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Research Article

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Plant-Based Silver Nanoparticles: A Sustainable Antimicrobial Innovation from *Symplocos racemosa* & *Nerium oleander*

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Abstract: Green synthesis provides a reliable and cost-effective way to create AgNPs, which makes it ideal for use in medicines and other medical applications. This method offers a viable alternative to conventional nanoparticle production methods by lessening the impact on the environment. "*Symplocos racemosa*" and "*Nerium oleander*" plant extracts, both methanolic as well as aqueous, had been evaluated for the presence of phytochemical ingredients in this study. In this work, a simplified process for producing silver nanoparticles (AgNPs) using extracts from these therapeutic plants and silver nitrate ($AgNO_3$) is presented. A systematic investigation was conducted utilising UV-visible spectrophotometry to analyze the resulting AgNPs. The growth rates of bacterial cells treated with powdered Ag-NPs were examined at different Ag-NPs concentrations. Consequently, it had been demonstrated that Ag-NPs significantly reduced the antibacterial action of S. aureus, E. coli, E. faecalis, as well as P. aeruginosa. These outcomes suggest that Ag-NPs could be employed as an effective antibacterial material.

Keywords: Green synthesis, *Symplocos racemosa*, *Nerium oleander*, phytochemical, silver nanoparticles, UV-visible spectrophotometry, antibacterial action.

Introduction

Pathogenic microbes' growing resistance to antimicrobial agents has become a significant problem for the healthcare industry, leading to extensive medical research into the matter. [1] The usage of silver-based antiseptics, which may have a broad spectrum of action and a far lower propensity to cause microbial resistance than antibiotics, has increased as a result of these issues and demands. [2] Bacterial plasma or cytoplasmic membrane, which is linked to several crucial enzymes and DNA, is a crucial target location for silver ions as they trigger release of K+ ions from bacteria. [3] [4] The application of botanical extracts in the creation of nanoparticles has demonstrated significant promise among several biosynthetic methods. Green synthesis has recently become a prominent and quickly improving area of nanotechnology. [5] "*Symplocos racemosa*" and "*Nerium oleander*" were chosen for this work because of their proven therapeutic benefits, which make them excellent choices for the manufacture of nanoparticles.

A well-known medicinal plant, *Symplocos racemosa* is a member of the Symplocaceae family. Although it is commonly known in English as Lodhra, it is known by a number of regional names throughout India. Flavonoids, phenolic glycosides, triterpenoids, tannins, loturine, loturidine, colloturine, linoleic acid, salireposide, and benzoylsalireposide are among the many bioactive substances it contains. [6]

Nerium oleander, sometimes known as oleander, is a well-known medicinal plant that is a member of the Apocynaceae family. Because of its many medicinal qualities, which include anti-inflammatory, antibacterial, anticancer as well as antioxidant actions, it is well known for its wide range of pharmacological uses. Bioactive substances including flavonoids, phenolic compounds, alkaloids, triterpenoids, cardiac glycosides, tannins as well as saponins are abundant in oleander. [7]

This work describes an effective and optimal process for creating nanocomposites using *Nerium oleander* and *Symplocos racemosa* plant extracts. [8] Furthermore, it investigates and assesses how well these nanocomposites suppress bacterial strains like both Gram-positive & Gram-negative bacteria. [9]

Material And Method

Materials

As shown in Figure 1, dried "*Symplocos racemosa*" bark and dried "*Nerium oleander*" leaves were meticulously procured from Batliwala, Dhan Mandi, Udaipur, a wholesale supplier. The specimens that were gathered were carefully recorded and kept at the Pharmacognosy Laboratory. Figure 2 shows comprehensive authentication procedure carried out by the Professor (Pharmcognosy).

To improve their stability and processing effectiveness, the plant materials were first allowed to naturally condition for three days at room temperature before being moved to an incubator set at 37°C for twenty-four hours. As shown in Figure 3, the dried samples were incubated and then crushed into homogeneous powders using a sterile mortar and pestle. To preserve their integrity for upcoming experimental uses, the powdered extracts were then placed in airtight jars and kept at room temperature.

