

## **Biogenic Synthesis of Silver and Iron Oxide Nanoparticles Using *Aronia prunifolia* Leaf Extract and Its Inhibitory Action Against Pathogenic Fungi**

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### **Abstract**

Because of the environment and abundant renewable resources, exploiting plant extracts to form metallic nanoparticles has become a promising alternative to chemical and physical methods. Numerous studies have shown that nanoparticles of silver (AgNPs) and iron oxide (Fe<sub>2</sub>O<sub>3</sub>NPs) have inhibitory effects against pathogenic fungi. In this study, we used the leaf extract of *Aronia prunifolia* to generate biogenic AgNPs and FeNPs, aiming to demonstrate the impact of nanoparticles on pathogenic fungi. Nanoparticles are characterized by UV-Vis, X-ray diffraction, EDX spectrum, and SEM techniques. Leaf extracts used for nanosynthesis yielded silver and iron oxide nanoparticles with distinct color changes and absorption peaks, showcasing tetragonal, pentagonal, and hexagonal shapes (15-50 nm) for silver and spherical morphology (16-60 nm) for iron oxide. The antifungal effectiveness of nanoparticles against *Aspergillus fumigatus*, *Rhizoctonia solani* Ag4 HgII, and *Aspergillus flavus* was investigated using a well diffusion method. Inhibition zones, ranging from 12.5 to 35.0 mm for AgNPs and 7.1 to 17.1 mm for FeNPs at concentrations of 10 to 30 µg/ml respectively, demonstrated the superior inhibitory potential of AgNPs over FeNPs. This study aims to address a gap in the literature by examining the inhibitory effects of green AgNPs and FeNPs on pathogenic fungi. Encased nanoparticles can be very useful in treating fungal infections; this will be the first investigation into the production of nanoparticles from *A. prunifolia* leaves.

**Keywords:** Well diffusion method, Nanoparticle characterization, *Aronia prunifolia*, Green synthesis, Antifungal activity.

## ***Aronia prunifolia* Yaprağı Ekstresi Kullanılarak Gümüş ve Demir Oksit Nanopartiküllerinin Biyojenik Sentezi ve Patojenik Funguslara Karşı İnhibitör Etkisi**

### **Öz**

Çevre ve yenilenebilir kaynakların bolluğu nedeniyle, metalik nanopartiküller oluşturmak için bitki özlerinden yararlanmak, kimyasal ve fiziksel yöntemlere umut verici bir alternatif haline geldi. Çok sayıda çalışma, gümüş (AgNP'ler) ve demir oksit (Fe<sub>2</sub>O<sub>3</sub>NP'ler) nanopartiküllerinin patojenik funguslara karşı önleyici etkiler olduğunu göstermiştir. Bu çalışmada *Aronia prunifolia*'nın yaprak ekstrektü kullanılarak biyojenik AgNP'ler ve FeNP'ler üretilerek yaprak ekstrektü ve nanopartiküllerin patojenik funguslar üzerindeki etkileri gösterilmiştir. Nanopartiküller UV-Vis, X-ışını kırınımı, EDX spektrumu ve SEM teknikleriyle karakterize edilmiştir. Nanosentez için kullanılan yaprak ekstrektüleri, gümüş için dörtgen, beşgen ve altıgen şekiller (15-50 nm) ve demir oksit için küresel morfoloji (16-60 nm) sergileyen, farklı renk değişimleri ve absorpsiyon zirveleri olan gümüş ve demir oksit nanopartikülleri verdi. Nanopartiküllerinin *Aspergillus fumigatus*, *Rhizoctonia solani* Ag4 HgII ve *Aspergillus flavus*'a karşı antifungal aktivitesi, iyi bir difüzyon yöntemi kullanılarak incelenmiştir. 10 ila 30 µg/ml konsantrasyonlarda AgNP'ler için 12.5 ila 35.0 mm ve FeNP'ler için 7.1 ila 17.1 mm arasında değişen inhibisyon bölgeleri, AgNP'lerin FeNP'lere göre üstün inhibitör potansiyelini ortaya koydu. Bu çalışmada, yeşil AgNP'ler ve FeNP'nin patojen fungus izolüne karşı önleyici aktivitesini karşılaştırarak literatürdeki bir boşluğu doldurmayı umuyoruz. Kaplanmış nanopartiküller fungus enfeksiyonlarının tedavisinde çok faydalı olabilir; bu, *A. prunifolia* yapraklarından nanopartiküllerin üretimine yönelik ilk araştırma olacak.

**Anahtar Kelimeler:** Well difüzyon yöntemi, Nanopartikül karakterizasyonu, *Aronia prunifolia*, Yeşil sentez, Antifungal aktivite.

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## 1. Introduction

Nanotechnology has emerged as the most innovative and revolutionary scientific field in decades, dealing with matter in the nanoscale range (1–100 nanometers). A rapidly developing branch of research, nanotechnology has several applications in a variety of industries, including agriculture and medicine (Khan and Rizvi, 2014; Sahu and Tiwari, 2023). Because of the biocompatibility, low toxicity, and environmentally benignity of both the technique and the nanoparticle (NPs) products, the biosynthesis of nanoparticles or green synthesis of nanoparticles has garnered much attention recently (Mohammadlou et al., 2016). One advantage of employing biological materials to synthesis nanoparticles is that it uses less energy and modest technology without the use of harmful chemicals; examples of these materials include bacteria, yeast, mold, microalgae, and plant extracts (Mie et al., 2014; Mohammadlou et al., 2016; Aseel et al., 2023).

