspension of colonies are made in 5mL of sterile Saline at 0.145mol/L. Turbidity of suspension is modified to 0.5 McFarland standard. The resulting suspension will yield 1 x 10^6 to 5 x 10^6 cells per mL and a semi-confluent growth can be expected with different isolates of Candida. After making a suspension of the candida isolates, a sterile cotton swab is taken and them emersed into the solution. The inoculation of SDA surface is done by sweeping the cotton swab onto the surface by rotating the disk at 90° after each time.

4.6 Antifungal Assay against Candida Species:

The fungistatic activity of the produced Ag-NPs was done by disk diffusion method which is used to acquire the inhibitory zone of the synthesized Ag-NPs hampering the growth of the tested Candida strains which are common source of healthcare associated infections.

4.6.1: Antifungal assay against Candida parapsilosis:

C. Parapsilosis was cultured on SDA using Fluconazole and Voriconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.5 and Table 2 respectively.

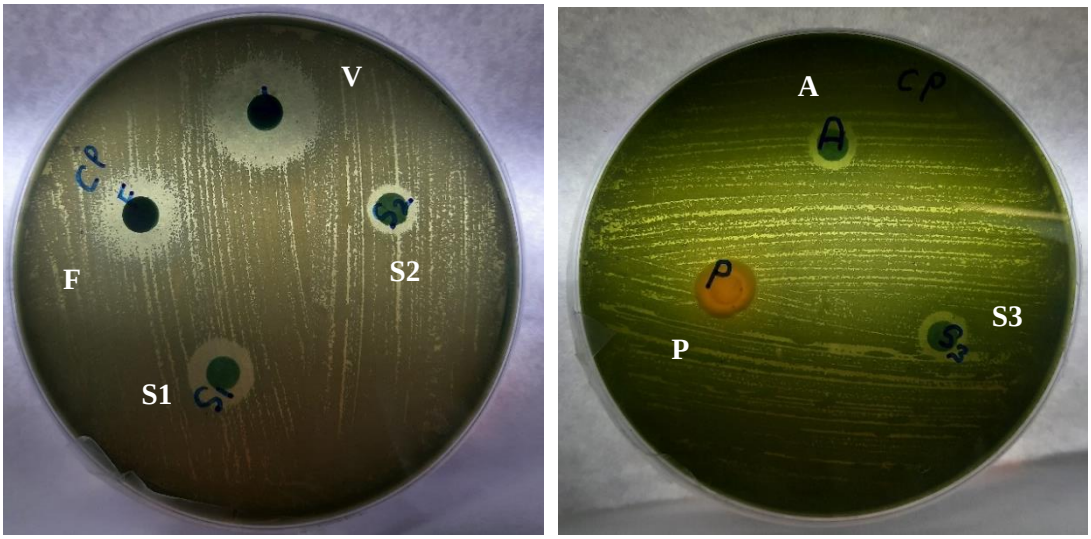


Fig: 4.5: Antifungal assay against Candida parapsilosis

Table 2: Antifungal treatment of Candida parapsilosis

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	10
2	V (Voriconazole)	13
3	S1	5.9
4	S2	4.4
5	S3	4.5
6	P (Plant Extract)	3
7	A (AgNO3)	4

Where; S1: 20 µL Ag-NPs from S.T extract

S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.6.2: Antifungal assay against Candida krusei

C. krusei was cultured on SDA using Fluconazole and Voriconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.6 and Table 3 respectively.

