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Biogenic Silver Nanoparticles: Utilizing *Vitex negundo* and *Eclipta prostrata* for Antimicrobial and Antibiofilm Activity

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Abstract

This research unveils a straightforward and environmentally friendly method for producing silver nanoparticles by utilizing leaf extracts from Vitex negundo and Eclipta prostata plants. These nanoparticles are garnering significant interest due to their incorporation of biologically active plant compounds, making them ideal for green synthesis. The study highlights the efficacy of this process in creating nanoparticles with unique biological applications. Furthermore, the synthesized nanoparticles demonstrate promising antimicrobial properties and show potential in combating biofilm formation. Stability of silver nanoparticles was observed by colorimetric observation. The biosynthesized silver nanoparticles from the leaf extracts of Vitex negundo and Eclipta prostata were tested separately for Antibacterial activity. The study evaluated the antibacterial activity of the synthesized silver nanoparticles against both gram-positive bacteria, including Bacillus and Staphylococcus aureus, and gram-negative bacteria, such as E. coli and Klebsiella. Additionally, their antifungal properties were examined. Furthermore, the efficacy of the silver nanoparticles in inhibiting biofilm formation was assessed using E. coli and Staphylococcus organisms.

Keywords: Staphylococcus aureus, biosynthesized silver nanoparticles, eclipta prostata, gram-positive bacteria, gram-positive bacteria colorimetric

INTRODUCTION

Nanoscience and nanotechnology involve the study and application of exceptionally minute entities and have widespread applicability across various scientific disciplines, including chemistry, biology, physics, material science, and engineering.

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The roots of nanoscience and nanotechnology can be traced back to a seminal talk titled “There's Plenty of Room at the Bottom” delivered by physicist Richard Feynman at a meeting of the American Physical Society held at the California Institute of Technology (CalTech) on December 29, 1959. In this groundbreaking presentation, Feynman envisioned a future where scientists would have the capability to manipulate and control individual atoms and molecules, long before the term “nanotechnology” was even coined. It wasn't until over a decade later that Professor Norio Taniguchi, while exploring the realm of ultraprecision machining, officially introduced the term “nanotechnology” to describe this field of study and innovation. Nanoparticles are defined as a ultrafine unit with dimensions measured in

nanometers (nm: 1 nm = 10^{-9} meter). They exists naturally in the world and are also been created by human activities. Because of their microscopic size and having unique characteristics and manufactured nanoparticles are applied in various fields including medicine, engineering, catalysis, and environmental remediation.

Two factors that are important to decide the properties of nanomaterials are quantum effects and structures. The minute structures means they have greater surface area than other materials and can alter or improve their properties such as strength and reactivity and can affect the performance of nanoparticles [1].

Silver nanoparticles have been attracting much attention in the past few decades in fields such as biomedicine, catalysis, energy storage, and sensors due to their unique properties. Silver nanoparticles are famous for broad spectrum and high efficient antimicrobial and anticancer properties also promoting bone healing, wound repair, enhancing the immunogenicity of vaccines etc. [2].

Green synthesis of silver nanoparticles is done using the stems of caramulla fimbriata plant and characterization of silver nanoparticles with the help of SEM analysis it showed silver nanoparticles at absorbance of 400-500 nm [3]. Green synthesis of silver nanoparticles and their characteristics is done with the plant extract of terminalia chebula plant as the plant is said to have plant metal interactions by using XDR, FT-IR, SEM, EDAX and TEM [4]. The synthesis of silver nanoparticles is done with azadiracta indica aqueous extract and characterization of nanoparticles using UV-Visible spectrophotometer, TEM, and also toxicity of silver nanoparticles was carried out using MMT dye assay [5].

APPLICATIONS

Nanotechnology and nanomaterials can be applied in all kinds of industrial sectors. There are usually found in these areas:

- Electronics
- Energy
- Biomedicine
- Environment
- Food
- Textile

Biosynthesis of Nanoparticles

The demand for nanoparticle biosynthesis arose due to the high costs associated with physical and chemical processes. In the pursuit of more cost-effective pathways for nanoparticle synthesis, researchers turned to microorganisms and later utilized plant extracts. Nature has evolved diverse mechanisms for synthesizing nano- and micro-scale inorganic materials, leading to the emergence of a relatively novel and largely unexplored research area focused on the biosynthesis of nanomaterials

The evaluation of nanoparticle preparation from a green chemistry perspective involves scrutinizing three key steps: the selection of a solvent medium for synthesis, the choice of an environmentally friendly reducing agent, and the selection of a non-toxic material for nanoparticle stabilization. Many reported synthetic methods heavily rely on organic solvents, primarily due to the hydrophobic nature of the capping agents utilized. Utilizing bio-organisms for synthesis aligns with the principles of green chemistry, as the organisms themselves are eco-friendly, serving as both the reducing and capping agents in the reaction. In contrast, chemical synthesis methods may introduce toxic chemical species onto the nanoparticle surface, potentially posing risks in medical applications. Biosynthesized nanoparticles, however, are inherently eco-friendly and biocompatible, making them suitable for pharmaceutical applications without such concerns.

Biosynthesis of silver nanoparticles using Anisomeles malabarica plants extracts for larvicidal and repellence effect of the mosquito (aedes ageypti) on cotton fabric using aqueous and methanol extracts of plant at different concentrations of silver nanoparticles was observed and concluded to have larvicidal and repellent effect on aedes mosquitos and also said to have control over aedes mosquito [6].

Biosynthesis of silver nanoparticles with Neem plant extract and characterisation is done using UV-Visible spectrophotometer which indicated formation of silver nanoparticles at 427 nm absorbance and also their antimicrobial activity is tested with E coli [5].