There is a vast array of uses for biosynthetic nanotechnology in energy research, electronics, mechanics, food and feed, medicinal science, cosmetics, chemical industries, medication, and gene delivery. A diverse range of unique physicochemical features and a wide variety of possible applications, including material science and healthcare applications, are achieved by the decrease in material size (Sivasankarapilai et al., 2019).

Silver, which has high antibacterial, antifungal, and antiviral properties, is one of the materials commonly used to make nanomaterials, along with gold, copper, magnesium, and zinc (Abou El-Nour et al., 2010; Faisal and Kumar., 2017). Silver nanoparticles, often known as AgNPs, have several applications, such as coatings, biological labelling, optical receptors, intercalation material in electrical batteries, and catalysts in chemical processes. Researchers think that because AgNPs have a huge surface area and interact directly with bacteria, this contributes to their great antimicrobial activity (Mira et al., 2015; Stabryla et al., 2023).

It is widely known that silver nanoparticles can function as a catalyst for a variety of antibacterial processes (Salam et al., 2012; Hai et al., 2022), healthcare, and sensors (Kuppusamy et al., 2016). Its ability to suppress bacteria found in a variety of industrial and medicinal processes has been demonstrated. By adhering to the cell wall and causing it to become permeable, they allow themselves to enter bacteria cells (Javed et al., 2021). The interaction of nanoparticles with microbial DNA, proteins, or enzymes inhibits cell growth (Singh et al., 2010; Saxena and Raj., 2021). Metallic nanoparticles have proven useful in a variety of applications, including biomedicine (Sulaiman et al., 2013, Singh et al., 2021), catalysis (Raj et al., 2020), antimicrobial agent (Al-Otibi et al., 2020), antiplasmodial agent (Okaiyeto et al., 2021), textile engineering (Dubas et al., 2006), drug delivery (Khalid and El-Sawy., 2017).

Iron nanoparticles (FeNPs) are one type of metal nanoparticle that has found extensive use in medicine delivery, solar energy conversion, and biological applications (Khlebtsov and Dykman, 2011; Iv et al., 2015; Kisimba et al., 2023). Furthermore, FeNPs exhibit significant toxicity against a wide range of pathogenic fungi and bacteria (Mahmoud et al., 2011; Golipour et al., 2019). The production of FeNP utilizing bio-based materials has gained significant attention in nanoscience and technology due to its exceptional features and applications (Goswami et al., 2018). There have been reports of the biosynthesis of FeNPs using plants, microbes, and enzymes (Devatha et al., 2016; Raj et al., 2018). Plant-based materials are the most beneficial among biological sources because they include secondary metabolites and/or higher bioactive compounds that may be efficiently reduced to serve as a capping agent for the synthesis of FeNPs (Charbgoon et al., 2017).

Metal oxide nanoparticles have shown promise as a substitute for organic nanomaterials in the investigation of several approaches to combat microbial resistance to antibiotics because they are more stable, selective, and have better endurance than organic nanomaterials (Stankic et al., 2016). Since plant extracts are environmentally benign, biocompatible, and extremely stable, iron nanoparticles made from them can be used in a variety of applications (Makarov et al., 2014; Yadi et al., 2018). The plant biomass acts as a reducing agent as well as a support material during the nanoparticles' template-assisted synthesis (Herlekar et al., 2014); Additionally, they are inexpensive, sustainable, renewable, readily removed, and may help in the production of nanoparticles (Zhou et al., 2011; Herlekar et al., 2014).

Over the past twenty years, there has been a significant surge in interest in the biosynthesis of metallic nanoparticles as a result of the increasing need to create safe, dependable, biocompatible, benign, and environmentally friendly procedures for material creation (Thakkar et al., 2010; Akhtar et al., 2013). Numerous techniques utilizing biological organisms, including viruses, fungi, bacteria, yeast, and plants, have been developed and successfully used to manufacture nanoparticles (Ildiz et al., 2017; Wani et al., 2023). Because it is ecologically beneficial, plant extract-directed synthesis has been regarded as one of the most dependable and promising techniques for creating NPs (Demirbas et al., 2016; Cuong et al., 2022).

Plant extracts contain active ingredients called flavonoids, polyphenols, phenolic acids, and terpenoids that function as stabilizing and reducing agents during the formation of nanoparticles. It has been determined that phenols are potent antioxidants with significant antioxidant activity. The leaves of *A. prunifolia* are abundant in flavonoids, phenolic acids, and anthocyanins, among other phenolic components (Szopa et al., 2017).

