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Green Synthesis of Silver Nanoparticles Using *Salacia Chinensis* Root and *Ammania Baccifera* Leaf Extracts and Evaluation of Their Antimicrobial Activity.

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KEYWORDS

Green synthesis, silver nanoparticles, Salacia chinensis, Ammania baccifera ,characterization techniques, antimicrobial activity

ABSTRACT:

Extracting AgNPs (silver nanoparticles) from plant materials through green methods is less harmful for the environment compared to using chemicals. In this study silver nanoparticles were produced using the roots Salacia chinensis and the leaves of Ammania baccifera and performed the green synthesis using these two plant materials. The synthesis was monitored for colour development which was confirmed using UV-visible spectroscopy. Further analysis through FT-IR, XRD, SEM, and TEM confirmed the presence of crystalline, spherical nanoparticles of sizes ranging from 10 to 33 nm. The AgNPs exhibited strong antibacterial and antifungal activities; notable antimicrobial activities against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Salmonella typhi were observed through standard well-diffusion and MIC/MBC tests. This approach to synthesis is not only inexpensive, but is easily scalable and favourable in temperature, avoiding high temperatures and toxic chemicals, supporting its use for medicinal and industrial purposes. As highlighted in this study, the plant mediated AgNPs demonstrated great potency against infection.

Introduction

Nanotechnology is a rapidly developing branch of biomedical research that has been helpful in a number of key technological achievements (1). It emerged in the 21st century and has been rapidly increasing ever since (2). Biotechnology in combination with nanotechnology is a burgeoning field of interdisciplinary research that is bridging the gap between the fields of materials science, nanotechnology, and biological science (3).Among the nanomaterials that have been investigated, silver nanoparticles (AgNPs) are considered to be among the most remarkable due to their excellent physical, chemical, and biological properties (4). Nanoparticles are very tiny particles, often ranging in size from one to one hundred nanometres. They do possess a number of beneficial qualities, such as antibacterial, anticancer, and catalytic functions (5). Because of this, they are valuable in a wide variety of fields, including health, scientific studies on the environment, cosmetics, and electronics. Silver nanoparticles are known to possess antibacterial properties; they effectively inhibit the growth of a variety of microorganisms with their ability to inhibit their multiplication (6). The synthesis of nanoparticles is accomplished by the utilisation of two separate types of biological and physicochemical processes. The top-down technique is somewhat different from the bottom-up strategy, which is the other option (7). Biosynthetic techniques utilizing microbes or plant extracts have arisen as a straightforward and feasible alternative to chemically manufactured and other physical processes (8). The aforementioned literature suggests that the synthesis of AgNPs from *Salacia chinensis* and *Ammania baccifera* is minimal. This study examines the synthesis of AgNPs through the reduction of aqueous Ag+ ions using the roots of *Salacia chinensis* and the leaf extract of *Ammania baccifera*. The green synthesized Ag-NPs were characterized through multiple microscopic and analytical methods, including X-ray powder diffraction (XRD), Fourier-transform infrared spectroscopy (FT-IR), ultraviolet-visible

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absorption (UV-vis) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) (9). The antibacterial and antifungal properties of green-synthesized Ag-NPs were compared with conventional antibiotics using standard susceptibility testing methods (10).

Materials and method

Chemicals, reagents and plant source

The reagents used were of analytical grade and is duly procured from Loba Chemical.*Salacia chinensis* and the leaf extract of *Ammania baccifera* were collected from Indore,India All chemicals, reagents, and culture media used in this study were of analytical grade. Silver nitrate (Sigma-Aldrich), aluminium chloride (Sigma-Aldrich), quercetin (Sigma-Aldrich), gallic acid (Sigma-Aldrich), sodium phosphate buffer (HiMedia), sodium chloride (HiMedia), sodium carbonate (HiMedia), ferric chloride (HiMedia), Folin-Ciocalteu reagent (Qualigens), dimethyl sulfoxide (Merck), methanol (Merck), ethanol (Merck), hydrochloric acid (Merck), sulphuric acid (Merck), Whatman filter paper No. 41 (GE Healthcare Life Sciences), carbopol 934 (Loba Chemie), and triethanolamine were used for the study.

