Comparative Evaluation of Antimicrobial Efficacy of AgNPs synthesized from leaf extracts of *Laurus nobilis* and *Persea americana*

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Abstract

Silver nanoparticles (AgNPs) possess a broad spectrum of antibacterial, antifungal and antiviral properties. Silver nanoparticles have the ability to penetrate bacterial cell walls, changing the structure of cell membranes and even resulting in cell death. Their efficacy is due not only to their nanoscale size but also to their large ratio of surface area to volume. They can increase the permeability of cell membranes, produce reactive oxygen species, and interrupt replication of deoxyribonucleic acid by releasing silver ions. In this investigation,we have synthesized nanoparticles from the leaf extracts of *Laurus nobilis* and *Persea americana* and characterize the final product, and evaluated their antimicrobial activities.Both the leaf extracts were found to be effective against the *Escherchia coli* , *Staphylococcus aureus, Bacillus aureus* and *Pseudomonas aeruginosa*

Key Words

Silver nanoparticles, *Laurus nobilis, Persea americana*,antimicrobial activity

Introduction

Silver nanoparticles are one of the promising products in the nanotechnology industry. AgNPs can be biosynthesized by bacteria, fungi, yeast, actinomycetes and plant, thus avoiding the use of toxic substances and enabling for further application in medical and pharmaceutical industries[1].Plants contain a wide range of metabolites that can aid in reducing silver ion, stabilizing and capping AgNPs[2], therefore the concentration and composition of AgNPs will vary depending on the plant type[3]. this is particularly the medicinal plant's case, given that it is a rich source of phytochemicals and antioxidants. The Polyphenols, carotenoids, and vitamins are the principal antioxidant components of medicinal plants. the curative plants.The medicinal plants display a wide range of anti-inflammatory, antibacterial, antiviral, anti-aging, and anti-cancer activities[4] The intricate biomolecules in medicinal plants help to reduce metal ions and nanoparticle stabilisation into the correct form and size[5]. The production of AgNPs by plants involves the process of reduction. It is quite easy to do because all that is needed is plant extract and silver salt, and then it is reduced.[6]. It is known that nanoparticles have a large surface area that either penetrates the cell or attaches itself to the cell wall[7] generating a change in the membrane's permeability that makes it porous[7], which results in further cell content leakage.[8,9,10,11] Moreover, the appearance of pores on membrane result to diffusion of nanoparticles into the cell where it binds with sulfur and phosphorus-containing proteins, thus leading to the inactivation of proteins and DNA[12]. Another theory intends that the release of Ag+ ions during the oxidation dissolution process is what causes AgNPs' antibacterial action. The main interaction between the oxidised silver ions from AgNPs and the thiol groups of several enzymes and proteins is what interferes with the respiratory chain and damages the bacterial cell wall. Additionally, the formation of reactive oxygen species (ROS), which is thought to be the primary factor in the majority of cell deaths via the through the inactivation of DNA replication and production of ATP[13].The present study is aimed to synthesize silver nanoparticles with leaf extracts of *Laurus nobilis* and *Persea americana* and characterize the final product, and evaluate their antimicrobial activities

Materials and Methods

Reagents and Materials

Silver nitrate (Merck) and all other chemicals purchased were of high purity grade. All solvents used were of analytical grade. Double distilled water was used for the preparation of stock solutions.

Preparation of leaf extract of *Laurus nobilis*

Freshly plucked leaves of *Laurus nobilis* are taken and crushed using a blender and water is added and again blended. This extract is then filtered. The leaf extract is taken in a beaker and heated up to 20 minutes and cooled. Again the extract is filtered using a Whatman paper No.1.

Preparation of leaf extract of *Persea americana*

Freshly plucked leaves of *Persea americana* are taken and crushed using a blender and water is added and again blended. This extract is then filtered. The leaf extract is taken in a beaker and heated up to 20 minutes and cooled. Again the extract is filtered using a Whatman filter paper No.1.

