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Article

Green Synthesis of Silver Nanoparticles from Various Medicinal Plants

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Abstract: Chemical solvents are commonly used to prevent microbial growth; dangerous to human health and have limited antibacterial properties. On the other hand, Nanoparticles made of metallic elements (such as copper, silver, and gold) have several uses in the field of biotechnology**.** Silver nanoparticles are more efficient in their antimicrobial, antibacterial, anti-inflammatory and anti-cancer properties. The current study aimed to determine the green synthesis of silver nanoparticles and their antibacterial activity from the aqueous extracts of leaves of *Couroupita guianensis, Punica granatum, Vitex negundo, Cirtrus maxima.* AgNPs of plant extracts were prepared using silver nitrate with the respective plant extract. Then they were characterized by FTIR analysis. The respective functional groups in the synthesized silver nanoparticles were confirmed with FTIR Spectra. The antibacterial activities of the synthesized nanoparticle extract were observed by zone of inhibition. From the results, the nanoparticles synthesized from the plants extract could pave a way to formulate a drug to treat microbial infection.

Keywords: Silver nanoparticles, Anti-bacterial activity, Leaves extracts, Zone of inhibition

1. Introduction

The use of medicinal plants is safe, as there are no or very few adverse effects. The major advantage of these medicines is that they are in tune with nature. Medicinal plants are said to be a rich source of components that are used to make pharmacopoeial, non-pharmacopoeial, or synthetic medications. Apart from that, these plants have played an important role in the evolution of human cultures all across the world. Therefore, some have vital sources of nourishment, and as a result, they are suggested for their medicinal properties. [1]

Nanotechnology includes the creation, characterization, and manipulation of components with a length of 1 to 100 nanometers in one dimension. When particle size is reduced below to this dimension, the chemical and physical properties of the resulting materials appear to be very different from large scale components. Silver is a precious metal that occurs in nature, and most commonly used as a mineral ore in combination with other elements. Because of its unique features (high electrical and thermal conductivity), it has been used in a wide range of applications include antibacterial agents, pharmaceutical and food industries, in diagnostics, orthopaedics, drug delivery, and also the anticancer agents, which has the tumor-killing effects of anticancer drugs. [2]

Punica granatum commonly known as pomegranate, is a fruit-bearing shrub of the Lythraceae family, subfamily Punicoideae that grows to a height of 5 to 10 meters (16 to 33 feet). They are grown abundantly in the Middle East and also commonly found in the Caucasus, north and tropical Africa, Iran, the Indian subcontinent, Central Asia, Southeast Asia's drier regions, and the Mediterranean Basin. They are rich in antioxidants and antimicrobial agents. [6,7]

Vitex negundo (Chinese chaste tree) commonly called the five-leaved chaste tree, is a big fragrant shrub with quadrangular, thickly white, tomentose branchlets. This plant is used in a traditional medicine, especially in South and Southeast Asia. It has a wide range of beneficial plant-based substances with antioxidant and anti-inflammatory properties. Flavonoids and phenols, have cardio-protective characteristics for heart health, while terpenoids and organic fatty acids have relaxing and analgesic properties to reduce mental stress, joint pain, and muscular pains. [2]

Couroupita guianensis, a family of Lecythidaceae with significant therapeutic potential. It's also known as Ayahuma, Kailaspati, Nagalingam, and other names. The flowers have taken on religious importance and are donated to Buddhist temples and shrines. This plant have been used in treatment for variety of diseases, including cold, gas in the intestines, and stomach aches. Antioxidant, anthelmintic, immunological modulator, and antinociceptive activities have all been discovered in *Couroupita guianensis* leaves. [3]

The Pomelo, scientifically known as *Citrus maxima* or *Citrus grandis*, is the biggest citrus fruit in the Rutaceae family and the grapefruit's main progenitor. It is a non-hybrid citrus fruit that is native to Southeast Asia. The pomelo, which has a flavour similar to that of a sweet grapefruit, is widely consumed and utilized for celebratory events across Southeast Asia. It has the potential for medication interactions. [16]