Preparation of plant extract for synthesis of Ag-NPs

Gathering plant materials for extraction and doing first phytochemical analysis

For the purpose of producing the aqueous plant extract, the dried (powdered) plant material, which included the roots of *Salacia chinensis* and the leaves of *Ammania baccifera*, was brought to a boil with 100 millilitres of deionised water for a period of ten minutes [11]. AgNPs were produced by using the filtrate as a greenource of reducing agent in the production process, as the phytochemicals in the extract are responsible for the bioreduction of Ag+ ions to metallic silver (Ag0) [12]. Filtration of the extract using Whatmann filter paper no. 41 resulted in the production of the filtrate. During the preliminary phytochemical testing of this filtrate, it was determined that the presence of carbohydrates, proteins, flavonoids, and polyphenols was verified using standard analytical methods [13].

3.3.2 Synthesis of AgNPs

In the beginning, a solution with a concentration of 1 millimoles was made by dissolving 16.9 milligrammes of silver nitrate in one hundred millilitres of doubly deionised water [14]. To create silver nanoparticles (AgNPs), an aqueous solution of silver nitrate was combined with an aqueous plant extract [15,17]. A number of different volume ratios of silver nitrate solution to plant extract were optimised in order to generate the required quantity of silver nanoparticles (AgNPs). These volume ratios included 10:1, 10:2, 10:3, and 10:4 [18]. After an initial assessment that used visual colour change, the existence of silver nanoparticles (AgNPs) was confirmed by the use of ultraviolet-visible spectroscopy (Hitachi, Japan) [16]. Using the finalised ratio of silver nitrate solution to aqueous plant extract, the effects of stirring at a speed of 300 revolutions per minute, heating at a temperature of sixty degrees Celsius, and adjusting the pH to between two and twelve were tested [15,17].

3.3.3 Purification of AgNPs

AgNPs that had been synthesised were put through a cooled high-speed centrifuge (Kubota 6500, Japan) in order to get rid of any impurities that were present [19]. A centrifuge was used to spin the biomatrix for twenty minutes at a speed of seventeen thousand revolutions per minute and a temperature of four degrees Celsius [19]. Isolated settling AgNPs were then stored in a separate location for further in vitro antioxidant tests to be performed in the supernatant (RSL) taken from the centrifuge [21]. Verifying the UV-Visible spectra both before and after centrifugation was done in order to ensure that the synthesised AgNPs were of a very high purity [19][22]. For the purpose of characterisation, the AgNPs were produced by a procedure known as lyophilization, which included freeze-drying [20][23].

3.3.4 Characterization of AgNPs

Fourier transforms infrared spectroscopy (FT-IR): The FT-IR spectra of lyophilised silver nanoparticles (Shimadzu, Japan) were investigated. Examining the samples with a resolution of 4 cm, in the range of 450 to 4000 cm, was accomplished by the use of the KBr pellet method.[28]

X-ray diffraction: For the purpose of acquiring X-ray diffraction data of AgNPs, a diffractometer, namely a Philips PRO expert diffractometer that was manufactured in the Netherlands, was used. Cu Ka radiations that were filtered with nickel were used to

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acquire the data at a temperature of thirty degrees Celsius. A voltage of 40 kilovolts, a current of 30 milliamperes, and a range of 70 to 700 twenty were used to run the system.[29]

Scanning electron microscopy-Energy dispersive X-ray Spectroscopy (SEM- EDAX/EDS): In order to scan the materials for the SEM-EDAX experiments, the SEM Quanta 200, which is compatible with the EDAX system, was used. After applying a tiny quantity of AgNPs colloid to a carbon grid and allowing it to dry in a low vacuum (10-130pa), the voltage was maintained at 20 kev throughout the process. Initially, a magnification of three thousand times was used in order to scan the substance. Following the completion of the repair, the EDAX equipment was used to investigate whether or not silver was in fact present. Immediately after the confirmation, scanning was carried out at 6000x and 12000 xs.[30]

Transmission electron microscopy (TEM): To get crisp pictures from a TEM (Hitachi - H-7500) 120 kV equipped with CCD camera, samples of AgNPs colloid were spotted onto a carbon-coated copper grid and scanned via 1,50,000x to 3,00,000x magnification.