Synthesis of silver nanoparticles from *Laurus nobilis*

Aqueous solution of silver nitrate (1 mM) was prepared and mixed with fresh plant extract of *Laurus nobilis* at a ratio of 9:1. This solution was placed on a stirrer with a magnetic pellet in the room temperature at 27 ± 2°C for 30 minutes. Silver ions are reduced to silver metal of nano dimensional range. During reduction process the temperature was kept at 30-35º C.

Synthesis of silver nanoparticles from *Persea americana*

Aqueous solution of silver nitrate (1 mM) was prepared and mixed with fresh plant extract of *Persea americana* at a ratio of 9:1. This solution was placed on a stirrer with a magnetic pellet in the room temperature at 27 ± 2°C for 30 minutes. Silver ions are reduced to silver metal of nano dimensional range. During reduction process the temperature was kept at 30-35º C.

Sample Characterization

The UV-visible spectra were measured on a SHIMADZU UV-1800 spectrophotometer operating in the range of 200-1100 nm. X-ray diffraction measurements were carried out using BRUKER D2 PHASER II Generation instrument with CuKα radiation in θ-2θ configuration. All measurements were performed at room temperature.

Antimicrobial Analysis

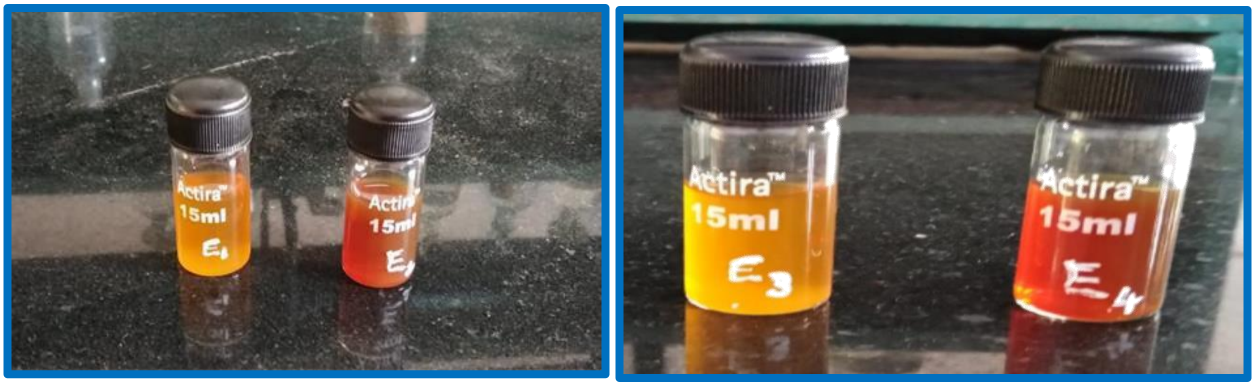
For the evaluation of antibacterial activity five bacterial strains were selected namely *Escherichia coli*, *Pseudomonas aeruginos*a, *Bacillus aureus* and *Staphylococcus aureus*.

Antibacterial Activity was studied using The Kirby-Bauer Method

Results and Discussion

Color change of solution

In this study, the plant extracts of *Laurus nobilis* and *Persea americana* are used for considering their potential for reduction of Ag^+ ions. The fresh extract of *Laurus nobilis* was yellow in color, but after addition of $AgNO_3$ solution and stirring at room temperature, gradually the solution color changed into brown.In other words, by passing the incubation time, the color intensity increased, which confirmed Ag ion reduction and the formation of AgNPs.



(a) **(b)**

Fig.1 **Colour change in the (a)*Laurus nobilis* and (b) *Persea americana* extract after adding AgNO3 solution**

The fresh extract of *Persea americana* was yellow in color, but after addition of AgNO3 solution and stirring at room temperature, gradually the solution color changed into red.In other words, by passing the incubation time, the color intensity increased, which confirmed Ag ion reduction and the formation of AgNPs.

