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RESEARCH ARTILCE

Comparative Study of Antibacterial and Antifungal Activities of Silver Nanoparticles and Capsicum Annum Leaves Extracts

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ABSTRACT

Researchers are becoming more interested in the eco-friendly bio-synthesis of Ag-NPs because they could be used in many areas of science and technology. The Capsicum annuum L. extract was used to prepare silver nanoparticles (Ag-NPs) by treating silver nitrate with it. To make the Ag-NPs, a water-based extract of capsicum annum leaves was used to change the Ag^+ ions in silver nitrate to Ag^0.The synthesized particles was characterized using SEM, EDX, FTIR, and UV visible spectroscopy. The synthesized particles and extract were then tested for their ability to kill bacteria and fungi by testing them against species like Escherichia coli, Pseudomonas florescens, Bacillus subtilis, Staphylococcus aureus, Pseduomonousaliginoza, Candida albicans, Rhizopus oryzae, Aspergillus Niger, Aspergillus Parasiticus, and Aspergillus flavus. The comparison of antibacterial and antifungal activity against microbes was carried out and it was found from the growth curves of bacteria and fungi that the silver nanoparticles showed more zone of inhibition than the plant extract.

1. Introduction

Nanoscale materials structures, which are usually between 1 and 100 nanometers (nm), are being used in new ways in nanoscience and nanotechnology. Nanomaterials might be able to help solve technology and environmental problems in biology, biomedical science, solar energy conversion, and water treatment. Nanoparticles have a very large surface area to volume ratio. In science, where a lot of surface area is needed, this feature can be used. One example is the catalytic industry, where some nanoparticles have been shown to work well as catalysts [1]. In addition, the nanoparticles have the ability to kill bacteria. Silver nanoparticles (AgNPs) are thought to have these important properties. Nano biotechnology study has paid a lot of attention to AgNPs because they have special physical, chemical, and biological properties and can be used in medicine, optics, and electronics. Also, silver nanoparticles can be used for many other important things. For instance, they can be used as spectrally selective coatings to absorb solar energy, as an intercalation material for batteries, and as optical sensors for bio marking. AgNPs are well known for their biological properties. But in the last few years, pathogenic germs have become resistant to antimicrobials. This is a big problem for the health care business, and many people have looked into it [2]. Because they have special

physical and chemical properties, AgNPs are found to be effective against microbes [3]. AgNPs made from the extract of Chrysophyllum oliviforme are resistant to various bacteria [4]. AgNps made from Morinda citrifolia leaf extract were also effective against various microbes [5]. Furthermore, AgNPs made from Phoenix sylvestris showed better antibacterial activity [6]. AgNPs also showed antioxidants [7]. AgNPs have great antimicrobial action, even at low concentrations, because they have a large surface area to volume ratio. In addition, they have low cytotoxicity and immune reaction. AgNPs have been used in dentistry to make antibacterial products that make the quality of dental work better [8]. Nanoparticles made from metabolites have a lot of biological functions [9]. Phytochemicals in plant can also bind to metals and have a chelating effect. When it comes to natural antioxidants, flavonoids, coumarins, tocopherols, and cinnamic acid products are some of the most important ones [10,11]. A lot of natural items have been used to make nanoparticles. It was found that aromatic and medicinal plants could help make silver nanoparticles, which had a lot of cellular effects. The bark of the Salacia chinensis plant was used to make silver nanoparticles. The green nanoparticles that were made showed strong action against cancer cell [12]. Dittrichia graveolens was used to make silver nanoparticles that showed powerful antioxidant action [13]. Scientists used medical plants to make silver nanoparticles that showed strong antioxidant properties [14,15,16]. Also, the Sideritis montana leaves used to make AgNPs were said to have a lot of antioxidant activity [17]. Extracts from plants work as both stabilisers and reducers when nanoparticles are being made [18]. Due to the harmful effects of manufactured products, there is a need to make nanoparticles that are safe, cheap, and can be turned into products quickly [19]. Green chemistry principles can be used for synthesis of nanomaterials from plants, like austenite and silver nanoparticles made from live Lucerne [20]. The biosynthesis of $CaCO_3$ crystals from chickpea seeds that are sprouting [21], and the broth of geranium and lemongrass leaves to make Au and Ag NPs [22,23]. The plant Capsicum annuum L. is grown and popular in many countries It's cheap and easy to find in stores. It's also not harmful like some other reducing agents, which makes it a great all around agent for making nanomaterials. Some of the biomolecules found in Capsicum annuum L. extract are proteins, enzymes, polysaccharides, amino acids, and vitamins. These can be used to mix with silver ions and help shape the formation of silver nanoparticles in solution [24].