2. Materials and Methods

2.1. Biosynthesis of Silver Nanoparticles

The leaves of *Punica granatum, Vitex negundo, Couroupita guianensis, Citrus maxima* were collected from the herbal garden in Bannari Amman Institute of Technology, Erode. These leaves were washed and dried in shade dry at room temperature. Dried leaves were crushed into a fine powder and stored in a sealed container. Typically, 2g of each of the powdered leaf sample was dissolved in 50ml of distilled water. The mixture was boiled at 60^0C for 10 min and filtered using Whatmann No.1 paper. To the extract filtrate, freshly prepared 0.1 M of silver nitrate solution was added with the ratio of 1:1. The mixtures were incubated in the dark at room temperature for 1 hour. The color change from pale yellow to dark brown was observed in 20-30 minutes; indicates the silver nanoparticles were synthesized from the plant extracts. Then the extracts were centrifuged at 5000 rpm for 15 minutes. The pellet is dried and stored for further analysis. [9, 10]

2.2. Characterization of Silver nanoparticles

Fourier Transform Infrared analysis is an important technique in chemistry which identifies certain functional groups in a molecule by the infrared spectroscopy. Through the collection of various absorption bands from the sample, a specific functional group of a molecule or impurities in a sample could be analyzed. [10]. Prepared nanoparticles were analyzed by FTIR SHIMADZU instrument in the wave length range of 4000 to 800 cm^{-1}. [11]

2.3. Antibacterial Activity of green synthesized Silver Nanoparticles

Antibacterial activity of the silver nanoparticles was studied by the well diffusion method. A grown bacterial culture *Bacillus subtilis* is used for this activity. Green synthesized Silver Nanoparticles at a concentration of 10 μg/mL were dissolved in deionized water and used for the antibacterial activity. Muller- Hinton agar solution was prepared and 100 μL of *Bacillus subtilis* was spread equally on the surfaces of the agar plates. The agar was punctured with 6 mm size and the each of the wells were loaded with the synthesized AgNps followed by incubation at 37 °C for 24 h. The antibacterial activity was observed based on formation of clear area (Zone of Inhibition ZOI) in millimeters around the wells and was measured by using meter scale. [12]

3. Results and Discussion

3.1. Characterization of AgNps

Plant extracts act as reducing agent and capping agent in the synthesis of the silver nanoparticles. There was a reduction of Ag^+ ions with the biomolecules found in the plant extracts like polysaccharides, amino acids, enzymes, proteins etc., Proteins in the plant extract helped in the reduction of Ag^+ ions and controlled the nanoparticle synthesis. The green synthesis of silver nanoparticles from various medicinal plants (*Punica granatum, Vitex negundo, Couroupita guianensis, and Citrus maxima*) was studied. The color change from pale yellow to dark brown was observed in the leaves extracts; when it is treated with the 0.1 M $AgNO_3$ within 20 mins (Figure 1); and then the maximum intensity is obtained after 12hrs with the increasing intensity in the time of incubation of the silver nanoparticle synthesis [3]. The color change in the mixture is due to the excitation of surface plasmon vibrations; it indicated the silver nanoparticles were synthesized [13].