(a) *(b)*

Figure 1: Pictures of dried (a) Symplocos racemosa and (b) Nerium oleander samples

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AUTHENTICATION CERTIFICATE
TO WHOMSOEVER IT MAY CONCERN

This is to certify that the following botanical species selected for Ph.D Research work of Devanshi Vaghela have been identified and authenticated by Professor Dr.Y. S. Sarangdevot, Professor in Pharmacognosy, B.N.College of Pharmacy, Udaipur, Rajasthan.

Sr. No.	Common Name	Plant part	Botanical Species	Family	Authentication Number
1.	Chakwad	Seeds	*Cassia tora*	*Fabaceae*	BNCP/24/DV-1
2.	Kuth	Stem	*Saussurea lappa*	*Asteraceae*	BNCP/24/DV-2
3.	Daruhaldi	Bark	*Berberis aristata*	*Berberidaceae*	BNCP/24/DV-3
4.	Haldi	Rhizome	*Curcuma longa*	*Zingiberaceae*	BNCP/24/DV-4
5.	Mulethi	Roots	*Glycyrrhiza glabra*	*Fabaceae*	BNCP/24/DV-5
6.	Lodh	Bark	*Symplocos racemosa*	*Symplocaceae*	BNCP/24/DV-6
7.	Chameli	Leaves	*Jasminum grandiflorum*	*Oleaceae*	BNCP/24/DV-7
8.	Kaner	Leaves	*Nerium oleander*	*Apocynaceae*	BNCP/24/DV-8

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Figure 2: Letter of authentication

(a) *(b)*

Figure 3: Sample of dried (a) Symplocos racemosa and (b) Nerium oleander powdered

Method

Preparation of botanical extracts

Aqueous Extracts

In a 250ml flask, 100ml of distilled water was mixed with 10g of precisely weighed powdered plant material. After that, the mixture had been heated for 20minutes at 80°C in a water bath to maximize extraction of bioactive components. After the solutions had cooled to room temperature, they were meticulously filtered, first through a sterile stainless-steel strainer and then through Whatman filter paper for optimal purity. To maintain their stability and effectiveness, the resultant extracts were separately collected and refrigerated at 4°C. [10] Strict sterility procedures were strictly followed during the experiment to ensure accuracy, dependability, and contamination-free processing.

Methanolic Extracts

After combining the dry powders with 30% methanol (1:10), they were incubated for 24hrs at 37°C. The methanol extracts had been first strained employing a sterile stainless-steel strainer and then filtered by means of Whatman filter paper after incubation. [11] After that, the resultant extracts were collected and kept for later use at 4°C in a refrigerator. The existence of secondary metabolites was assessed using the produced extracts.

Assessment of qualitative Phytochemicals

Botanical extracts are subjected to phytochemical analysis for identifying presence of several organic metabolites. [Table 1] Alkaloids, terpenoids, glycosides, resins, saponins, tannins, flavonoids, phenols as well as amino acids had been among phytochemicals that were screened in the methanolic and aqueous extracts. [12]

Table 1: Assessment of qualitative phytochemicals

Phytochemicals	Test Procedures
Flavanoids	When 0.1ml of a plant-derived solution is mixed with 2ml of diluted sodium hydroxide, result is a bright yellow color.
Saponins	2.5ml of distilled water and 0.5ml of natural plant extract were combined and forcefully stirred. Saponins are indicated by froth.
Tannins	1mL of plant extract and 1ml of distilled water were combined to create a solution, then a few drops of 5% ferric chloride were added. The presence of tannins in the solution is confirmed by a noticeable green precipitate.
Steroids	To create a red color in the lower layer, which indicates the presence of steroid chemicals, 0.1ml of natural plant concentrate was combined with 1 ml of chloroform, 1ml of acetic anhydride, and a few drops of strong sulfuric acid.
Quinone	5mL of herbal extract was added to 0.2ml of strong hydrochloric acid. The existence of quinones is confirmed by the color yellow.
Terpenoids	One mL of plant extract, 0.4 ml s of chloroform, and 0.2 ml s of pure sulfuric acid were combined to create the combination. Terpenoids are indicated by a reddish-brown hue.

