iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 **Issue:** XII **Month of publication:** December 2023

DOI: https://doi.org/10.22214/ijraset.2023.57370

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 11 Issue XII Dec 2023- Available at www.ijraset.com

Natural Green Synthesis of Silver Nanoparticles using Leaf Extract of Guazuma Ulmifolia Lam and Analysis of its Antimicrobial Property

G Rohan Hugar[1], Dr. M. L. Pruthvi[2], Dr. M. K. Mahesh[3]
[1] *Student,*[2]*lecture,* [3]*Professor, Post Graduate Department of Botany, Yuvaraja's College, Mysore*

Abstract: ***In the field of life science, nanoparticles are one of the leading adverse technologies. Here, the particles are seen in nanometers of 1–100 nm. In small-size particles, many useful applications are seen in the fields of medicine, pharma, agriculture, and many more industry sectors. In the present study,*** **Guazuma *ulmifolia*, a member of the Malvavceae family,** **was** ***a test plant. In this study, silver nanoparticles were synthesised from plant extract. Characterization using SEM, XRD, FTIR, and UV spectra confirmed the presence of nanoparticles. Silver nanoparticle sizes were measured using SEM, and the sizes ranged from 132nm to 352 nm. The XRD size of silver nanoparticles calculated using Debye-Scherrer's equation was found to be 15.31 nm. Four drop-down FT-IR peaks were observed, which are 3369.60, 2162.44, 1643.53, and 635.04. N-H and C-I bonds were observed, and UV-Vis nanoparticles synthesised showed maximum absorptions between 300 nm to 700 nm. The sharp bands of silver nitrate nanoparticles were around 535nm for fresh leaves. The antimicrobial activity of synthesised nanoparticles against bacterial test pathogens, viz., Escherichia coli, Salmonella typhi, Bacillus subtilis, and Staphylococcus aureus, showed significant antibacterial activity. The average percentage of inhibition of bacteria is 38.83%, 0%, 10%, and 18.5%. Antifungal activity was also observed against Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus, and Alternaria solani. The average percentage of inhibition in fungi is 15.68%, 58.53%, 0%, 32%, and 22.2%.***

Keywords: Natural Green synthesis of silver nanoparticles, Characterization, antimicrobial activity, G.ulmifolia.

I. INTRODUCTION

In 1974, professor Norie Taniguchi of the University of Tokyo in Japan is credited with coining the term "nanotechnology." [1]. The manipulation of particles with one of their size dimensions less than 100 nm and possessing particular properties that can be used in specific applications is one definition for it[2]. The Greek prefix "nano" designates a "dwarf" or extremely small object, and it represents one thousand millionth of a metre (109 m). Nanoscience and nanotechnology should be distinguished from one another [3]. The reason nanoparticles are so interesting is that they serve as a link between the bulk material and the atomic/molecular structure by exhibiting entirely new or improved properties based on particular attributes like size, shape, distribution, ionic strength, capping agent, and morphology [2],[4].

Many Greeks, Romans, Persians, and Egyptians stored food items in silver containers some 5000 years ago[9]. Because of its known antimicrobial properties, cutlery was widely used throughout ancient times by different dynasties to store and consume a wide variety of food and drink items. As early as 300 BC, there are documented cases of silver being used therapeutically in literature. In Hinduism, silver utensils are still favoured for preparing "panchamrit," a dish that includes curd, ocimum sanctum, and other ingredients. The ancient Indian Aurvedic medicine book known as the "Charak Samhita" contains references to the medicinal properties of different metals. Silver was widely used as an antimicrobial until Alexzander Flemming's discovery of antibiotics. [10],[11]

A reducing biological agent and a silver metal ion solution are the two main ingredients needed for the green synthesis of AgNPs. There is typically no need to add external capping and stabilising agents because reducing agents or other components already present in the cells function as these substances' stabilising and capping agents.[14]

Utilising plant extracts, microbial cell biomass, cell free growth medium, and biopolymers, green syntheses of AgNPs have been carried out. Algae and angiosperms are among the plants used to synthesise AgNps; however, there are few reports on lower plants, so angiosperms are the best option. AgNP synthesis has been carried out using parts such as leaves, bark, roots, and stems.[14]

The presence of numerous organic compounds, such as carbohydrates, fats, proteins, enzymes and coenzymes, phenols, flavanoids, terpenoids, alkaloids, gum, etc., capable of donating electron for the reduction of Ag+ ions to Ag^0, is what allows biological entities to synthesise AgNP. The active component that lowers Ag+ ions varies based on the organism or extract that is utilised. The dehydrogenation of acids (ascorbic acid) and alcohols (catechol) in hydrophytes, keto to enol conversions (cyperaquinone, dietchequinone, remirin) in mesophytes, or both mechanisms in xerophytes plants are thought to provide the electrons needed for the AgNPs' nano-transformation.[12],[13].

