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Research Article

Antibacterial Activity of Green Synthesis of Iron Nanoparticles Using *Lawsonia inermis* and *Gardenia jasminoides* Leaves Extract

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Recently, development of reliable experimental protocols for synthesis of metal nanoparticles with desired morphologies and sizes has become a major focus of researchers. Green synthesis of metallic nanoparticles has accumulated an ultimate interest over the last decade due to their distinctive properties that make them applicable in various fields of science and technology. Metal nanoparticles that are synthesized by using plants have emerged as nontoxic and ecofriendly. In this study a very cheap and simple conventional heating method was used to obtain the iron nanoparticles (FeNPs) using the leaves extract of *Lawsonia inermis* and *Gardenia jasminoides* plant. The iron nanoparticles were characterized by thermal gravimetric analysis (TGA), Fourier transform infrared spectroscopy (FT-IR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and X-ray diffraction (XRD). The antibacterial activity was studied against *Escherichia coli, Salmonella enterica, Proteus mirabilis*, and *Staphylococcus aureus* by using well-diffusion method.

1. Introduction

Nanoparticles are considered as important structural masses of nanotechnology. The unique and most important property of the nanoparticles is that they unveil superior activity. There are remarkable applications of metal nanoparticles in the areas of diagnostic biological probes, catalysis, display devices, and optoelectronics [1]. The widespread practical application of metal nanoparticles (<100 nm) is attributable to a number of their unique properties [2–5]. Metal nanoparticles are widely synthesized using physical and chemical processes, which allow one to acquire particles with the preferred characteristics [6–8]. Several methods like hydrothermal [9], conventional heating [10], anodization [11], deposition precipitation [12], wet oxidation [13], electrodeposition [14], and sonication [15] are being applied to synthesize the nanoparticles. However, these production methods are usually expensive and labor-intensive and are potentially hazardous to the environment and living organisms.

Green synthesis has advances over chemical and physical method as it is cost operative, atmosphere friendly, and easily scrabbled up for large scale synthesis and in this method there is no need to use high energy, temperature, and toxic chemicals. Green synthesis offer better influence, control over crystal growth and their steadiness. Green synthesized nanoparticles are cheap and economical and have many applications in science [16–19].

From the dawn of civilization, human beings have used various medicinal plants to fight diseases [20]. *Lawsonia inermis* is a dwarf shrub, commonly known as "Mehndi or Henna" in Pakistan. It is renowned worldwide due to its cosmetic use for the reason of exclusive active principles in the leaves. It contains different variety of molecules which are bioactive. It is believed to decrease body temperature in situation of high fever and give beautiful and healthy hair. *Lawsonia inermis* is grown in various dry tropical and subtropical areas of North Africa, South Asia, South East Asia, and the Middle East [21]. Strong antimicrobial, anticancer, anti-inflammatory, analgesic, antiparasitic, and virucidal properties of this plant have been reported [22]. *Lawsonia inermis* leaves were studied for their antimicrobial prospective and they exhibited notable antibacterial activity against Gram-negative bacterial strains [23].

Gardenia jasminoides Ellis is a flowering plant, which has its place in genus *Gardenia* and family Rubiaceae. Traditionally, in many Asian countries it has been used as a folk medicine [24]. This plant has numerous medicinal uses for treating hemorrhage, jaundice, toothaches, hepatitis, sprains, wounds, and skin conditions [25–27]. As a hemostatic agent, *Gardenia* is very active as well in handling injuries of the joints, tendons, and muscles. "Crocetin" is an extracted chemical compound from the *Gardenia* berry, from which a yellow-silk dye has been made for this treatment [28].