Silver nanoparticles was synthesized using biological, chemical, physical methods along with many different reducing agents. The reduction of silver nitrate solution is done using concentrate of kinnow a variety of mandarin citrus fruit in which the fruit extract is added to the solution of silver nitrate and silver nanoparticles were synthesized and characterised using UV spectrometer and SEM to find the efficacy of biomaterials to synthesize nanoparticles [7].

Use of Plants to Synthesize Nanoparticles

Utilizing plants for nanoparticle synthesis offers several advantages, including easy availability, safe handling, and a wide array of metabolites that facilitate reduction processes. Currently, numerous plants are under investigation for their nanoparticle synthesis potential. For instance, gold nanoparticles ranging from 2 to 20 nm have been successfully synthesized using live Alfa alfa plants. Additionally, nanoparticles of silver, nickel, cobalt, zinc, and copper have been produced. Some plants are known to accumulate higher concentrations of metals compared to others, leading to their classification as hyperaccumulators.

Silver nanoparticles were synthesized from Lantana camara flower extract with the assistance of phytochemical constituents. The extract was used to synthesize silver nanoparticles and also the antibacterial efficacy was observed against Acinetobacter and Enterococcus bacteria [8].

Biosynthesis of silver nanoparticles from Pleurotus florida (mushroom) was done with cell free extract of pleurotus is able reduce silver ions in aqueous system of silver hydrosol in a single step method at room temperature using Diffused light as a source of energy. The nanoparticles exhibited high absorption peak for surface plasmon resonance at 450-500 nm of wavelength.[9]

The silver nanoparticles synthesized from the leaf extract of Erythroxylum monogynum using diamine silver solution later the silver nanoparticles were characterised using SEM and FT-IR. The nanoparticles were also used to test viability of MCF-7 cancer cell lines along with their toxicity with the use of MTT assay and it was fond to decline the growth of MCF-7 cell lines due to silver nanoparticles [10].

PLANT DESCRIPTION

1. *Vitex negundo*
2. *Eclipta prostata*

VITEX NEGUNDO

Vitex negundo, commonly referred to as the Chinese chaste tree, five-leaved chaste tree, or horseshoe vitex, is an aromatic deciduous shrub characterized by its quadrangular, densely whitish, tomentose branchlets. Typically, it grows as an erect large shrub, occasionally resembling a small slender tree, reaching heights ranging from 2 to 8 meters.

Vitex negundo (Figure 1a), with its diverse therapeutic properties, finds application in various medicinal contexts:

- It serves as a remedy for coughs and boils and is known to be beneficial in treating conditions such as leprosy, asthma, and rectal prolapse.
- The plant is also recognized for its ability to regulate the menstrual cycle, offering relief to individuals experiencing irregularities.
- Additionally, it aids in reducing pain and resolving hard swellings of the spleen.
- *Vitex negundo* is valued for its effectiveness in alleviating backaches and headaches. Moreover, the juice extracted from its green leaves is believed to enhance vision when applied to the eyes.

Figure 1. (a) *Vitex negum* (b) *Eclipta prostrata*.
Source: "Botanical Gardens" of University College of Science, Saifabad, Osmania University

ECLIPTA PROSTATA

Eclipta prostrata (Figure 1b), commonly referred to as false daisy, Gunta kalagaraku/Gunta galagaraku, karisalankanni, and bhringraj, belongs to the sunflower family. It is widespread across the world.

Uses

1. It prevents aging
2. Best way to use bhringraj is in oil form. Bhringraj oil offers a multitude of remarkable benefits for hair care. It aids in preventing hair fall and premature greying while also addressing issues like split ends. By strengthening the hair roots, this oil promotes robust hair growth, contributing to overall hair health and vitality.
3. Due to its antimicrobial properties, this plant is utilized in the treatment of various bacterial infections, offering effective remedies against microbial ailments.

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

The antimicrobial activity of silver nanoparticles were observed using agar well diffusion method, in which the wells consists of different concentrations of silver nanoparticles and drugs to observe their antimicrobial activity.

In on the antimicrobial study of Ag NPs synthesized from viridibacillus species extracts by green synthesis have noted to have remarkable antibacterial activity especially on gram negative bacteria like E coli and P aeruginosa [11]. The silver nanoparticles synthesized from azadirachta and alma plant extracts by producing characteristic light yellow color and had antimicrobial activity without

compromising their antimicrobial properties [12]. Antibacterial activity of silver nanoparticles in combination with conventional antibiotics against MDR enteric human pathogens like E coli, klebsiella, Salmonella, and Shigella and the results showed that E coli had high prevalence and the susceptibility induced in bacterial cultures was about 20-50 percent analysed antibiotics [13].

Silver nanoparticles synthesized at 6 hour exposure time showed maximum antibacterial activity yersinia enteroclolitica, aeromonas, salmonella, klebsiella, and staphylococcus reported that antimicrobial activity of silver nanoparticles on gram positive and gram negative organisms depends on different concentrations of nanoparticles [10]. The AgNP's synthesized from Vernonia plant extract antibacterial activity was tested with disk diffusion method against gram positive and gram negative bacteria and observed high inhibition zones were observed [14]. The another study in which the AgNP's synthesized from carduus crispus and its antimicrobial activity using well diffusion method with Penicillin G and Chloramphenicol, AgNP's and distilled water as control and surprisingly it was found to have exhibited effective inhibition against both gram positive and gram negative bacteria [15].

The aqueous extracts of solanum melongena AgNP's was tested for antibacterial activity against E coli, Shigella, Pseudomonas, klebsiella and Staphylococcus aureus and found to have effective inhibition in the growth of bacteria proving that the SM-AgNP's having antibacterial activity [16]. The silver nanoparticles synthesized from plant extracts of Hagenia abyssinica its antimicrobial activity was tested against klebsiella, salmonella, pneumonia and streptococcus and found to have higher inhibition in the growth of salmonella and concluded that gram negative bacteria have high effective inhibition with silver nanoparticles[17]. The recent study of silver nanoparticles synthesized from Aloe barbadensis miller leaf extracts were tested for antimicrobial activity against S aureus, S citrus, klebsiella and E coli were observed and noted that E coli had high zone of inhibition [18].

