

Research Article

Comparative Analysis of Synthesis and Characterization of Silver Nanoparticles Extracted Using Leaf, Flower, and Bark of Hibiscus rosasinensis and Examine Its Antimicrobicidal Activity

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Nanotechnology is emerging with the biosynthesis of silver nanoparticles which is toxic free and friendly with the environment. Out of all the biosynthesis methods, plant extracts are more predominantly used which is readily available, and it is a least cost method. The plants have secondary metabolites such as phenolic compounds named as flavonoids; tannins are turned to be the reluctant to synthesize silver nanoparticles (AgNPs) from silver nitrate. The plant chosen to synthesize silver nanoparticles is the *Hibiscus rosasinensis* for the reason of the presence of various biomolecules. The reduction and stabilization process are performed due to the biomolecules. Silver nanoparticles produced from the Hibiscus plant are easier than the conventional method. The formation of nanoparticle of silver is indicated by the maximum absorbance peak at a wavelength of 458 nm obtained from the leaf and the bark extracts. The antimicrobicidal effects on various microbes were examined, and it is proved that bark produced better results.

1. Introduction

In the recent years, nanotechnology is growing exponentially with extensive applications in the area of science, engineering, and technology [1, 2]. The nanotechnology is the field of production of nanoparticles for wide applications and the study of its properties. Nanoparticles are of 0D, 1D, 2D, or 3D ranging from 1 to 100 nm. There are different physical methods such as chemical vapour deposition and ball milling and chemical methods such as sol-gel synthesis and polyol synthesis available for the nanoparticle synthesis. The synthesis using chemical or physical methods is not eco-friendly since it involves many harmful and poisonous chemicals for the synthesis process [3]. This leads to the development of nanoparticles using green synthesis approach. The green-facilitated synthesis of nanoparticles is however ecologically friendly and does not use any toxic chemicals. The green mediators derived from plant extracts, fungi, and



FIGURE 1: Hibiscus rosasinensis plant.

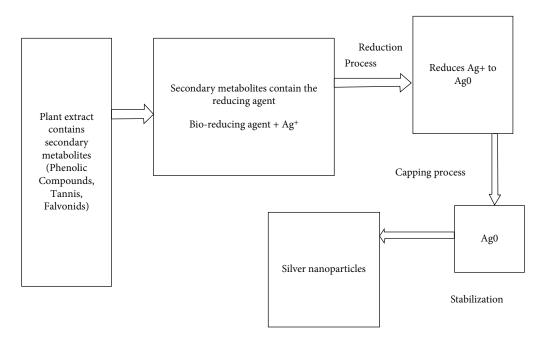


FIGURE 2: Green synthesis method of AgNPs.

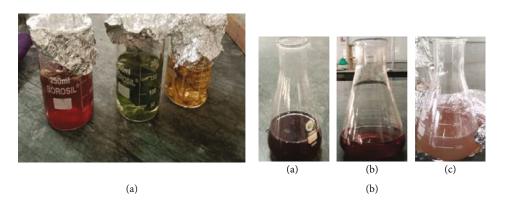


FIGURE 3: (a) Extracts of Hibiscus plant—A: flower; B: leaf; C: bark. (b) AgNPs formation—A: flower; B: leaf; C: bark.

bacteria can be used as the reductant and stabilizers for the nanomaterial synthesis [4, 5]. Among the green mediators, the plant extracts are rich in secondary metabolites such as phenolic compounds, tannins, and flavonoids. These secondary metabolites contain the reducing agent. The unique properties of the nanoparticles led to the applications of such nanoparticles in different fields. One such wide spread and remarkable possessing material is the silver nanoparticle. These silver nanoparticles are having the significant and inherent characteristics of acting as an