In nanometer size metallic nanoparticles, iron has received special attention because of its physical and chemical properties which are determined by its size, shape, composition, crystallinity, and structure [29]. Bimetallic iron and silver containing nanoparticles (Fe-Ag NPs) have numerous applications in optical, medical, and remediation fields [30]. Iron nanoparticles can also be used as oxidant for the synthesis of multiwalled carbon nanotubes- (MWCNTs-) core/thiophene polymer-sheath composite nanocables in the presence of cationic surfactant, decyl trimethyl ammonium bromide (DTAB) [31]. Against the bacterial strains causing digestive problems, iron nanoparticles of corn flakes-like morphology gave excellent antibacterial activity [32].

Bacterial resistance to various antibiotics is a serious clinical dilemma, so different antimicrobial activities were performed using plants as a source. The development in the field of green chemistry has delivered different nanomaterials as substitute antibacterial agents. In this present study, an effort is made to synthesize iron nanoparticles using leaves extract of *Lawsonia inermis* and *Gardenia jasminoides* as reducing agent. The characterization of green synthesized iron nanoparticles was characterized by thermal gravimetric (TGA), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and atomic force microscopy (AFM). Also antibacterial activity was studied against human pathogenic Gram-negative (*Escherichia coli*, *Salmonella enterica*, and *Proteus mirabilis*) and Gram-positive (*Staphylococcus aureus*) bacterial strains.

2. Materials and Methods

Fresh leaves of Henna and *Gardenia* plants were collected from various botanical gardens of Lahore, Pakistan. Sulfuric acid (conc.) and iron sulphate ($FeSO_4$) were purchased from Merck and all chemicals were used short of any further purification. CORNING (PC-420D) hot plate was used to maintain the temperature in the synthesis process. Various techniques were used to characterize the synthesized samples, that is, Fourier transform infrared spectroscopy (FTIR) on MIDAC M2000 which identified various functional groups present in extract and which determined the presence of metal, powder X-ray diffractometer (XRD) of X'pert PRO, and PANalytical, equipped with a copper anode source generating X-rays having wavelength equal to 1.54 Å. The elemental composition and morphology were investigated by using scanning electron microscope-energy-dispersive X-ray spectroscopy (SEM-EDX) on Hitachi S3400 on an accelerating voltage of 15.0 kV. The size of synthesized sample particles was determined by transmission electron microscope (TEM) of Phillip CM12 microscope.

2.1. Preparation of Powder. Fresh leaves of Henna and *Gardenia* plants were softly eroded in deionized water by which the dust particles were removed and plants material was then placed to dry under sunlight for seven days. All of the dried leaves of plants were ground using grinder, pastel, and mortar. After the process of grinding the leaves powder went through sieving to get very fine particles of uniform size. Nanoparticles were synthesized by using sieved powder.

2.2. Preparation of FeNPs. A simple conventional heating method was used in the synthesis of iron nanoparticles (FeNPs) by using plant extract. Plant extract was prepared by dissolving 2 gm of the sieved powder in 50 mL of deionized water and the resulting mixture kept on stirring for 3 hours by using the magnetic stirrer. The resulting solution was placed to stable for 1 hour and then filtered. 10 mL of 0.01 M $FeSO_4$ solution was used in which plant extract (filtrate) was added after every interval of 5 minutes using 2 mL in each interval until 50 mL; resulting mixture was stirred at 70°C. The difference of temperature was noted after every interval of 5 minutes. The solution was placed to cool down and the product was parted by centrifugation (10,000 rpm) for 2 minutes. The product was dried at 50°C for 3 hours. The plant extract (filtrate) acts as reducing, capping, and stabilizing agent in iron nanoparticles synthesis [33].

2.3. Antibacterial Studies. Three human pathogenic Gram-negative (*Escherichia coli*, *Salmonella enterica*, and *Proteus mirabilis*) and one Gram-positive (*Staphylococcus aureus*) bacterial strains were used for antimicrobial study of iron nanoparticles by well-diffusion method [34–36]. The bacterial strains were grown in Luria-Bertani (LB) at 37°C with continuous shaking at 200 rpm for 24 hours. 100 μL from each bacterial culture was spread on LB agar plates with the help of L-shaped glass spreader. Three wells were developed in each plate with the help of sterilized steel borer of 8 mm diameter and 30 μL sample suspension was loaded in each well. The plates were incubated for 24 hours at 37°C. Diameter of the inhibition zones was recorded in mm. The experiment was repeated thrice and the average values were calculated for antibacterial activity.