MATERIALS AND METHODS

- Mature leaves of *Vitex negundo* and *Eclipta prostrata* were collected from the surroundings.
- Methanol (70%), $AgNO_3$ (99.98%), Nutrient agar and Muller Hinton agar media.
- All aqueous solutions were prepared by using distilled water.
- All reagents used were of analytical grade.

METHODOLOGY

Sample Collection

Vitex negundo and Eclipta alba green leaves were washed and dried in an oven dryer at 40°C for 48 hours. The dried leaves were then ground into powder, stored in dark glass bottles and kept at -20°C until further analysis[13].

Aqueous Extract

10 gms of leaves powder is added in the conical flask containing 100 ml of distilled water and heated in heating mantle for 5 mins at 50°C and kept aside for overnight. Then it is filtered by using Whatman No 1 filter paper to obtain pure aqueous extraction [13]

To prepare the methanol extract, 10 grams of leaf powder is added to a conical flask containing 100 ml of 70% methanol, and the mixture is left overnight. Subsequently, it is filtered using Whatman No. 1 filter paper to obtain pure methanol extraction.

Preparation of 1 mM $AgNO_3$ Solution

The concentration of silver nitrate solution used for the green synthesis of silver nanoparticles from *Vitex negundo* and *Eclipta prostata* is 1 mM $AgNO_3$ solution. To prepare a 1 mM solution of $AgNO_3$, 0.016987 g of $AgNO_3$, with a molecular weight of 169.87 g/mol, is weighed and dissolved in a conical flask containing 100 ml of distilled water. The resulting solution is then stored in an amber bottle for further use.

Standardisation of Concentration of Plant Extract by Using $AgNO_3$ Solution

Standardisation of Aqueous Extract

A series of three test tubes were taken and to them different concentrations of aqueous plant extract and different concentrations of $AgNO_3$ solution are added. In the first test tube, 2 ml of plant extract and 6 ml of $AgNO_3$ solution is added and in the second test tube, 3 ml of plant extract and 7 ml of $AgNO_3$ solution is added. In the same way, of plant 3 ml extracts and 8 ml $AgNO_3$ solution are added in the third test tube respectively. After adding, these test tubes are kept in a dark place and the color change is observed and noted after 1 hour and 72 hours of incubation time in all the test tubes.

Standardisation of Methanol Extract

A series of four test tubes were taken and to them different concentration of methanol plant extract and different concentrations of $AgNO_3$ solution are addedIn the first test tube, 3 ml of plant extract is combined with 7 ml of $AgNO_3$ solution, while in the second test tube, 2.5 ml of plant extract and 7.5 ml of $AgNO_3$ is added. In the same way, 2 ml, 1 ml of plant extracts and 8 ml, 9 ml of $AgNO_3$ solution are added in the third and fourth test tubes respectively. After adding, these test tubes are kept in a dark place and the color change is observed and noted after 4 hours and 6 hours of incubation time in all the test tubes.

Standardisation of pH

pH for Aqueous Extract

The PH is adjusted by adding KOH. The standard concentration of aqueous extract is 2 ml. A series of four test tubes are taken and to that 2 ml of aqueous extract and 8 ml of $AgNO_3$ is added in all the four test tubes. For knowing the standard pH value, a solution from the first tube is taken with the help of Pasteur pipette and a drop is added on the pH paper and there the color change is observed and is checked on the pH scale, the color observed is lime yellow which indicates the pH-6. This process is continued for the remaining three test tubes. The color observed in the remaining test tubes are green, dark green and pale blue which indicates the pH-7, pH-8, pH-9 respectively. From the above process the standard pH is 8 for Aq.extract [19]

pH for Methanol Extract

The PH is adjusted by adding KOH. The standard concentration of methanol extract is 2.5 ml. A series of four test tubes are taken and to that 2.5 ml of methanol extract and 7.5 ml of $AgNO_3$ is added in all the four test tubes. For knowing the standard pH value, a solution from the first test tube is taken with the help of Pasteur pipette and a drop is added on the pH paper and the color change is observed and is checked on the pH scale, the color observed is lime yellow which indicates the pH-6. This process is continued for the remaining three test tubes. The colors observed in the remaining test tubes are green, dark green and pale blue which indicates the pH-7, pH-8, ph-9 respectively. From the above process the standard pH is 8 for methanol extract [19].

Synthesis of Ag/Vitex Negundo Emulsions (Silver Nanoparticles)

Emulsion of Aqueous Extract

For the above prepared aqueous extract, the standard concentration is 3 ml, and the pH is 8. For the preparation of emulsion, 10 ml of aqueous extract is added in the 40 ml of 1mM $AgNO_3$ solution in the beaker or conical flask. The pH should be adjusted to 8 by using KOH solution. These emulsions is kept aside and after one day the emulsion is centrifuged at 4000 rpm for 5 minutes to obtain a pure dense pellet for three times which is then washed with ethanol and again centrifuged at 4000 rpm for 5 minutes and the pellet is removed from the tube and is kept for drying. After drying the pellet, a powder is obtained and it should be stored in the bottle which is used for the antibacterial activity and bio film activity.

Emulsion of Methanol Extract

For the above prepared methanol extract, the standard concentration is 3 ml, and the pH is 8. For the preparation of emulsion, 12.5 ml of methanol extract is added in the 37.5 ml of 1 mM $AgNO_3$ solution

in the beaker or conical flask. The pH should be adjusted to 8 by using KOH solution. These emulsions is kept aside and after one day the emulsion is centrifuged at 4000 rpm for 5 minutes to obtain a pure dense pellet for three times which is then washed with ethanol and again centrifuged at 4000rpm for 5 minutes and the pellet is removed from the tube and is kept for drying. After drying the pellet, a powder is obtained and it should be stored in the bottle which is used for the antimicrobial and bio film activity.