AgNPs have found widespread application as anti-bacterial agents in various environmental applications, food storage, textile coatings, and the health industry. It is significant to remember that there is still uncertainty regarding the toxicity of silver, even after decades of use. Numerous recognised organisations, such as the US FDA, US EPA, SIAA of Japan, Korea's Testing and Research Institute for Chemical Industry, and FITI Testing and Research Institute, have approved products made with AgNPs. [8],[3]. AgNPs electrochemical qualities allowed for their incorporation into nanoscale sensors, which have a lower detection limit and quicker response times[20]Because of their numerous uses in a range of industries, including drug delivery, cancer therapy, carbon nanotube (CNT), carbon quantum dots (CQD), epoxy resin-coated CNTs, biomarkers, cell labelling, diagnostics, and antimicrobial agents, nanoparticles have drawn a lot of attention in recent years.[16]Nanoparticles can be toxic by either transferring electrons from molecules of oxygen or by obstructing the electron transport chain via an unidentified mechanism[17],[18].

Many researchers have used the disc diffusion method, which is the most widely used technique to access a liquid's antimicrobial activity, to confirm the AgNPs solution's antimicrobial action. This technique involves covering the surface of the targeted microbe that has been inoculated on nutrient medium plates with a disc of adsorbent material that is uniformly sized and dipped in a solution of increasing AgNP concentration. The formation of an inhibition zone surrounding the disc is indicative of the antimicrobial activity of the nanomaterials and well diffusion [13],[5],[6],[7],[8]. Nanoparticles capacity to generate reactive oxygen species (ROS) makes them highly promising as antibacterial agents. Reactive oxygen species generation damages the cell membrane mechanically, impairs ATP synthesis, and weakens the antioxidant defence system[15].

A. Objectives

1) Synthesis of silver nanoparticles from Guazuma ulmifolia.
2) Characterization of silver nanoparticles from Guazuma ulmifolia.
3) Antibacterial activity of silver nanoparticles from Guazuma ulmifolia
4) Antifungal activity of silver nanoparticles from Guazuma ulmifoila.

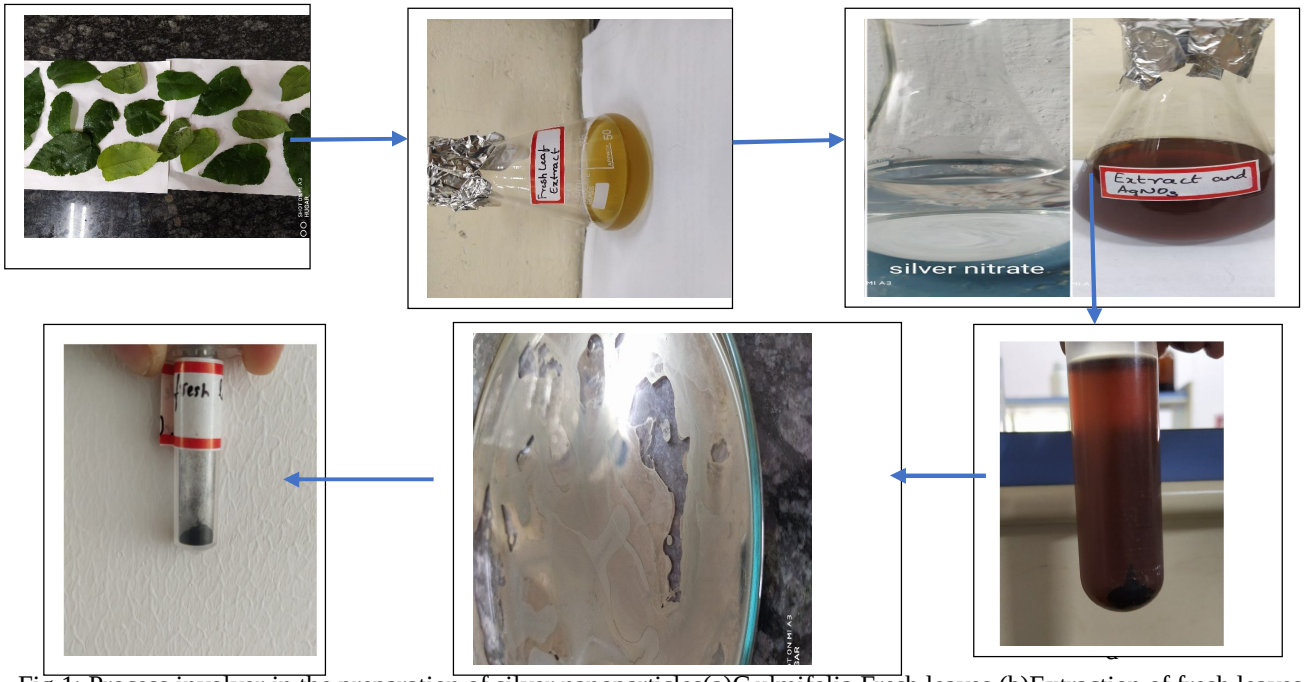


Fig 1: Process involver in the preparation of silver nanoparticles(a)G.ulmifolia Fresh leaves (b)Extraction of fresh leaves (c)silver nitrate in 1M and Synthesised AgNPs of fresh leaves (d)Centrifuge of Synthesised AgNPs (e)Air dry of pellet (f)Powder of silver nanoparticles.

II. MATERIAL AND METHOD

A. *Chemicals*

Analytical grade silver nitrate ($AgNO_3$) was used in this study. With out further purification the chemicals where used in this study.