Antibacterial activity

The antibacterial phytosynthetic activity of silver nanoparticles against four standard pathogens which includes *Staphylococcus aureus*, *Pseudococcus aeruginosa*, *Escherichia coli* and *Salmonella typhi*, was researched [31]. For initial screening, the agar well diffuse method was used [32]. Muhler Hinton agar was prepaired and sterilized [32]. The rest of the process was completed under a 37 degree incubator to avoid the contamination of microorganisms [33]. Following this step, the wells, measuring 6mm in diameter, were loaded with ciprofloxacin, AgNPs and silver nitrate [34]. The loaded plates were also left to incubate for 37 degree for a day [33]. The wholes were measured with a Hi-Media scale [34]. The experiment was calculated in triplicate and calculated with standard deviation [35]. The MIC and MBC was calculated with the serial dilution method. The AgNP phytosynthesized was defined the lowest concentration with no visible bacterial growth . The phytosynthesized AgNPs MBC was also the lowest concentration with no growth on Muhler Hinton plates after 24 hours [36].

Results and discussion

Synthesis of AgNPs

The synthesis of silver nanoparticles (AgNPs) was accomplished by adding silver nitrate (AgNO3) solution to aqueous SC and AB plant extract in different proportions. The formation of brown colored nanoparticles was observed as a visible color change from colorless to brown (Fig.1). It is greatly due to the excitation of surface plasmon resonance (SPR) in AgNPs. Colour changes that were similar have been reported in previous green synthesis studies, like in Ahmed et al. (2016), who described brown coloration as a form of rapid indicator of AgNP formation.[37]

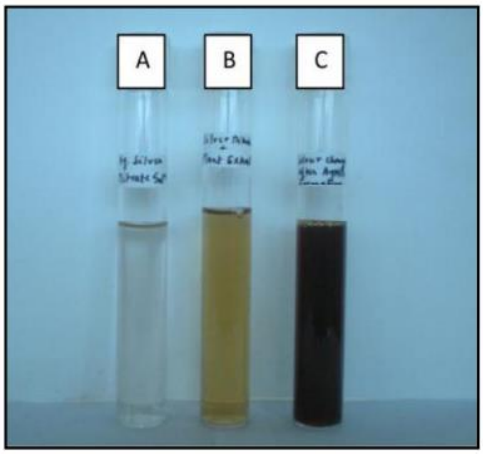

Figure 1: Typical image illustrating colour variations before to and during AgNP creation, where A: colourless aqueous silver nitrate solution B: aqueous plant extract C: brown colour formation following AgNP formation

Characterization of AgNPs

FTIR Analysis

The synthesis of phytochemical coated lyophilized silver nanoparticles (AgNPs) was characterized by FT-IR. SC mediated AgNPs (Fig. 2) displayed a FT-IR spectrum with characteristic peaks 3637, 3429, 3313, 3178, 2926, 1728, 1614, 1585, 1317 and 1191 cm^{-1}, demonstrating clear cut peaks characteristic of AgNPs suggesting phytochemical mediated synthesis. It is also interesting to note the absence of the hydroxyl group peaks in the stirring / heating synthesis, which was weakly under alkaline conditions. The peaks of AB mediated AgNPs (Fig. 2) showed the characteristic peaks of 3488, 3047.3,