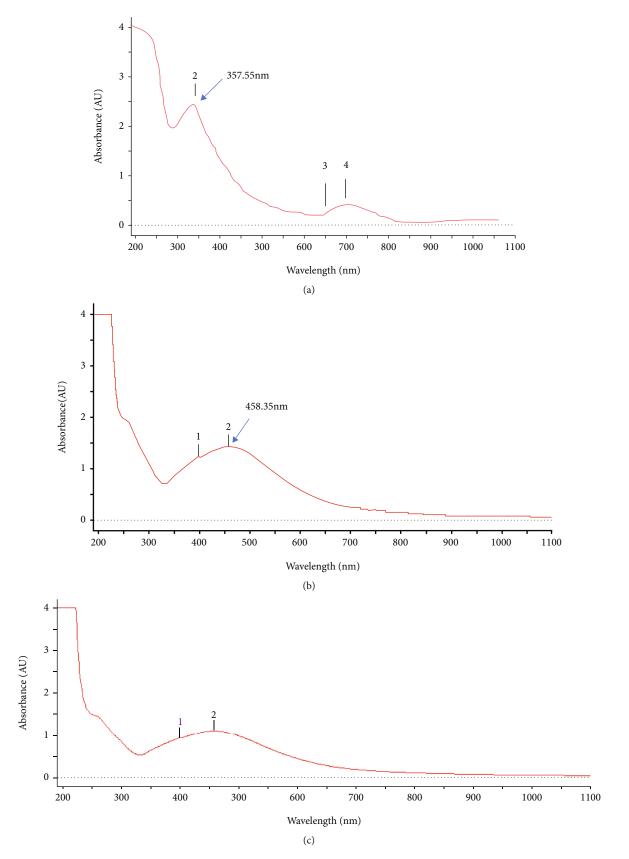


FIGURE 4: UV-visible spectrum of AgNPs using Hibiscus plant extract: (a) flower, (b) leaf, and (c) bark.

antimicrobial and antibacterial agent [6]. This property is used in the field of medicine to produce drugs against such microbial infections. The silver nanoparticle synthesis using different plants such as waste tea leaves, *Cucumis prophetarum*, *Azadirachta indica*, *Myrmecodia pendans*, *Rhynchotechum ellipticum*, and *Murraya koenigii* has been reported [7–12]. Each plant extract is giving the silver nanoparticle with its excellent sizes of various nanometer scales. *Hibiscus rosasinensis* is one such medicinal plant whose extract is used in the process of silver nanoparticle synthesis. In this work, the *Hibiscus rosasinensis* plant parts such as leaves, flowers, and bark have been chosen to synthesize silver nanoparticles. Extraction of the plant parts (leaf, flower, and bark) acts as the reductant for the silver nitrate solution to produce silver nanoparticle.

2. Materials and Methods

2.1. Plant Material. The Hibiscus rosasinensis is a small tree or shrub having glossy leaves shown in Figure 1. The colour of the flowers may vary from pink, red, or orange. The classification of the plant is as given as follows:

Family: Malvaceae Subfamily: Malvoideae Genus: Hibiscus Species: rosasinensis Botanical name: *Hibiscus rosasinensis*

The bark, leaves, and flowers of Hibiscus plant which are free from diseases were freshly collected from the local garden at Karur, Tamil Nadu, India.

2.2. Synthesis of AgNPs. The green synthesis method of synthesis of AgNPs is shown in Figure 2. The leaves, flowers, and bark of the Hibiscus plant were cleaned with normal tap water first and with distilled water again to clear away the impurities. 2.5 g of flower, leaves, and bark was taken in a beaker separately with 100 ml of distilled water each and boiled at 80-90°C for about an hour [13]. The prepared leaf and flower extracts were filtered with Whatman paper. The extract from the filtered solution acts as reductant and stabilizer. The 0.34 gm of silver nitrate (AgNO3) powder is mixed with 100 ml of distilled water to make 0.02 N of AgNO3 solution. 5 ml of the leaf extract is added to the 50 ml of the prepared silver nitrate solution drop wise. The same process is done for the flower and bark also. The colour of the flower, leaf, and bark extracts were purple, green, and brown. When these extracts of Hibiscus plant were added with the required amount of silver nitrate solution, the change of colour to dark brown from purple, green, and brown was noticed as shown in Figures 3(a) and 3(b). This colour change indicates the presence of AgNPs in the sample.