Anthocyanins are the most common kind of flavonoid found in *A. prunifolia* leaves (Kähkönen and Heinonen., 2003; Reddy et al., 2014), there is abundant evidence of their antimicrobial (Tkáčiková et al., 2013; Chen et al., 2014) anti-inflammatory, (Youdim et al., 2000; Reddy et al.,

2014) antitumorogenic, (Zhang et al., 2000) antiobesity (Norberto et al., 2013) and neuroprotective effects (Andres-Lacueva et al., 2005).

In spite of significant progress in nanoparticle production and their utilization as antifungals, there remains a pressing need for environmentally sustainable methods that can effectively combat fungal pathogens. Our study focuses on the synthesis of AgNPs and FeNPs derived from *A. prunifolia* leaves, assessing their antifungal efficacy against major fungal strains such as *Aspergillus fumigatus*, *Rhizoctonia solani* Ag4 HgII, and *Aspergillus flavus*. This research not only advances nanoparticle synthesis but also explores the potential of *A. prunifolia* leaf-based nanoparticles in combating fungal pathogens.

## 2. Materials and Methods

### 2.1. Materials

The *A. prunifolia* plant was obtained from the Department of Biology, Faculty of Science, Ondokuz Mayıs University, Samsun, Türkiye. Experimental chemicals were obtained from Sigma Aldrich Chemical Co, USA. Fungal pathogens (*A. fumigatus*, *R. solani* Ag4 HgII, and *A. flavus*) were acquired from the Department of Microbiology, Ondokuz Mayıs University, Samsun, Türkiye.

### 2.2. Preparation of Leaf Extract

Freshly harvested leaves were utilized to prepare the *A. prunifolia* leaf extract. Initially, the leaves were surface cleaned with running tap water, then treated with Milli-Q water, and then let dry in the shade for five days in order to remove any remaining moisture. Subsequently, using a kitchen blender, a fine powder was obtained. After weighing and adding the 10 g of leaf powder to 100 ml of Milli-Q water, the mixture was boiled for 30 minutes at 60°C. After that, the extracts were run through Whatman No. 1 filter paper and left to settle at room temperature in preparation for additional research.

### 2.3. Synthesis of Silver Nanoparticles

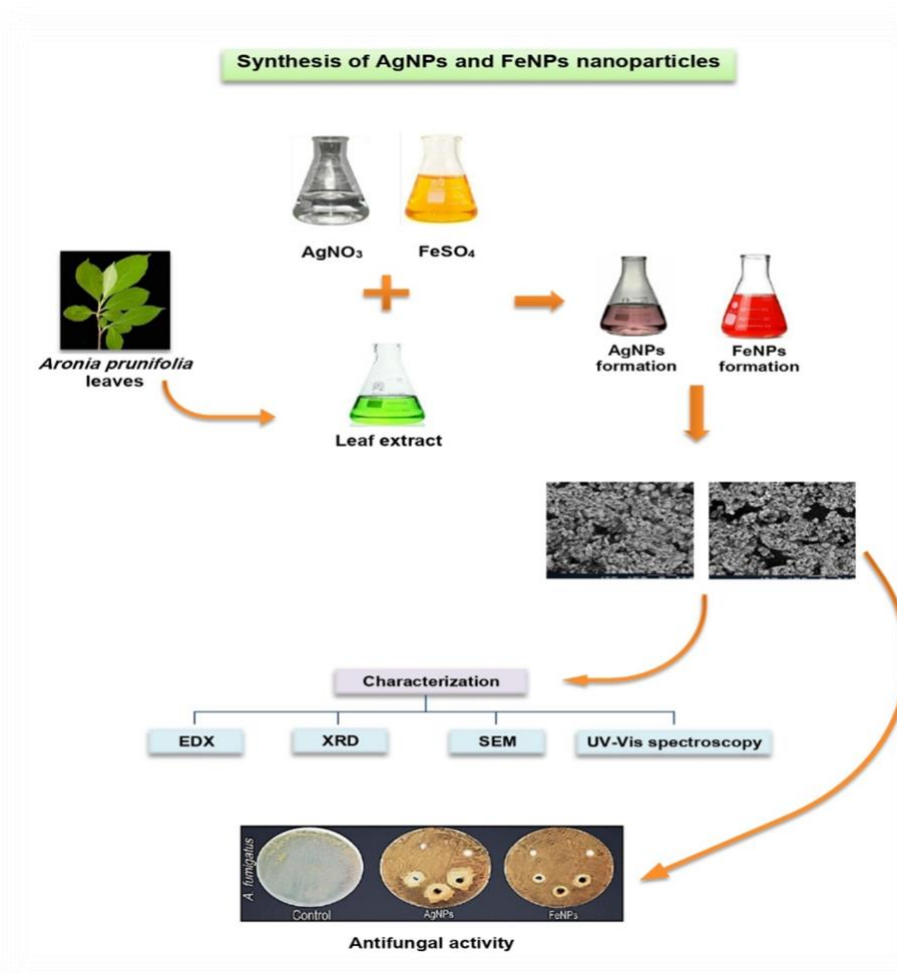
In order to synthesize AgNPs, a 100 ml Erlenmeyer flask containing 50 ml of 1 mM AgNO<sub>3</sub> solution was first created. Next, using a magnetic stirrer, 10 ml of *A. prunifolia*'s aqueous extract was added and properly stirred. *A. prunifolia* leaf extract was not used in the control setup. The two flasks were kept at 37°C in a dark environment to prevent silver nitrate from being photoinactivated. This

was similar to the approach used by Saxena et al. (2012), who also employed a similar synthesis technique.