Glycosides	0.4 ethanoic acid, 0.2 ml ferric chloride solution, and 1 ml plant extract were combined. Purified sulfuric acid (0.2 ml) was added to it. The presence of glycosides is indicated by a reddish-brown ring.

Silver nanoparticle preparations

***Symplocos racemosa* nano silver particle preparation**

In a 250ml container, a solution of silver nitrate ($AgNO_3$) with a concentration of 1mM was made. It was left at room temperature in the dark for 15minutes while being constantly swirled on a magnetic stirrer. To aid in the reduction of Ag^+ ions, aqueous extracts from *Symplocos racemosa* were applied in tiny aliquots at 1-minute intervals along with silver nitrate at a 1:5 ratio. A discernible color shift from deep yellow to bright yellow was seen when the mixture was further agitated for 20minutes at 60°C. To stabilize the process, the pH was then brought down to 9 as well as the solution was kept in a dark place for a whole day. After the incubation period was over, the solution was centrifuged for 10 minutes at 5000rpm and then washed twice in succession to guarantee purity. [13] A UV-Visible Spectrophotometer was then employed to evaluate the resultant silver nanoparticles (AgNPs), recording absorbance in the 250–700 nm range.

***Nerium oleander* nano silver particle preparation**

To guarantee even mixing, a 250ml container containing a 1mM silver nitrate ($AgNO_3$) solution was set on a magnetic stirrer and constantly swirled for 10minutes at room temperature. Aqueous plant extract as well as silver nitrate were applied in modest increments at 1minute intervals under controlled circumstances to aid in reduction of Ag^+ ions. The ratio was 1:5. After stirring the reaction mixture for a further 20 minutes at 60°C, a noticeable color shift from dark-yellow to light-yellow was seen, signifying that the silver nanoparticles had successfully formed. [14] The pH was brought down to 10 to stabilise the solution, and the combination was kept in a dark environment for a whole day to maximise the production of nanoparticles.

To eliminate contaminants and improve the quality of nanoparticles, solution had been centrifuged for 10minutes at 5000rpm after incubation. This was followed by two rounds of washing. After that, the produced silver nanoparticles (AgNPs) underwent UV-visible spectrophotometric examination as well as absorbance readings in the 250–700 nm wavelength range were noted.

UV-visible spectral analysis

Using the Systronics 199 Dual Beam Spectrophotometer, a UV-Vis spectrophotometric examination had been conducted to investigate produced silver nanoparticles in the wavelength range of 200-1000nm. After being combined with distilled water as a solvent, the green-synthesised silver nanoparticles from *Symplocos racemosa* were put in a Quartz cuvette (10mm) for spectrum measurement, and the outcomes were noted appropriately. [15] Similar steps were taken to create silver nanoparticles from *Nerium oleander* using distilled water as a solvent in a 10 mm Quartz cuvette, and their spectral data were recorded.

Nano silver particles' antibacterial action

Using 1.5% Mueller-Hinton Agar, antibacterial activity of silver nanoparticles was evaluated. [16] “Pseudomonas aeruginosa”, “Escherichia coli”, “Enterococcus faecalis”, and “Staphylococcus aureus” overnight cultures were made at a 1:20 dilution and equally distributed onto agar plates. Silver nanoparticles (5,10,15 well as 20µL) synthesized from *Symplocos racemosa* and *Nerium oleander* were carefully added to individual wells after precise 6 mm wells were made using a cork borer. For 12 to 24 hours, the plates had been incubated at 37°C, which allowed the bacterial strains and nanoparticles to sufficiently interact. [17] Using the zone of inhibition approach, antibacterial activity of silver nanoparticles (AgNPs) made from *Nerium oleander* and *Symplocos racemosa* was methodically investigated.