B. *Collection of leaves*

Fresh Leaves of Guazuma ulmifolia were collected from Yuvaraja's College campus, University of Mysore, Mysuru.

C. *Preparation of Fresh Leaves Extract*

Plucking the fresh leaves from the plant measured it for 20gm. Washed it cleanly under tap water for few minutes again washer under double distilled water for few more minutes and dried. leaves were finely chopped as small pieces then transferred into 1000ml borosil Beaker. Added 150ml of double distil water to 20gm of fresh leaves and boiled it for 20 minutes. Then filtered it through filter paper (Whatman no: 1 filter paper, pore size 25µm) and used immediately for the biosynthesis of Silver Nanoparticles.

D. *Green synthesis of silver nanoparticles*

Silver nitrate was provided in the department of botany, Yuvaraja's college Mysuru. For synthesis of silver nitrate (4mg of silver nitrate in 25ml of double distil water) 1M of $AgNO_3$. Adding of 10ml of fresh leaves extract to the 90ml Silver nitrate in 250ml of borosil conical flask stir it for few minutes, slowly it changes colourless to dark brown in colour indicates that formation of silver nanoparticle's reaction carried out in shade inside a BOD incubator for 24 hours.

E. *Extraction of Nanoparticle Samples*

The incubated samples where centrifuged in 10,200 rpm for 25 minutes continued for several times. The residue was dark brown in colour as pellet, pored it in sterilized Petri plate. Dried in room temperature to observe nanoparticles powder.

F. *Characterization Techniques*

Ultraviolet-visible spectrum (UV-vis) was conducted and noted on the Beckman coulter DU730 UV-vis spectrophotometer. The absorption spectra of the prepared NPs were recorded All ultraviolet- visible (UV-vis) spectra were recorded on the Beckman coulter by taking the aqueous dispersion of the NPs and scanned in the range of 300- 700 nm operated at resolution 1nm at IOE, Mysore. Distilled water was taken to adjust the baseline.

Scanning electron microscope (SEM)was used to study the morphological features of synthesized nanoparticles from fresh leaf extract of Guazuma ulmifolia. SEM images were recorded using Carl Zeiss Germany, model: EVO MA 15 SEM instrument at IOE, Mysore. X-ray diffraction the particle size and nature of the silver NPs were determined using Bruker Eco D8 advance X-pert PRO operating at a voltage of 40kV, a current of 20mA with silver Kα radiation at 2θ angle ranging from 10* to 80*. A thin film of the silver nanoparticles was made by dipping a glass plate in a solution and carried out for X-ray diffraction studies. The crystalline silver nanoparticle was calculated from the width of the XRD peaks and the average size of the nanoparticles can be estimated using the Debye Scherrer **$D= k\lambda/\beta\cos\theta$.** FT-IR analysis. Spectrum Two FT-IR 1600 spectrophotometer was used to IR spectra of Ag. NPs in the λ range of 600 to 4000 cm−1 and 4 cm−1(%T). Spectrum Two universal disc method was followed for the analysis.

G. *Antibacterial Assay*

The selected bacteria are Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, which were sub cultured from the pure culture in an inoculation tube containing nutrient agar media for antibacterial study. The pure culture was provided by the P.G department of Microbiology, manasagangothri campus, Mysore. The nutrient agar high medium was prepared as per the requirement according to the number of plates. A small amount of agar is added to solidify. Then the media was kept for sterilization. After sterilization the NA media was poured into sterile Petri plates under aseptic condition and allowed for solidification. Well diffusion method by agar plates was used for calculating the zone of inhibition. These 4 bacterial pathogens were then coated over a agar plate with the help of sterile swab of cotton. Then these plates were permitted to dry. After those 5 wells were bored by sterile cork borer measuring 8.0mm diameter in each agar plate. Subsequently, 25µl,50µl,75µl, positive control and negative control was taken. The antibiotic Ampicillin was taken as positive control distilled water as negative control 25µl,50µl, and 75µl synthesis silver nanoparticles. Then the plates were kept for complete diffusion followed by incubation at 37 ^{0}C for 24 hrs and measured the diameter of inhibitory zones in mm.

H. Antifungal Assay

The selected fungi are Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus and Alternaria solani which were sub cultured from the pure culture for further study. The pure culture was provided by the P.G department of Biotechnology manasagangothri campus, Mysore. PDA high medium was prepared as per the requirement according to the number of plates. A small amount of agar is added as solidifying agent and kept for sterilization. After sterilization the PDA media was poured into sterile Petri plates under aseptic condition and allowed for solidification. The antifungal activity of the NPs was determined by well diffusion method. The fungal inoculums prepared were used to test the antifungal potential. The PDA media was poured into sterile Petri plates in aseptic condition then plates were allowed to solidify in laminar air flow chamber. The 5 fungal pathogens were then coated over a media containing plates with the help of sterile swab of cotton. Then these plates were permitted to dry. After those 5 wells were bored by sterile cork borer measuring 8.0mm diameter in each agar plate. Subsequently, 25µl,50µl,75µl, positive control and negative control was taken. The antibiotic Bavistin was taken as positive control distilled water as negative control 25µl,50µl,and 75µl synthesis silver nanoparticles. Then the plates were sealed and incubated at room temperature for 5-7 days and finally antifungal activity was calculated by measuring the diameter of inhibitory zones in mm.