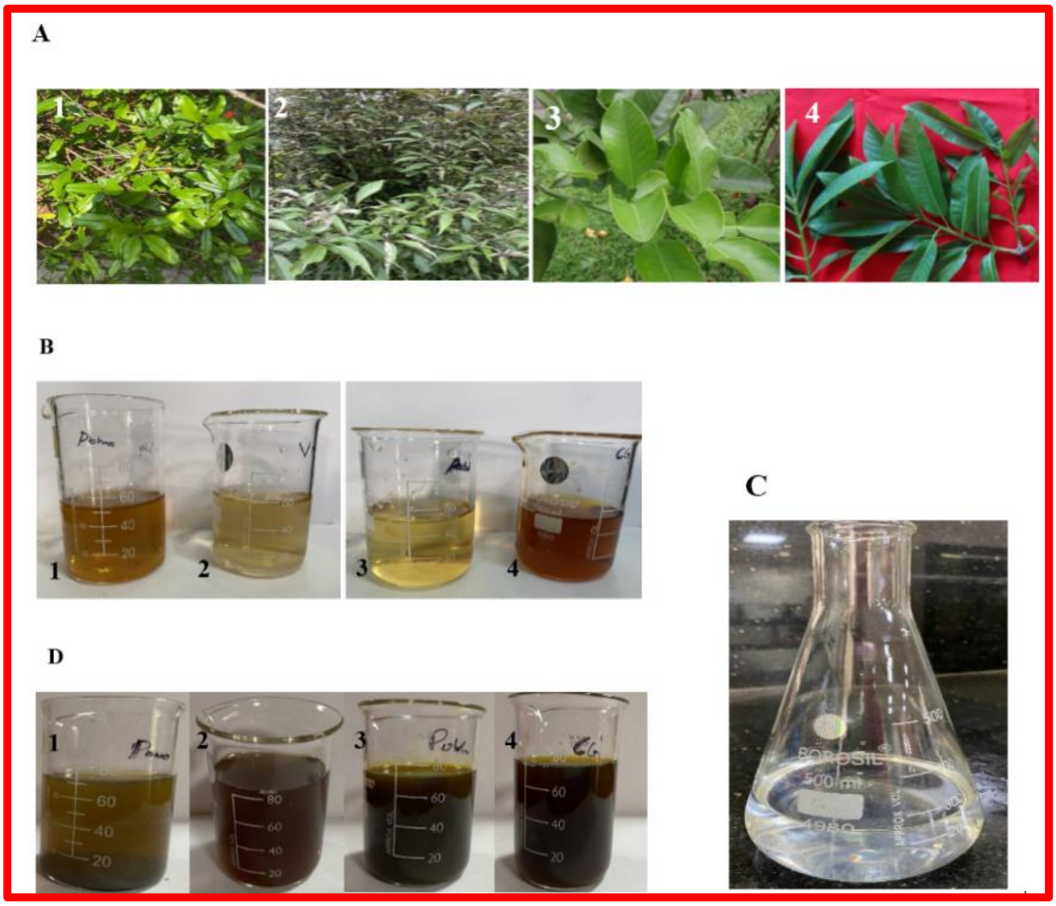

Figure 1. Green synthesis of AgNps. **(A)** Plants leaves of Punica granatum (1), Vitex negundo (2), Couroupita guianensis (3), Cirtrus maxima (4); **(B)** Leaves Extracts of Punica granatum (1), Vitex negundo (2), Couroupita guianensis (3), Cirtrus maxima (4); **(C)** Silver nitrate aqueous solution(control); **(D)** Synthesis of AgNps with Punica granatum (1), Vitex negundo (2), Couroupita guianensis (3), Cirtrus maxima (4) characteristic the color change after an hour.

3.2. FTIR analysis

FTIR spectra analysis determined the green synthesized silver nanoparticles of *Punica granatum* (Figure 2) revealed bands at 3421.72cm^{-1} corresponding to –OH stretching, proved the presence of alcohol class and appeared as a strong bond; 2926.01 cm-1 and 1637.56 cm^{-1} corresponding to C-H stretching and C=C stretching showed the presence of alkene groups and appears to be medium, strong bond; 1689.64 cm^{-1} corresponding to C=O stretching showed the presence of ketone conjugated strong appearance; 1388.75 cm^{-1} corresponding to C-H bonding group proved the presence of aldehydes respectively. Hence, the obtained phenolic compounds in the pomegranate leaf extract showed the stabilization and also the reduction of the nanoparticles. [9]