Antibacterial Activity Testing by Using Agar Well Diffusion Method

Antibacterial activity was performed for *Vitex negundo*, *Eclipta prostata* and silver nanoparticles. Prepare Muller-Hinton agar medium (dissolve 6.84g of MH agar in 180 ml of distilled water in 250 ml conical flask) for *Vitex negundo* and *Eclipta prostrata*. Autoclave the medium and pour into the petriplates while still it is in molten state. Allow it to solidify in cool place. Aseptically puncture the plates using a borer and create four wells, each with concentrations of 50 µl, 75 µl, and 100 µl. These wells will contain aqueous and methanol emulsion extracts of both plant extracts. Inoculate the plates by swabbing with E coli, klebsiella, bacillus, staphylococcus cultures (gram positive and gram negative control each plant extract samples. Add 10 µl and 20 µl of Ciprofloxacin to two wells each of the positive and negative control plates, and 50 µl and 100 µl of DMSO to another two wells each. Ensure the plates are kept upright during incubation at 37°C for 48 hours. Following incubation, examine the plates for the presence of clear zones of inhibition around each well.

Micro Titre Plate Bio Film Assay (Quantitative Method)

Bio film assay is conducted in triplicate in 12 wells micro titre plates (tissue culture treated, flat-bottom wells-TPP). In the microtitre plate biofilm assay, the EMJH broth serves as the negative control. The procedure entails daily extraction of liquid culture over a span of 10 days, with intervals of 24 hours. Following this, the wells are gently rinsed with distilled water to eliminate non-adherent planktonic cells. After rinsing, the wells are left to air dry for 15 minutes before fixation using 2% sodium acetate. Once the sodium acetate solution is removed, the wells are allowed to air dry once more. Subsequently, the cells are stained using 500 µl of 1% crystal violet solution, followed by removal of excess stain. The cells are then rinsed three times with distilled water. Finally, the remaining crystal violet solution in the wells is dissolved in 750 µl of ethanol/lacetone (80/20, v/v solution) before measuring the optical density at 600 nm (OD 600). To correct the background staining of crystal violet, the mean official density at 600 nm of the negative control is subtracted from the mean optical density at 600 nm of the bio film formation of pathogen.

RESULTS AND DISCUSSIONS

Synthesis of Nanoparticles

Synthesis of Silver Nanoparticles using medicinal plants such as *Vitex negundo* and *Eclipta prostata* have shown formation of nanoparticles when reacted with 1 Mm Silver nitrate ($AgNO_3$) is shown in Figure 2 (a, b). Fresh suspension of *Vitex negundo* and *Eclipta prostrata* was taken in volumes of 2 ml, 3 ml, 4 ml which was first yellowish green in colour. However after addition of $AgNO_3$ and observed after 1 and 72 hrs respectively. The emulsion turned dark brown/black in colour as shown in the (Figure 2b).

After eclipta aqueous solution was prepared the dark brown colour was observed more in 3 ml volume at both 1 hr span and also after 72 hrs. dark brown or blackish brown indicates the formation of nanoparticles. Whereas the other tubes haven't shown as much as deposits or dark colour. Similarly 4 ml volume also shown deposits but not as much as 3 ml. So, 3 ml is considered as standard here (Table 1).

Eclipta alba methnol extract was added with $AgNO_3$ and observed for deposits in which the 3 ml sample shown the highest deposition. So the 3 ml sample is taken as standard which showed the highest deposition after 72 hrs incubation (Table 2).

Vitex negundo mixed with distilled water and $AgNO_3$ is added. The 3 ml conc. at 1 hr shows deposits immediately. After 72 hr it turns to black colour. The highest deposits were observed in 3 ml test tubes

than other two test tubes. So, the 3 ml is taken as standard for the volumes of both plant extracts and $AgNO_3$ concentrations (Table 3).

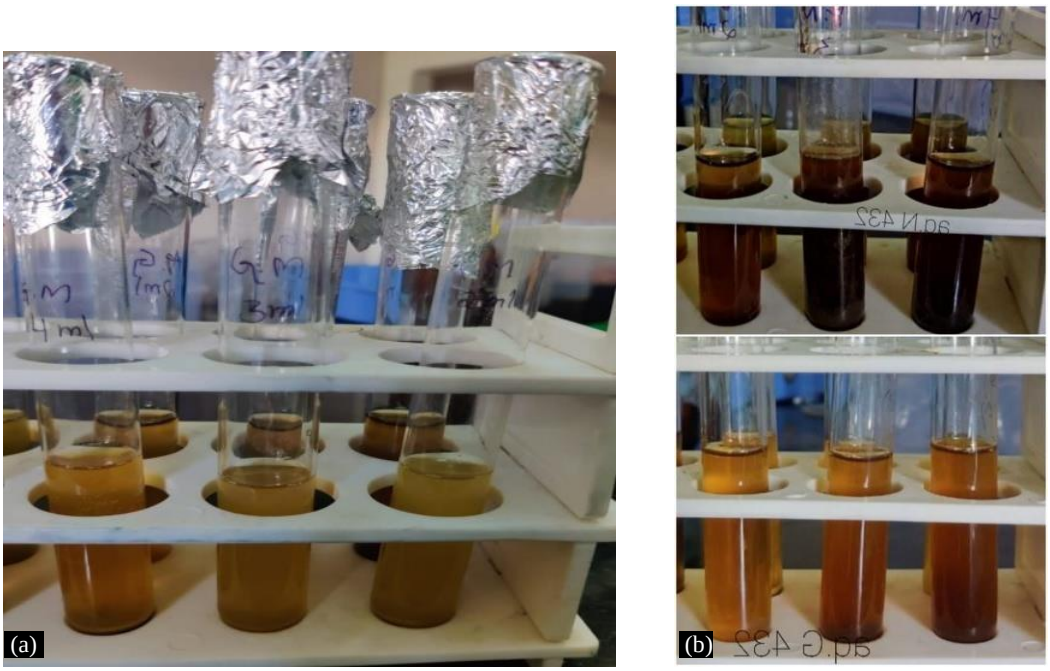

Figure 2. (a) Yellowish green colour. (b) Dark brown/black colour.

