



Article Green Synthesis and Antibacterial Activity of Ag/Fe₂O₃ Nanocomposite Using Buddleja lindleyana Extract

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Abstract: In the study reported in this manuscript, silver/iron oxide nanocomposites (Ag/Fe₂O₃) were phytosynthesized using the extract of *Buddleja lindleyana* via a green, economical and ecofriendly strategy. The biosynthesized Ag/Fe₂O₃ nanocomposites were characterized using UV-Vis spectrophotometry, FTIR, XRD, TEM, DLS and SEM-EDX analyses. The particulates showed a triangular and spherical morphology having sizes between 25 and 174 nm. FTIR studies on the nanoparticles showed functional groups corresponding to organic metabolites, which reduce and stabilize the Ag/Fe₂O₃ nanocomposite. The antimicrobial efficacy of the phytosynthesized Ag/Fe₂O₃ against bacterial pathogens was assessed. In addition, Ag/Fe₂O₃ exhibited broad spectrum activities against *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* with inhibition zones of 23.4 ± 0.75, 22.3 ± 0.57, 20.8 ± 1.6, and 19.5 ± 0.5 mm, respectively. The Ag/Fe₂O₃ composites obtained showed promising antibacterial action against human bacterial pathogens (*S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*), making them candidates for medical applications.

Keywords: Buddleja lindleyana; Ag/Fe₂O₃; nanocomposite; phytosynthesis; antimicrobial activity

1. Introduction

In recent years, leaf extract-mediated biosynthesis of nanomaterials has been extensively studied [1–4]. Leaf extract-mediated synthesis of nanomaterials is more eco-friendly and economical than other methods of synthesis such as chemical reduction and physical methods [5–7]. Due to the growing demand for ecologically friendly material synthesis techniques, the biosynthesis of nanomaterials has gained attention as an emerging feature of the interface of nanotechnology and biotechnology [8–17]. The biosynthesis of inorganic materials, particularly metal nanoparticles, utilizing microbes and plants has received a lot of attention [18–24]. Nanosilver has many important applications [25–28]. Antimicrobial agents have been utilized with it [29,30]. The study reported in the first paper on green leaf cell extract solution-mediated synthesis of Ag/Fe2O3 hybrid nanoparticles successfully employed a green method to synthesize Fe_2O_3 as well as Ag/Fe₂O₃ nanoparticles. The synthesized nanoparticles showed excellent antibacterial, antifungal and anticancer activities [31]. Utilizing various reducing agents as well as no reducing agents, it was successful to produce nano-hybrid Ag/Fe₂O₃NPs depending on carboxymethyl–chitosan. Their fairly reversible magnetization curves show that γ -Fe₂O₃ NPs transition to a superparamagnetic state [32]. Antibacterial testing on nanocomposites revealed good antimicrobial action against both Gram-positive and Gram-negative bacteria, as well as good activity