Results And Discussions

Assessment of qualitative Phytochemicals

The findings showed that both extracts contained active ingredients. The table indicates that flavanoids, quinone, and glycosides are absent from *Symplocos racemosa* methanol and aqueous extracts, saponins, steroids, tannins, and

terpenoids are present. Likewise, *Nerium oleander*'s aqueous extract shows the absence of saponins and terpenoids but the presence of flavanoids, steroids, tannins, quinone, and glycosides. *Nerium oleander*'s methanolic extract shows that flavanoids, steroids, tannins, and quinone are present but saponins, terpenoids, and glycosides are absent.

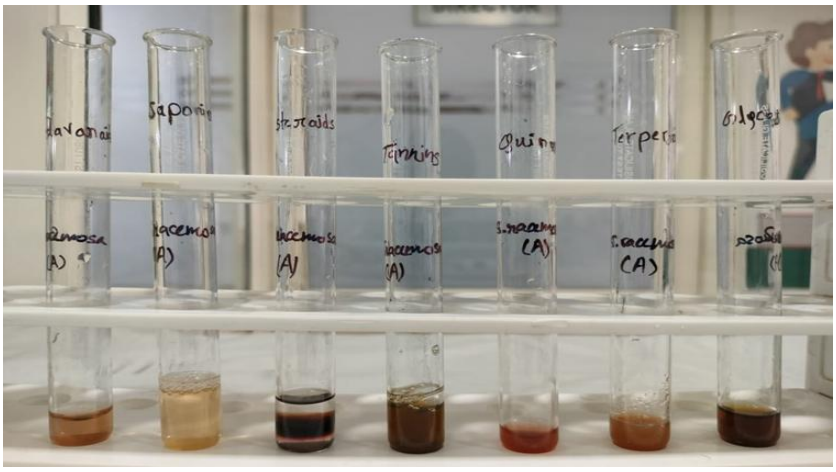


Figure 4: Qualitative phytochemical results from an aqueous plant extract of Symplocos racemosa

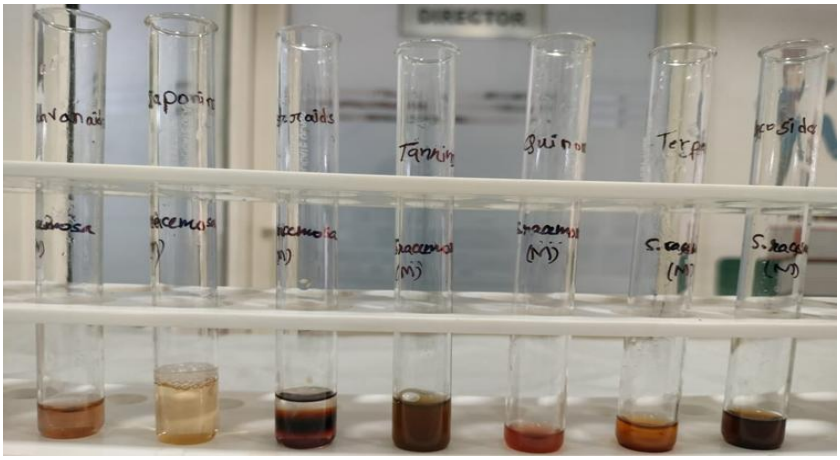


Figure 5: Qualitative phytochemical results from an methanolic plant extract of Symplocos racemosa

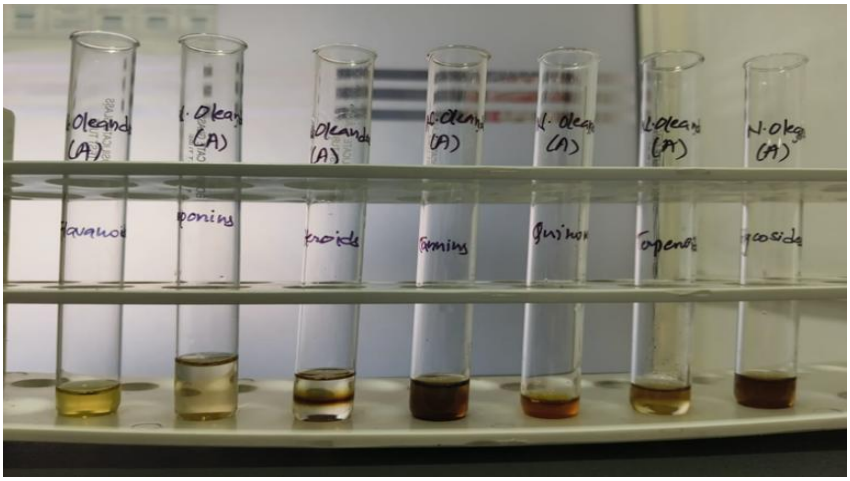


Figure 6: Qualitative phytochemical results from an aqueous plant extract of Nerium oleander