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1749 and 1577.77 cm^{-1} confirming phytochemical plant signatures. The suppression or shift of hydroxyl bands under alkaline conditions suggests that there is the presence of these functional groups in silver reduction, that are again in equilibrium with findings by Mittal et al. (2013). [38] AB-mediated AgNPs showed prominent peaks at 3488, 3047, 1749, and 1577 cm^{-1}, that confirms similar phytochemical interactions. The absence of strong hydroxyl peaks under these conditions suggests that there is consumption during AgNP formation. The peak assignments and shifts suggest sililarity with what was reported by Song & Kim (2009), thus validating our known observations.[39]

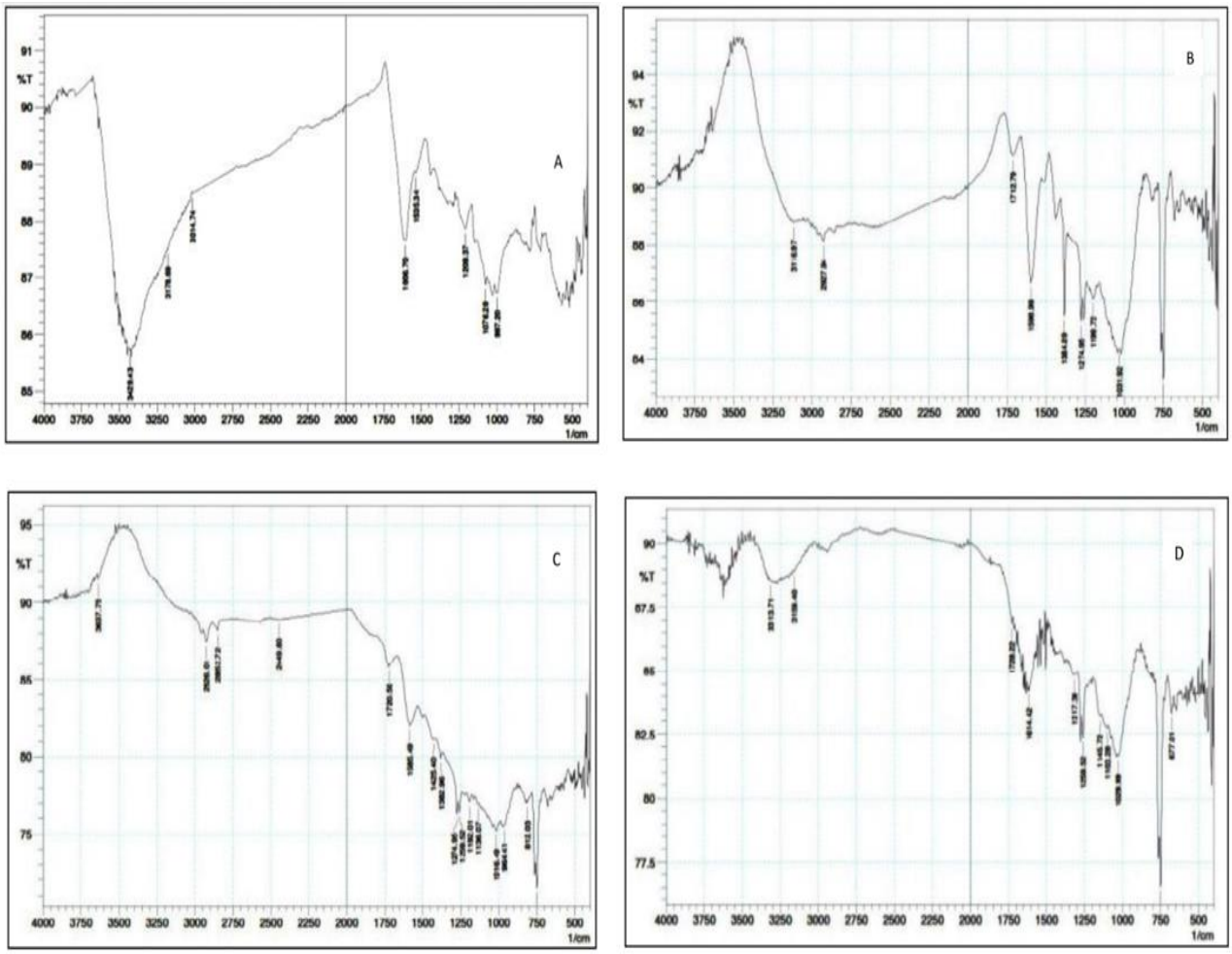

Figure 2 : FT-IR spectrum of SC mediated synthesis of AgNPs at(A) room temperature (B) by stirring (C) by heating (D) alkaline condition