2.3. SEM Analysis of AgNPs. The sample was subjected to SEM (scanning electron microscope) analysis to study the surface morphology [14]. The sample was made as thin films, and a least quantity of the sample is poured on the grid which is made of carbon-coated copper [15]. The grid was placed with the sample, and the sample was dried for analysis.

TABLE 1: Absorbance peak for different extracts.

Extract	Wavelength (nm)	Absorbance (AU)
Flower	357.55	2.2307
Leaf	458.35	1.4279
Bark	458.35	1.1062

2.4. UV-Visible Spectroscopy. The AgNPs formation was observed through the UV-visible spectrometer. The UV-visible spectrophotometer has a quartz cuvette with path length of 1 cm. The AgNP sample obtained from leaf, flower, and bark extracts of Hibiscus was placed in the cuvette. The UV-visible spectrum is produced between the wavelength ranges of 300–700 nm [16].

2.5. *FTIR Analysis.* The secondary metabolites present in the plant extract and the functional groups [8] on the AgNPs were identified by using FTIR characterization technique. The different functional groups present in the sample are responsible for the stabilization and the reduction process for the AgNPs synthesis. The capping agents stabilize and control the excess growth of nanoparticles, and it is important to find the appropriate capping agent [17].

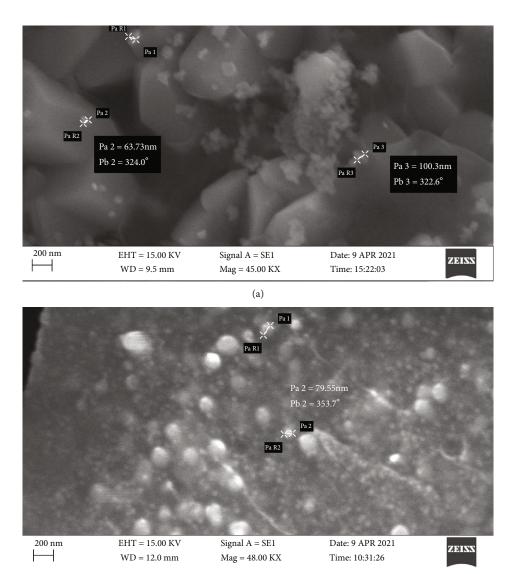
3. Results and Discussion

The majority of the world's population is using herbal medicines for many diseases nowadays. From the ancient days, the herbs were used as the medicinal drugs for number of diseases. Many herbs are having pharmacological actions, which are abundant in nature. The medicinal herbs are having compounds like secondary plant metabolites like flavonoids and tannins [18] which have been used for thousands of years for the healthcare.

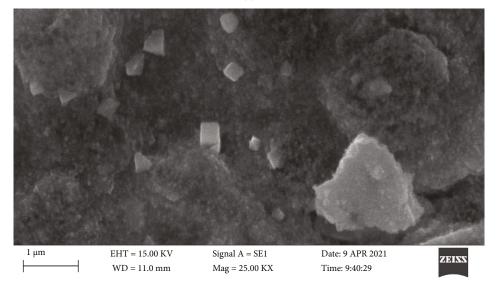
The *Hibiscus rosasinensis* is a medicinal herb which is rich in secondary plant metabolites, and these compounds are acting as reductant and the capping agent in the formation of silver nanoparticles. In this green chemistry synthesis method of silver nanoparticles, the metal salts are reduced into the aqueous metal ion precursors [19]. As a result of this reduction, the colour changes in the mixture. This colour change of the solution indicates the formation of the silver nanoparticles [20].

3.1. Characterization Analysis of AgNPs. The silver nanoparticles were synthesized using the leaves, flower, and bark of the *Hibiscus rosasinensis* plant. When silver nitrate solution is added with the plant extract, colour change of the extracts to dark brown showed the development of the silver nanoparticles. This property is called as the surface plasmon resonance of silver [21]. The UV-visible spectrophotometer was used to analyse the formation and stability of the AgNPs in the colloidal solution. The maximum absorbance peaks found for the flower, leaf, and bark extracted AgNPs were 357-400 nm, 440-460 nm, and 400-460 nm, respectively, shown in Figure 4.