Table 1. Aqueous extract of *eclipta prostata.*

E.AQ	$AgNO_3$	Time	Observation	Colour
2 ml	8 ml	1 hr	No	Yellowish brown
		72 hrs	No	Greenish yellow
3 ml	7 ml	1 hr	Yes	Brown
		72 hr	Yes	Brown
4 ml	6 ml	1 hr	Yes	Brown
		72 hr	Yes	Brown

Table 2. Methanol extract of *eclipta prostata*.

E.M	$AgNO_3$	Time	Observation	Colour
2 ml	8 ml	1 hr	No	Yellow
		72 hrs	No	Yellow
3 ml	7 ml	1 hr	Yes	Dark brown
		72 hrs	No	Yellow
4 ml	6 ml	1 hr	Yes	Yellow
		72 hrs	No	Yellow

Table 3. Aqueous extract of *vitex negundo*.

V.AQ	$AgNO_3$	Time	Observation	Colour
2 ml	8 ml	1 hr	No	Yellow
		72 hrs	No	Yellow
3 ml	7 ml	1 hr	Yes	Dark brown
		72 hrs	Yes	Black
4 ml	6 ml	1 hr	No	Brown
		72 hr	Yes	Dark Brown

Table 4. Methanol extract of *vitex negundo*.

V.M	$AgNO_3$	Time	Observation	Colour
2 ml	8 ml	1 hr	No	Yellow
		72 hrs	No	Yellow
3 ml	7 ml	1 hr	Yes	Yellow
		72 hrs	Yes	Dark yellow
4 ml	6 ml	1 hr	Yes	Yellowish Brown
		72 hrs	No	Yellow

Vitex negundo was mixed with methanol and prepared and $AgNO_3$ is added to this. The 4 ml concentration at 1 hr showed deposits immediately. This is taken as standard for the *vitex negundo* methanol extract and used for further process (Table 4).

Standardisation of Ph

The samples which shown deposition partially is taken and their ph are adjusted. One sample which shown highest deposition is taken as standard already i.e. *vitex negundo* aqueous. For the adjustment of ph the partially deposition shown samples and deposition shown samples are taken and divided into equal amounts into three test tubes and are adjusted to ph 6, 7, and 8 respectively.

For *Eclipta prostata* the 3 ml sample is taken because it shown the high deposition by showing dark colour and was divided into equal parts and adjusted to 6 7 8 pH in which at pH 8 of the sample shown formation of nano particles (Table 5).

E.M is heated after adding 1.5 Mm $AgNO_3$ for 15 minutes and also heated after adjusting the ph for 15 minutes at 70 degrees Celsius. The methanol extract Eclipta aqueous was standardised at 2 ml so it is taken into equal volumes and adjusted to ph 6 7 8 and at ph 8 deposits are shown in (Table 6).

Vitex negundo methanol extract also shown deposits at ph 8. It is observed that after standardising the ph all the samples showed formation of nanoparticles at PH 8 and were taken for further process (Table 7).

Antimicrobial Activity

This field holds significance across various domains, including medicinal chemistry. The silver nanoparticles synthesized in this study displayed notable antibacterial properties against a range of pathogens, including Escherichia coli, Klebsiella, Bacillus, and Staphylococcus aureus. The zone of inhibition caused by the silver nanoparticles at different concentrations is shown in the tables. Similarly antifungal activity was also done and silver nanoparticles showed antifungal activity given in the table along with the concentrations.

Antibacterial Activity

For antimicrobial activity Mullen Hinton agar medium was prepared and wells were made and plant samples were added to the wells and different organisms are streaked on to the plates and incubated for 24 hrs. After incubation zone of inhibition was measured and noted as shown (Figures 3–6).

Table 5. Standarization of ph of aqeous extract of *eclipta prostata*.

E.AQ	$AgNO_3$	PH	Observation	Colour
3 ml	7 ml	6	No	Yellow
3 ml	7 ml	7	No	Yellow
3 ml	7 ml	8	Yes	Black

Table 6. Standardisation of ph Methanol extract of *eclipta prostata*.

E.M	$AgNO_3$	PH	Observation	Colour
2 ml	8 ml	6	No	Yellow
2 ml	8 ml	7	No	Yellow
2 ml	8 ml	8	Yes	Black

Table 7. Standardisation of ph Methanol extract of *vitex negundo*.

V.M	$AgNO_3$	PH	Observation	Colour
3 ml	7 ml	6	No	Yellow
3 ml	7 ml	7	Yes	Brown
3 ml	7 ml	8	Yes	Black

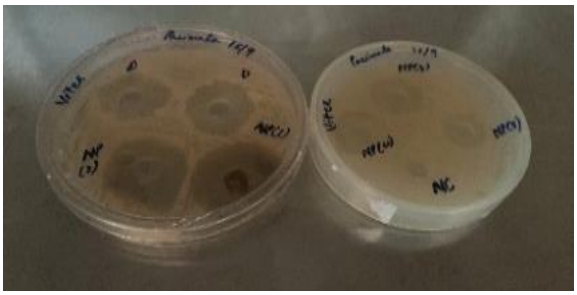

Figure 3. Antibacterial activity of vitex negunda against *Klebsiella* and e.coli.