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). against yeast and S. aureus resistant strain [33]. The Ag/Fe_2O_3 -Graphene oxide (GO) nanocomposites have shown superior long-term antibacterial capabilities against bacteria (Gram-negative and Gram-positive bacteria), demonstrating their particular potential as a promising long-term biocide with minimal environmental hazard. These abilities were compared to plain Ag nano and Ag/Fe₂O₃ [34]. The creation of Ag on magnetic-Fe₃O₄ NPs surfaces modified using *Stachys lavandulifolia* extract as a reducing and stabilizing magnetic agent was proposed as an effective and environmentally friendly method. Furthermore, the 4-nitrophynol reduction using the nanocomposite and its bactericidal activity was evaluated [35]. Nanocomposites such as Fe₃O₄ or Ag/Fe₂O₃ are covered by porous silica oxides serving as magnetic cores to carry anticancer drugs [36]. Nanomaterials which have magnetic behavior, such as Fe_3O_4 or Ag/Fe_2O_3 , are good candidates for superparamagnetism [37,38]. Magnetic resonance imagining (MRI) investigations also utilize iron oxide nanoparticles [39]. The combination of Ag and Fe_2O_3 achieves the properties of both metals [40]. Due to these properties, researchers have been interested in making a hybrid composite of Ag/Fe₂O₃ offering better catalytic, bactericidal, and bio-imaging properties [41–43]. To prepare metal nanoparticles, different techniques such as physicochemical, chemical, and green synthesis by using plant, algae, fungi, and bacterial extracts can be employed [44–55]. Plant-based NPs are highly active against different epidemic microbial diseases and are nontoxic to humans [56,57]. The easiest method to synthesize Ag/Fe₂O₃ nanocomposite by utilizing Algaiia Monozyga extract as a natural reducing and capping agent was studied [41]. Numerous experimenters have utilized *Rumix acetosa*, Hordeum vulgar, and Azadiracta indica in the green fabrication of Fe₂O₃NPs [58,59]. Leaf extractmediated synthesis of Fe_2O_3NPs is gaining the attention of researchers because of its lower toxicity compared to other chemical reduction methods [28]. Due to their potential utility in targeted drug administration and magnetic hyperthermia cancer treatment, extensive study has been completed on the anticancer properties of Fe_2O_3NPs [60–63]. There have been very few reports of investigations on the creation of Ag/Fe₂O₃ NPs. According to Chen et al., several heteromers of Ag/Fe_2O_3 NPs may be synthesized and exhibit bactericidal properties [43]. It is hypothesized that a key factor in the antibacterial action of Ag/Fe_2O_3 NPs is the release of Ag^+ ions [41]. Pathogens including bacteria and fungus, which are multi-drug-resistant pathogens, are more easily defeated by Ag/Fe_2O_3 NPs than by Ag NPs alone. To the best of our knowledge, this is the first publication to examine the biosynthesis of the Ag/Fe₂O₃ by *Buddleja lindleyana* leaves. The present study aims to study the green synthesis of nanocomposite Ag/Fe₂O₃ NPs-based safe natural Buddleja lindleyana leaves as a capping and reducing agent. Additionally, the impact of Ag/Fe_2O_3 NPs on harmful bacteria was investigated.

2. Materials and methods

2.1. Materials

Buddleja lindleyana leaves were collected in the UK. Fe₂ (SO₄)₃·6H₂O and silver nitrate (AgNO₃, >99.98%) were purchased from Sigma-Aldrich. All aqueous solutions were prepared using distilled deionized (DI) water.

2.2. Biosynthesis of Ag/Fe_2O_3

Firstly, *Buddleja lindleyana* leaves were carefully washed with DI water to remove impurity and then dried for 2 days at room temperature. The *Buddleja lindleyana* leaves were then weighed out at 10 g and added to 100 mL of DI water. After being heated for 30 min at 90 °C, the mixture was filtered to filter out the broth [31]. The final extract was collected for further usage. For the synthesis of Ag/Fe₂O₃, a suitable aqueous solution of *Buddleja lindleyana* extract was gradually added into the mixture containing 1 g Fe(SO₄)₃·6H₂O and 0.1 g silver nitrate (AgNO₃) in an Erlenmeyer flask while stirring at 300 rpm with a magnetic stirrer at 70 °C for 3 h.

2.3. Characterization of Ag/Fe₂O₃

Preliminary characterization of the Ag/Fe₂O₃ prepared was carried out using UV-Vis spectroscopy. The measurement was carried out using a Jasco dual-beam spectrophotometer (model V-530, Tokyo, Japan) having an operational wavelength range of 300 to 800 nm. Thereafter, the formed pellet was used to identify functional groups present using FTIR spectroscopy. In addition, FTIR was employed to identify the functional groups present in the aqueous leaf extract that led to the formation of nanocomposite. For that, the dry leaf powder of Buddleja lindleyana was pelletized, and their FTIR (Jasco 460 plus, Tokyo, Japan) spectra were recorded between 4000 and 400 cm⁻¹. UV-Vis spectroscopy was used to perform preliminary characterization of the Ag/Fe₂O₃ produced. With an operating wavelength range of 300 to 800 nm, the measurement was performed using a Jasco dual-beam spectrophotometer (model V-530, Tokyo, Japan). The produced pellet was then utilized to analyze the functional groups using FTIR spectroscopy. Additionally, FTIR was used to pinpoint the functional groups in the aqueous leaf extract that contributed to the creation of the nanocomposite. In order to do that, pellets of Buddleja lindleyana's dry leaf powder were made, and their FTIR (Jasco, 460-plus, Tokyo, Japan) spectra were taken between 4000 and 400 cm⁻¹. The FTIR spectra for *Buddleja lindleyana* extract and the spectrum for the aqueous extract of Ag/Fe₂O₃ were assembled. The size and morphological characterization of the synthesized and lyophilized Ag/Fe₂O₃ was assessed using transmission electron microscope (TEM). TEM (JEOL-1010, Tokyo, Japan) was used to describe Ag/Fe₂O₃ in order to determine their sizes and morphologies. The outcome was obtained by injecting the carbon-coated copper grid with Ag/Fe_2O_3 solution and placing it on a sample holder. Furthermore, the X-ray thin film diffraction measurement for the bio-reduced Ag/Fe₂O₃ was also performed using a Goniometer = PW3050/60 using Cu k α radiation at 40 kV and 25 °C. Subsequently, relevant X-ray patterns were obtained in the range of 20–80 °C. DLS (dynamic light scattering) particle sizing was employed to study the dispersion of Ag/Fe₂O₃ in colloidal utilizing the Zetasizer Nanosizer (Malvern-Instruments, Worcestershire, UK). For the particle size analysis, the Ag/Fe₂O₃ sample was re-suspended in purified water at 25 ppm and vortex-mixed to obtain a homogenous solution. A field emission scanning electron microscope (FESEM) (Quanta, 250-FEG, Taipei, Taiwan) was connected to an energy-dispersive X-ray analyzer (EDX, Unit). EDX and mapping were used to determine the surfaces of the prepared Ag/Fe_2O_3 .

