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Research Article

Microwave Assisted Biosynthesis of Silver Nanoparticles Using the Rhizome Extract of *Alpinia galanga* and Evaluation of Their Catalytic and Antimicrobial Activities

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Biomediated methods are considered to be a safer alternative to conventional physicochemical methods for the fabrication of nanomaterials due to their eco-friendly nature. In the present study, silver nanoparticles (AgNPs) were synthesized by microwave irradiation using aqueous rhizome extract of the medicinal plant *Alpinia galanga*. The nanoparticles were also synthesized under ambient condition without the assistance of microwave radiation and the former method was found to be much faster than the latter. The silver nanoparticles were characterized by UV-vis., FTIR, XRD, and HR-TEM analysis. UV-vis. spectroscopic studies provided ample evidences for the formation of nanoparticles. The FTIR spectrum confirmed the presence of plant phytochemicals as stabilizing agent around the AgNPs. XRD and HR-TEM analyses clearly proved the crystalline nature of the nanoparticles. From the TEM images, the nanoparticles were found to be roughly spherical in shape with an average diameter of 20.82 ± 1.8 nm. The nanoparticles showed outstanding catalytic activity for the reduction of methyl orange by NaBH₄. The AgNPs were also evaluated for their antimicrobial activity by well diffusion method against *S. aureus*, *B. subtilis*, *V. cholera*, *S. paratyphi*, and *A. niger*. They were found to be highly toxic against all the tested pathogenic strains.

1. Introduction

Nanomaterials have generated immense interest in recent times because of their promising applications in various areas of science and technology. Among these, metal nanoparticles are most capable owing to their properties that depend on their size and morphology which makes them a proper aspirant in various applications [1–4]. Several methods are available for nanoparticle synthesis such as chemical [5], photochemical [6], electrochemical [7], and biological methods [8]. Many of these production routes involve the use of toxic chemicals and require harsh reaction conditions. Chemical method of nanoparticle synthesis is still most common because of its short reaction time. However, in this method, the chemical reagents used as reducing and capping agent are usually toxic and lead to environmental pollution.

With increasing focus on green chemistry, biological synthesis of metal nanoparticles is gaining more attention recently because of its simplicity, environment benign nature, and cost-effectiveness. Plant extracts and several microorganisms such as bacteria, fungi, and yeast have been used for nanosynthesis [9–11]. Many successful reports are obtainable on the biological synthesis of nanoparticles using plant extract as both reducing and stabilizing agent [12–16]. Even though plant mediated biosynthesis can be carried out at ambient conditions, the time required for nanosynthesis is much longer than the chemical methods. Microwave assisted biosynthesis is a remedy to this problem. The reaction time can be reduced to a greater extent by microwave irradiation. Microwave assisted synthesis using plant extracts as both reducing and capping agent is a viable way for the rapid and facile green synthesis of silver nanoparticles. It has

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several attractive features such as shorter reaction time, lower energy consumption, and better product yield [17]. Microwave irradiation offers rapid and uniform heating of the reaction medium and thus provides uniform nucleation and growth conditions for nanoparticles. Renugadevi and coworkers synthesized silver nanoparticles using leaf extract of *Baliospermum montanum* with the aid of microwave heating [18]. Abboud et al. prepared silver nanoparticles by a microwave assisted method using aqueous onion (*Allium cepa*) extract [19].

Alpinia galanga Willd is an important Ayurvedic medicinal plant belonging to the family Zingiberaceae. It is a perennial herb found commonly throughout the Western Ghats of India. The plant has been found to possess various therapeutic activities like anti-inflammatory, antiallergic, antifungal, antibacterial, and antidiabetic [20]. It is used as a constituent in various Ayurvedic preparations like Dasamoolarishtam, Rasnadi Choornam, Aswagandharishtam, Balarishtam, and so forth [21]. The rhizome is also used against rheumatism, bad breath, ulcers, throat infections, stomach disorders, and skin diseases [22]. The rhizome contains tannins and flavonoids such as kaempferol, galangin, and alpinin. In addition to that, they are also found to contain acetoxy chavicol acetate, acetyl euginol acetate, p-coumaryl alcohol, methyl cinnamate, cineole, pinenes, and camphor to a certain extent [20].