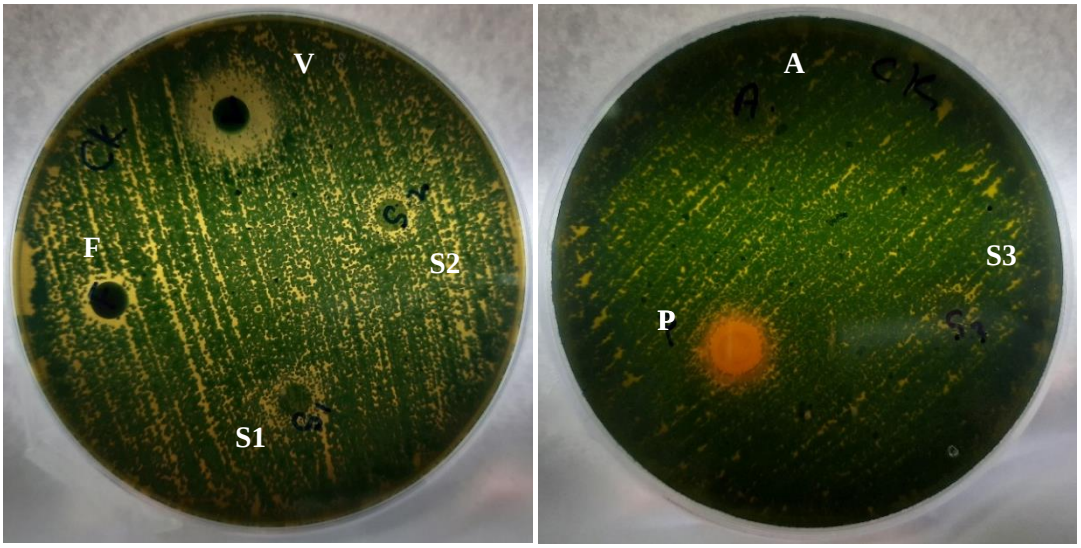


Fig: 4.6: Antifungal assay against Candida krusei

Table 3: Antifungal treatment of Candida krusei

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	4.1
2	V (Voriconazole)	9
3	S1	2.3
4	S2	2
5	S3	1.9
6	Plant Extract	3
7	AgNO3	0.5

Where; S1: 20 µL Ag-NPs from S.T extract

S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.6.3: Antifungal assay against Candida albicans

C. albicans was cultured on SDA using Fluconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.7 and Table 4 respectively.

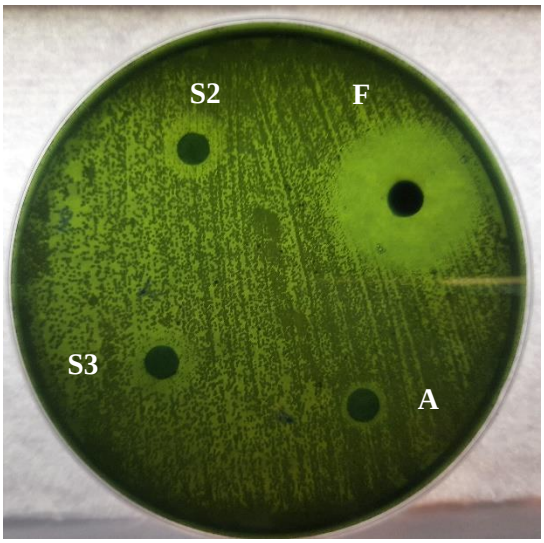


Fig: 4.7: Antifungal assay against Candida albicans

Table 4: Antifungal treatment of Candida albicans

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	13
2	S2	4.3
3	S3	5
4	AgNO3	2

Where, S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.5.5: Antifungal assay against Candida glabrata

C. glabrata was cultured on SDA using Fluconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.8 and Table 5 respectively.

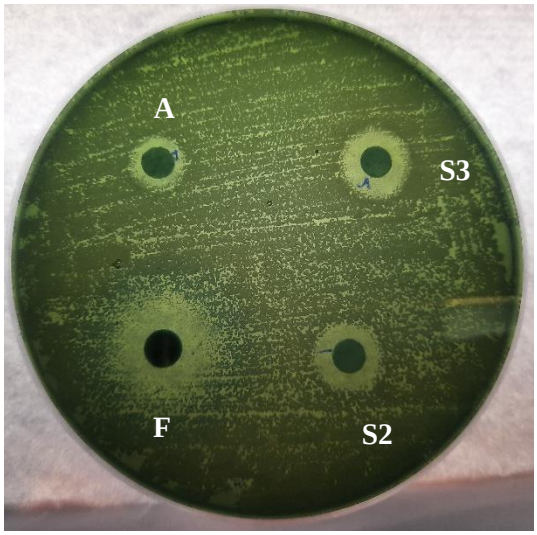


Fig: 4.8: Antifungal assay against Candida glabrata

Table 5: Antifungal treatment of Candida glabrata

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	9
2	S2	6.5
3	S3	6.7
4	A ($AgNO_3$)	3

Where, S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.5.5: Antifungal assay against Candida auris

C. auris was cultured on SDA using Fluconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.9 and Table 6 respectively.