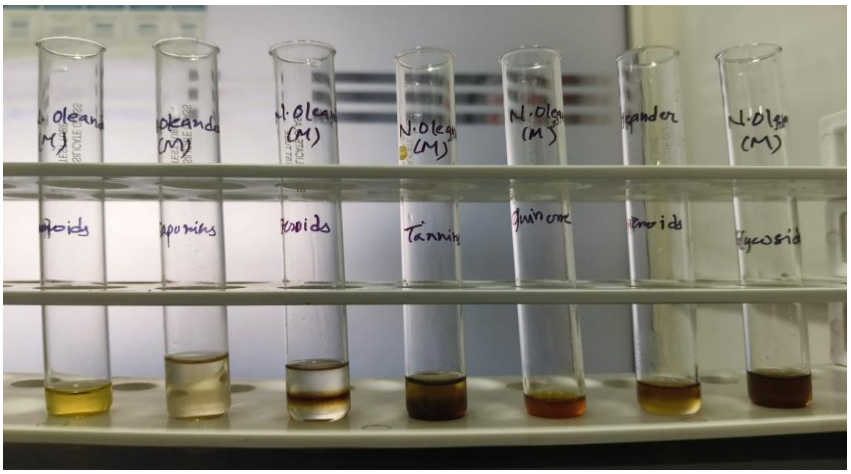


Figure 7: Qualitative phytochemical results from an methanolic plant extract of Nerium oleander

Table 2: Results of Secondary Metabolite test of *Symplocos racemosa*

Secondary Metabolites Test Components	Results	
	Aqueous Extract	**Methanolic Extract**
Flavanoids	Negative	Negative
Quinone	Negative	Negative
Glycosides	Negative	Negative
Saponins	Positive	Positive
Tannins	Positive	Positive
Steroids	Positive	Positive
Terpenoids	Positive	Positive”

Table 3: Results of Secondary Metabolite test of *Nerium oleander*

Secondary Metabolites Test Components	Results	
	Aqueous Extract	**Methanolic Extract**
Saponins	Negative	Negative
Terpenoids	Negative	Negative
Glycosides	Positive	Negative
Flavanoids	Positive	Positive
Tannins	Positive	Positive
Steroids	Positive	Positive
Quinone	Positive	Positive”

Silver nanoparticle preparations

Symplocos racemosa: The fluid was noticeably changed by the reaction, which produced silver nanoparticles (AgNPs). The solution had a discernible color change, turning from deep yellow to brilliant yellow. This modification confirmed that the extract and $AgNO_3$ had completed their reaction.

Figure 8: Symplocos racemosa nano silver particles

Nerium oleander: The dark-yellow solution turns light-yellow as a result of AgNP production, as shown in the figure below. The observed hue shift verifies that the extract and $AgNO_3$ have finished reacting.

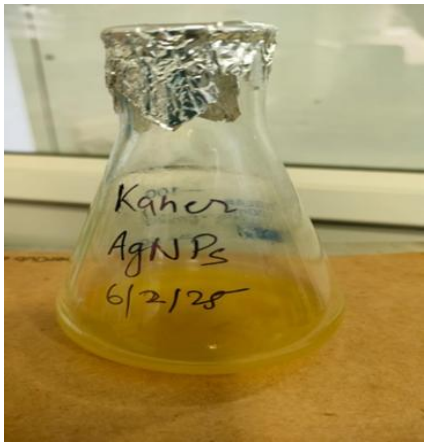


Figure 9: Nerium oleander nano silver particles

UV-Visible spectral analysis

Green-synthesised silver nanoparticles floating in distilled water were placed in a 10 mm quartz cuvette, and scanning was carried out between 240 and 700 nm in wavelength, as shown in Figures 10 and 11.