In this study AgNPs was produced using Capsicum annuum L. extract (Figure 1) and evaluated for the antimicrobial activities of selected microbes.

Figure 1. Capsicum annum

2. Materials and Methods

2.1 Chemicals

The Capsicum annuum L. used in this experiment was brand new and green. It came from the district of Swat in the province of Khyber Pakhtunkhwa Pakistan. Sigma Aldrich was used to buy the silver nitrate and methanol.

2.2 plant extraction

The Capsicum annuum L plant was gathered in Swat, which is a district of Khyber Pakhtunkhwa. Organic liquids and then distilled water were used to wash the leaves of capsicum annum. After being washed, the leaves were left to dry in the shade. Capsicum annuum L leaves that had been dried out were boiled in 200 mL of deionized (DI) water at 55 °C for 4 h. The solution was split into two equal parts after being filtered through Whatman filter paper. One was used to make silver nanoparticles, and the other part was used for the antimicrobial activity of extract [25].

2.3 Silver NPs synthesis

Some Capsicum annum leaves (10 g, 100 mL) were mixed with $AgNO_3$ (2 mM, 100 mL) in DI water and left at 60 °C for 2.5 h. It went from being light yellow to dark brown. UV–Vis measurements were used to track the progress of the process. Once the reaction was done, it was spun at 8,000 rpm for 20 min. The nanoparticles were then dried in a vacuum at 60 °C for 1 hour [25].

2.4 Microorganisms

Escherichia coli, Pseudomonas florescens, Bacillus subtilis, Staphylococcus aureus, and Pseduomonousaliginoza were

the types of bacteria used in the study. Different types of fungi were used in the research. These were Aspergillus flavus, Candida albicans, Rhizopus oryzae, Aspergillus Parasiticus, and Aspergillus Niger.

2.5 Characterization

A Shimadzu Corporation made FTIR spectrophotometer was used to identify the functional groups. SEM analysis were done to know about the size and morphology of the particles and to know about the elemental analysis of the synthesized particles an Oxford EDX (Inca-200) was used.

2.6 Evaluation of antifungal and antibacterial activity

The agar well diffusion method was used to test the antimicrobial activity of the extract and AgNPs. It took 100ppm of the culture of each strain to cover a Petri dish with Muller Hinton agar for bacteria and potato dextrose agar for fungus. A clean cork drill was used to make four holes in each plate. The first well has DMSO as a negative control. The 2nd well has sulfamethoxazole for bacteria and clotrimazole for fungi (100ppm) as a positive control. The third well has a extract (100ppm), and the fourth well has AgNPs (100ppm). For antibiotic activity, Petri plates were kept in an incubator at 37 °C for 24 h. For antifungal activity, they were kept in an incubator at 28 °C. We also found the nanoparticles minimum inhibitory concentration (MIC) against different diseases and got important results [26].

3. Results and discussion

3.1 UV spectroscopy analysis

Green production of AgNPs was done with plant extract, which helps break down Ag+ in an $AgNO_3$ solution. The AgNPs and extract are made, they show bell-shaped absorption peaks at 410 nm with an absorbance of 0.2 and 457 nm with 2.3, respectively (Figure 2). The fact that the absorption peak shows up in this range proves that silver nanoparticles were made. The phytoconstituents in the extract of capsicum annum leaves may be what cause the bioreduction because they take part in processes and help make AgNPs and keep them stable through different plant metaboliteS [27]. In the past, dried fruits of Lycium barbarum were utilized to make silver nanoparticles, which had a UV peak at 438 nm [28]. According to the earlier study, nanoparticles with an absorption peak between 390 nm and 420 nm were small. On the other hand, nanoparticles with an absorption peak near 450 nm or higher were of different sizes [29].

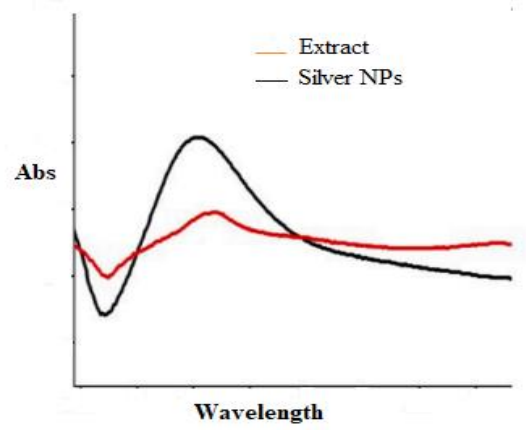

Figure 2. UV graph of extract and AgNPs.