Fig. 2 Colour change indicating the formation of nanoparticles in the extracts after 24 h

Silver nanoparticle surface plasmon excitation causes color change in the solution which is the primary and notable evidence for the formation of AgNPs.

UV-visible spectroscopic studies

Nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state and refractive index near the nanoparticle surface which makes UV-visible spectroscopy for identification structural characterization of AgNPs. The absorption spectrum of silver nitrate is indicated in Fig.3 The absorption spectrum of *Laurus nobilis* extract shows a peak at 270 nm as indicated in Fig.4. From the Fig.4(b) the absorption spectrum of the pale yellow – brown silver colloid prepared by *Laurus nobilis* leaf extract reduction showed a Surface Plasmon absorption band with a maximum of 450 nm indicating the presence of spherical or roughly spherical AgNPs. The absorption spectrum of *Persea americana* extract shows a peak at 315 nm as indicated in Fig. 5(a) The absorption spectrum of synthesized AgNPs from *Persea americana* extract is depicted in Fig.5(b) shows a peak at 434 nm.

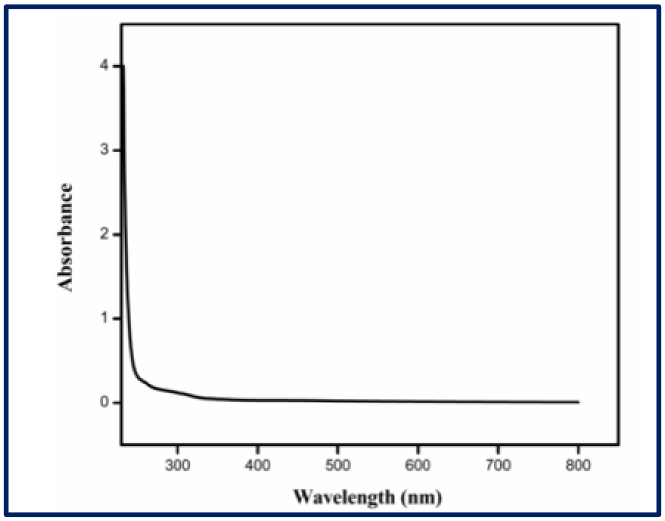


Fig.3. Absorption spectrum of silver nitrate

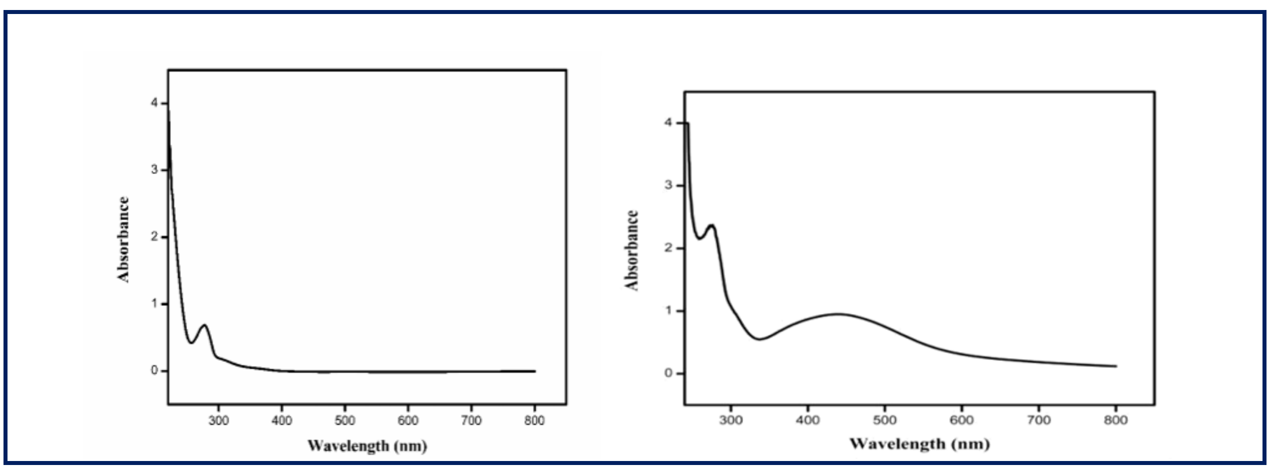


Fig.4. Absorption spectrum of4(a) *Laurus nobilis* extract and 4(b)AgNPs synthesized from *Laurus nobilis* extract