The silver nanoparticle using leaf and bark exhibits the maximum absorbance peak exactly at 458 nm listed in







(c)

FIGURE 5: Surface morphology of AgNPs using Hibiscus plant extract: (a) flower, (b) leaf, and (c) bark.

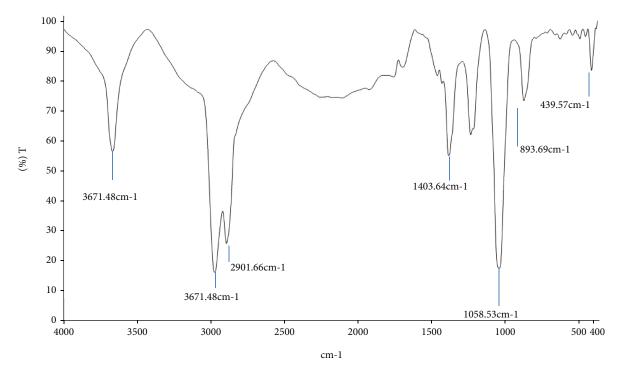


FIGURE 6: FTIR of AgNPs using Hibiscus rosasinensis flower.

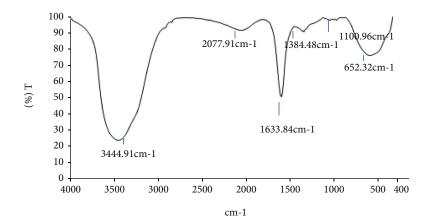


FIGURE 7: FTIR of AgNPs using Hibiscus rosasinensis leaf.

Table 1. But the flower extract is having peak at 357 nm whose wavelength is less than that of the silver nanoparticle from leaf and bark extracts. This is because of the presence of some silver ions in the sample particle formed from flower extract. It is also due to the reason that the silver nanoparticle depends on the particle size and shape [22].

The silver nanoparticle exhibits different morphological structures. There are various synthesizing ways like chemical, physical, ultrasonic, hydrothermal, and biological methods available for different applications. The silver nanoparticles are normally available in different shapes as dendrites, flower-like, nanorods, nanoplates, nanocubes, nanospheres, and nanowires. Shenashen et al. [23] reported that silver nanoparticles synthesized from plant extracts exhibit mostly spherical shaped. Raghava et al. [24] reported that silver nanoparticles formed by *Rivina humilis* leaf extract were also spherical in shape with average size of 51 nm. Similar to this, the silver nanoparticle formation using plant (*Hibiscus rosasinensis*) mediation showed that majorly, the nanospheres and some particles are nanocubes in this work.

The SEM was used to analyse the morphological structure of the nanoparticles. The scanning electron microscope images of the AgNPs synthesized by Hibiscus flower, leaf, and bark by reducing silver nitrate solution are shown in Figure 5. It is found that the silver nanoparticles synthesized were mostly spherical [8, 25] in shape and the size is found as 200 nm for flower, 200 nm for leaf, and 1 μ m for bark. This morphological structure chains the result obtained using UV-visible spectroscopy for the synthesized silver nanoparticles. The properties of the silver nanoparticles are

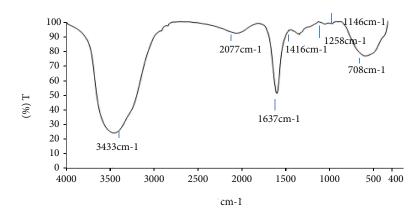


FIGURE 8: FTIR of AgNPs using Hibiscus rosasinensis bark.