After five minutes of stirring, the mixture was allowed to cool to room temperature. The creation of AgNPs is confirmed by the emergence of the characteristic yellow (Figure 2). Following that, the colloid solution was put into an amber container and kept at room temperature. After centrifuging the collected AgNPs for 20 minutes at 10,000 rpm, the pellet was redispersed in deionized water to further purify them.

## 2.4. Iron Oxide Nanoparticle Synthesis

A ferrous sulfate solution,  $\text{FeSO}_4$ , was created using deionized water. 1mM of Ferrous sulphate was prepared using 100 ml of deionized water. 100 ml of plant extract was mixed into this precursor solution. After adding 10 ml of *A. prunifolia* leaf dropwise to the solution mixture while stirring constantly, the brown hue that resulted verified the creation of  $\text{Fe}_2\text{O}_3$  nanoparticles (Figure 3). For two hours, the mixture was agitated at  $55^\circ\text{C}$ .



**Figure 1.** Workflow for green synthesis of nanoparticles using plant leaf extract.

Following that, the particle was dried in a hot air oven, and the supernatant was discarded. A brownish-black powder was obtained and kept in sterile bottles for further investigation (Madhavi et al., 2013). Figure 1 shows the steps for preparing the nanoparticles.

## 2.5. Characterization

The nanoparticles underwent characterization at the Ondokuz Mayıs University Black Sea Advanced Technology Research and Application Center, Samsun, Türkiye. The Thermo Evolution 220 UV-Visible Spectrophotometer was utilized to explore the wavelength range of 200–900 nm, confirming the presence of nanoparticles. X-ray diffraction (XRD) patterns of nanoparticles were obtained using a RIGAKU SMART LAB diffractometer, operating at 40 kV and 30 mA with  $\text{CuK}\alpha$  radiation. This method, employing zero background sample holders and scanning at  $2\theta$  from  $0^\circ$  to  $80^\circ$  at room temperature, provided insights into the crystalline structure.

Morphological characterization was conducted at the Chemical Analysis Lab (CA), Baghdad, Iraq, using a JEOL JSM-7001F Schottky Emission Scanning Electron Microscope (SEM), which was crucial in revealing the size and shape of the synthesized nanoparticles.

## 2.6. Antifungal Assay

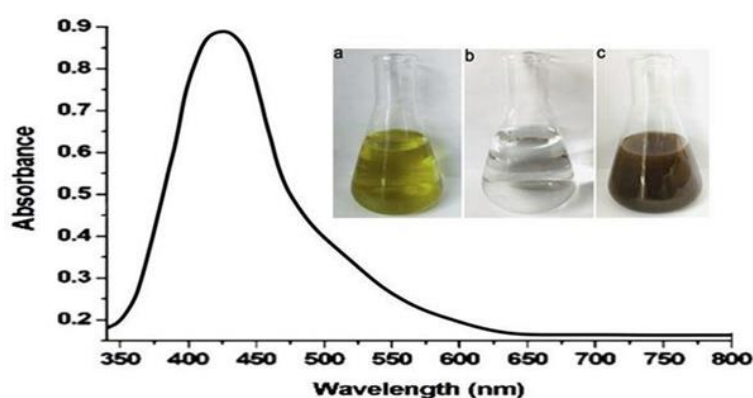
The concentration-dependent antifungal activity of the synthesized AgNPs and FeNPs nanoparticles was assessed using the well diffusion method. For the purpose of the study, the fungal strains were cultured in potato dextrose broth (PDB) for 72 hours. Using a spreader, 100  $\mu\text{L}$  of culture (containing  $10^4$  cells  $\text{ml}^{-1}$ ) was placed on potato dextrose agar (PDA) substrate to prepare the microorganism.

A total of 50  $\mu\text{L}$  of Ag NP and  $\text{Fe}_2\text{O}_3\text{NP}$  (10, 20, and 30  $\mu\text{g}/\text{ml}$ ) suspension was placed into the center of the well with 6 mm of dia. For the antifungal test, 70% ethanol alcohol and distilled water were utilized as positive and negative controls. The petriplates were incubated at  $25^\circ\text{C}$  for 72 h in an incubator, during which activity was evidenced by the presence of a zone of inhibition (mm) surrounding the well. Every test was conducted three times, and the results presented below are the averages of those three trials (Jayaseelan et al., 2012). Data on mean zones of inhibition were analyzed using OriginPro 2024. Descriptive statistics were reported as mean  $\pm$  SEM. Statistical significance ( $p < 0.05$ ) was determined via one-way ANOVA and Tukey's post hoc test.