In this work, we report a novel one-pot, microwave assisted method for the synthesis of silver nanoparticles using the rhizome extract of *Alpinia galanga* as both reducing and capping agent. This is a simple, green, and cost-effective method for the rapid and facile synthesis of silver nanoparticles. The catalytic activity of the silver nanoparticles synthesized by microwave irradiation using *Alpinia galanga* (AgNP-*Alpinia*) was examined using the reduction reaction of the azo dye methyl orange by NaBH₄. Its antimicrobial potential was tested against some selected pathogenic microbial strains.

2. Materials and Methods

- 2.1. Materials. Silver nitrate (AgNO $_3$), methyl orange, and sodium borohydride (NaBH $_4$) of analytical grade were purchased from Merck (India) and used as such without further purification. Nutrient broth, potato dextrose agar (PDA), and Mueller Hinton agar (MHA) were obtained from Himedia Chemicals (Mumbai, India). All aqueous solutions were prepared using double distilled water.
- 2.2. Preparation of Alpinia galanga Rhizome Extract. Fresh rhizomes of Alpinia galanga were collected and identified taxonomically. They were washed thoroughly with distilled water. They were sliced into small pieces, air-dried, and then powdered. Dried powdered sample (5 g) was taken in a round bottom flask fitted with condenser and boiled for 10 min with 100 mL of double distilled water. It was cooled and filtered through Whatman number 1 filter paper. The extract thus obtained was stored in a refrigerator for further use.
- 2.3. Synthesis of Silver Nanoparticles (AgNP-Alpinia). In a typical microwave synthesis, 90 mL of 1 mM silver nitrate

solution was taken in a 250 mL beaker. To this, 10 mL *Alpinia galanga* rhizome extract was added and stirred well. It was then placed in a domestic microwave oven (Sharp R-219T (W)) operating at a power of 800 W and frequency 2450 MHz. The solution was then subjected to microwave irradiation for 90 sec. The formation of AgNPs was monitored using UV-vis. spectrophotometer by analyzing the reaction mixture after 30, 60, and 90 sec of microwave action. The silver nanoparticle solution was then centrifuged at 10000 rpm for 10 min. The supernatant was decanted and the nanoparticles were redispersed in distilled water. The above process was repeated three times. The purified sample thus obtained was freeze-dried to get dry sample.

In order to synthesize AgNP-*Alpinia* at room temperature, 10 mL of *A. galanga* extract was added to 90 mL of 1 mM aqueous solution of silver nitrate and allowed to react at room temperature for 3 hours. The reaction mixture was subjected to intermittent UV-vis. analysis at an interval of 30 minutes to examine the formation of AgNP-*Alpinia*.

- 2.4. Reduction of Methyl Orange. The reduction of methyl orange by NaBH₄ was used to look into the catalytic efficiency of AgNP-Alpinia. To track this reaction, 0.5 mL freshly prepared NaBH₄ solution (0.06 M) was added to 2 mL of methyl orange solution (0.1 \times 10⁻³ M) taken in a quartz cell. Then 0.5 mL of AgNP-Alpinia solution was added to start the reaction. The change in the concentration of methyl orange with time was monitored by UV-vis. spectrophotometry. The absorption spectra were recorded at one-minute intervals in the range of 200–600 nm at ambient temperature (30°C).
- 2.5. Antimicrobial Study by Well Diffusion Method. The antimicrobial activity of the AgNPs was evaluated by well diffusion method. Clinical isolates of human pathogenic bacterial strains of Staphylococcus aureus, Bacillus subtilis, Vibrio cholerae, and Salmonella paratyphi and fungal strain of Aspergillus niger isolated from contaminated food were used in this study. The bacterial and fungal strains were subcultured in liquid nutrient agar and potato dextrose agar (PDA), respectively, prior to the experiment. Mueller Hinton agar (MHA) medium and potato dextrose agar (PDA) medium were prepared following manufacturer's instruction, sterilized, and transferred into autoclaved Petri dishes placed in a laminar air flow. When the medium was solidified, the culture of test bacteria was uniformly seeded over the surface of MHA medium and that of fungus on the PDA medium using sterile cotton swabs. Using a sterile gel puncture, wells of 8 mm diameter were made on the MHA and PDA plates. To these wells, solutions of *A. galanga* extract, silver nitrate, and different volumes of AgNP-Alpinia were poured using a micropipette. All the MHA plates were incubated at 37°C for 24 hours and the fungal plate was kept at room temperature for 48 hours. Finally, the zone of inhibition around the wells was measured in mm.
- 2.6. Characterization. UV-vis. spectral studies were carried out on a Shimadzu UV-2450 spectrophotometer. FTIR spectrum was recorded using Perkin Elmer-400 spectrometer