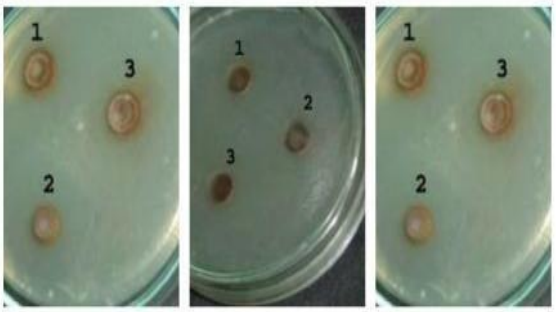

Figure 4. Antibacterial activity against bacillus

Figure 5. Antibacteral activity of *eclipta prostata* against *Klebsiella* and e.coli.

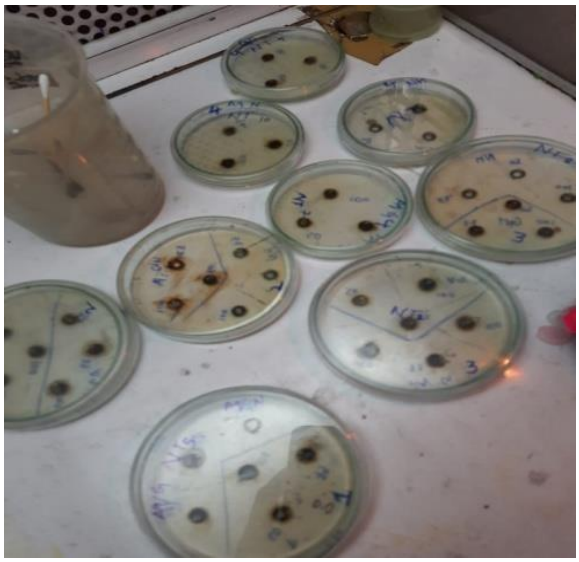

Figure 6. Staphylococcus.

Escherichia coli

In Escherichia coli *Vitex negundo* methanol extract 75 µl concentration and 100 µl concentration and also aqueous *Vitex negundo* of 100 µl concentration has shown highest inhibition of all for E coli ().

Bacillus

According to the observations shown the aqueous *Vitex negundo* at concentrations of 100 µl and Eclipta methanol extract of 100 µl also have same capability ().

Klebsiella

For klebsiella, Eclipta methanol extract showed no activity but, aqueous *Vitex negundo* shows highest inhibition of all at concentration 100 µl (Table 10).

Staphylococcus Aureus

For staphylococcus aureus the Eclipta alba has shown no activity as similar to klebsiella and aqueous *Vitex negundo* shown the highest inhibition at 100 µl concentration (Table 11). Aqueous *Vitex negundo* showed the highest inhibition for all the organisms when compared to all other samples.

Antifungal Activity

Antifungal activity was also observed for samples in which the aqueous Eclipta alba shown no activity and aqueous *Vitex negundo* shows highest activity among the other samples as shown in table (Table 12) and (Figure 7).

Stability

Stability of nanoparticles was observed by colorimetric observation. Aqueous *Vitex negundo* and Eclipta alba were standardised with PH 8 as earlier known and O.D readings were taken with time interval of 1 hr. Results shown are in the following table (Table 13 and Table 14).

Table 8. Antibacterial activity of V.M,E.M,AQ.E,AQ.V for E.Coli.

Sample	50 µl (100 mg/ml)	75µl (100 mg/ml)	100µl (100 mg/ml)
V.M	0.5 cm	1 cm	1.3 cm
E.M	0.3 cm	0.4 cm	0.6 cm
AQ.E	0.2 cm	0.5 cm	0.8 cm
AQ.V	0.6 cm	0.7 cm	1 cm

Table 9. Antibacterial activity of V.M,E.M,AQ.E,AQ.V for Bacillus.

Sample	50µl (100mg/ml)	75µl (100mg/ml)	100µl (100mg/ml)
AQ.E	0.3 cm	0.5 cm	0.7 cm
AQ.V	0.3 cm	0.5 cm	0.8 cm
V.M	0.2 cm	0.4 cm	0.5 cm
E.M	0.1 cm	0.3 cm	0.8 cm

Table 10. Antibacterial activity of V.M,E.M,AQ.E,AQ.V for Klebsiella.

Sample	50µl (100mg/ml)	75µl (100mg/ml)	100µl (100mg/ml)
AQ.E	0.1 cm	0.3 cm	0.4 cm
AQ.V	0.5 cm	0.7 cm	0.9 cm
E.M	No activity	No activity	No activity
V.M	0.3 cm	0.3 cm	0.7 cm

Table 11. Antibacterial activity of V.M,E.M,AQ.E,AQ.V for Staphylococcus Aureus.

Sample	50µl (100mg/ml)	75µl (100mg/ml)	100µl (100mg/ml)
AQ.E	0.1 cm	0.3 cm	0.4 cm
AQ.V	0.5	0.7	0.9 cm
E.M	No activity	No activity	No activity
V.M	0.3 cm	0.3 cm	0.7 cm