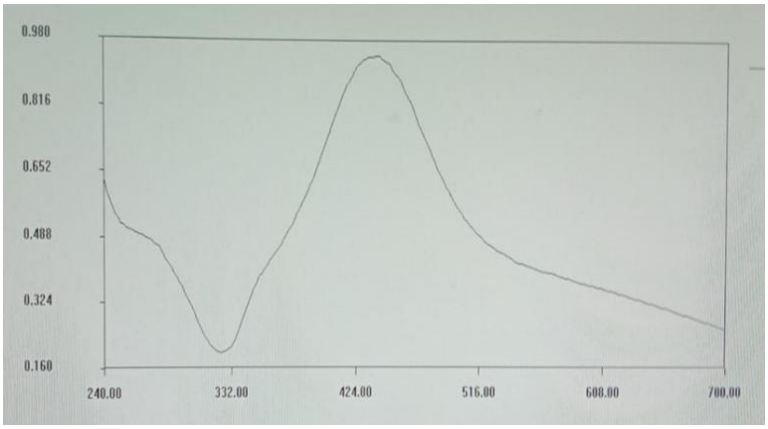


Figure 10: Characterization of Symplocos racemosa silver nanoparticles using a UV-visible spectrophotometer

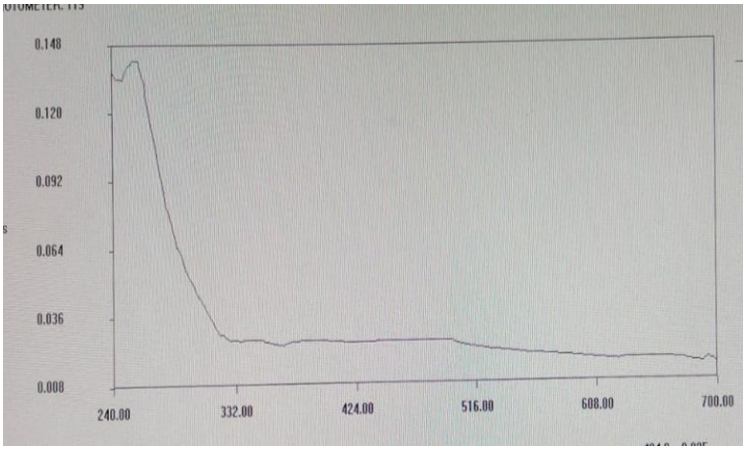


Figure 11: Characterization of Nerium oleander silver nanoparticles using a UV-visible spectrophotometer

Nano silver particles' antibacterial action

The zone of inhibition for *Nerium oleander* nano silver particles and *Symplocos racemosa* nano silver particles is depicted in the graphs Figures 12 and 14 respectively. It was discovered that 20 µL was the highest inhibitory concentration of *Symplocos racemosa* nano silver particles. For Nerium, at different doses, bacteriostatic potential against every pathogen was noted.

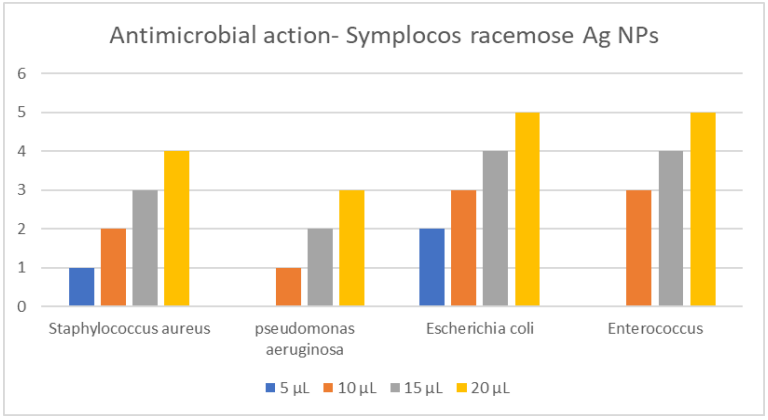


Figure 12: The proportion of the zone of inhibition against common microbial infections is graphically represented