2.4. Assessment of the Inhibitory Activity of Ag/Fe₂O₃ against Pathogenic Microbes

The inhibitory activity of the Ag/Fe₂O₃ against bacteria and fungi was assessed using a well-diffusion test. Four bacteria including *B. subtilis, S. aureus, E. coli*, and *P. aeruginosa* were employed for the tests. Using the streaking method, test bacteria were inoculated into sterile Petri plates containing 20 mL of Mueller–Hinton. Sterile cork borer (6 mm) was used to prepare wells, and then, 200 μ L of Ag/Fe₂O₃ at a concentration of 5 mg/mL was added in the well. The inoculated plates were kept in a refrigerator for 45 min to achieve adequate diffusion of Ag/Fe₂O₃, which was followed by incubation at 37 °C for 24 h for the bacteria test. The inhibition zone around each well was measured.

3. Results and Discussion

3.1. Synthesis of Ag/Fe₂O₃ Nanoparticles

One of the goals of this study was to use *Buddleja lindleyana* plant sources for the preparation of nanomaterials. The *Buddleja lindleyana* plant, which belongs to the Loganiaceae family, was chosen and used in this study for Ag/Fe₂O₃ synthesis. The main chemical components of *Buddleja lindleyana* include flavonoids, triterpenoids, phenylethaoids, and iridoid glycosides [64–66]. The primary idea behind this procedure was to develop a clean, environmentally friendly method of creating nanomaterials using *Buddleja lindleyana* extract, which has the ability to function as both a reducing and a stabilizing agent in the production of a Ag/Fe₂O₃ nanocomposite (Figure 1).



Figure 1. The mechanism of the reduction process of the Ag/Fe₂O₃ nanocomposite using *Buddleja lindleyana* extract.

The synthesis of Ag/Fe₂O₃ was conducted by adding Fe(SO₄)₃·6H₂O and AgNO₃ as a precursor to the *Buddleja lindleyana* leaf extract until a gradual change in the reaction color was observed. After 3 h of incubation at 70 °C, the color of the reaction mixture changed from pale green to dark brown (Figure 2). Such a change of color of the reaction mixture represents the formation of Ag/Fe₂O₃ by the *Buddleja lindleyana* extract. UV-Vis spectra of the Ag/Fe₂O₃ nanocomposite after 3 h were measured within the range of 300–800 nm and are presented in Figure 2. The strong peak formed at 320 nm, as visible in the UV-Vis spectra, was used to identify the formation of Ag/Fe₂O₃ nanocomposite. Similarly, UV-Vis studies of AgFeO₂NPs by Berastegui et al. revealed high-level absorptions from 300 to 650 nm [67]. The presence of Ag/Fe₂O₃ nanocomposite caused the widening of absorbance at 320 nm (Figure 2).



Figure 2. Color change and UV-Vis absorption spectra of Ag/Fe₂O₃ nanocomposite synthesis by *Buddleja lindleyana* extract.