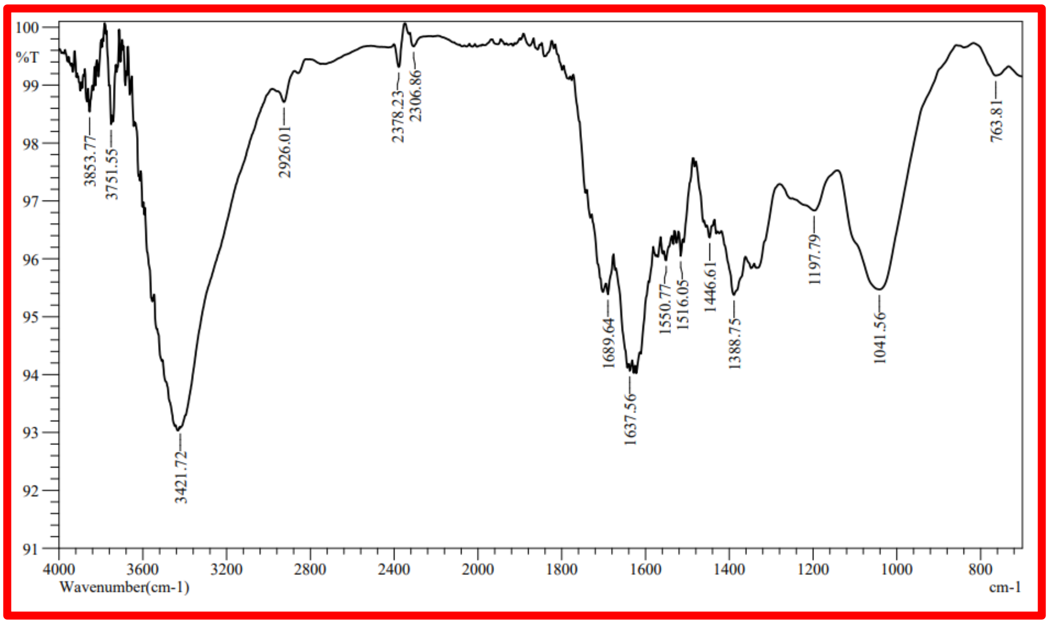

Figure 2. FT-IR spectra of green-synthesized AgNPs of Pomegranate (*Punica granatum*) showing probable functional groups in synthesized nanoparticles.

FTIR spectra analysis determined the green synthesized silver nanoparticles of *Vitex negundo* (Figure 3) revealed bands at 3751.55 cm^{-1}, 3435.22 cm^{-1}and 1381.03 cm^{-1} corresponding to-OH stretching proved the presence of alcohol group and appears medium sharp; 1381.03cm^{-1} corresponding to C-H bonding showed the presence of aldehyde group; 1622.3 cm^{-1} corresponding to C=C stretching which is the α,β-unsaturated ketone functional groups respectively[2]. The functional groups obtained in the FTIR spectrum of this leaf extract could be resulted and involved in the reduction of silver nanoparticles.