UV–vis spectra analysis

AgNPs were separated from the residual plant biomatrix by repeating centrifugation at 17000RPM for 20 minutes at 4 degrees C. The obtained pellets were then redissolved in deionized water. The washing procedure was performed 3 times to remove phytochemical impurities. The AgNPs were characterized and the purified samples were confirmed with UV spectroscopy showing no secondary peaks of plant phyto and silver ions. Only characteristic AgNP absorption peaks were seen (Figs. 3). Therefore, the effective purification was performed and the monodispersity of the nanoparticles were confirmed before proceeding with further analyses.

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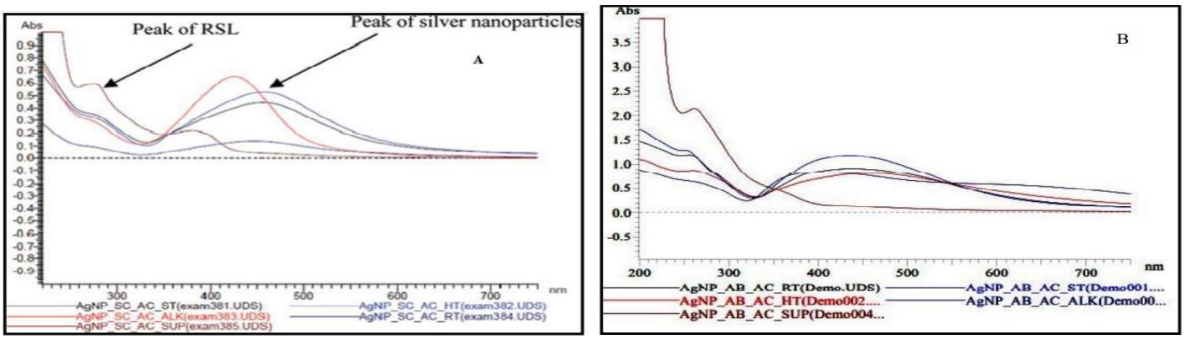

Figure 3. (A).UV-visible overlay spectra of SC mediated synthesis of AgNPs.(B).UV-visible overlay spectra of AB mediated synthesis of AgNPs at all optimized conditions

SEM and TEM analysis

Fig 4 show the SEM micrographs with the corresponding synthesis conditions which include Alkaline Conditions, Stirring, Heating and Ambient. Heating was noted to give larger and better dispersed particles when compared to room temperature and solid-state synthesis. Alkaline conditions did show silver nanoclusters which could be seen in the SEM micrographs. TEM analysis did confirm the polymorphic (triangular, hexagonal, spherical) morphologies .(Fig 5). The observed size changes is due to the differences in nucleation and growth rates under different synthesis environments. Xie et al. (2007) also showed similar in report that temperature elevation impacts agglomeration but also promotes the quality and regularity of crystal.[40] The particle diameters in our study lies within 20–100 nm.These are consistent with literature values for green-synthesized AgNPs(Bhainsa & D'Souza, 2006), reported with a 50–80 nm range using yeast extract.[41]

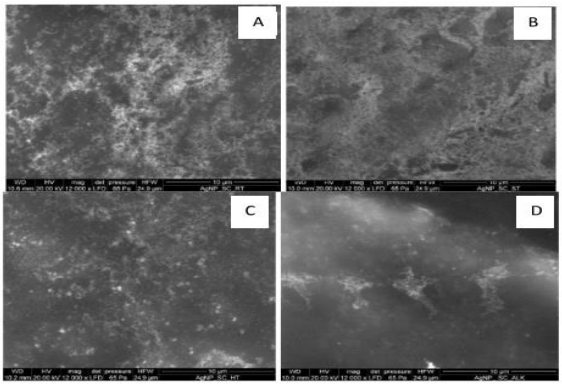

Figure 4. SEM image of SC mediated synthesis of AgNPs at room temperature (A) by stirring (B) by heating (C) in alkaline condition (D)