In metal nanoparticle such as silver, the conduction band and valence band lie very close to each other. The reaction mixture showed colour change from yellowish brown to reddish brown which indicated the formation of AgNPs. The absorption peak obtained in the visible range 420 nm wavelength is a clear evidence of formation of AgNPs from the silver nitrate solution. The frequency and width of the Surface Plasmon absorption are dependent on the size and shape of the metal nanoparticles as well as the dielectric constant of metal itself and the surrounding medium. Broad bell-shaped spectrum curve was obtained from UV–Vis spectral analysis.

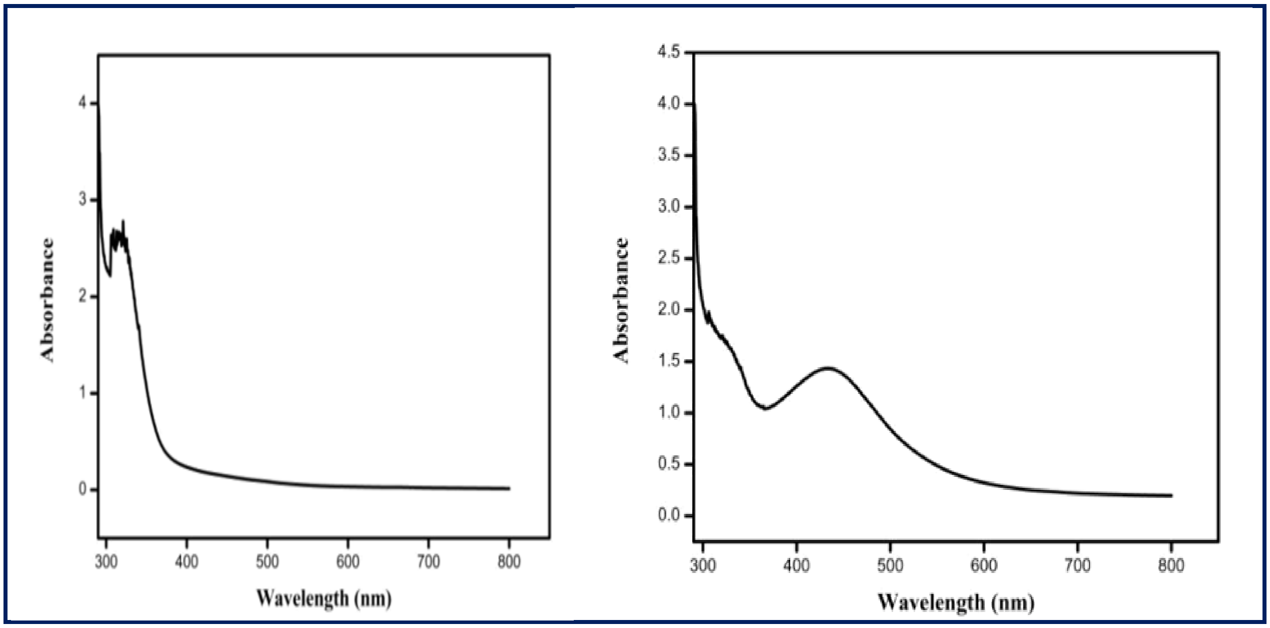


Fig.5 Absorption spectrum of 5(a) *Persea americana* extract and 5(b)AgNPs synthesized from *Persea americana* extract

Various metabolites from plant extract introduced to solution make the plasmon band broad because they may be read in this spectrophotometric range, too. Surface plasmon resonance (SPR) of silver occurs at 450 nm. This peak increased with time up to 360 min. According to Mie theory, spherical nanoparticles show only a single SPR band. The number of peaks increases by increasing diversity of particles shapes. Thus, it can be concluded that the synthesized AgNPs are unanimously spherical in nature.