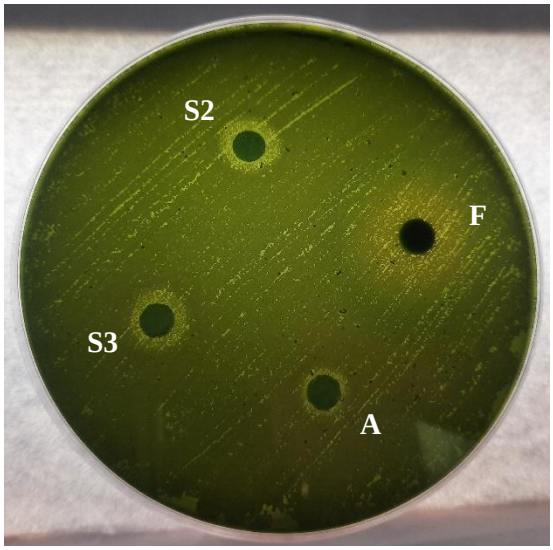


Fig: 4.9: Antifungal assay against Candida auris

Table 6: Antifungal treatment of Candida auris

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	9.7
2	S2	4
3	S3	4.3
4	A (AgNO3)	1.7

Where, S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.5.5: Antifungal assay against Candida tropicalis

C. tropicalis was cultured on SDA using Fluconazole as controls, and incubated for 3 days at 28 ± 2 ˚C. Colony growth and zone of inhibition for Ag-NPs, $AgNO_3$ and plant extract was shown in fig 4.10 and Table 7 respectively.

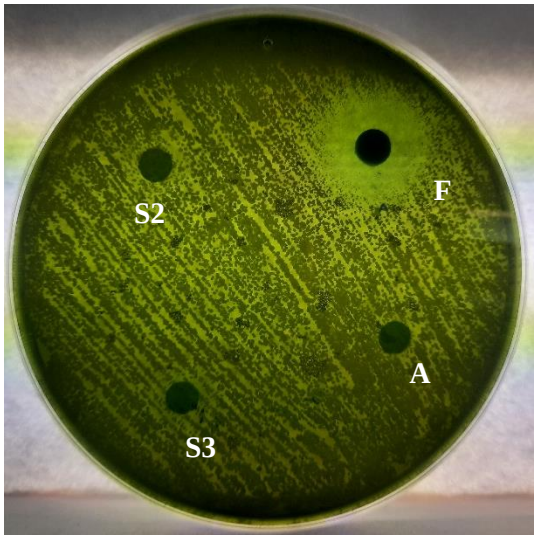


Fig: 4.10: Antifungal assay against Candida tropicalis

Table 7: Antifungal treatment of Candida tropicalis

Sr. No.	Sample	Zone of inhibition(mm)
1	F (Fluconazole)	14
2	S2	4.2
3	S3	4.6
4	A (AgNO3)	0.6

Where, S2: 10 µL Ag-NPs from S.T extract

S3: 10 µL Ag-NPs from C. Asiaticum extract

4.5.6: Experimental results:

From table 2 and table 3 we observe that Voriconazole is more resistant than Fluconazole when used as controls against C. parapsilosis and C. krusei. Both these controls are less effective on C. krusei as compared to C. parapsilosis.

From particle size distribution curves S2 have an average size of 12 nm (fig.4.4(b)) and S3 have an average size of 11 nm (fig.3.9(b)). Since S2 and S3 have almost similar size their zone of inhibition is also almost similar. A slight deviation in their zone of inhibition can be seen, this is because smaller nanoparticle penetrates the cell wall deeper and disrupt the cell structure. Higher the concentration of Ag-NPs bigger will be the zone of inhibition. This general trend is followed in all the candida species.

$AgNO_3$ also shows a slight resistance against Candida species. The orangish colour caused by the plant extract observed in fig.4.5 and fig.4.6 this colour change can be caused by bacterial present in plant extract. Hence plant extract was not used further in this study.

CHAPTER 5:

CONCLUSION:

Ag-NPs were synthesized successfully with biological agents and without the conventional usage of any toxic chemical reducing agents. The biomolecules present in the plant extract of *Crinum Asiaticum* and *Sphagneticola trilobata* is responsible for the production of Ag-NPs. TEM images were studied for morphology information and zeta potential analysis was done for checking the electrostatic and dynamic stability. Ag-NPs were able to degrade the dye in about 20 minutes, hence we can say that the synthesized Ag-NPs exhibit catalytic properties. The fungistatic activity of biosynthesized Ag-NPs was done by disk diffusion method on SDA, giving the inhibitory zone of the Ag-NPs resisting the growth of the tested Candida strains which can cause life threatening infections. Fluconazole was used as control throughout the study. Therefore, we can conclude that the green method used in the synthesis of Ag-NPs is a reliable synthesis route and can be used for fungal treatment, reducing toxic dyes efficiently and it is inexpensive.