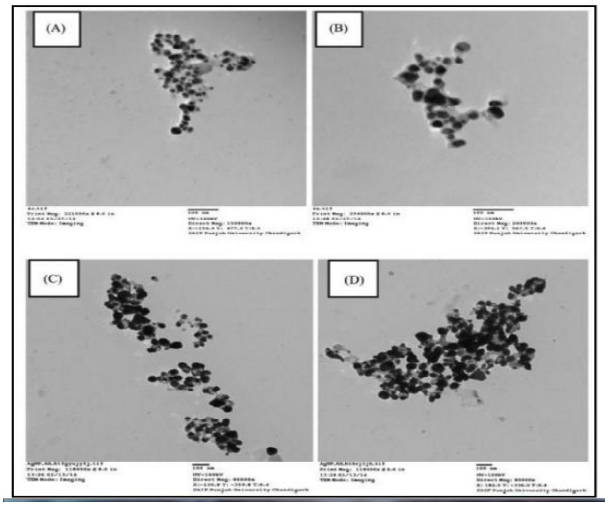

Figure 5: TEM image of SC mediated synthesis of AgNPs at room temperature (A) by stirring (B) by heating (C) in alkaline condition (D)

XRD analysis

XRD confirmed the crystalline nature. The SC synthesized AgNPs (Fig.) showed peaks at 32.82°, 38.82°, 44.22° and 64.32° which are (111), (200), (220) and (311) of fcc structure. While AB synthesized AgNPs (Fig.6) showed characteristic peaks at 32.26° and 46.20°. Observed values matched with standard silver (Fig 7) verifying phase purity. This study confirms the crystalline nature and phase purity of the synthesized nanoparticles suggesting similar observations by Song & Kim (2009), who suggested that fcc silver is standard for AgNPs of plant origin.[42]

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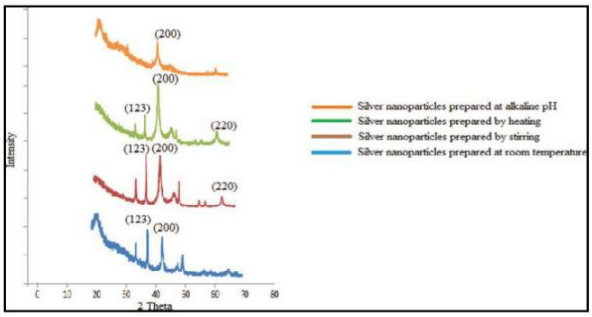

Figure 6: X-RD spectrum of SC mediated synthesis of AgNPs

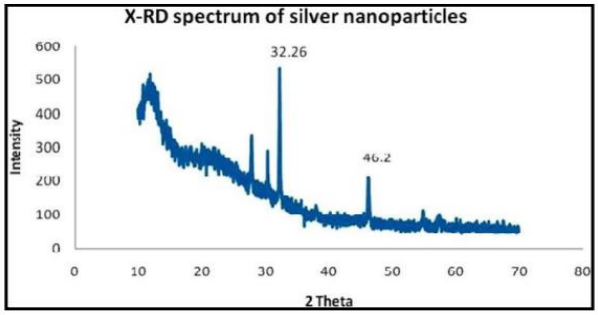

Figure 7: X-RD spectrum of AB mediated synthesis of AgNPs

Antimicrobial assays (in vitro)

As seen in Figure 8, both the 0.2% silver nitrate gel (Silverex™ ionic) and 0.03% AgNPs gel displayed the same zones of inhibition (17 mm) suggesting no difference in activity. The AgNPs gel formulation (F2) showed the best efficacy against S. aureus, P. aeruginosa, and methicillin resistant S. aureus (MRSA). The comparative analysis consisted of (1) 0.03% AgNPs colloidal dispersion, (2) 0.03% AgNPs gel formulation and (3) 0.2% silver nitrate gel. The most important outcome was that the phytosynthesized AgNPs gel achieved complete inhibition of all the tested strains.