X-ray Diffraction Analysis

The XRD pattern of the prepared AgNPs from the leaf extracts of *Laurus nobilis* and *Persea americana*.are shown in Fig6 and 7 shows The peaks at 2θ=38.215º, 44.420º, 64.594º, 38.184º, 44.321º, 64.520º, 64.427º, 77.497º, reveal that it is a face centre cubic (FCC) structure. The discernible peaks can be indexed to (100), (111), (200), (220) planes of a cubic unit cell, which corresponds to cubic structure of silver (JCPDS card No.89-3722).

Crystallite size calculation was done using Debye-Scherrer formula ,2dsin θ =n λ

where 'λ' is wave length of X-ray (0.15406 nm), 'β' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter size.

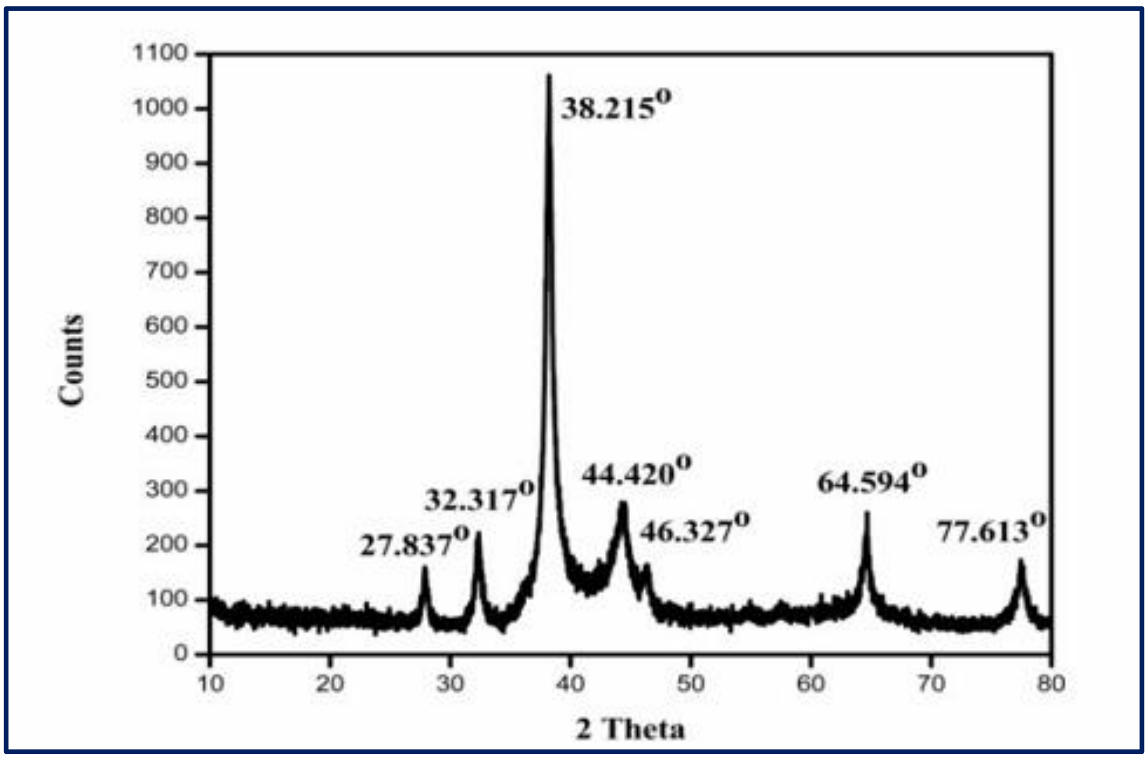


Fig.6 XRD profile of synthesized AgNPs from the leaf extracts of *Laurus nobilis*