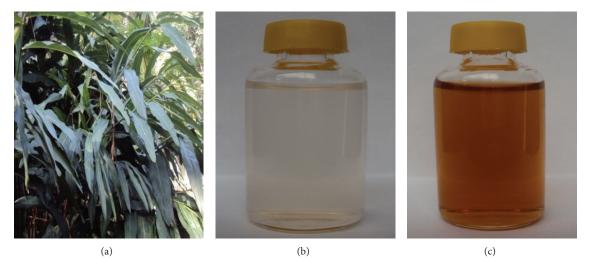


FIGURE 1: Photographs of (a) Alpinia galanga plant, (b) reaction mixture containing 1 mM AgNO₃ and rhizome extract before microwave irradiation, and (c) after microwave synthesis.

with ATR attachment. XRD measurement was made on a PANalytic X'PERT-PRO X-ray spectrometer. High resolution-transmission electron microscopic (HR-TEM) images were obtained using a JEOL JEM-2100 microscope.

3. Results and Discussion

3.1. Synthesis and UV-Vis. Spectroscopic Analysis of Silver Nanoparticles. UV-vis. spectrophotometric analysis is used to follow and confirm the formation of silver nanoparticles. The reduction of Ag⁺ ions into Ag nanoparticles was monitored by recording the absorption spectrum of the reaction mixture with time in the range of 250–700 nm. The UV-vis. spectrum of Alpinia galanga extract does not show any peak in the range from 250 to 700 nm. The first evidence for the formation of AgNP is obtained from the change in colour of the reaction mixture. The addition of Alpinia extract to silver nitrate solution does not cause any appreciable change in the colour of the solution. The photograph of Alpinia galanga plant and that of the reaction mixture before and after microwave irradiation are shown in Figure 1.

Upon microwave irradiation, the colour of the reaction mixture gradually changes from colourless to yellowish brown. The UV-vis. spectra of the reaction mixture recorded at 30 sec intervals are shown in Figure 2. A peak was observed at about 417 nm after irradiation for 30 sec, the intensity of which increased with increasing reaction time without much change in the wavelength. The microwave synthesis was completed in just 90 sec. The band at 417 nm is due to the surface plasmon resonance (SPR) of silver nanoparticles. The peak is almost symmetrical and there are no peaks in the range of 450-700 nm indicating the absence of silver nanoparticle aggregation [23]. The SPR band arises due to the collective oscillations of the conduction electrons of nanoparticles in presence of visible light which is highly influenced by shape and size of the nanoparticles [24]. The UV-vis. spectral studies propose that the nanoparticles are uniformly

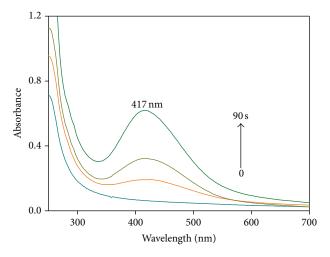


FIGURE 2: UV-vis. absorption spectrum of AgNP-*Alpinia* recorded at different microwave irradiation time.

distributed and are more or less spherical in shape. The main attraction of microwave synthesis is that it yields small, uniform sized nanoparticles in much lesser reaction time. The speedy consumption of starting materials reduces the formation of agglomerates in microwave assisted methods and provides nanoparticles with narrow size distribution [17].