3. Results and Discussion

3.1. TGA of FeNPs Synthesized Using Henna and Gardenia Leave Extract. The weight losses were 12% at 60–205°C and 31% at 280–510°C which were due to the removal of moisture, hydrogen, and three oxygen molecules present in coumaric acid (chemical constituent in Henna), respectively, as shown in Figure 1.

Figure 2 represents the TGA for the FeNPs synthesized using *Gardenia* plant extract. Initial weight was lost at 7%

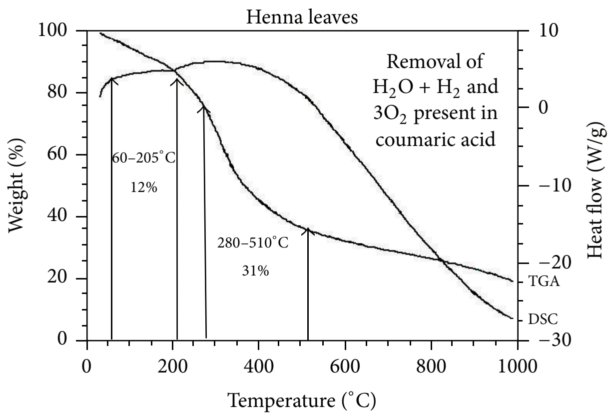


Figure 1: TGA of FeNPs synthesized using Henna leaves extract.

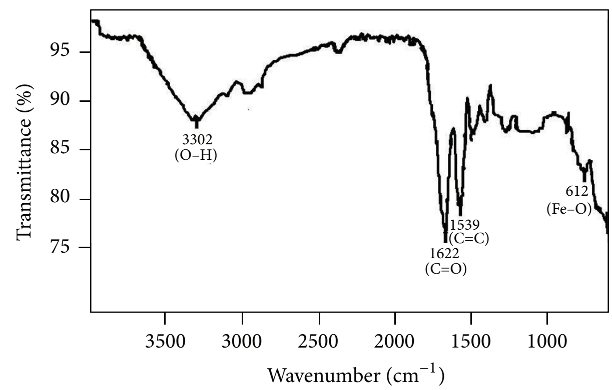


Figure 3: FTIR spectra of FeNPs synthesized using Henna leaves extract.

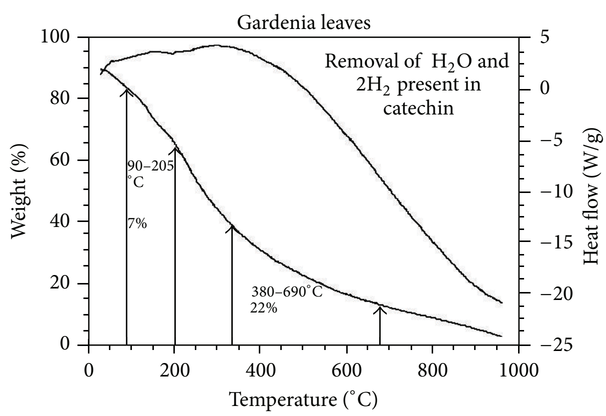


Figure 2: TGA of FeNPs synthesized using *Gardenia* plant extract.