Table 1. XRD data of AgNPs synthesized from *Laurus nobilis* extract

2θ	d	$1000/d^2$	$(1000/d^2)/54.23$	hkl
38.215	2.35	180.60	3	111
44.420	2.04	240.78	4	220
64.594	1.44	481.23	9	221

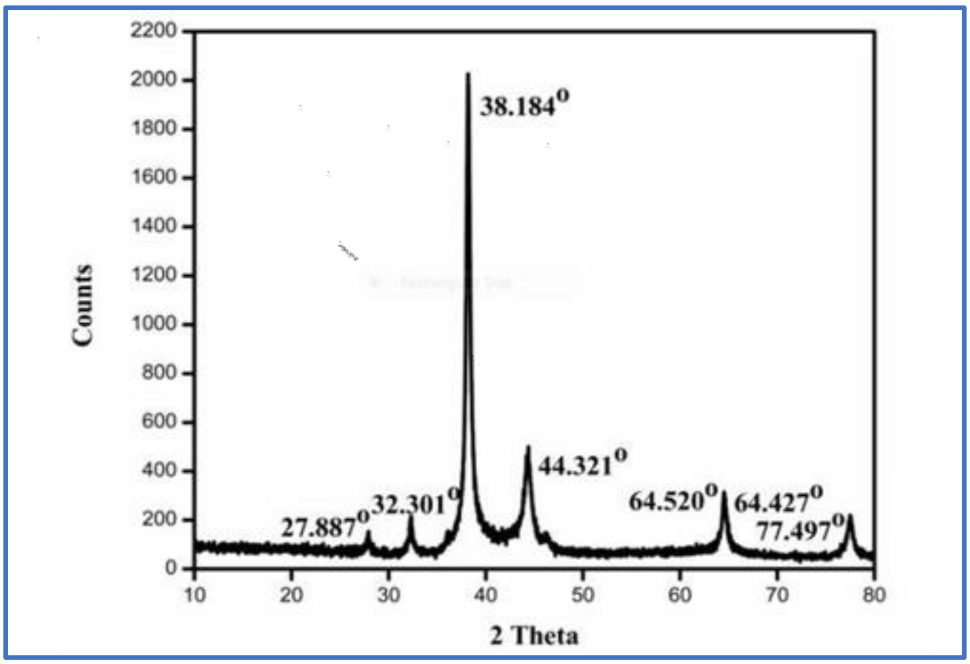


Fig7 XRD profile of synthesized AgNPs from the leaf extracts of *Persea americana*

Table .2 XRD data of AgNPs synthesized from *Persea americana* extract

2θ	**d**	**$1000/d^2$**	**$(1000/d^2)/57.78$**	**hkl**
38.184	2.35	180.31	3.1	111
44.321	2.04	239.80	4.1	200
64.520	1.44	480.30	8.3	220
64.427	1.44	478.92	8.2	220
77.497	1.23	660.50	11.4	311

Antibacterial Activity Analysis

In the present investigation the antibacterial effect of synthesized AgNPs is studied on different types of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa, Bacillus aureus* and *Staphylococcus aureus.*The antibacterial activities of the AgNPs synthesized from leaf extracts of both *Laurus nobilis* and *Persea americana* are tabulated in Table.3

Table .3 Zone of Inhibition exhibited by AgNPS Synthesized from leaf extracts

Bacteria	**Sample antimicrobial activity (mm)**		**Positive Control (Amikacin) (mm)**
	Laurus nobilis	***Persea americana***	
Escherichia coli	15	14	20
Pseudomonas aeruginosa	10	12	23
Bacillus cereus	11	13	28
Staphylococcus aureus	12	10	24