III. RESULT AND DISCUSSION

A. Synthesis of Silver Nanoparticle

The fresh leaf extract which was prepared, taken10ml of sample and added to 90ml of 1M concentration of silver nitrate in the ratio of 1:9 in 250 ml borosil conical flask at room temperature. The reaction is carried out in shade inside a BOD incubator for 48 hours. The colour change was observed colourless to dark brown in colour. The colour change suggests the synthesis of silver nanoparticles.

B. UV-Vis Spectroscopy

UV-Vis analysis is one of the most important characterization methods to study nanoparticles. The surface plasmon resonances (SPR) of synthesized nitrate nanoparticles have been studied by UV-Vis Spectrophotometer. The absorption of visible radiations due to the excitation of SPR, imparts various colours to nanoparticles. As the nanoparticles size changes, colour of the solution is also supposed to change. So, UV-Vis absorption spectrum is quite sensitive to the formation of nanoparticles. All the nanoparticle samples are subjected to UV-Vis study. Fig. 2 shows the UV-Vis spectrum of the 1sample. The nanoparticles synthesized showed maximum absorptions between 300 nm to700 nm. The sharp bands of silver nitrate nanoparticles were around 535nm for fresh leaf.

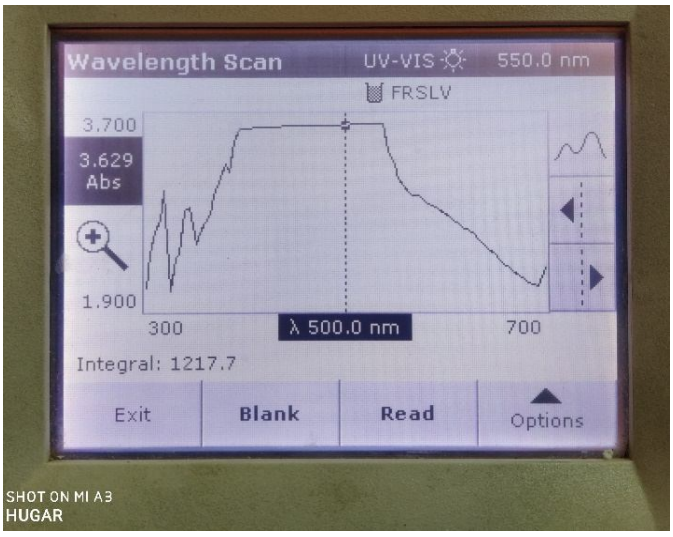


Fig 2: (a) Fresh leaf

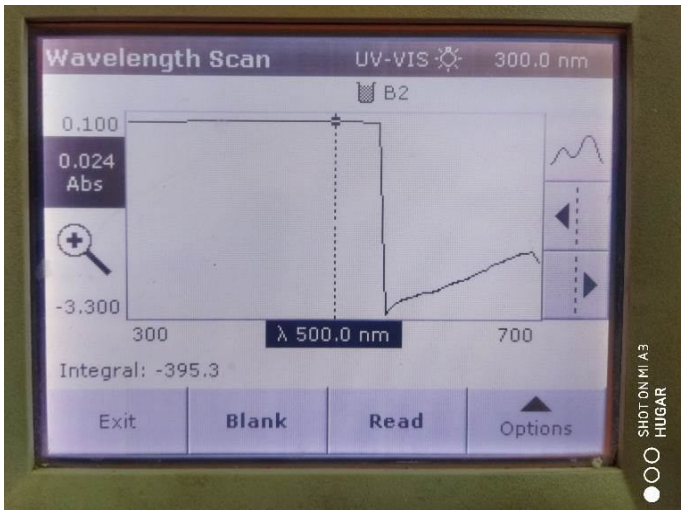
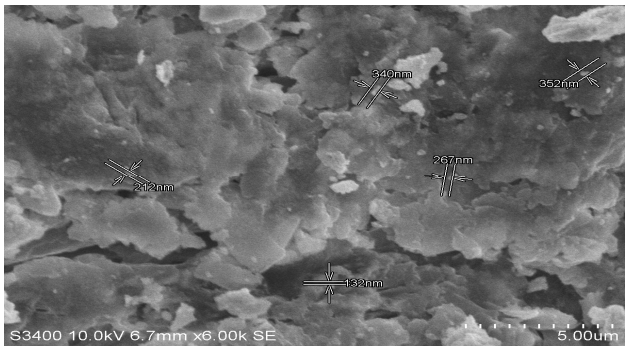


Fig :(b)Blank

C. SEM (Scanning Electron Microscopy)

Scanning Electron Microscopy provided further insight into the morphology and size details of the synthesized nanoparticles. The typical SEM image shown individual silver nanoparticles as well as number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into large irregular structure with not well-defined morphology was observed. The nanoparticles were measured from the SEM image with the help of Image J software. SEM images of silver nanoparticles of fresh leaf extract were 132nm, 212nm, 267nm, 340nm, 352nm.