FTIR spectroscopic analysis was used to investigate the structural features of the bioactive constituents in *Buddleja lindleyana* extract as well as the probable chemical alterations due to the formation of Ag/Fe₂O₃. As indicated in Figure 3, the bonding arrangements of *Buddleja lindleyana* were investigated using FTIR in the spectrum range of 400–4000 cm⁻¹ (Figure 3). The H-bonded and -OH stretch vibrating of hydroxyl and phenolic groups of *Buddleja lindleyana* extract was found in a wide range 3367.9–3216.9 cm⁻¹ [31]. The -CH

group is responsible for the bands at 2918.5 cm⁻¹ and 2850.6 cm⁻¹. The presence of C-O of the ester group is indicated by the strong peak seen at 1728.7 cm^{-1} . The existence of NH amine is shown by the peak at 1603.8 cm^{-1} [20]. The existence of functional groups such as carboxylic acid and ether is further confirmed by FTIR analysis, which provides a peak value of 1008 cm^{-1} . The bioactive chemicals in *Buddleja lindleyana* extract are employed to convert ions into their appropriate metal forms. Furthermore, these phytochemicals have extremely reactive hydroxyl groups, which give hydrogen and so help to reduce the quantity of free radicals. The findings support the theory that these phytochemicals play a role in the bio-reduction process that leads to the production of nanomaterials [68,69]. According to the IR spectra of Ag/Fe₂O₃, the suppression of aliphatic molecules in Ag/Fe₂O₃ can be linked to redox changes of phytochemicals during Ag/Fe_2O_3 formation (Figure 3). Furthermore, IR studies revealed that chemical groups from the extract were bound to the Ag/Fe₂O₃ layer, indicating that Buddleja lindleyana extract worked as a stabilizer for the development of nanocomposite. The bending vibration of AgO and FeO interactions in Ag/Fe₂O₃ may explain the occurrence of peaks at 611.4 and 561.4 cm⁻¹, respectively, in Figure 3.



Figure 3. FTIR spectral analysis of *Buddleja lindleyana* extract and Ag/Fe₂O₃ nanocomposite.

3.2. Crystalline Structure of Ag/Fe₂O₃ Nanoparticles

The crystalline structure of the Ag/Fe₂O₃ NPs prepared was validated using XRD analysis, as shown in Figure 4. The primary strong angles in the diffractogram of the photosynthesized Ag/Fe₂O₃ were visible in the XRD patterns, showing that the Ag/Fe₂O₃ nanocomposite was crystallographic in nature. Bands at 31.5°, 35.4°, 38.4°, 42.4°, 44.4°, 52.6°, 57.1°, 64.6°, and 77.6° corresponded to Ag/Fe₂O₃ diffraction peaks. The values of silver appeared at the angles 38.4°, 44.4°, 64.6°, and 77.6° [20], while the peaks of the angles for iron oxide appeared at 31.5°, 35.4°, 42.4°, 52.6°, and 57.1° [31]. Therefore, the results clearly support the successful synthesis of nano Ag/Fe₂O₃. Diffraction patterns of the Ag/Fe₂O₃ nanocomposite did not show the presence of any contaminants, which verifies the purity of the Ag/Fe₂O₃ nanocomposite obtained.



Figure 4. X-ray diffraction (XRD) of the Ag/Fe₂O₃ synthesized by Buddleja lindleyana extract.

The most widely used technique for determining the morphological features and sizes of nanostructures is the transmission electron microscope (TEM). Ag/Fe₂O₃ nanocomposites have been created in various forms such as triangular and spherical with an average size range of 25–174 nm, as seen in the TEM image (Figure 5A). These different forms are a representation of the majority of nanocomposites found in Ag and Fe_2O_3 . The planes of the nanocomposite, as well as the degree of crystallinity of Buddleja lindleyana Ag/Fe₂O₃ particles, are shown by the bright circular areas in the SAED (selected area electron diffraction) pattern (Figure 5B). The SAED pattern's ring patterns match the Fe_2O_3 NPs' (104), (110), (113), (024), and (122) planes [70]. The SAED pattern further displays planes (100), (200), (220) and (311) that correspond to silver nanoparticles in addition to the aforementioned planes [71]. The average diameter of the Ag/Fe_2O_3 nanocomposite was determined using dynamic light scattering (DLS) analysis. The produced Ag/Fe_2O_3 nanocomposites were a poly-dispersed aggregate with an average diameter of 270.9 nm (Figure 5C). However, biomaterials deposited on the Ag/Fe₂O₃ surface by *Buddleja lindleyana*, such as organic compounds associated as stabilizers, as well as the metal core (Ag and Fe) of the Ag/Fe₂O₃ nanocomposite, alter the size determined by DLS [40,72].