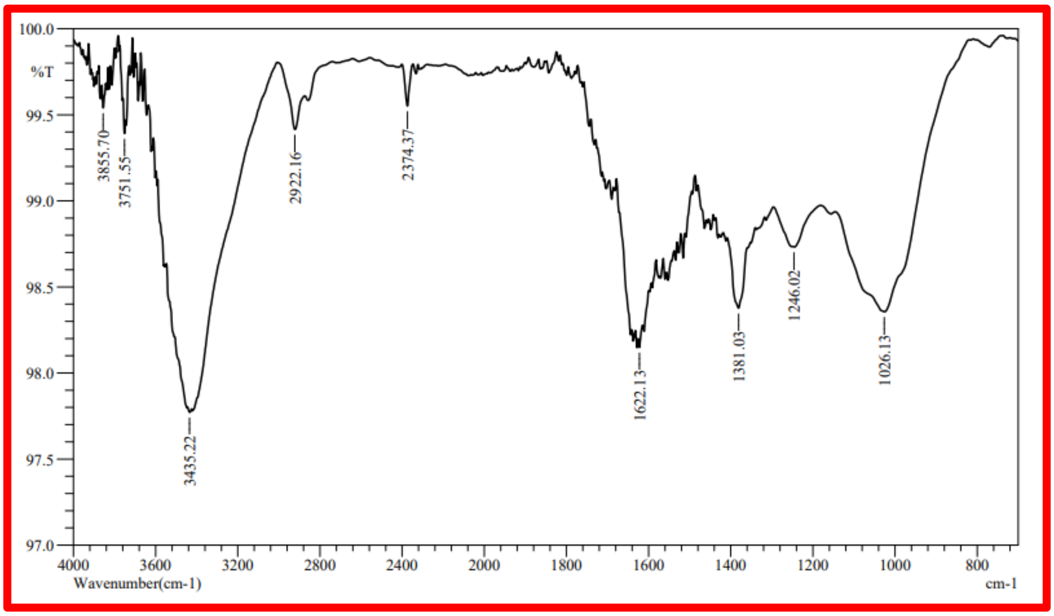

Figure 3. FT-IR spectra of green-synthesized AgNPs of Vitex showing probable functional groups in synthesized nanoparticles.

FTIR spectra analysis determined the green synthesized silver nanoparticles of *Couroupita guianensis* (Figure 4) revealed bands at 3425.58 cm^{-1} corresponding to –OH stretching proved the presence of alcohol class and appears strong bond; 2924.09 cm-1 and 1710.86 cm^{-1} corresponding to C-H stretching and C=O stretching showed the presence of alkene groups and conjugated aldehyde appears to be medium, strong bond; 1382.96 cm^{-1} corresponding to C-H bonding proving the presence of aldehyde with medium appearance ; 1521.84 cm^{-1} corresponding to N-O stretching group proving the presence of nitro compounds respectively. C=O bonds and Ag-N which is perpendicular to nano surface and involved in the formation of capped on nanoparticles [3]. Hence the obtained functional compounds in this leaf extract was found to play a vital role in the nanoparticles capping.