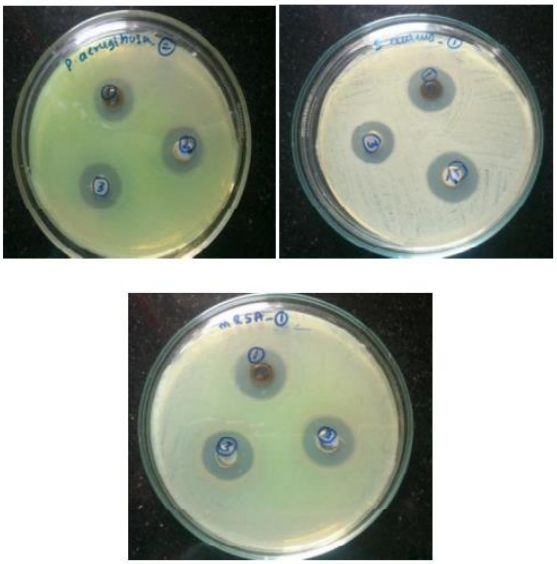

Figure 8: Antibacterial efficacy of AgNPs gel formulation (F2) against S. aureus, P. aeruginosa, and methicillin-resistant S. aureus was 1: AgNPs colloidal dispersion (0.03%). 3: Commercially available gel with 0.2% silver nitrate (SilverexTM ionic) and 2: AgNPs gel (0.03%)

Table 1: Comparative AgNPs zone of inhibition and synergistic effect with ciprofloxacin made using SC and AB aqueous plant extracts

Sr. No	Component	Zone of inhibition (mm)			
		P aeruginosa	*E. Coll*	*S. typhi*	*S. aureus*
1.	Ciprofloxacin (5 mg/ml)	24±1.52	34±0.57	28±1.52	20±0.57
2.	Ciprofloxacin(5mg/ml) + AgNPs_SC(100|10|10	24±0.57	34±0.57	28±1.00	18±1.52
3.	AgNPs_SC(100 mg/ml)	13±1.0	NZI	NZI	13±0.57
4.	AgNPs AB (100 mg/ml)	15.66±0.577	NZI	NZI	NZI
5.	$AgNO_3$(100 pg/ml)	NZI	NZI	NZI	NZI

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6.	SC plant extract	NZI	NZI	NZI	NZI
7.	AB plant extract	NZI	NZI	NZI	NZI

NZI- No Zone of Inhibition

Conclusions

The use of plant extracts for the synthesis of nanoparticles is an environmentally friendly method that deviates from the traditional chemical approaches. This method is still in the process of being developed. As discussed in [35–42] , the leaf extract of R. officinalis can be considered for the green synthesis of Ag-NPs since it reduces silver salts. Ultraviolet–visible spectroscopy indicates the surface plasmon properties of the metallic nanoparticles. R. Officinalis is easily accessible in the locality of my region which is why I selected it. With regard to the influence of time on the formation of Ag-NPs, it was found that the reaction was quite fast shortly after the reaction was initiated. FT-IR confirmed the active reduction agents in the extract are the phenolic compounds. the photomicrographs obtained from SEM and TEM confirmed the Ag-NPs were nanometer in size, while the XRD peaks obtained confirmed the (FCC) crystalline structure. Ag-NPs have been found to possess strong antimicrobial properties against multiple strains of varied microbes. This work shows that the leaves of R. officinalis can be utilized for the synthesis of nanoparticles by reduction methods. One of the merits of this work is the synthesis of Ag-NPs without the requirement of high pressures or temperatures, and without the use of toxic chemicals and extensive energy.

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