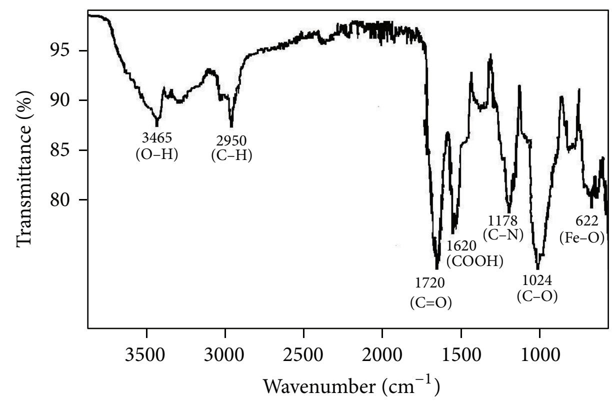


Figure 4: FTIR spectra of FeNPs synthesized using *Gardenia* leaves extract.

at 90–205°C because of removal of moisture, two hydrogen molecules present in catechin (chemical constituent of *Gardenia*). Second weight loss was 22% at 340–690°C and represents the removal of two oxygen molecules present in ferric sulphate used.

3.2. FTIR Analysis of FeNPs Synthesized Using Henna and Gardenia Leaves Extract. The main constituent of Henna extract is Lawson (2-hydroxy-1,4-naphthoquinone). It contains benzene unit, p-benzoquinone unit, and phenolic group. The Henna extract was evaporated to dryness to get a solid mass. Its FTIR spectrum is shown in Figure 3 [37].

The phenolic O–H stretch appeared at 3302.38–3264.84 cm^{-1}. The aromatic C=C stretching frequency which appeared at 1539.81 cm^{-1} is due to p-coumaric acid present in the sample. The C=O stretching frequency appeared at 1622.64 cm^{-1}. Thus Lawson was characterized by IR spectroscopy. It was inferred that Lawson has coordinated with Fe^0 through the phenolic oxygen, aromatic ring, and C=O group of the p-benzoquinone resulting in the formation of Fe^0 which have Lawson as capping and stabilizing agent on the anodic sites of the metal surface. The band at 612.63 cm^{-1} was due to Fe as shown in Figure 3. Thus FTIR spectral study leads to the conclusion that the fingermark film consists of Fe^0 Lawson complex [38].

The FTIR spectra of Fe nanoparticles synthesized with *Gardenia* leave extract are given in Figure 4. The broad absorption band in the region from 3400 to 3200 cm^{-1} represents –OH group stretching and another peak at 2700 cm^{-1} represents C–H stretching. The bands at 1647.28 and 1521.71 cm^{-1} may be assigned, respectively, to a C=O stretching vibration band (C=O) and a coupled vibration involving the bending and the C–N stretching modes of the amido bond of the biomass. The peak at 1032.47 cm^{-1} is corresponding to the vibrations of C–O in C–CCOOR. The band at 612.63 cm^{-1} was due to Fe vibrations.

3.3. XRD Analysis of FeNPs Synthesized Using Henna and Gardenia Leaves Extract. The nature and phase composition of FeNPs were identified by X-ray powder diffractometer with Bragg's angle ranging from 10° to 70°. The presence of Fe in nanopowder was confirmed by a series of reflection angles (2θ) at 44.34° and 64.43° having hkl values (111), (200), and (202) [39], respectively, with cubic plane of Fe [40] as shown in Figure 5.

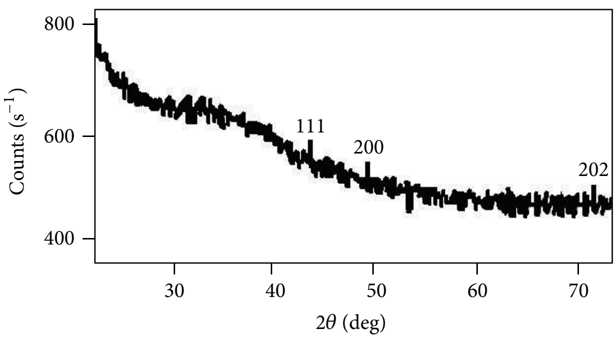


Figure 5: XRD pattern of FeNPs synthesized using Henna leaves extract.