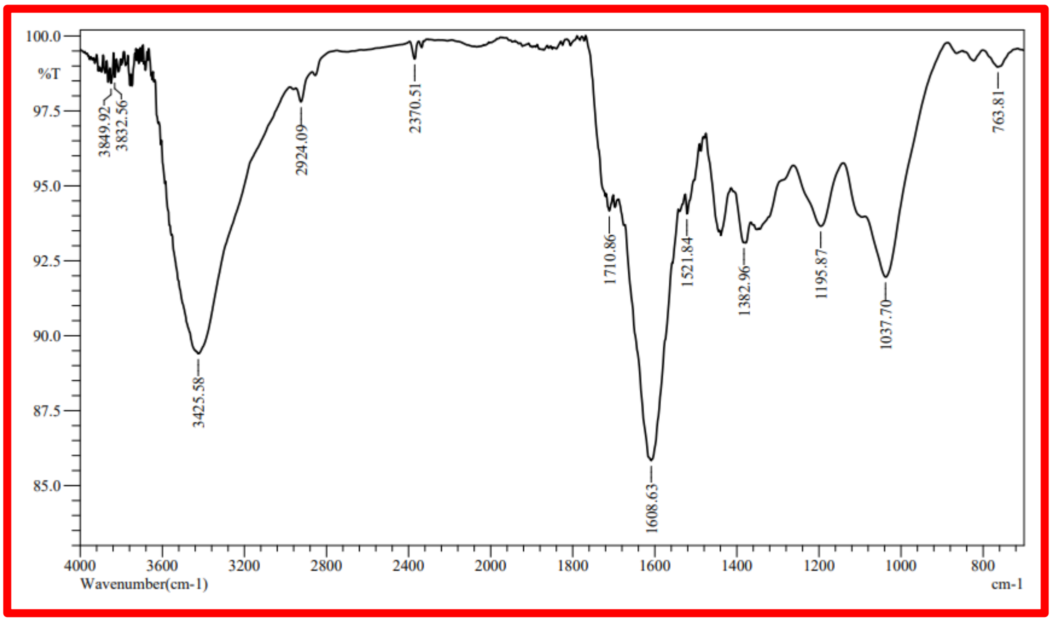

Figure 4. FT-IR spectra of green-synthesized AgNPs of *Couroupita guianensis* showing probable functional groups in synthesized nanoparticles.

FTIR specrtra analysis determined the green synthesized silver nanoparticles of *Cirtrus maxima* (Figure 5) revealed bands at 3417.86cm^{-1} and 1321.24cm^{-1} corresponding to –OH stretching groups proved the presence of alcohol class, phenol groups and appears strong bond; 2926.01 cm-1 and 1643.35 cm^{-1} corresponding to C-H stretching and C=C stretching showed the presence of alkene groups and appears to be medium, strong bond; 1384.89cm^{-1} corresponding to C-H stretching proved the presence of aldehyde group with medium appearance ; 1242.16 cm^{-1} corresponding to C-N stretching group proving the presence of aldehydes; Absorption peaks at 827.46cm-1 represents strong C–Cl stretching in the presence of halogen compounds respectively. Hence, the binding of carbonyl groups compounds where it binds the metal ions and acts as a capping agent for silver in the pomelo leaf extract helps to stabilize the nanoparticles [16]

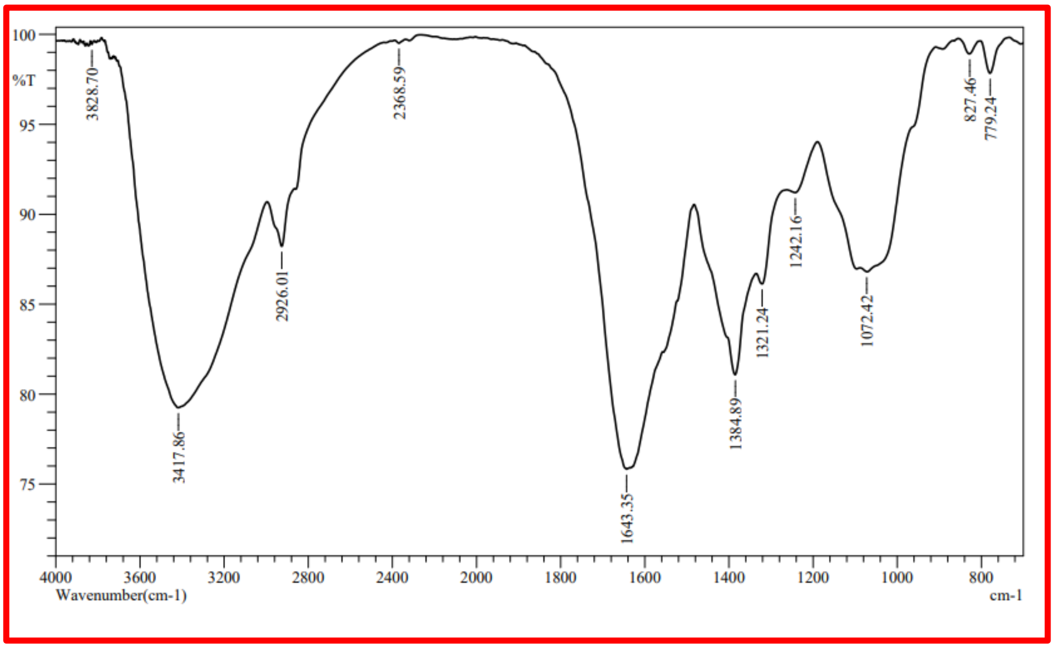

Figure 5. FT-IR spectra of green-synthesized AgNPs of Pomelo showing probable functional groups in synthesized nanoparticles.