### 3. Findings and Discussion

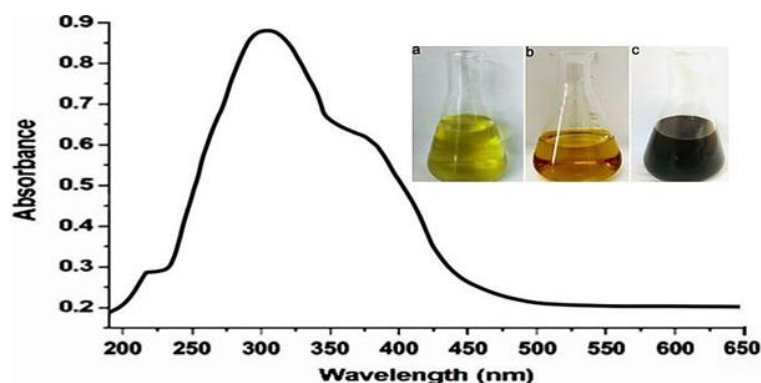
#### 3.1. Nanoparticle Characterization

After 48 hours, an aqueous solution's color changed from green to thick brown, indicating that the silver ions in contact with the leaf extracts had reduced into silver particles. The pale yellow color of Ag nanoparticles intensified with an increase in plant extract content (Figure 2). In an aqueous solution, Ag nanoparticles were previously observed to appear yellowish (Perera et al., 2013). An absorbance spectra peaking at around 425 nm in Figure 2 indicates the production of Ag nanoparticles (Ahmad et al., 2003). Ag atom surface plasmon oscillations are excited, which results in the color and absorbance spectra at 425 nm (Twu et al, 2008).



**Figure 2.** Spectrum of UV-Vis absorption for silver nanoparticles. (a) *A. prunifolia* leaves extract without AgNO<sub>3</sub> (b) Silver nitrate solution (c) 1mM AgNO<sub>3</sub> with *A. prunifolia* leaves extract.

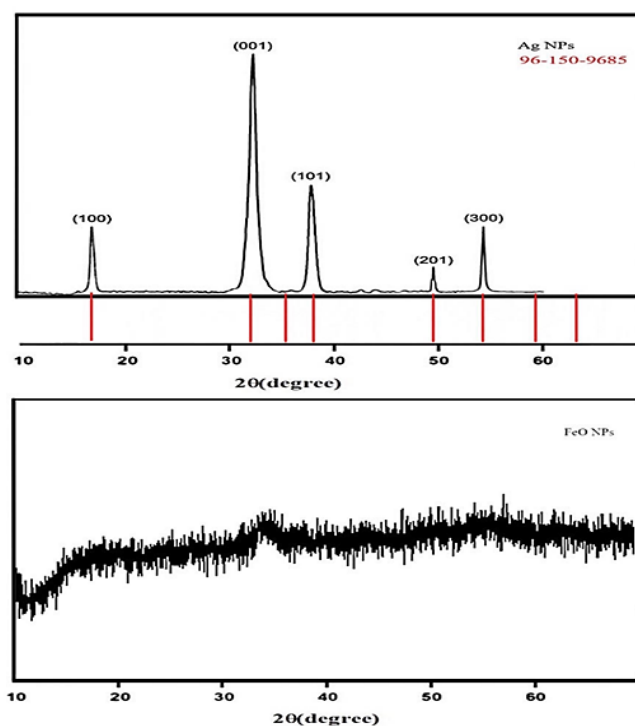
The Fe<sub>3</sub>O<sub>4</sub> nanoparticles Figure 3 varied in color from light brown to dark brown and finally to black with an increased concentration of plant extract. As a characteristic of iron oxide nanoparticles, the UV-Vis in Figure 3 indicated that the material became excited at 300 nm, confirming the creation of Fe<sub>3</sub>O<sub>4</sub>. Iron oxide peaks are often observed between 300 and 350 nm, a finding that was further supported by Kiwumulo et al. (2022). This also relies on the phytochemicals that are employed in the production of green-derived iron oxide nanoparticles as reducing agents. Iron nanoparticles are known to absorb a certain amount of UV light, which is brought on by electrons moving from the valence band to the conduction band.



**Figure 3.** UV- Vis absorption spectrum of iron oxide. (a) *A. prunifolia* leaves extract without  $\text{FeSO}_4$  (b) Ferrous sulphate solution (c) 1mM  $\text{FeSO}_4$  with *A. prunifolia* leaves extract.

Figure 4, illustrates the X-ray diffraction patterns of the synthesized AgNPs. The distinctive peaks observed correspond to the trigonal (hexagonal axis) crystal structure of AgNPs. Specifically, the (011) peak is identified at  $2\theta = 38.16^\circ$ , and the (300) peak is positioned at  $64.5^\circ$ . These peak positions are consistent with the crystallographic information found in the PDF card number 96-150-9685. The presence and location of these peaks provide valuable insights into the crystalline structure of the synthesized silver nanoparticles, further supporting the characterization of the material.