3.2 FTIR analysis

The FTIR test was used to describe the green synthesized AgNPs. The FTIR (Figure 3) study showed the bioactive compounds functional groups that are in charge of reduction, stabilization, and capping. In the spectrum, the peak at 3197 and 2928 cm^{-1} were due to the OH stretching [30]. The peak at 2101 cm^{-1} showed the CH bending vibration, and at 1583 cm^{-1}, the C=C stretching signal was seen (Figure 3). The 1371 cm^{-1} peak is for OH bending and the 1240 cm^{-1} peak is for CN stretching of the amine. The CO bond was seen at 1043 cm^{-1} and the CH bond was seen at 765 cm^{-1}. The FTIR values were the same as the values of AgNPs made from S. ebulus seeds [31].

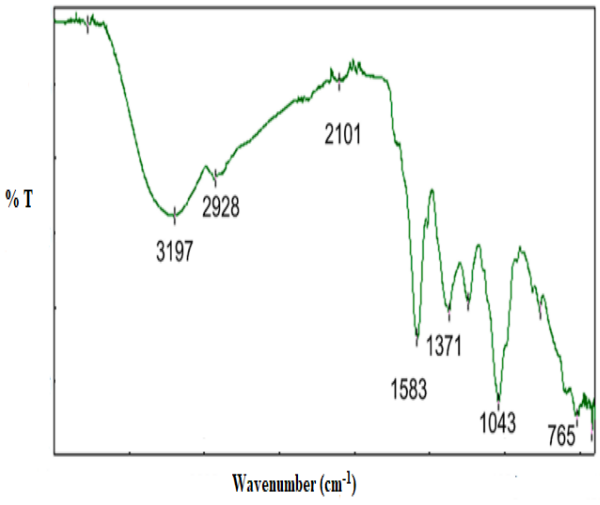

Figure 3. FTIR spectrum of AgNPs

3.3 SEM Analysis

The SEM was done for the surface morphology of the synthesized silver nanoparticles and showed circular morphology with a grapes like distribution (Figure 4). The particle size was measured using image j software and the size was found to be in the range of 60 to 80nm. In the past, Khan et al. did a Green synthesis of AgNPs from Saliva Sclarea leaf extract and discovered that the surface of the synthesized nanoparticles is uniform, but different nanopores can be seen [32]. In a different study, Karan et al. made silver nanoparticles using an extract from Sambucus ebulus leaves. They used SEM to see that the AgNPs@Se had a high density, a uniform distribution, and less clumping together. It was found that the green synthesized AgNPs@Se particles had an average size of 18.6 nm and a spherical shape [25].

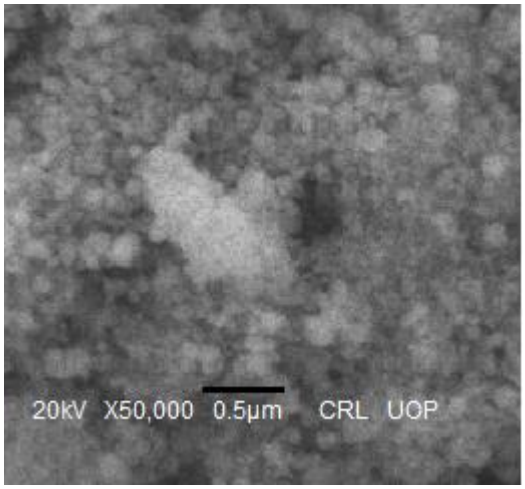

Figure 4. SEM image of the AgNPs

3.4 EDX spectrum

An EDX spectrum confirmed the elements that made up the synthesized AgNPs. The EDX spectrum of the AgNPs is shown in Figure 5. The elements present are the oxygen, silver ,and carbon (Table 1).