As stated earlier, the rhizome extract of *A. galanga* is rich in various phytochemicals like tannins, terpenoids, and flavonoids such as kaempferol, galangin, and alpinin. When *A. galanga* extract is added to AgNO₃ solution, these flavonoids effectively reduce Ag⁺ ions into Ag atoms which then join to form silver nanoparticles. The phytochemicals also act as capping agents and thus protect and stabilize the nanoparticles by preventing agglomeration.

To study the effect of microwave irradiation upon rate of formation of nanoparticle, silver nanoparticles were also synthesized using plant extract at room temperature without microwave irradiation. The UV-vis. spectra of the reaction

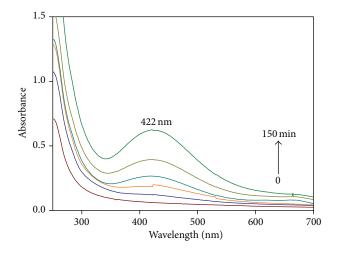


FIGURE 3: UV-vis. spectra of silver nanoparticles at different stages of room temperature synthesis.

medium recorded at 30 min intervals are shown in Figure 3. A peak was observed at 422 nm due to surface plasmon resonance of silver nanoparticles after a reaction time of 1 hour which increased with time till about 150 min of reaction time. This SPR value is higher than that obtained in case of microwave assisted synthesis. It is observed that the SPR band shifts to longer wavelength with increasing particle size [25, 26]. Thus in this synthetic method, microwave assisted synthesis yields relatively small nanoparticles in much less reaction time than room temperature synthesis. This observation is further confirmed by TEM analysis. The reduction of Ag⁺ ions into Ag and the concomitant formation of stable nanoparticles happened so rapidly within 90 sec in microwave method making this a faster method for nanoparticle synthesis.

3.2. FTIR Spectroscopy. FTIR spectroscopic study was conducted to examine the association of plant extract with the nanoparticles. Figure 4 shows the FTIR spectrum of A. galanga stabilized silver nanoparticles synthesized by microwave irradiation. The broadband appearing in the range of 3420–3220 cm⁻¹ results from the stretching vibrations of the hydroxyl (–OH) group of various metabolites present in Alpinia extract. A weak band observed at 2920 cm⁻¹ is associated with aliphatic O–H stretching vibrations. The absorption peak at 1607 cm⁻¹ is attributed to C=C stretching vibrations of aromatic ring. An intense band at 1386 cm⁻¹ can be assigned to –C–O stretching vibrations in carboxyl group. The strong absorption band at 1030 cm⁻¹ is characteristic of C–O stretching vibrations of alcohols. The peak at 824 cm⁻¹ is due to the presence of aromatic ring.

3.3. X-Ray Diffraction (XRD) Analysis. X-ray diffraction studies were conducted to get information about the crystalline nature of silver nanoparticles. Figure 5 shows the X-ray diffraction pattern of AgNP-Alpinia obtained by microwave method. The XRD image shows four peaks at 2θ values of

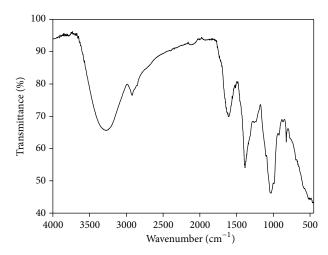


FIGURE 4: FTIR spectrum of *A. galanga* stabilized silver nanoparticles synthesized by microwave irradiation.

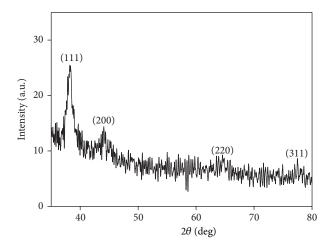


FIGURE 5: XRD pattern of AgNP-Alpinia synthesized by microwave method.

38.30°, 44.17°, 64.56°, and 77.53°, respectively. Comparing this with JCPDS file number 04-0783, the above peaks can be assigned to (111), (200), (220), and (311) reflections of face centered cubic silver nanoparticles. The X-ray diffraction studies undoubtedly demonstrate that the silver nanoparticles formed in this method are crystalline in nature.