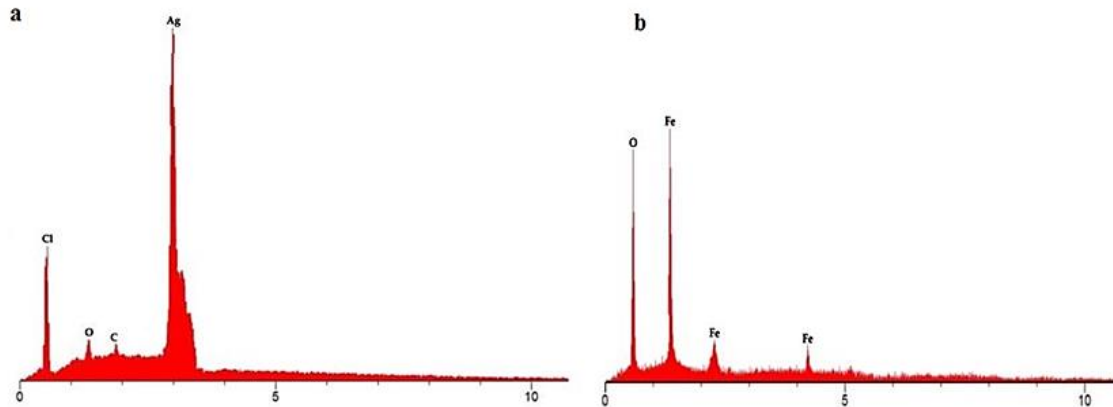
The absence of distinct peaks in the X-ray diffraction patterns for the iron nanoparticles suggests that a crystalline form of iron particles was not formed under the conditions of the study. The absence of crystallinity in the FeONPs could be attributed to factors such as synthesis conditions, size effects, or surface modifications that prevented the formation of a crystalline structure.



**Figure 4.** X-ray diffraction (XRD) pattern of synthesized: (a) silver (b) iron oxide nanoparticles from leaf extract of *A. prunifolia*.

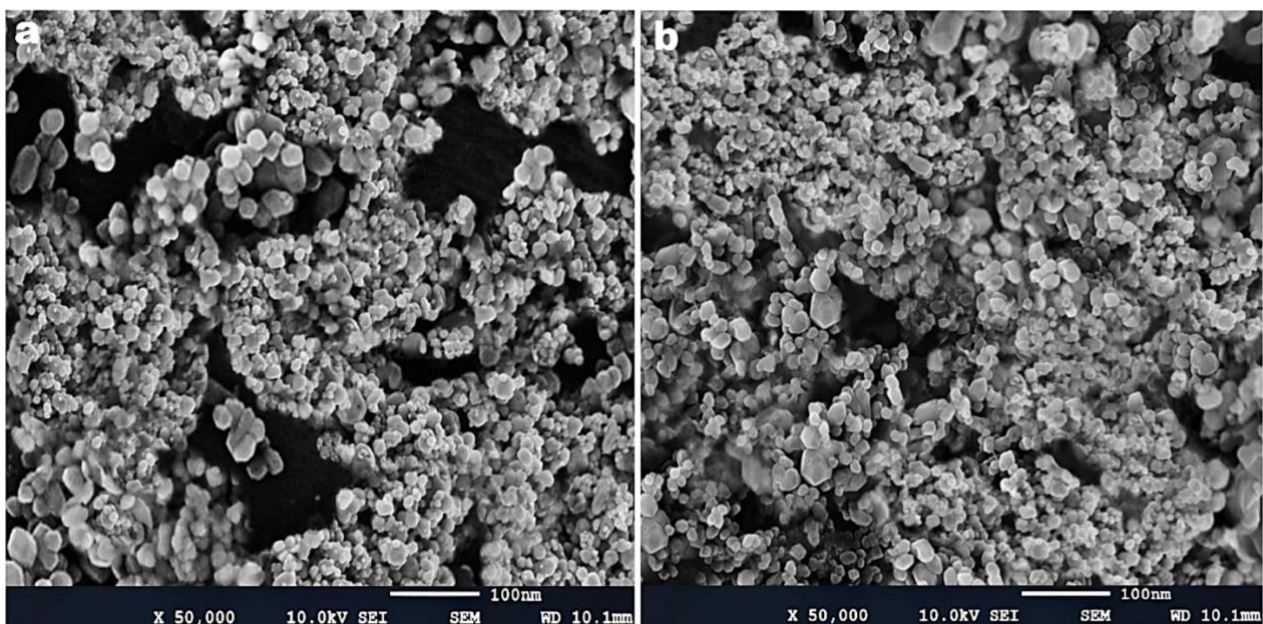


From the EDX spectrum, it was understood that *A. prunifolia* leaf extract with  $\text{AgNO}_3$  had a recorded weight of 74.25% Ag nanoparticle (Figure 5a). However, the EDX study presented in Figure 5b revealed that the iron and oxygen percentages on the surface of iron oxide nanoparticles were 75.9% and 24.1%, respectively. Only the peaks for the Fe and O atoms were visible in the EDX data, confirming that there were no contaminants present when the required material was being prepared.



**Figure 5.** Energy dispersive X-ray spectroscopy analysis of (a) silver (b) iron oxide nanoparticles.

The produced silver nanoparticles were tetragons, pentagons, and hexagons with sizes ranging from 15 to 50 nm, according to SEM examination (Figure 6a) (Rajakumar and Rahuman, 2011). FeNPs Figure 6b's SEM images were primarily spherical as well, with an average size of 16 nm and a range of 16 to 60 nm (Ankamwar et al., 2010).