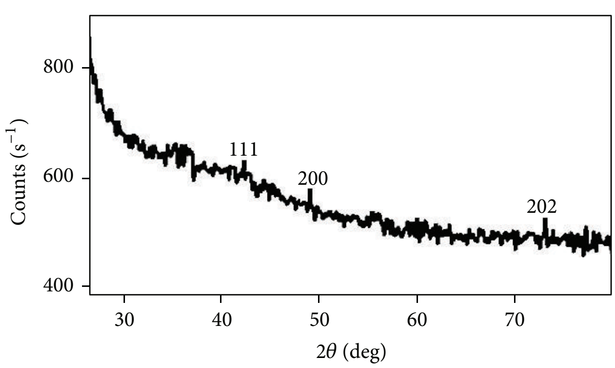


Figure 6: XRD pattern of FeNPs synthesized using *Gardenia* leaves extract.

The presence of Fe in nanopowder synthesized by *Gardenia* leave extract was confirmed by a series of reflection angles (2θ) at 44.34° and 64.43° having hkl values (111), (200) and (202) [39], respectively, with cubic plane of Fe [40] as shown in Figure 6.

3.4. TEM Image of FeNPs Using Henna and Gardenia Leaves Extract. TEM analysis of the FeNPs was performed which were formed by using the Henna leaves extract and $FeSO_4$ salt solution. The size of iron nanoparticles synthesized using Henna leaves extract was calculated as 21 nm as shown in Figure 7. While particle size of the same was observed as 32 nm when synthesized using the *Gardenia* leaves extract as shown in Figure 8.

3.5. SEM-EDX Analysis of FeNPs Synthesized Using Henna and Gardenia Leaves Extract. FeNPs synthesized using extract of leaves of Henna plant are studied under SEM. It indicates that nanoparticles formed are agglomerated because of the adhesive nature having morphology of distorted hexagonal-like appearance as shown in Figure 9(a).

Elemental composition of FeNPs synthesized using Henna leaves extract was determined by using EDX analysis. It was observed that the percentage of iron is 6.86%, carbon is 54.59%, oxygen is 36.57%, magnesium and phosphorus are 0.68%, and potassium is 0.63% as shown in Figure 9(b). Mg and carbon are due to plant constituents.

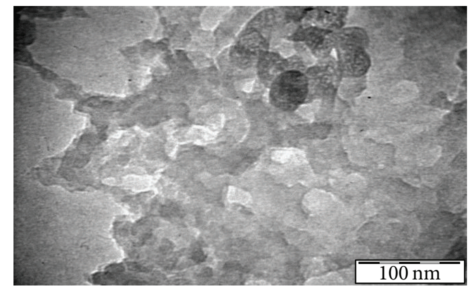


Figure 7: TEM analysis of FeNPs synthesized using Henna leaves extract.

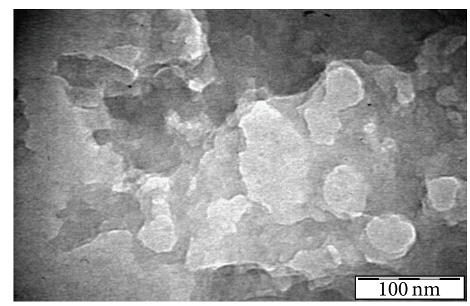


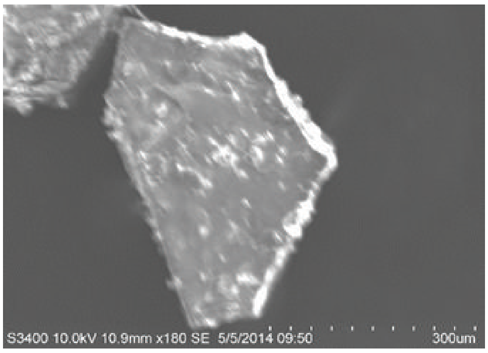
Figure 8: TEM image of FeNPs synthesized using *Gardenia* leaves extract.