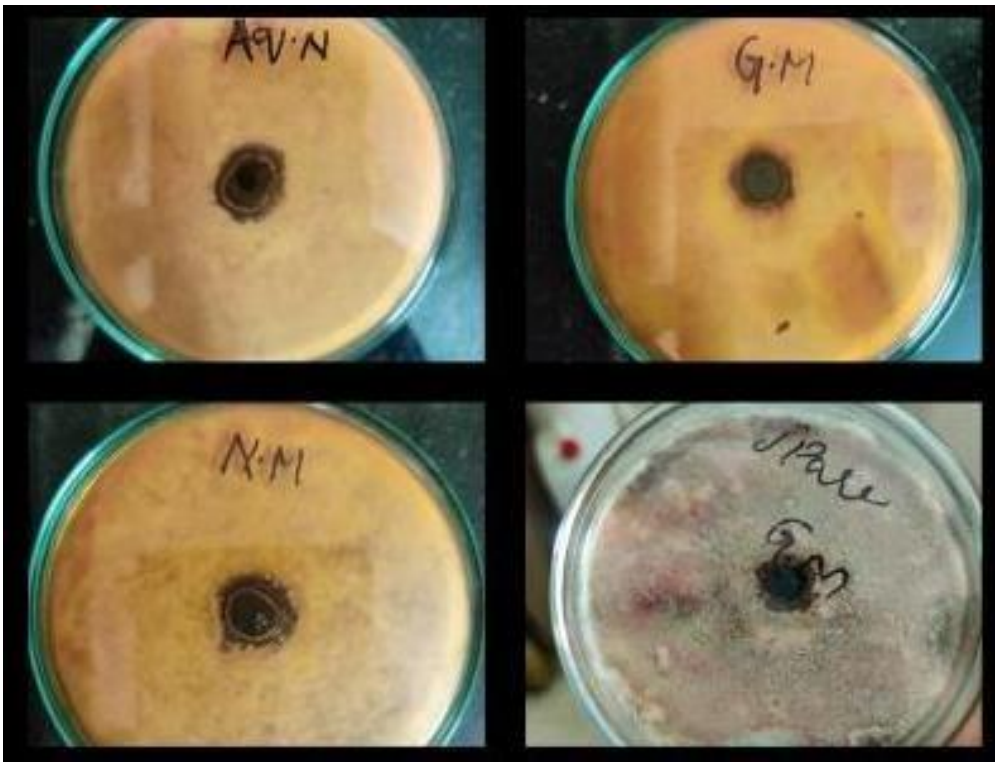

Figure 7. Antifungal activity of AQ.V,E.M,V.M,AQ.E.

Table 12. Antifungal activity of *eclipta prostata* and *vitex negundo*.

Sample	100µl(100mg/ml)
AQ.E	No activity
AQ.V	0.5 cm
E.M	0.4 cm
V.M	0.4 cm

Table 13. O.D readings of Aqueous *eclipta prostata*.

Wavelength	12:30pm	1:30pm	2:30pm	3:30pm
420 nm	1.80	1.95	1.93	1.96
490 nm	1.79	1.93	1.92	1.90
540 nm	1.59	1.96	1.86	1.76
590 nm	1.44	1.84	1.83	1.68
650 nm	1.37	1.67	1.80	1.64

Table 14. O.D readings of *Vitex negundo*.

Wavelength	12:30pm	1:30pm	2:30pm	3:30pm
420 nm	1.99	1.97	1.95	1.93
490 nm	1.98	1.92	1.93	1.92
540 nm	1.96	1.84	1.87	1.87
590 nm	1.92	1.80	1.76	1.86
650 nm	1.87	1.76	1.74	1.69

When the readings are observed the peak for both of the samples and at all times at 420 nm and decreased gradually in Figure 8, 9.

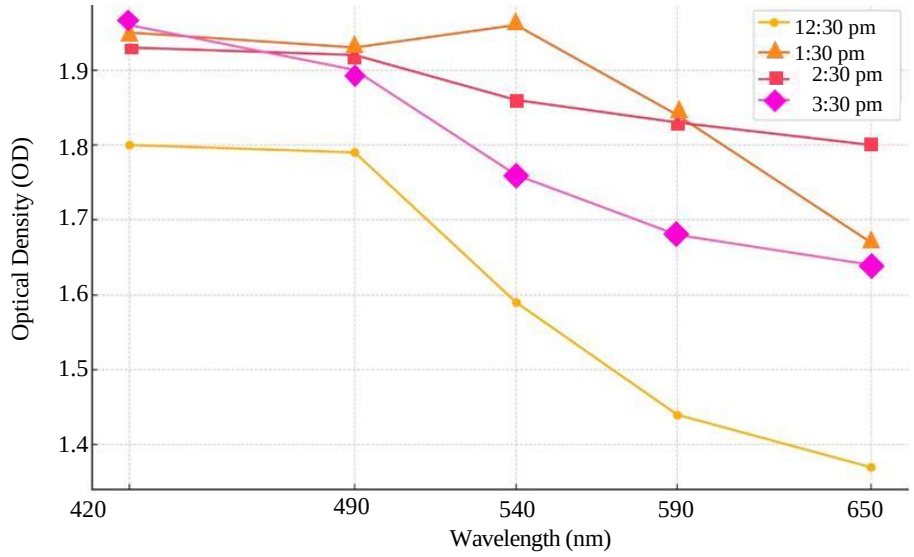

Figure 8. Optical density Vs. Wavelength at different time for eclipta alba.

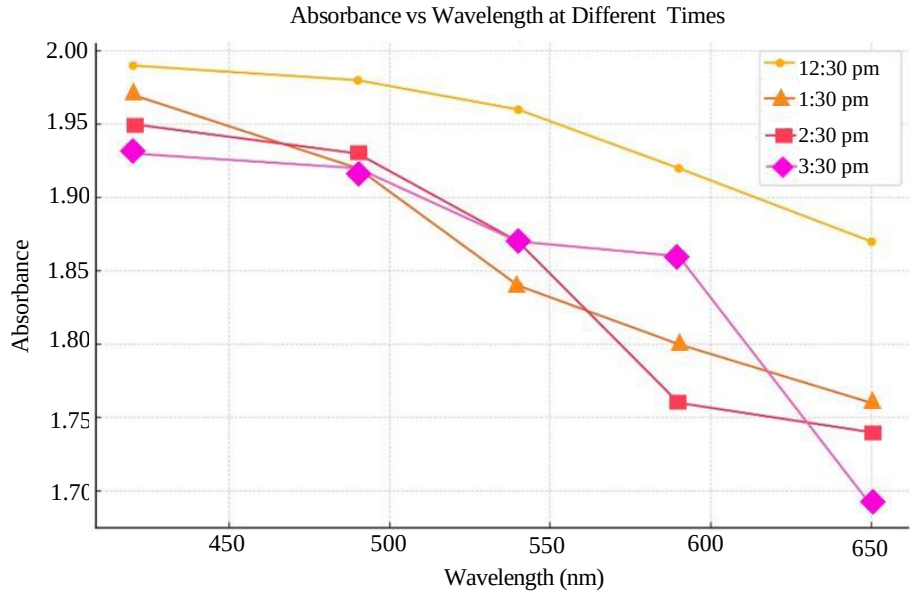

Figure 9. Optical density Vs. Wavelength at Different Times. in *vitex neguda*.