**Figure 6.** (a) Silver nanoparticles made from an extract from *A. prunifolia* leaves are seen under a scanning electron microscope. (b) Iron oxide nanoparticles under a microscope.

### 3.2. Biosynthesised Nanoparticles Antimicrobial Properties

Using the well diffusion technique, the antibacterial activity of biologically biosynthesized AgNPs and FeNPs against a variety of pathogenic fungi was assessed. *A. fumigatus*, *R. solani* Ag<sup>+</sup>, Hg<sup>II</sup>, and *A. flavus* were effectively inhibited by AgNPs, as shown in Table 1. The inhibitory activity of Fe<sub>2</sub>O<sub>3</sub>NPs was manifested against the same fungi in Table 2. In the current investigation, an extract of *A. prunifolia* leaves was used to produce the iron oxide and silver nanoparticles. The Aronia plant is a significant source of terpenoids, flavonoids, polyphenols, and phenolic acids, which are important components of plant extracts and serve as stabilizing and reducing agents during the synthesis of NPs. In the process of creating NPs, leaf extract serves two purposes: first, it converts metallic salts into NPs; second, it stabilizes the NPs by preventing them from aggregating (Kuppusamy et al., 2016).

The findings show that the various concentrations of iron oxide and silver nanoparticles utilized in this investigation inhibited the radial development and spore germination of every tested fungal pathogen (Figure 7). According to the results of our investigation, the maximum concentration proved to be more beneficial than the lowest (Wani et al., 2012). The inhibitory effects of AgNPs and Fe<sub>2</sub>O<sub>3</sub> nanoparticles against *A. fumigatus*, *R. solani*, and *A. flavus* have been documented in a number of investigations, which supports our findings. (Ankanna and Savithamma, 2011; Krishnaraj et al., 2012; Balashanmugam et al., 2016; Saqib et al., 2022).

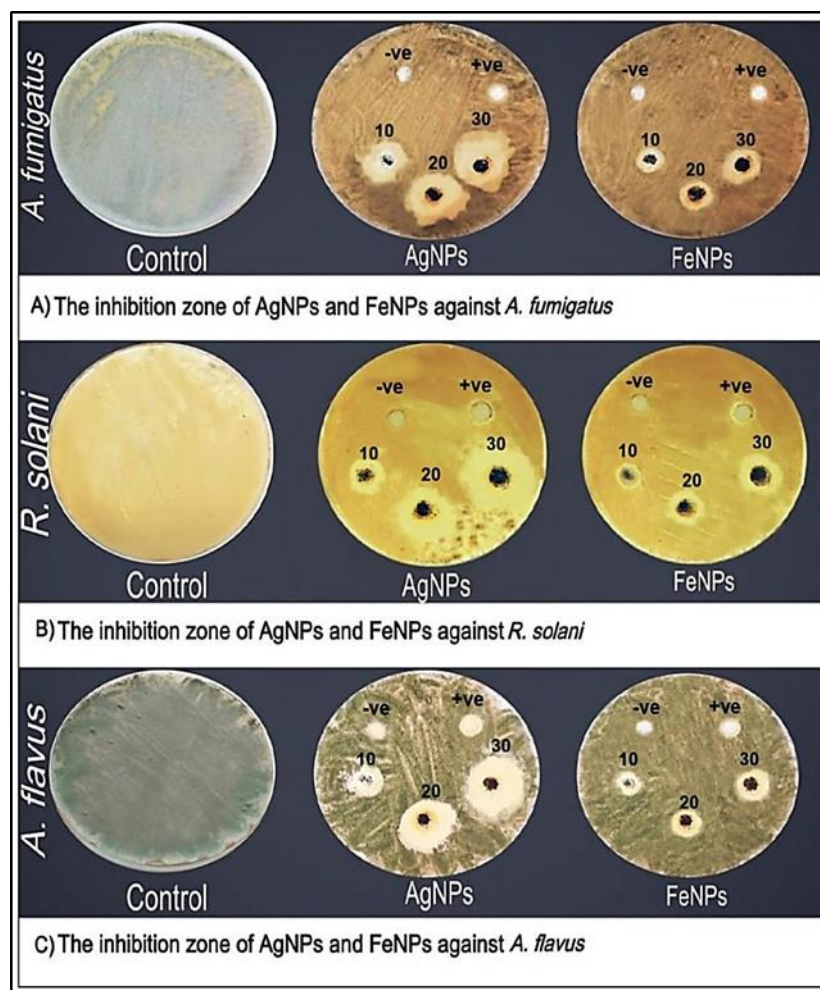
Because of their small size and high area-to-volume ratio, nanoparticles exhibit important changes in characteristics from bulk forms of the same material (Issa et al., 2013; Khan et al., 2019). These NPs are utilized in pharmaceutical goods, medical diagnostic imaging, and therapy regimens because of their distinct physiochemical and biological characteristics (Khan et al., 2019). When exposed to 10 to 30 µg/mL of biosynthesized NPs, the proliferation of all species was reduced by the antifungal activity of AgNPs and FeNPs.