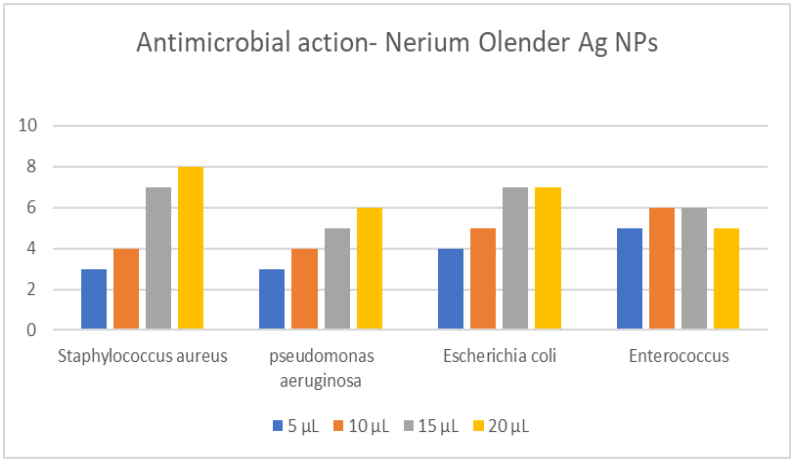
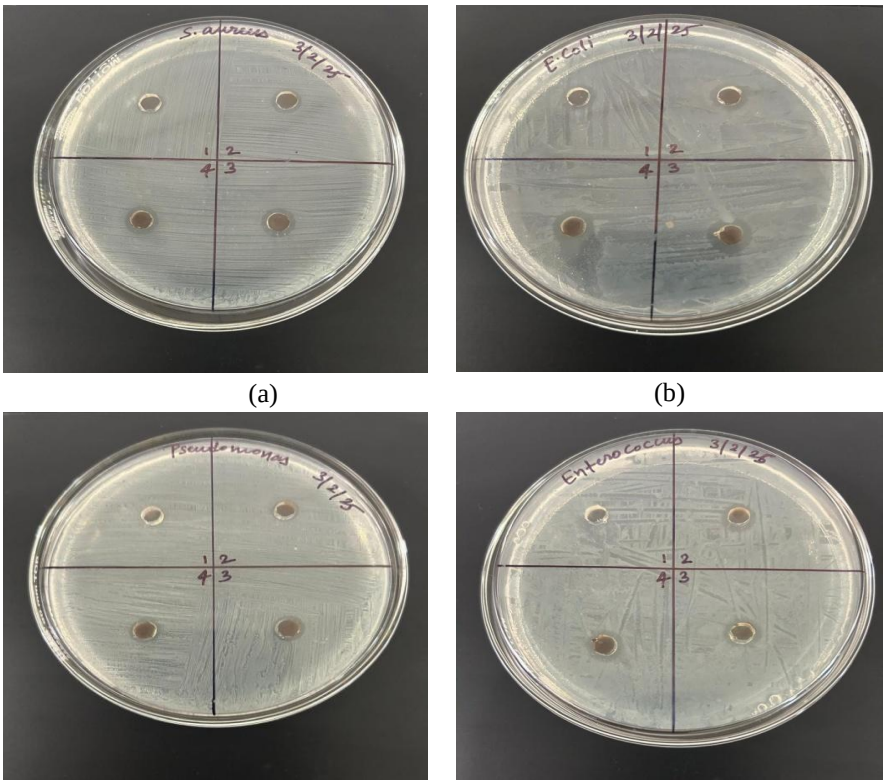


Figure 13: The proportion of the zone of inhibition against common microbial infections is graphically represented



(a) (b)