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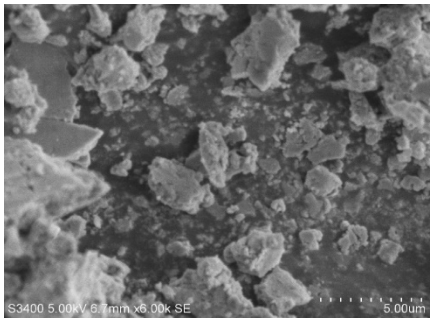

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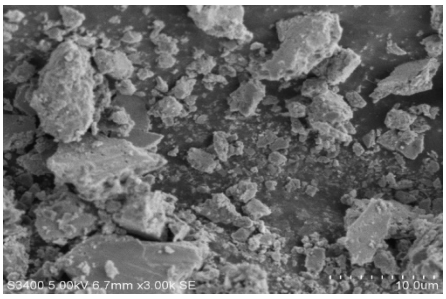


c

Fig 3: SEM images of Fresh leaves extract

D. X- Ray Diffraction (XRD) Analysis

Fresh leaf X-Ray diffraction pattern of synthesized AgNPs showed 8 distinct peaks with 2θ values.27.27, 32.22, 38.15, 44.26, 46.26, 54.43, 64.64, 77.56. these 8 values shows 23.33, 19.29, 15.07, 6.92, 16.25, 16.07, 15.23 and 10.33.can be seen in to sets of face centric planes (012),(202),(001),(020),(001),(001),(001) and (020) respectively. These sets of planes may be indexed to the face centered cubic (FCC) lattice structure of the silver nanoparticles. The mean size of silver nanoparticles calculated using Debye-Scherrer's equation was found to be 15.31nm

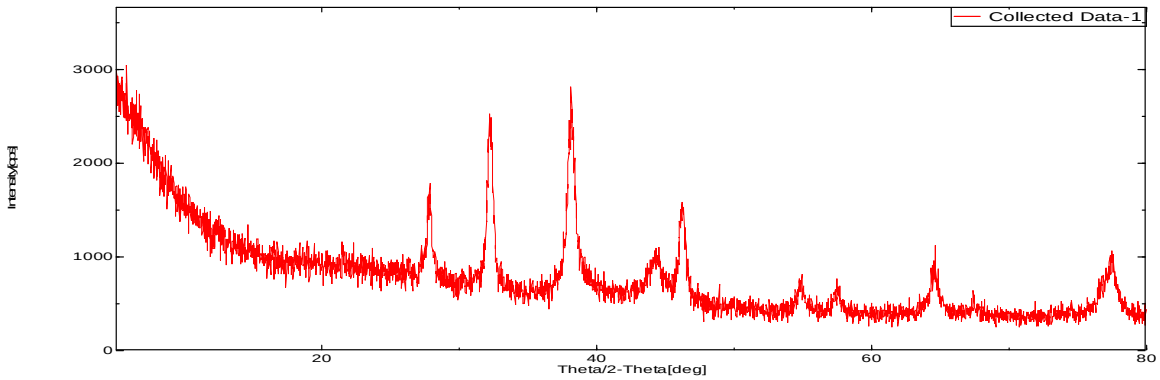


Fig : 4 X-RAY diffraction graph of fresh leaf extract

E. FTIR (Fourier-transform infrared spectroscopy) Analysis

Fresh leaf extract The AgNPs were analysed using FTIR spectrum. The sample of fresh leaf extract of *G. ulmifolia*, 2-5ml of extract was used for analysis. Certain compounds were present, like aliphatic secondary amine, transition metal carbonyls, and aliphatic iodo compounds were found. The 4 drop down peaks were observed that is 3369.60, 2162.44, 1643.53 and 635.04. N-H and C-I bonds were observed.

Peaks (drop down peak)	X in (cm^{-1})	Y in (%T)	Organic compounds
1	3369.60	73.46	Aliphatic secondary amine
2	2162.44	91.14	Transition metal carbonyls
3	1643.53	84.23	Organic nitrates
4	635.04	55	Aliphatic iodo compounds