Antibacterial Activity by Disc Diffusion Method

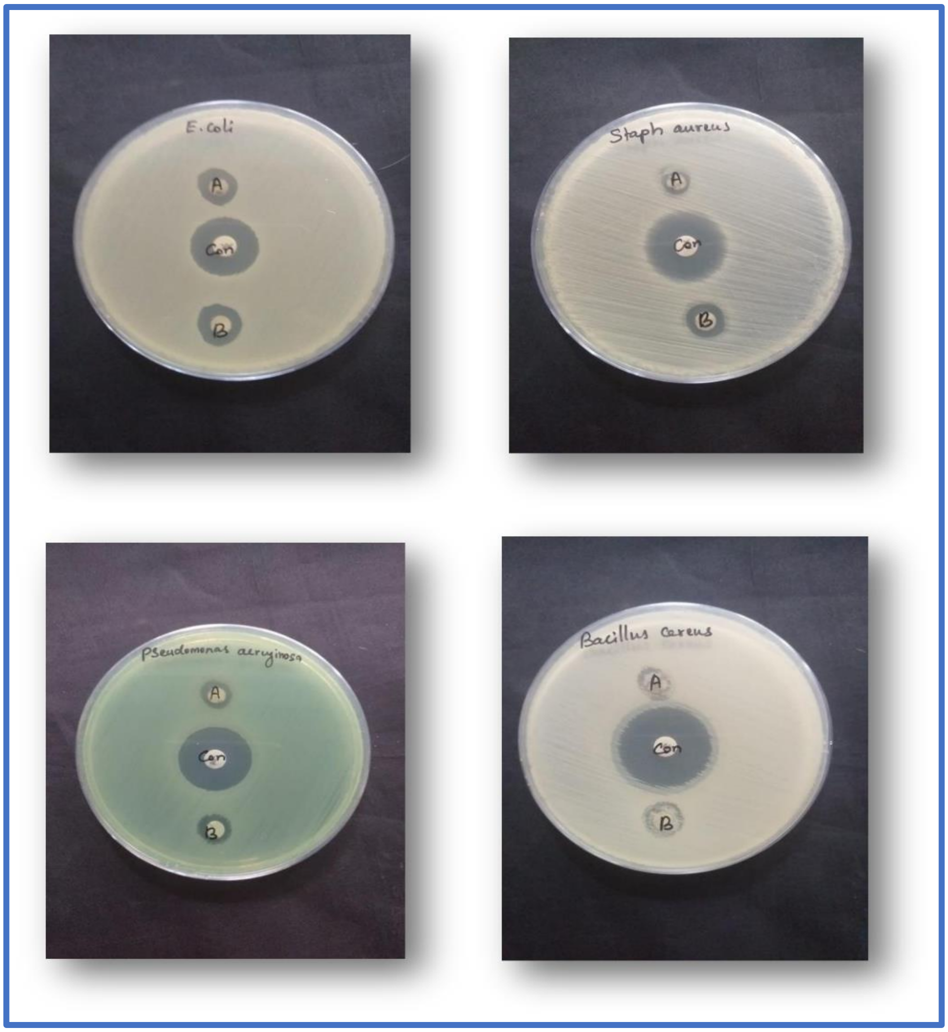


Fig.8 Zone of inhibition exhibited by AgNPs synthesized from plant extracts(A)*Persea americana* and (B)*Laurus nobilis*

Both the leaf extracts of *Laurus nobilis* and *Persea americana* have exhibited high degree of antibacterial potential against *E. coli*.

The order of antibacterial activity for *Laurus nobilis* is

Escherchia coli > *Staphylococcus aureus*> *Bacillus aureus*> *Pseudomonas aeruginosa*

The order of antibacterial activity for *Persea americana* is

Escherchia coli > *Bacillus aureus* > *Pseudomonas aeruginosa* > *Staphylococcus aureus*

Conclusion

The AgNPs synthesized from both the leaf extracts have high antibacterial potential against all the four strains. The synthesis of silver nanoparticles using *Laurus nobilis* and *Persea americana* leaves extract provides environmentally friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles from *Laurus nobilis* were of spherical and estimated sizes were 27-80nm. The synthesized nanoparticles from *Persea americana* were of spherical and estimated sizes were 20-50 nm surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-Vis spectrophotometer and XRD techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance whereas the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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