SEM was used to analyze the morphology of Ag/Fe₂O₃ prepared using a simple and single-step process, as demonstrated in Figure 6A. The Ag/Fe₂O₃ seemed to have a mono-dispersed and aggregation-free micrograph. The EDX spectra revealed the existence of several well-defined peaks in the Ag/Fe₂O₃ nanocomposite that were due to silver, iron, oxygen, and carbon components (Figure 6B). Furthermore, EDX spectra revealed the production of a very pure Ag/Fe₂O₃ nanocomposite with no additional impurity-related peaks. The SEM image and the EDX spectra of the nanocomposite prepared revealed that the Ag/Fe₂O₃ nanostructures were well distributed in the *Buddleja lindleyana* extract [73]. In addition, elemental EDX mapping was used to determine the Ag and Fe elemental distribution (spatial/lateral) of the Ag/Fe₂O₃ nanocomposite prepared. The mapping of Fe, Ag, O, and C elements can be seen in the picture (Figure 6C). Both Fe and Ag were uniformly distributed throughout the Ag/Fe₂O₃ sample, according to the EDX elemental analysis shown in the Ag, Fe, O, and C element mapping images of Ag/Fe₂O₃ (Figure 6) [41].

3.3. Antimicrobial Activity

Recently, several pathogenic microorganisms have developed resistance to currently available commercial antibiotic agents and also caused adverse impact on human health. Thus, new active and safe antimicrobial agents are required. Because nanomaterials have

antibacterial capabilities, they have recently received attention [74,75]. According to Figure 7, the well diffusion technique was used in this investigation to evaluate the Ag/Fe₂O₃ nanocomposite's antibacterial efficacy against human bacterial pathogens (*S aureus, E. coli, B subtilis,* and *P. aeruginosa*). The maximal Ag/Fe₂O₃ dose (5 g/mL) showed a high level of inhibitory activity against the pathogens *B. subtilis, S. aureus, E. coli,* and *P. aeruginosa* with inhibition zones of 23.4 ± 0.75 , 22.3 ± 0.57 , 20.8 ± 1.6 and 19.5 ± 0.5 mm, respectively (Figure 7). A previous study showed that silver particles have a good antimicrobial effect on harmful bacteria, whether they are Gram-positive or Gram-negative bacteria [43]. The antibacterial activity of Ag/Fe₂O₃ nanocomposite was investigated in earlier investigations, and it showed good activity against Gram-negative and Gram-positive bacteria [34,40,76]. Ag/Fe₂O₃ has an antibacterial impact on bacteria because it damages cell walls, disrupts structural proteins, inactivates enzymes, inhibits electron transport chains, damages nucleic



acids (DNA), and causes oxidative stress brought on by the generation of reactive oxygen species (ROS) [41,72,77]. As a result, Ag/Fe_2O_3 has emerged as a viable antibacterial

treatment option and is useful in the medicinal field.



Figure 5. TEM image (**A**), SAED pattern (**B**), and DLS (**C**) of the synthesized Ag/Fe₂O₃ by *Buddleja lindleyana* extract.



Figure 6. (A) SEM image, (B) EDX spectrum, and (C) element mapping of Ag/Fe_2O_3 nanocomposites.



Figure 7. Antimicrobial activity of the synthesized Ag/Fe₂O₃ by Buddleja lindleyana extract.

4. Conclusions

Buddleja lindleyana extract was used for the phytosynthesis of Ag/Fe₂O₃NP through an eco-friendly route. The fabricated Ag/Fe₂O₃ nanocomposites were characterized using UV-Vis, FTIR, XRD, TEM, DLS and SEM-EDX analyses. The Ag/Fe₂O₃ nanocomposites obtained are triangular–spheroidal and exhibit a high absorbance band around 320 nm. According to the observation made from the FTIR analysis, it is possible to conclude that the compounds present in *Buddleja lindleyana*'s extract play an important role in the reduction and stabilization of nanocomposites. The Ag/Fe₂O₃NPs obtained are stable in colloidal solution. Results also revealed that the Ag/Fe₂O₃NP nanocomposites fabricated have outstanding antimicrobial activity against pathogenic strains (*S aureus, E. coli, B subtilis,* and *P. aeruginosa*). It was found that the antimicrobial activity of the Ag/Fe₂O₃ nanocomposites is dependent on the structure of the nanocomposites, which have significant activity against all bacterial strains tested. Finally, the Ag/Fe₂O₃ nanocomposites biosynthesized using the extract of *Buddleja lindleyana* were also shown to have antibacterial properties, making them promising materials for usage in the medical field.

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