Fungal growth inhibition often increases with increasing NP concentration. At 30 µg/ml, the growth of the fungus was significantly inhibited. Elevated NP concentrations in solution may have the ability to adhere to and saturate fungal hyphae, hence causing disruption of the fungal cells (Dawoud et al., 2021). In our investigation, AgNPs have a greater inhibitory capacity than FeNPs. Ag<sup>+</sup> is responsible for this inhibitory action, which mainly impacts the activity of membrane-associated enzymes like those in the respiratory chain. Ag<sup>+</sup> has also been shown to impair DNA replication, which may impact the production of some microbial proteins and enzymes. (Morozova, 2021).

For field applications, further research is required to confirm AgNPs' antifungal efficacy. Additionally, AgNPs can interact with substrates by competitive inhibition, which renders the enzymes inactive and stops them from producing the products needed for cell function. The

concentration of fungal spores and nanoparticles determines how much inhibition occurs. Iron oxide NP has demonstrated potential antibacterial action against a number of human diseases, according to a related study (Abdeen et al., 2013). (Pulit et al., 2013) investigated the antifungal activity of green-produced silver nanoparticles against *Aspergillus niger* and *Cladosporium cladosporioides* and found that these NPs exhibit potent biocidal effects even at low concentrations. (Nehra et al., 2018) Described how iron oxide nanoparticles inhibited the growth of *Fusarium solani*, *Aspergillus niger*, *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*. Additionally, they mentioned that iron oxide nanoparticles have the potential to be employed as antifungal agents against a variety of fungal plant infections and that they might be used as efficient antimicrobial agents.

Iron is a potent reducing agent that causes lipopolysaccharides and membrane proteins' functional groups to break down. Additionally, iron nanoparticles oxidize intracellular oxygen, which results in oxidative damage through the Fenton reaction. These nanoparticles cause further harm and cell death by penetrating through damaged membranes (Lee et al., 2008; Cheeseman et al., 2020).



**Figure 7.** Photographs of antifungal test against *A. fumigatus*, *R. solani* Ag4HgII, and *A. flavus* (agar-well diffusion method) for AgNPs and FeNPs. Concentrations of AgNPs and FeNPs used are 10, 20, and 30 µg/ml. +ve and -ve positive and negative control.

**Table 1.** Antifungal activity of silver nanoparticles synthesized from leaf extract of *A. prunifolia* at 10, 20, and 30 µg/ml (Each value is an average of three replications).

| Pathogenic fungi             | Inhibition zone (mm diameter) |          |          |
|------------------------------|-------------------------------|----------|----------|
|                              | 10 µg/ml                      | 20 µg/ml | 30 µg/ml |
| <i>Aspergillus fumigatus</i> | 14.3±0.3                      | 28.0±0.5 | 33.1±0.5 |
| <i>Rhizoctonia solani</i>    | 12.7±0.6                      | 30.0±0.3 | 35.0±0.7 |
| <i>Aspergillus flavus</i>    | 12.5±0.3                      | 32.5±0.7 | 34.1±0.4 |

**Note:** Values of Mean Zones of Inhibition are expressed as Mean ± SEM.

**Table 2.** Antifungal activity of iron oxide nanoparticles synthesized from leaf extract of *A. prunifolia* 10, 20, and 30 µg/ml (Each value is an average of three replications).

| Pathogenic fungi             | Inhibition zone (mm diameter) |          |          |
|------------------------------|-------------------------------|----------|----------|
|                              | 10 µg/ml                      | 20 µg/ml | 30 µg/ml |
| <i>Aspergillus fumigatus</i> | 9.3±0.8                       | 12.1±0.5 | 15.0±0.9 |
| <i>Rhizoctonia solani</i>    | 7.1±0.8                       | 14.0±0.8 | 17.1±0.5 |
| <i>Aspergillus flavus</i>    | 9.1±0.3                       | 15.4±1.0 | 14.0±0.5 |

**Note:** Values of Mean Zones of Inhibition are expressed as Mean ± SEM.

#### 4. Conclusions and Recommendations

In this work, we have successfully characterized and synthesized silver and iron oxide nanoparticles utilizing *A. prunifolia* leaf extract as a dual-function reducing and stabilizing agent. The antimicrobial efficacy of the resulting nanoparticles was systematically assessed against various pathogenic fungi, namely *A. fumigatus*, *R. solani* Ag4 HgII, and *A. flavus*. Both AgNPs and FeNPs exhibited discernible inhibitory effects on spore germination and radial growth of the tested fungal strains. The inhibitory potency demonstrated a concentration-dependent response, with AgNPs manifesting superior effectiveness to FeNPs. Our study provides substantive evidence regarding the efficacy of *A. prunifolia* leaf extract in the green synthesis of silver and iron oxide nanoparticles, thereby accentuating their viability as antimicrobial agents against fungal pathogens. However, it is imperative to acknowledge the need for further research to elucidate and validate their safety and efficacy profiles, ensuring a comprehensive understanding of their practical applications across diverse academic and industrial domains.

#### Authors' Contributions

All authors contributed equally to the study.

## Statement of Conflicts of Interest

There is no conflict of interest between the authors.

## Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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