FeNPs synthesized using extract of leaves of *Gardenia* plant are studied under SEM and shown in Figure 10(a). It indicates that nanoparticles formed are agglomerated because of the adhesive nature having morphology of shattered rock-like appearance.

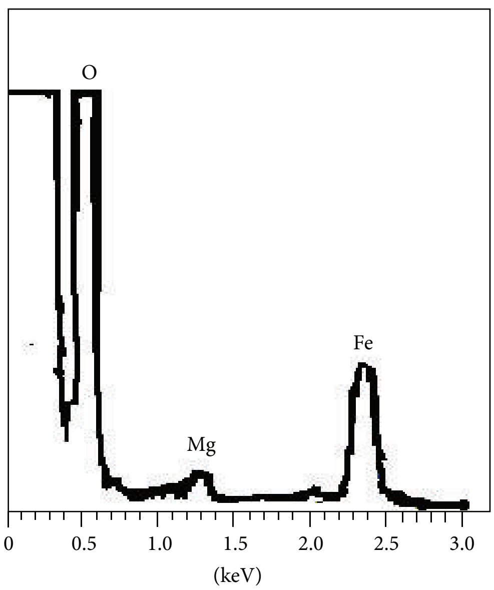
Elemental composition of FeNPs synthesized using *Gardenia* leaves extract was also determined by using EDX analysis.

Elemental composition was found as percentage of iron is 4.68%, carbon is 50.79%, oxygen is 41.37%, aluminium is 0.76%, silicon is 1.57%, and potassium is 0.83% as shown in Figure 10(b).

3.5.1. Antibacterial Results. Iron nanoparticles were synthesized using five different plants, that is, *Lawsonia inermis*, *Gardenia jasminoides*, *Azadirachta indica*, and *Camellia sinensis* leaves extract and *Cinnamon zeylanicum* barks extract and it was found that all are susceptible to all bacterial strains. Here we are representing the results of mainly the two of them, that is, iron nanoparticles synthesized using *Lawsonia inermis* and *Gardenia jasminoides* (Table 1) and the comparison is given in Figure 11. FeNPs of *Gardenia jasminoides* were more potent against *Staphylococcus aureus* with zone of inhibition (ZOI) 16 mm, whereas for *Lawsonia inermis* it was 15 mm as shown in Figure 12. Against *Escherichia coli*, *Salmonella enterica*, and *Proteus mirabilis* the ZOI of iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* leaves extract was 14 mm and 15 mm, 9 mm and 12 mm, 11 mm and 13 mm, respectively.

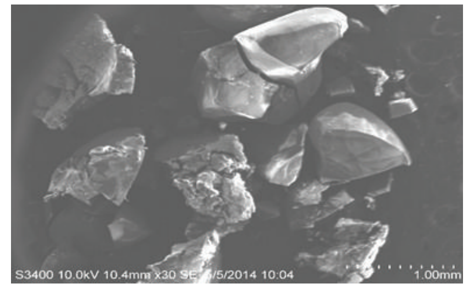


(a)

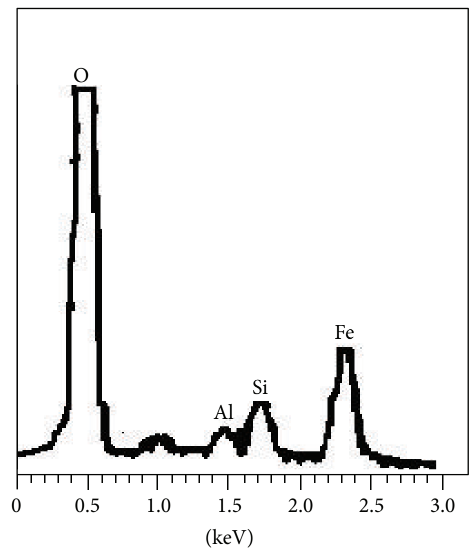


(b)

FIGURE 9: (a) SEM image and (b) EDX graph of FeNPs synthesized using Henna leaves extract.