3.4. HR-TEM Analysis. The size and shape of the synthesized silver nanoparticles were examined using transmission electron microscopy (TEM) analysis. The TEM images of AgNPs synthesized by microwave method are given in Figure 6. It is clear that the nanoparticles are almost spherical in shape. The particle size distribution histogram drawn after ignoring any tiny particle (Figure 6(b)) shows that the size of the particles comes between 15 and 27 nm and the average particle size is found to be 20.82 ± 1.8 nm. The HR-TEM image (Figure 6(c)) shows clear lattice fringes which indicates that the growth of silver nanoparticles takes place preferentially on the (111)

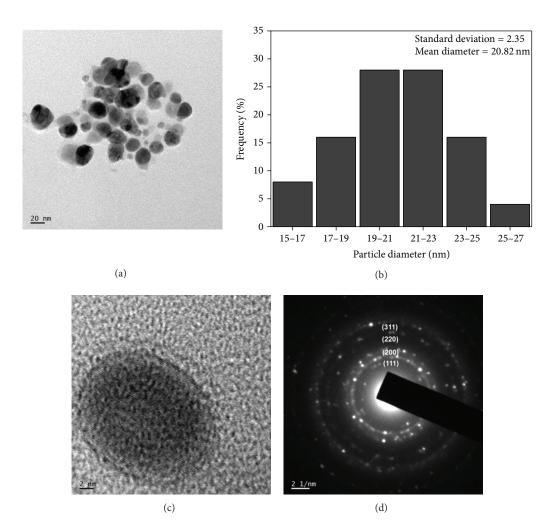
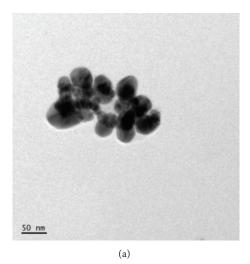


FIGURE 6: The TEM images of AgNP-Alpinia synthesized by microwave method: (a) image displaying particle distribution, (b) the corresponding particle size distribution histogram, (c) HR-TEM image, and (d) SAED pattern.

plane. The selected area electron diffraction (SAED) pattern (Figure 6(d)) of AgNP-*Alpinia* shows circular rings which can be attributed to the face centered cubic structure of silver nanoparticles. The more intense circular ring closer to the centre is due to (111) reflections. The second ring is indexed to the (200) reflections. The third and fourth rings belong to (220) and (311) reflections, respectively. The clear circular rings also suggest that the synthesized silver nanoparticles are polycrystalline in nature.

The TEM images of AgNP-Alpinia synthesized at room temperature are given in Figure 7. As is evident, the particles obtained in this case are a little more aggregated when compared to microwave method. Here, the average size of the nanoparticles is calculated to be 25.60 ± 1.90 nm which is higher than that obtained by microwave method. The results of TEM study are therefore in accordance with UV-vis. spectral study. Thus in this work, microwave irradiation provides smaller nanoparticles at a faster rate than room temperature synthesis.

3.5. Catalytic Reduction of Methyl Orange. The reduction of organic dyes by NaBH₄ has often been used for evaluating the catalytic efficiency of metal nanoparticles. A dye is considered to be suitable for catalytic study if it exhibits different colors in the oxidized and reduced forms and also if its absorption maximum does not interfere with the SPR band of metal nanoparticle [27]. Methyl orange is suitable for the catalytic study of silver nanoparticles because its aqueous solution is orange red in colour and upon reduction, it becomes colourless. In addition, the UV-vis. spectrum of methyl orange shows strong absorptions in the range of 200-600 nm with λ_{max} at 464 nm which is separated from the surface plasmon absorption of silver nanoparticles. The absorption maximum at 464 nm is due to the presence of azo group. The reduction of methyl orange by NaBH₄ in the absence of silver nanocatalyst is negligibly slow which is evident from the observation that the intensity of the peak remains nearly unchanged for several hours in its absence. On the other hand, a change in peak intensity as well as fading of the colour of methyl orange was observed immediately after



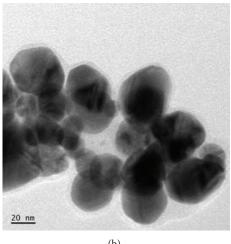


FIGURE 7: TEM images of AgNP-Alpinia synthesized at room temperature under different magnifications.