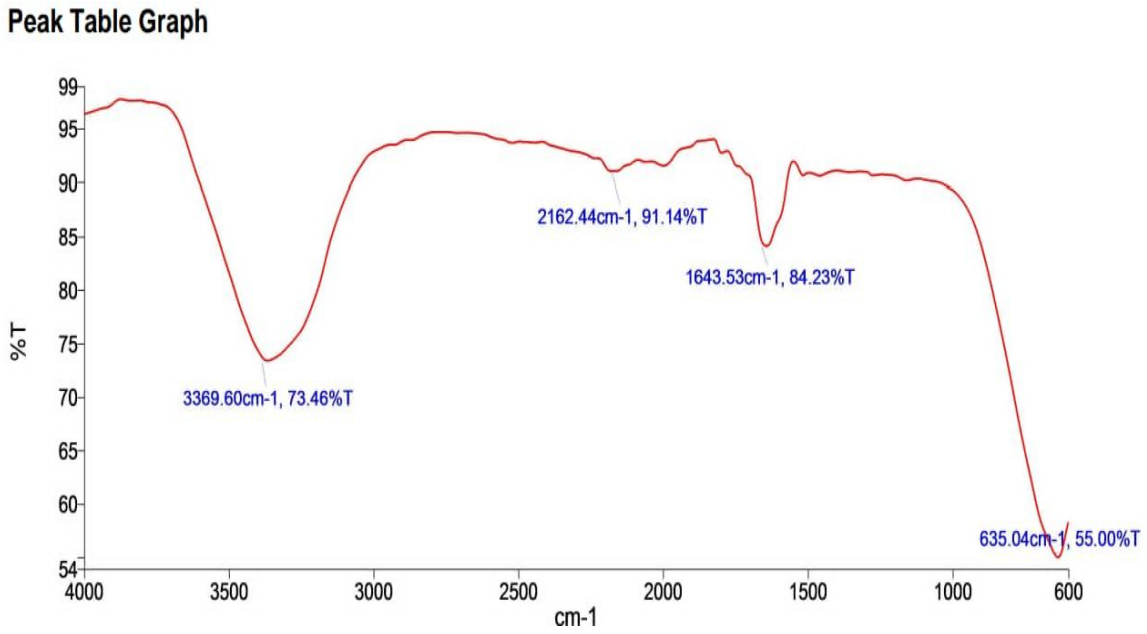


Fig 5:Graphical representation of FT-IR

F. Antibacterial Activity of Nanoparticles

Green synthesised nanoparticle suspensions with varying concentrations were tested for their antibacterial activity against gram-positive and gram-negative bacteria, including Staphylococcus aureus, Salmonella typhi, Bacillus subtilis, and Escherichia coli. The antimicrobial agent (NPs) was tested for its capacity to break apart bacterial cells using the well diffusion method. The antimicrobial activity against gram-positive and gram-negative bacteria was investigated at various sample concentrations. shown in table.

Table1: antibacterial activity of Ag NPs from *G. ulmifolia* fresh leaf extract

Bacterial Culture	Inhibition zones (mm)				
	+ve control	-ve control	25µl	50µl	75µl
Escherichia coli	30	0	10	11	14
Salmonella typhi	0	0	0	0	0
Bacillus subtilis	10	0	0	0	3
Staphylococcus aureus	9	0	0	1	4

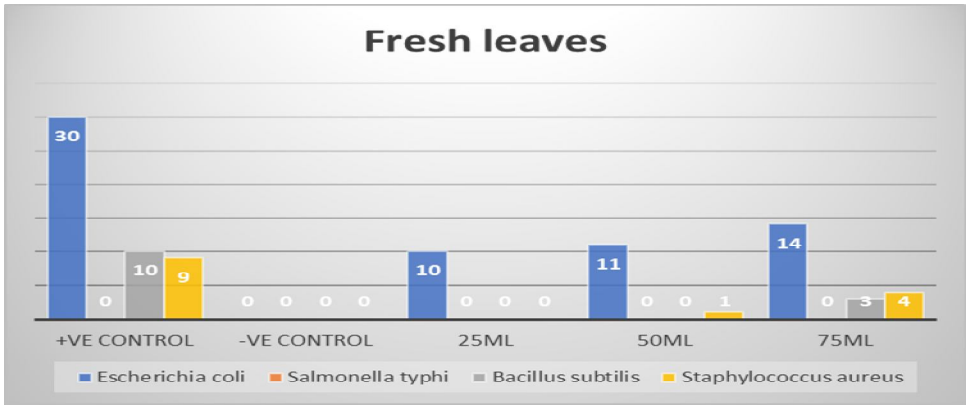
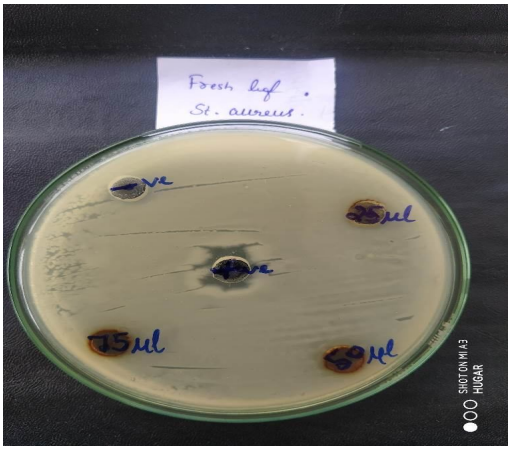


Fig 6: Graphical representation of antibacterial activity of Fresh leaf extract

A

B

C



D

Fig : 7(A-D) Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus.

According to other researchers, the reason why nanoparticles have antibacterial properties is because of their nanoscale size, which enables them to accumulate or deposit on the surface of bacterial strains under study.

Because of the presence of phytochemical components, plant extracts may also have antibacterial activity in addition to NPs. To precisely explain how NPs work against bacterial strains.

The presence of an inhibitory zone strongly implies that part of the biocidal activity of nanoparticles involves membrane rupture. Both the initial bacterial concentration and the concentration of nanoparticles affect the degree of suppression. Because the particles are smaller, they might stick to the bacterial cells' surface more firmly, rupturing the membrane and letting internal components escape, which would kill the germs.