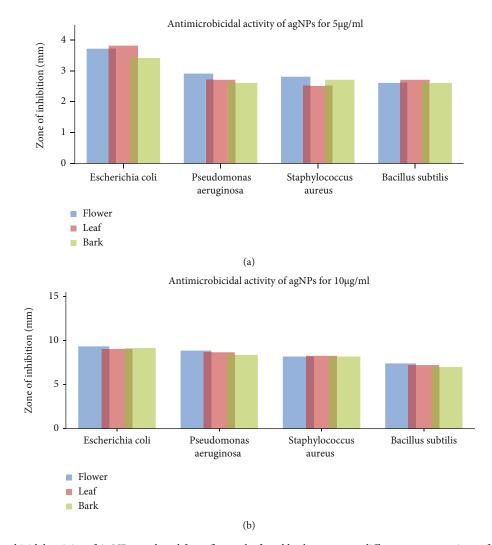


FIGURE 9: Antimicrobicidal activity of AgNPs produced from flower, leaf, and bark extracts at different concentrations of (a) $5 \mu g/ml$ and (b) $10 \mu g/ml$.

governed by the size and the shape, and thus, it is widely used in medicinal applications.

FTIR is used to analyse the bonding structures [8] formed due to the interactions of the metal particles and

the biomolecules. The transformation of simple inorganic silver nitrate to silver has been analysed with this FTIR [26] with the help of different phytochemicals. These phytochemicals are used as the reductant, stabilizer, and capping

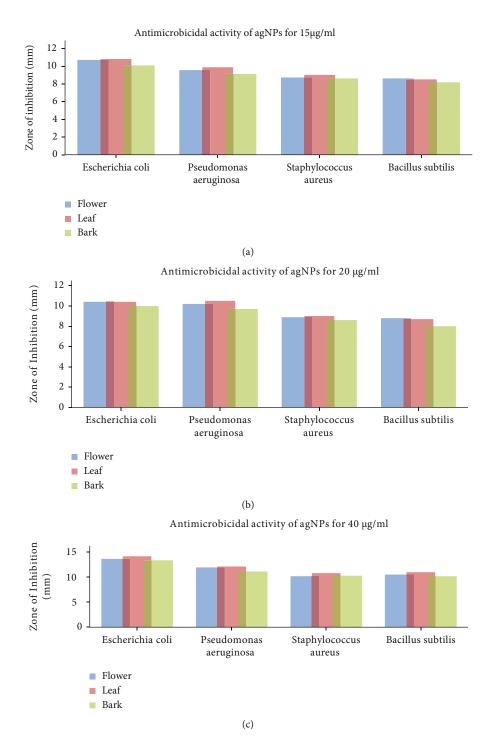


FIGURE 10: Antimicrobicidal activity of AgNPs produced from flower, leaf, and bark extracts at different concentrations of (a) $15 \mu g/ml$, (b) $20 \mu g/ml$, and (c) $40 \mu g/ml$.

agent. The FTIR results visibly show the biofabrication of silver nanoparticles synthesized using the Hibiscus plant extracts.

The FTIR spectrum analysis reveals the occurrence of dissimilar functional groups [27, 28] in the AgNPs solution for flower, leaf, and bark shown in Figures 6–8, respectively. The silver nanoparticles using flower extract is having the O-H stretch assigned at 3671 cm⁻¹, C-H stretch alkanes group assigned at 2983 cm⁻¹, C-H stretch aldehydes were

assigned at 2075 cm⁻¹, C=O bonding at 1711 cm⁻¹, C=C aromatic at 1480 cm⁻¹, C-O bonding at 1250 cm⁻¹, and =C-H alkenes assigned at 893 cm⁻¹ were predicted. The AgNPs formed by using the leaf extract have a peak at 3444 cm⁻¹ with the O-H stretch of the phenolic group, 1633 cm⁻¹ corresponds to the C=O stretch of the ketone group, C-O stretch was assigned at 1100 cm⁻¹, and C-Cl alkyl halide group has a peak at 652 cm⁻¹. The FTIR spectrum is showing the functional groups for the silver

nanoparticles using bark extract is 3433 cm^{-1} is at O-H stretch, 1637 cm^{-1} is assigned for C=C stretching alkenes group, C-O stretching is having peak at 1258 cm^{-1} and C=C bending is at 708 cm^{-1} .