Table 1. Elemental composition of AgNPs

Element	Weight%	Atomic%
Ag	68.23	71.34
O	20.63	19.17
C	9.27	6.49

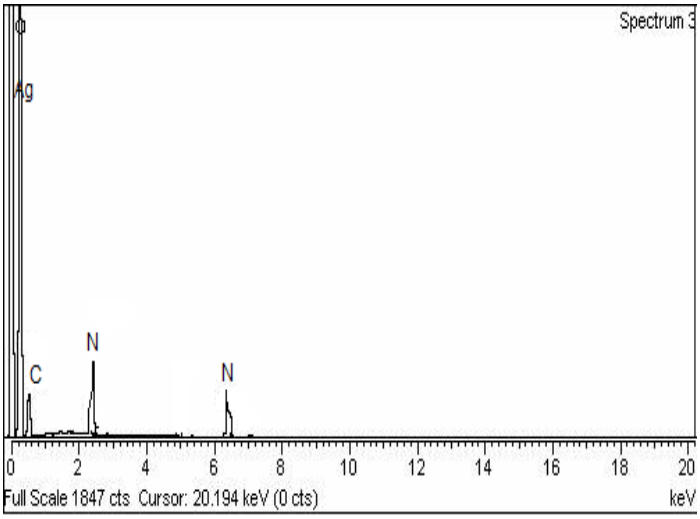

Figure 5. EDX spectrum of silver nanoparticles

3.5 Antibacterial activity

The Agar well diffusion method was used to evaluate the antimicrobial activities and to see how well the extracts and AgNPs worked against harmful microbes. With 50 mg/mL of extract, the zone of inhibition (ZOI) in table 2 for E.coli was 5.7 mm, for P. fluorescens it was 7 mm, for S. enterica it was 11 mm, for B. subtilis it was 6 mm, and for S. aureus it was 7 mm. It is possible to use AgNPs to kill germs because they are stable and can work against both gram positive and gram negative bacterial cells. Using AgNPs the zone of inhibition for E.coli was12 mm, for P. fluorescens was 13 mm, for S. enterica was 14 mm, for B. subtilis was 8 mm, and for S. aureus was found to be 9 mm. The antibacterial results are shown in the form of a graph in figure 6. The bacteria that were tested showed the highest ZOI gainst S. enterica for both the extract and the AgNPs. It is possible for small AgNPs to kill more bacteria than big ones because they can interact with more surfaces and be absorbed more easily. Based on the results of this study, the AgNPs works well against Salmonella enterica. It was also said what were the MIC of the extract and AgNPs. It was found that the NPs had much lower MICs than the extracts. The results clearly showed that the nanoparticles that were made have strong antibacterial properties. So, it can be used as a good source for making new drugs that kill germs that are resistant to them. Table 3 shows the MIC values that were found for different strains.

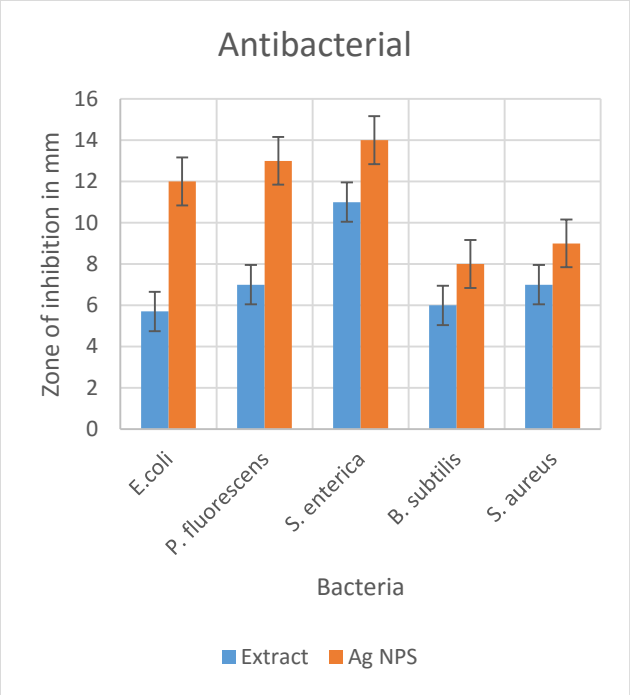

Figure 6. Error bar graph of antibacterial activity of extract and Ag NPs

Table 2. Zone of inhibition

Strain	Extract (mm)	Ag NPS (mm)
E. coli	5.7	12
P. fluorescens	7	13
S. enterica	11	14
B. subtilis	6	8
S. aureus	7	9