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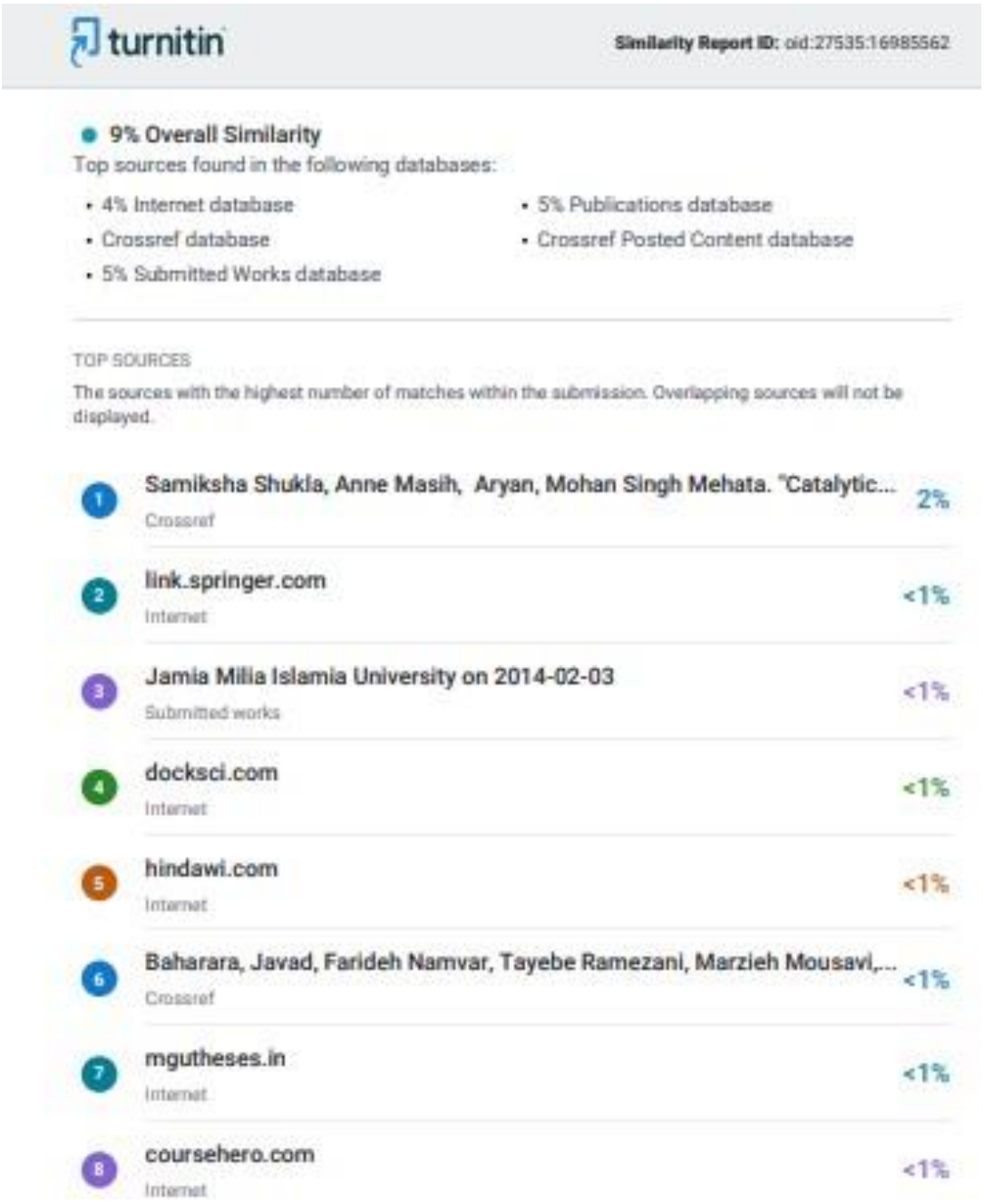
APPENDIX
PLAGIARISM REPORT:



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Catalytic activity of silver nanoparticles synthesized using *Crinum asiaticum* (Sudarshan) leaf extract

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Crinum asiaticum
Green synthesis
Catalytic activity
Silver nanoparticles
Absorption spectra