3.3. Antibacterial Activity of AgNPs by Well-Diffusion Method

Antimicrobial activities of synthesized silver nanoparticles against *Bacillus subtilis* were performed and the zone of inhibitions were observed using a well diffusion method (Figure 6). AgNps synthesized with *Punica granatum, Vitex negundo, Couroupita guianensis, Citrus maxima* were prepared at concentrations 10 µg/mL and used against *Bacillus subtilis* with 0.3 ± 0.4 mm, 0.4± 0.5mm, 0.2± 0.3 mm and 0.3± 0.6 mm respectively [15,16]. The positive control was kept; where the culture was grown properly and in the negative control there is no contamination had formed. These results showed that the synthesized Silver nanoparticles displayed an improved bacterial inhibition.

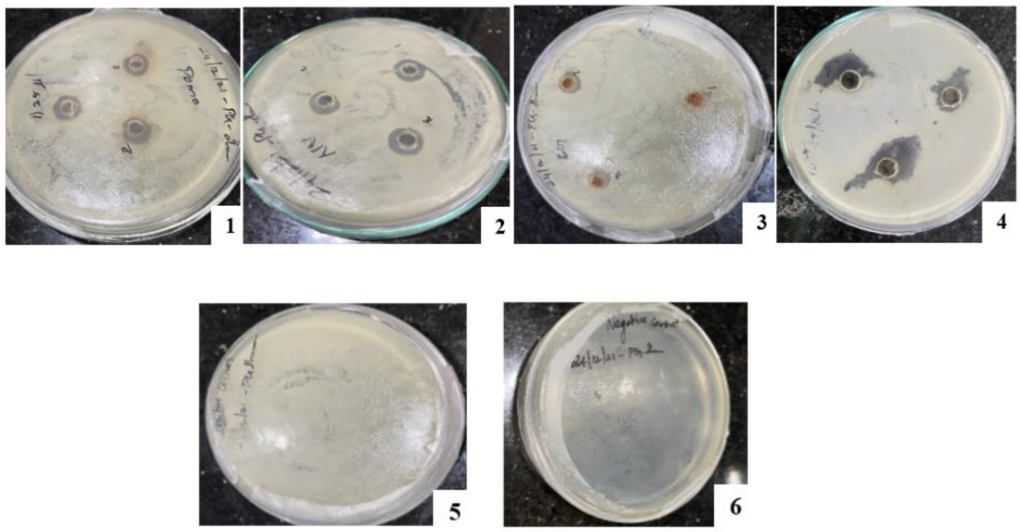

Figure 6. Growth of *Bacillus subtilis* forming the zone of inhibition in the presence of Silver Nanoparticles synthesized with leaves extracts of *Punica granatum* (1), *Vitex negundo* (2), *Couroupita guianensis* (3), *Cirtrus maxima* (4); A positive control (5), A negative control (6).

4. Conclusion

In this investigation, the reduction of Ag^+ ions by the leaves extracts of the *Punica granatum, Vitex negundo, Couroupita guianensis,* and *Citrus maxima* has been observed. We have studied that these medicinal plants could be a good source for the synthesis of nanoparticles; since they have showed an excellent anti-bacterial activity. The green synthesis of silver nanoparticles from various medicinal plants is became an excellent advantages in recent times. The green synthesized nano particles have been used in many versatile fields include medical field; since they are eco-friendly and used in bactericidal, skin treatments. The functional group of compounds in plant extracts were evaluated and the anti-bacterial property of these plant extracts have showed the better results against *Bacillus subtilis* through zone of inhibition. From the current study; it is revealed that the green synthesis of AgNps from medicinal plants could be used for the development and formulation of new drug or medication.

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