(a)



(b)

FIGURE 10: (a) SEM image and (b) EDX graph of FeNPs synthesized using *Gardenia* leaves extract.

TABLE 1: Antibacterial activity of iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* leaves extract (30 μL/mL).

Microorganisms	Leaves extract *Lawsonia inermis*	Leaves extract *Gardenia jasminoides*
	Zone of inhibition (mm)	
Bacterial strains		
E. coli (−)	14	15
S. enterica (−)	09	12
P. mirabilis (−)	11	13
S. aureus (+)	15	16

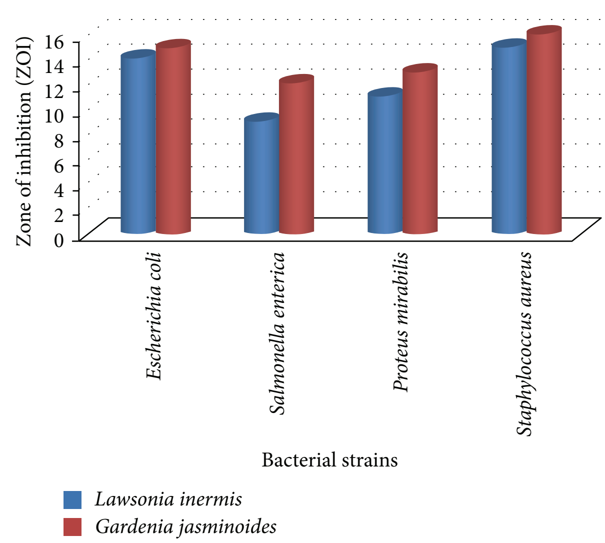
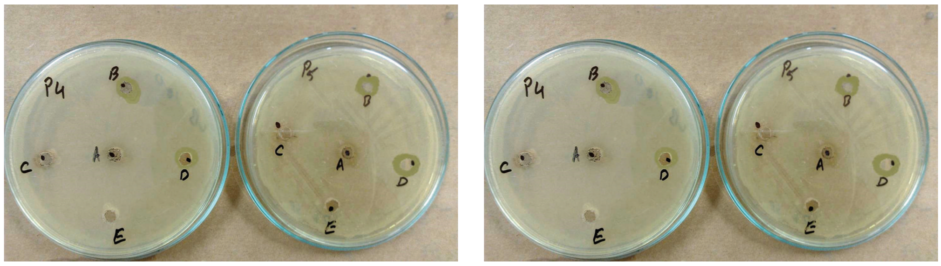


FIGURE 11: Comparison of antibacterial activity of iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* leaves extract against bacterial strains.

4. Conclusion

Due to the rich biodiversity of plants, the green world has potential for the synthesis of noble metal nanoparticles. Iron nanoparticles with an average size of 21 nm and 32 nm were synthesized using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract, respectively. Green synthesized iron nanoparticles in the present study show good antibacterial activity against the human pathogens *Escherichia coli* and *Staphylococcus aureus*. As *Lawsonia inermis* and *Gardenia*



(a) (b)

Figure 12: (a) Zone inhibition of FeNPs against P_4 = *Escherichia coli*, P_5 = *Salmonella enterica*, B = *Lawsonia inermis*, and D = *Gardenia jasminoides*. (b) Zone inhibition of FeNPs against P_4 = *Staphylococcus aureus*, P_5 = *Proteus mirabilis*, B = *Lawsonia inermis*, and D = *Gardenia jasminoides*.

jasminoides have been used in folk medicines, these green iron nanoparticles of *Lawsonia inermis* and *Gardenia jasminoides* have potential biomedical activities and have several paybacks such as suitability for medical and pharmaceutical submissions.

Conflict of Interests

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

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