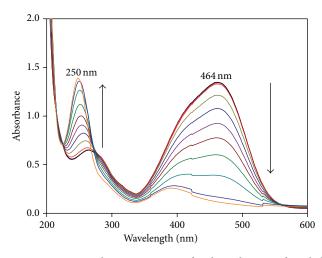


FIGURE 8: UV-vis. absorption spectra for the reduction of methyl orange catalyzed by 0.02 mg/mL AgNP-*Alpinia* measured at an interval of 1 min.

the addition of the catalyst indicating the enhancement in reaction rate in presence of the catalyst. The kinetics of this reaction was studied spectrophotometrically by monitoring the absorption peak at 464 nm. The UV-vis. spectra recorded in an interval of one minute during the reduction of methyl orange catalyzed by 0.02 mg/mL AgNP-*Alpinia* synthesized by microwave method at 30°C are given in Figure 8.

As is evident from Figure 8, the UV-vis. spectrum of methyl orange has absorption at 464 nm and 264 nm, respectively. The absorbance of the peak at 464 nm was found to decrease with time. At the same time, a new peak appeared at 250 nm whose intensity increased with time. The reaction was completed in 11 min as was evident from almost zero absorption at 464 nm. After completion of the reaction, an absorption band was observed at 396 nm. This is believed to be the SPR band of nanosilver. At intermediate stages of the reaction, this weak band is not observed as this peak is covered by the strong absorption band of methyl orange which extends over the range of 350–600 nm. But

the SPR band is blue shifted from 417 nm to 396 nm. This is because during the reduction process, the BH₄⁻ ions relay electrons to the surface of the catalyst resulting in its modification [28]. The main steps involved in this catalytic reaction are (a) the diffusion and adsorption of the electron donor borohydride ion and the electron acceptor methyl orange on the surface of AgNP catalyst, (b) the electron transfer between them, and (c) the diffusion of reaction products away from the catalyst surface. The capping agents surrounding the nanoparticles help the first step by attracting the reactants closer to the catalyst surface. The nanocatalyst plays the role of a moderator by passing on electrons from the donor to the acceptor. Since the amount of NaBH₄ used in this reaction is much higher than that of methyl orange, its concentration remains practically constant during the reaction and hence the reaction may be considered to follow pseudo-first-order kinetics. So the rate equation may be written as $k = 1/t \ln[A_o]/[A]$, where k is pseudo-firstorder rate constant, $[A_o]$ is the initial concentration of methyl orange, and [A] is the concentration at "t" time. Figure 9 shows the plots of ln[A] versus time obtained by using different amounts of AgNP-Alpinia catalyst. The good linear correlation between the variables reveals that the reaction strictly follows pseudo-first-order kinetics. Also, it is evident that the reaction rate increases with increase in the amount of catalyst. A small induction time was observed for all the reactions studied when carried out under air (inset of Figure 9) and this was found to decrease with increasing amount of the catalyst. A similar observation has been made by several other researchers for the nanosilver catalyzed reduction of 4-nitrophenol by NaBH₄ [29-31]. The induction time may be caused by the reduction of O₂ present in the reaction medium which takes place faster than the reduction of methyl orange [29] and also may be the time taken for the diffusion and adsorption of the reacting species on to the catalyst surface.

The first-order rate constants (k) obtained from the slope of the above linear plots are given in Table 1. It is apparent that

Amount of AgNP (mg/mL)	Reaction time (min)	$k (\text{min}^{-1})$	Correlation coefficient (R^2)
0.01	13	0.3550	0.9976
0.02	11	0.3884	0.9990
0.03	10	0.4229	0.9980
0.04	8	0.4553	0.9943

TABLE 1: Catalytic activity of AgNP-Alpinia for the reduction of methyl orange.

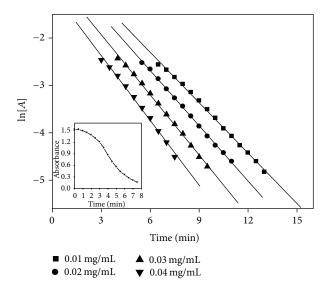


FIGURE 9: Plots of ln[A] versus time for the reduction of methyl orange by NaBH₄ using different amounts of the catalyst at 30°C.

the pseudo-first-order rate constants increase with increase in amount of the catalyst.