The bacteria like Escherichia coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus. shows different percent of zone of inhibition in different concentration of microliter,25µl, 50µl, 75µl. The average percentage of inhibition of bacteria is **38.83%, 0%, 10%, 18.5%.** in this way the fresh leaf extract of my plant shows the zone of inhibition on the bacteria.

I. Antifungal Activity of Nanoparticles

Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus, and Alternaria solani were the five different fungi species tested for the antifungal activity of green synthesised nanoparticle suspensions at varying concentrations. The antifungal agent's (NPs) effectiveness against the fungal cells was evaluated using the well diffusion method. The antifungal activity at varying concentrations against the fungal species.

Table2: Antifungal activity of Ag NPs from *G. ulmifolia* fresh leaf extract

Fungal Culture	Inhibition zones (mm)				
	+ve control	-ve control	25µl	50µl	75µl
Aspergillus niger	17	0	0	0	8
Fusarium oxysporum	15	0	6	8	11
Penicillium atramentosum	0	0	0	0	0
Aspergillus flavus	25	0	0	10	14
Alternaria solani	15	0	0	2	8

A

B

C

D

E

Fig :8 (A-E) Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus, and Alternaria solani.

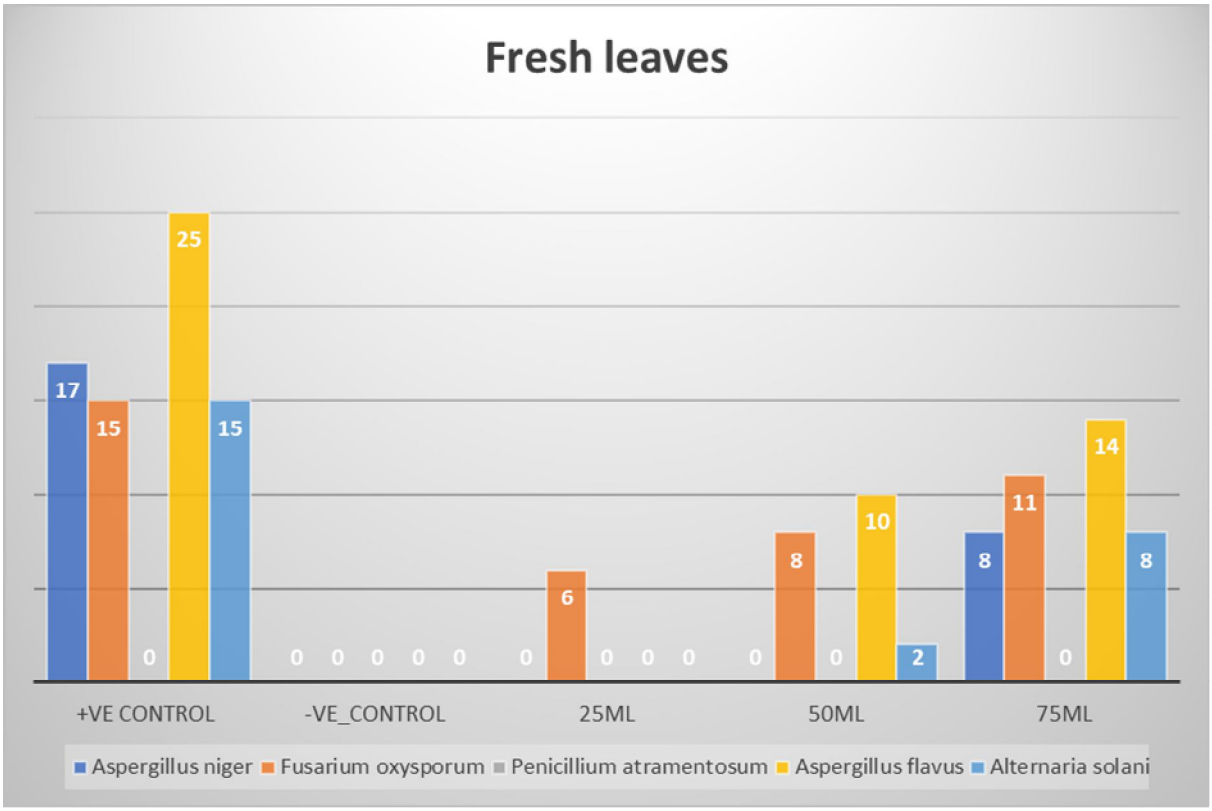


Fig 9: Graphical representation of antifungal activity of Fresh leaf extract

Additionally, it was discovered that the Ag-doped nanoparticles worked well against various plant pathogenic fungi. It is imperative to mention that the ability of the nanoparticle extract to prevent fungal growth was demonstrated.

NPs can adhere strongly to the surfaces of fungus cells and have a high surface-to-volume ratio. Moreover, because of its tiny size, it can efficiently enter the cell and harm the cell wall.

The fungi like Aspergillus niger, Fusarium oxysporum, Penicillium atramentosum, Aspergillus flavus, Alternaria solani**.** shows different percent of zone of inhibition in different concentration of microliter,25µl, 50µl, 75µl. the average percentage of inhibition in fungi is 15.68%, 58.53%, 0%,32%, 22.2%. In this way the fresh leaf extract of my plant shows the zone of inhibition on the fungi.