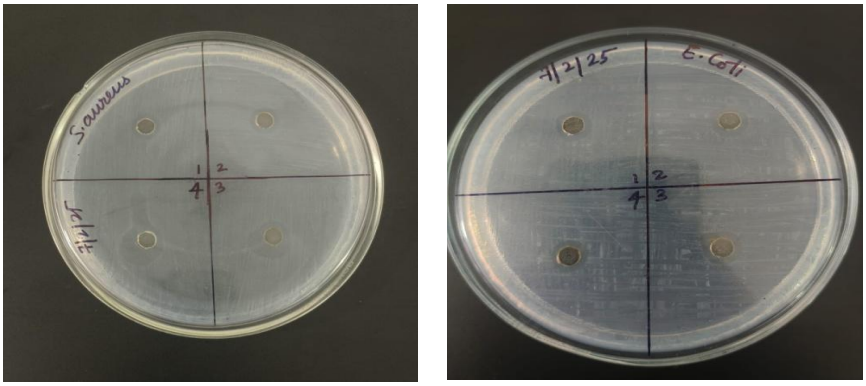
(c) (d)

Figure 14: shows the zone of inhibition for Symplocos racemosa silver nanoparticles evaluated against (a) Staphylococcus Aureus (b) Escherichia coli (c) Pseudomonas Aeruginosa (d) Enterococcus

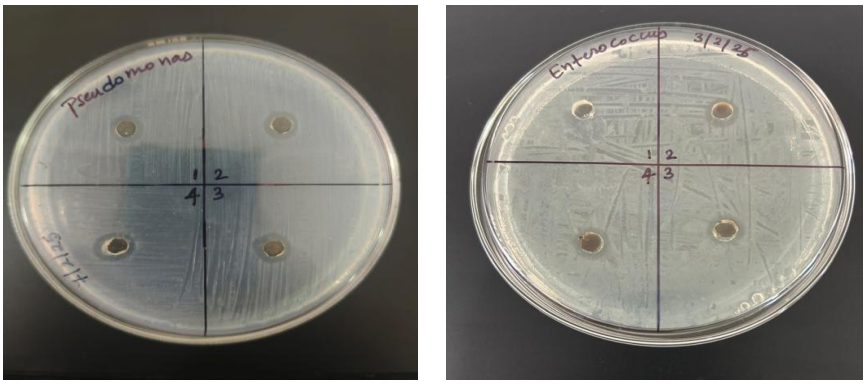
Table 4: Antimicrobial action of *Symplocos racemosa* Nano silver particles

Organism	**Nano silver particles of *Symplocos racemosa***			
	5 µL	**10 µL**	**15 µL**	**20 µL**
Staphylococcus aureus	1 mm	2 mm	3 mm	4 mm
pseudomonas aeruginosa	0 mm	1 mm	2 mm	3 mm
Escherichia coli	2 mm	3 mm	4 mm	5 mm
Enterococcus	0 mm	3 mm	4 mm	5 mm

For *Nerium oleander*:



(a) (b)



(c) (d)

Figure 15: shows the zone of inhibition for Nerium oleander nano silver particles evaluated against (a) Staphylococcus Aureus (b) Escherichia coli (c) Pseudomonas Aeruginosa (d) Enterococcus

Table 5: Antimicrobial action of *Nerium oleander* Nano silver particles

Organism	**Nano silver particles of *Nerium oleander***			
	5 µL	**10 µL**	**15 µL**	**20 µL**
Staphylococcus aureus	3 mm	4 mm	7 mm	8 mm
pseudomonas aeruginosa	3 mm	4 mm	5 mm	6 mm
Escherichia coli	4 mm	5 mm	7 mm	7 mm
Enterococcus	5 mm	6 mm	6 mm	8 mm

Conclusion

All things considered; our results show that plant extracts make it easier to create functional silver nanoparticles. When applied to a wide range of pathogens, these nanoparticles exhibit potent antibacterial qualities. At greater concentrations, the inhibitory effect of *Symplocos racemosa* silver nanoparticles on both Gram-positive & negative bacteria was more noticeable. Silver nanoparticles made from *Nerium oleander* shown a notable inhibition of the growth of both Gram-positive & Gram-negative bacteria. The study demonstrates the effectiveness of *Nerium oleander* and *Symplocos racemosa* silver nanoparticles against broad spectrum pathogens. Consequently, it encourages the use of extract of the *Symplocos racemosa* and *Nerium oleander* plant as a natural remedy for bacterial infections.

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