ABSTRACT

Synthesizing silver nanoparticles (Ag-NPs) from the green route has been gaining momentum in recent years since it is an eco-friendly, cost-efficient, one-basic and straightforward use of precursors. The study presents a green route to synthesize Ag-NPs using green leaves of *Crinum asiaticum* and examines how biomolecules present in *Crinum asiaticum* lead to the formation of Ag-NPs. Biosynthesized Ag-NPs were confirmed, and the effect of varying pH, medium temperature and plant extract concentration on the reduction of silver ions was observed. Ag-NPs exhibit an absorption peak at around 418 nm due to surface plasmon resonance. The size, morphology, crystalline structure and stability of Ag-NPs were examined using different techniques like absorption spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and zeta potential analysis. The shape of Ag-NPs was spherical and the average particle size was about 13 nm. Appreciable catalytic properties of Ag-NPs were also ascertained by reducing a toxic dye like methylene blue (MB) in the presence of $NaBH_4$ with a rate constant of 0.307 min^{-1}.

Selection and peer-review under responsibility of the scientific committee of the First International Conference on Design and Materials (ICDM)-2021.

1. Introduction

Currently, the interest in synthesizing and applying metallic nanoparticles (MNPs) is increasing progressively. This is attributable to the diversity of the disciplines where metal nanoparticles can be useful. Different ways are being researched to characterize the antibacterial, antimicrobial, cytotoxic, optical and various other properties of MNPs [1–8]. Typically, the synthesis of MNPs necessitates certain reducing and capping agents like sodium dodecyl sulphate (SDS) and hydrazine [9]. However, such synthetic chemicals for the synthesis process result in high production costs and frailtoxicness to biological applications due to their adverse environmental effects and toxicity. Hence, greener and eco-friendly ways of synthesizing MNPs can prove to be a very efficient and bio-friendly alternative to physical and chemical industrial ways.

Although the exact mechanism of green synthesis of MNPs haven't entirely been understood, compounds like alkaloids, phenols, terpenoids, flavonoids and proteins present in plants [10] are responsible for the successful capping and reduction of silver NPs. In the context of the synthesis of nanoparticles using microorganisms [11], the antioxidant [12] or reducing properties of microbes are considered to be responsible for the successful formation of NPs. However, fungi or bacteria-mediated synthesis requires elaborate microbe cell culturing and a purely aseptic environment. Therefore, it is safe to conclude that plant-mediated synthesis is the preferable alternative to chemical reduction.

Since ancient times, silver has been a precious noble metal due to its anti-corrosive and medicinal [13] properties. Plant-mediated method for synthesizing silver nanoparticles (Ag-NPs) is non-toxic, economical, eco-friendly, doesn't require high pressure or temperature conditions and provides a non-complicated one-step technique. The formation of Ag-NPs is straightforward to track due to noticeable colour change from colourless to brown, for which surface plasmon resonance (SPR) is responsible [14]. Ag-NPs can be used as an anti-platelet, anti-bacterial, anti-fungal, anti-

Abbreviations: Ag-NPs, Silver nanoparticles; TEM, Transmission electron microscopy; XRD, X-ray Diffraction; MB, Methylene blue; AgNPs, Metallic nanoparticles; SDS, Sodium dodecyl sulphate; SPR, Surface plasmon resonance; CA, *Crinum asiaticum*; $NaBH_4$, Sodium borohydride; $AgNO_3$, Silver nitrate; Ubiquitin UP: e-Electrons; FCC, Face centered cubic; DLS, Dynamic light scattering.

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https://doi.org/10.1016/j.matpr.2021.12.408
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the First International Conference on Design and Materials (ICDM)-2021.

Please cite this article as: S. Shukla, A. Masih, Aryan et al., Catalytic activity of silver nanoparticles synthesized using *Crinum asiaticum* (Sudarshan) leaf extract, Materials Today: Proceedings, https://doi.org/10.1016/j.matpr.2021.12.408

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Dear Authors,

Authors: Samiksha Shukla, Anne Masih, Aryan Aryan, Mohan Singh Mehata

Paper Title: Catalytic activity of silver nanoparticles synthesized using Crinum asiaticum (Sudarshan) leaf extract

Paper ID: MATPR-D-21-10958

This is to enlighten you that the above-mentioned paper is accepted and has been presented virtually at the 1st **International Conference on Design and Materials** organized at **Delhi Technological University**, Delhi, India held during **27-30th January 2022**.

The team of ICDM DTU and CIDE DTU would like to extend congratulations to you.

Dr Harish Kumar
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