3.6. Antimicrobial Efficacy of AgNP-Alpinia. The antibacterial activity of the silver nanoparticles synthesized by microwave irradiation was investigated against the Gram positive bacteria Staphylococcus aureus and Bacillus subtilis and against the Gram negative bacteria Vibrio cholerae and Salmonella paratyphi. The antifungal activity was tested against Aspergillus niger. All the antimicrobial studies were done by well diffusion method. The solutions of AgNO₃ and plant extract having concentration the same as that utilized for nanoparticle synthesis were used in this study. The photographs showing the antimicrobial activity are shown in Figure 10 and the values of zone of inhibition observed around the wells are given in Table 2.

The silver nanoparticles (AgNPs) were found to be more effective against Gram negative than Gram positive bacteria. In addition, the bactericidal activity of AgNP was observed to increase with increasing dosage. It has already been reported that the bactericidal activity of nanoparticles depends on their size and dose and they usually show more activity against Gram negative bacteria than against Gram positive bacteria [32–35]. The structural difference between Gram positive and Gram negative bacteria is mainly in the composition and thickness of the peptidoglycan layer

in the cell wall. The cell wall of Gram positive bacteria is generally composed of a three-dimensional peptidoglycan layer of thickness approximately 20-80 nm, while that of Gram negative bacteria is only about 7-8 nm. Gram negative bacteria which possess a thin peptidoglycan layer beneath their cell membrane are thus more susceptible to nanosilver attack than Gram positive bacteria. The synthesized silver nanoparticles also showed excellent antifungal activity against Aspergillus niger. The aqueous plant extract did not show any antibacterial activity at the concentration level used in this study but was found to have some antifungal activity. Many possible mechanisms have been proposed for explaining the antimicrobial activity of silver. It is believed that Ag interacts with the -SH groups of proteins on the cell walls, thereby blocking respiration and causing death [36]. Sondi and Salopek-Sondi reported that the antimicrobial activity of silver nanoparticles was closely associated with the formation of "pits" in the cell wall of bacteria, thereby causing permeability and resulting in death [37].

4. Conclusions

In the present study, we have reported a simple, fast, ecofriendly, and economic method for the synthesis of silver nanoparticles. Here, silver nanoparticles have been produced by a biomediated microwave assisted synthetic route using the rhizome extract of Alpinia galanga as both the reducing and stabilizing agent. They have also been produced at room temperature without the assistance of microwave radiation and it is found that microwave method yields smaller nanoparticles in much lesser reaction time. The formation of silver nanoparticles is confirmed by UV-vis., FTIR, XRD, and HR-TEM techniques. The catalytic competence of the synthesized nanoparticles is investigated using the reduction reaction of methyl orange by NaBH₄. The catalyst exhibits remarkable catalytic activity for this reaction. The zones of inhibition obtained in the antimicrobial test suggest that the nanoparticles produced by this method have efficient antimicrobial activity. This green chemistry approach for nanoparticle synthesis has several promising attractions and can be used for the large scale production of silver nanoparticles which find application as nanocatalysts and antimicrobial agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Microorganism	Zone of inhibition (mm)					
	A. galanga extract (50 μ L)	$AgNO_3$ solution (50 μ L)	AgNP (40 μ L)	AgNP (50 μ L)		
S. aureus	Nil	12	13	14		
B. subtilis	Nil	11	13	14		
V. cholera	Nil	14	17	19		
S. paratyphi	Nil	14	16	18		
A. niger	12	25	_	29		

TABLE 2: Values of zone of inhibition obtained by well diffusion method.











FIGURE 10: The antimicrobial activity against (a) *V. cholera*, (b) *S. paratyphi*, (c) *B. subtilis*, (d) *S. aureus*, and (e) *A. niger.* In antibacterial study, (1) 50 μ L *A. galanga extract*, (2) 50 μ L AgNO₃, (3) 40 μ L AgNP, and (4) 50 μ L AgNP. In antifungal study, (1) 50 μ L AgNP, (2) 50 μ L AgNO₃, and (3) 50 μ L *A. galanga* extract.

Acknowledgment

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