Table 3. MIC of extract and Ag NPs

Strain	Extract (mg/mL)	Ag NPS (mg/mL)
E.coli	13	2
P. fluorescens	14	1.6
S. enterica	3	4
B. subtilis	11	2
S. aureus	3	1.6

3.6 Antifungal activity

The Agar well diffusion method was used to see how well the extracts and AgNPs worked against harmful microbes. It took 50 mg/mL of extract to kill C. albicans (5.2 mm), A. niger (8.0 mm), R. oryzae (8.3 mm), A. paharisticus (6.8 mm), and A. flavus (8.3 mm) after it was incubated. AgNPs are used to kill living things because they are stable and can kill a wide range of fungus cells. The zone of blockage for AgNPs (50 mg/mL) grew to 6.3 mm for C. albicans, 11 mm for A. niger, 14 mm for R. oryzae, 9.6 mm for A. paharisticus, and 13.5 mm for A. flavus (Table 4). The antifungal results that were seen are shown in the form of a graph in figure 7. For the bacteria that were used, the highest ZOI was seen against R. oryzae and A. niger for the extract and against R. oryzae for the AgNPs. Because AgNPs have more surface area to interact with and are easier to absorb, small AgNPs can be more effective at killing fungi than big ones. It was also said what the MIC of the extract and nanoparticles were. It was found that the nanoparticles had much lower MICs than the extracts. The results clearly showed that the nanoparticles that were made have strong antifungal properties. So, it can be used as a good source for making new drugs that kill germs that are resistant to them. Table 5 shows the MIC values that were found for different strains.

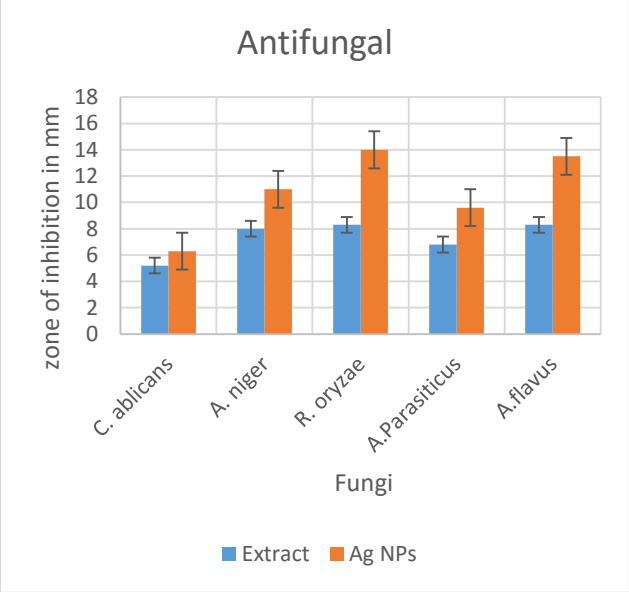

Figure 7. Error bar graph of antifungal activity of extract and Ag NPs.

Table 4. **Zone of inhibition**

Strain	Extract (mm)	Ag NPs (mm)
C. albicans	5.2	6.3
A. niger	8 .0	11.0
R. oryzae	8.3	14.0

A. Parasiticus	6.8	9.6
A. flavus	8.3	13.5

Table 5. MIC of extraxt and Ag NPS

Strain	Extract (mg/mL)	Ag NPs (mg/mL)
C. albicans	16	1.6
A. niger	17	3.0
R. oryzae	13	2.6
A.Parasiticus	14	2.8
A.flavus	16	3.0

4. Conclusion

The results showed that substantial amounts of silver nanoparticles (AgNPs) were produced and that this was verified using UV-visible Spectroscopy. We observed appearance of a Surface Plasmon Resonance (SPR) peak, which corresponds to the silver nanoparticles 410 nm. In conclusion and based on our results, extracts of plant leaves produce usable silver nanoparticles. To make the Ag-NPs, a water-based extract of capsicum annual leaves was used to change the Ag+ ions in silver nitrate to Ag^0. The silver nanoparticles was characterized using UV, FTIR, SEM, and EDX. The synthesized particles and extract were then tested for their ability to kill bacteria and fungi by testing them on species like Escherichia coli, Pseudomonas florescens, Bacillus subtilis, Staphylococcus aureus, Pseduomonousaliginoza, Candida albicans, Rhizopus oryzae, Aspergillus Niger, Aspergillus Parasiticus, and Aspergillus flavus. The growth curves of bacteria and fungi showed that silver nanoparticles showed more antimicrobial activities than the extract.

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