3.2. Antimicrobicidal Activity of AgNPs. The silver nanoparticles have a wide range of antimicrobicidal activity against different microorganisms. It has the tendency to penetrate into cell walls of the microorganisms and make the cell membrane inactive, thereby causing cell death. The nanoscale property enhances the efficiency of the antimicrobicidal activity. The silver nanoparticles have large surface area which can easily pierce into the cell membranes and disturbs the DNA replication of the microorganisms. This action of arresting the replication of DNA is due to the release of silver ions present in the silver nanoparticles. These silver ions that bind with the DNA molecules restrict the cell to replicate, and thus, it prevents the microorganisms to multiply itself. Thus, the growth of the microorganisms is stopped [29].

The silver nanoparticles had proved the antimicrobicidal agent [30] against different microorganisms. The silver nanoparticles were synthesized using green mediation process of using Hibiscus rosasinensis as a reductant. The prepared AgNPs were used to check the antimicrobial agent against the E. coli (Escherichia coli), P. aeruginosa (Pseudomonas aeruginosa), S. aureus (Staphylococcus aureus), and B. subtilis (Bacillus subtilis). The silver nanoparticles have the ability to kill bacteria and other microbes with the release of Ag⁺ ions [31]. The microorganisms have the ability to multiply itself rapidly, and the silver ions inhibits the multiplication capacity of the microbes. Thus, it prevents the further growth of the microbes. The different microorganisms were cultivated separately with different concentrations of AgNPs such as $5 \,\mu\text{g/ml}$, $10 \,\mu\text{g/ml}$, $15 \,\mu\text{g/ml}$, $20 \,\mu\text{g/ml}$, and $40 \,\mu\text{g/ml}$. There were different sets of cultivation plates for different pathogens with the silver nanoparticles prepared by flower, leaf, and bark extracts. The zones of inhibition against the microorganisms were noted for different concentrations. The graphical representation was shown for the zone of inhibition for different extracts and concentrations against different microbes in Figures 9 and 10.

D. Nayak et al. [32] investigated the antimicrobicidal activity of AgNPs prepared using Hibiscus flower and used against the *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Klebsiella pneumoniae* with 100 μ g/ml concentration. Vijayaraj and Sri Kumaran [33] proposed that the nanoparticle of silver was synthesized for the antibacterial activity against the fish pathogen named *Aeromonas hydrophila*, and the zone of inhibition is found to be 11 mm as the maximum. Similar to the above results, the silver nanoparticles extracted in the present study proved to be an antimicrobicidal agent against different pathogens.

It was observed that the minimum inhibitory concentration inhibits the growth of the microbes and its zone of inhibition value is minimum for $5 \mu g/ml$ and $10 \mu g/ml$. At this point, the agents restricted the growth of the microorganisms. The silver nanoparticle exhibited good resistant against the *Bacillus subtilis* for low concentration. It was found that *Staphylococcus aureus* had a strong resistant to the AgNPs in different concentrations. The zone of inhibition was consistently more for the *Escherichia coli* and *Pseudomonas aeruginosa* in all the concentrations of AgNPs. The inhibitory action was found quiet more for the silver nanoparticles prepared using the bark extract when compared with the silver nanoparticles prepared from flower and leaf extracts.

4. Conclusion

It is evident that the preparation of silver nanoparticles using green synthesis method is an easier and a cost-effective method. As it is a natural extract, it is not producing any toxic chemicals during the synthesis. It is proved that extract of the leaves, flowers, and bark of the Hibiscus plant is acting as a reductant in the silver nanoparticle synthesis process. The green-synthesized silver nanoparticles using flower, leaves, and bark of *Hibiscus rosasinensis* showed their antimicrobicidal activity against different microbes. Comparatively, the silver nanoparticles prepared from the bark extract showed a very good inhibition action against the microorganisms.

Data Availability

The data used to support the findings of this study are included within the article.

Disclosure

This study was performed as part of the Employment of Authors.

Conflicts of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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