IV. CONCLUSION

Guazuma ulmifolia fresh leaf eco-Fridley silver nanoparticles are synthesised sustainably and have a wide range of industrial, medicinal, and antibacterial uses. We can determine the presence of silver ion particles by the colour changing to brown.

By observing the FT-IR, SEM, XRD, and UV-vis spectroscopy characterization. With the aid of spectroscopy, the ionising agent can be observed. SEM technology was used to analyse the shape and size of the particles. There is still a lot to discover about green living. XRD was used to determine the silver NPs' nature and particle size. FT-IR reveals flavonoids and alkaline components.

Along with having antimicrobial qualities against bacteria and fungi, the plant also performed well in the zone of inhibition. This makes it simple to determine that it has inhibiting ability as well. This makes it suitable for use in pharmacological and ayurvedic medicinal applications, among many others.

Since plants have a good ability to inhibit environmental harm, further research can be done to compare the effects of AgNPs with those of common medications used to treat these diseases.

One of the innovations and a rapidly expanding material in a variety of fields, silver nanoparticles produced positive results in this medicinal plant.

V. ACKNOWLEDGMENT

The authors gratefully acknowledge faculty of Post Graduate department of Botany, Yuvaraja's College Mysore, University of Mysore, for providing their support and laboratory facility to conduct this research work.

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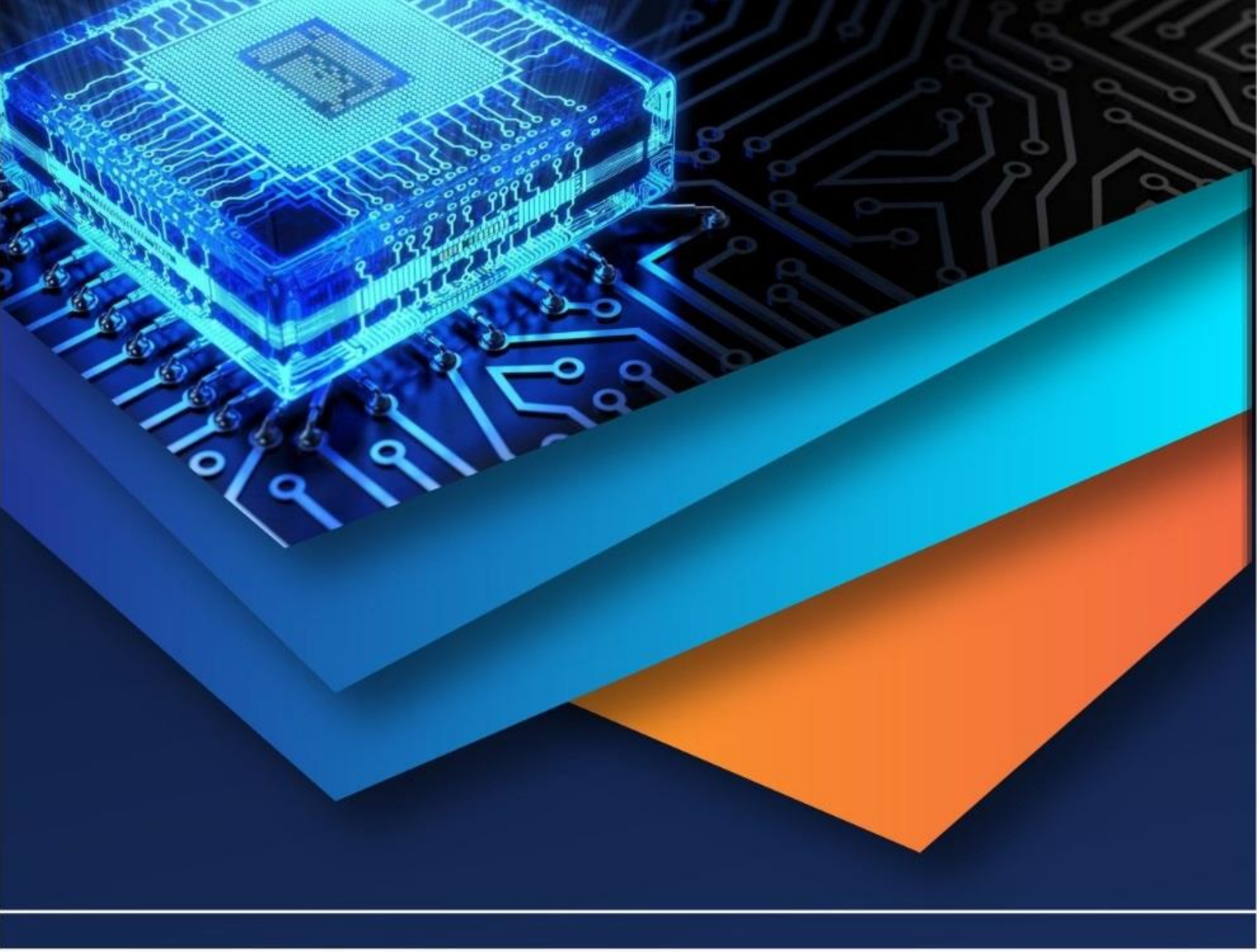

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