Biofilm Activity

Bio film activity is measured by taking 12 well plates and media and sample are added and are incubated and media is added alternative days and after 8 days the samples were washed and stained with gram iodine and are rinsed with alcohol and O.D readings are taken to measure the bio film inhibition activity and readings were taken at 600 nm and calculated by using the formula given below.

% of inhibition = O.D in control – O.D in treatment (100)

Results were obtained as shown below for ecoli and staphylococcus respectively.

Ecoli

From the above table *Vitex negundo* methanol extract and Eclipta alba methanol extract shown highest inhibition at the concentration of 100 µl, with an inhibition rate of 67.2% and 66.2% for E.coli. Whereas, aqueous extract of *Vitex negundo* has shown lowest inhibition of all as shown in Table 15 and Fig 10, 11.

Upper

Lower

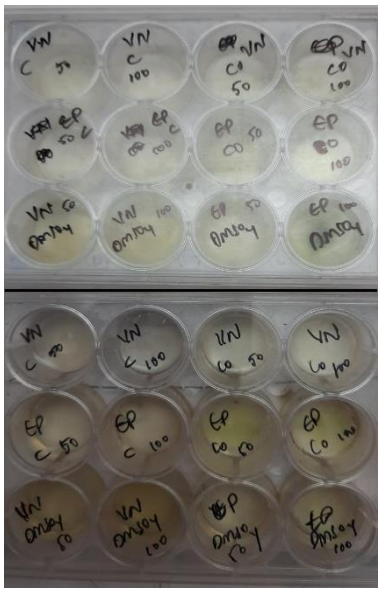

Figure 10. Upper-biofilm activity at different concentration for E.coli, lower-biofilm activity at different concentration for Staphylococcus aureous

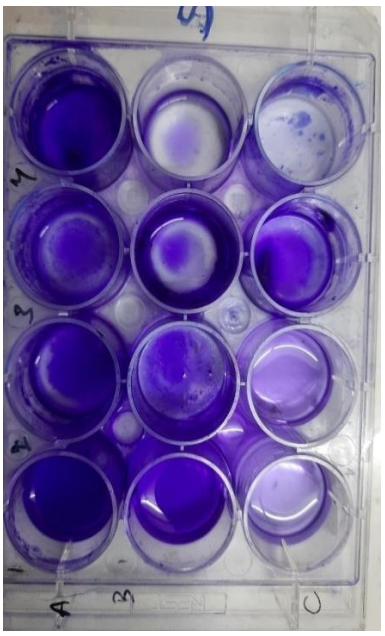

Figure 11. Staining by crystal violet for E.coli**.**

Table 15. Biofilm activity of e.coli at different concentration.

Sample	Concentration	% of inhibhition
Aq.V	50 µl	32
Aq.V	100 µl	51.6
Aq.E	50 µl	54.2
Aq.E	100 µl	68
V.M	50 µl	25.9
V.M	100 µl	67.2
E.M	50 µl	48.6
E.M	100 µl	66. 2
Ciproflaxin	10 µl	70

Table 16. Biofilm activity of Staphylococcus at different concentration.

Sample	Concentration	% of Inhibhition
AQ.V	50 µl	21.6
AQ.V	100 µl	35.2
AQ.E	50 µl	25
AQ.E	100 µl	41.3
V.M	50 µl	29
V.M	100 µl	51
E.M	50 µl	31
E.M	100 µl	58
Ciproflaxin	10 µl	40
Only Plant Extract	100 µl	25.6
Silver Nitrate	5 µl	10

Staphylococus

For staphylococcus, Eclipta methanol extract at 100 µl concentration shown highest inhibition, with an inhibition rate of 58%. Whereas Vitex methanol extract shows lowest rate of inhibition among the other samples as shown in Table 16 and Figure 10.

CONCLUSION

Utilizing plant extracts for the synthesis of silver nanoparticles embodies the principles of green chemistry, offering numerous benefits. This approach is economically viable, efficient, and environmentally friendly, promoting healthier workplaces and communities. It contributes to energy conservation and cost-effectiveness while safeguarding human health and the environment. Furthermore, it leads to reduced waste generation and the production of safer products, aligning with sustainable practices for a more sustainable future. The medicinal properties of certain plants, specifically the aqueous extracts derived from fresh leaves of *Vitex negundo* and *Eclipta prostrata*, serve as effective mediums for the production of silver (Ag) nanoparticles. The formation of these nanoparticles within the extracts was visually confirmed by the noticeable change in color. Such alterations in coloration serve as indicative evidence of the successful synthesis of Ag nanoparticles within the plant extracts.

These plant extracts play a crucial role in both the reduction and stabilization of silver, facilitating the formation of silver nanoparticles. The silver nanoparticles present within the extracts demonstrated significant inhibition of S. aureus, Bacillus, Klebsiella, and E. coli growth. Among the extracts, those containing silver nanoparticles from V. negundo exhibited the most potent antibacterial activity. Additionally, the antibacterial and antifungal efficacy of these plant extracts containing silver nanoparticles was notably higher compared to other tested formulations. Hence, the AgNps synthesized have greater anti-microbial activity. This synthesized silver nanoparticle also shows the bio film inhibition activity against the bacteria E.coli & staphylococcus.

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