# Green Synthesis and Evaluation of *Mentha piperita* meditated Iron Nanoparticles for in vitro Antibacterial Activity.

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## Abstract

Iron oxide nanoparticles ($Fe_2O_3$) stabilized with *Mentha piperita* obtained from Pakistan were synthesized. The study focuses on exploring the antimicrobial potential of $Fe_2O_3$ NPs using the agar well diffusion method. The physicochemical characteristics of the prepared $Fe_2O_3$ nanoparticles were studied using different techniques such as UV-Vis spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), and scanning electron microscope (SEM) analysis. The spectra obtained from FTIR, EDX, and SEM confirm the formation of $Fe_2O_3$. The analysis of UV-visible spectra absorption peak was found at 250 nm confirming the successful formation and stability of iron oxide nanoparticles. The NPs exhibited uniform spherical morphology with some irregular shapes and contained Fe, O, Ca, Si, Na, Mg, and Cl indicating the synthesis of $Fe_2O_3$ NPs. Additionally, the $Fe_2O_3$ NPs formed through biosynthesis demonstrated antimicrobial activities against *E.coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Stable $Fe_2O_3$ NPs were synthesized using *Mentha piperita* aqueous leaf extract and revealed antimicrobial activity on *E.coli, S. aureus, and P. aeruginosa*.

Keywords: *Mentha piperita*, $Fe_2O_3$ NPs, *P. aeruginosa*, UV-visible spectroscopy.

## Introduction:

Nanotechnology employs information from Physics, chemistry, biology, material science, health sciences, and engineering. It has several applications in practically all fields of science and human life (Khatra, Harikumar, and Nirmala 2013). Nanotechnology prompted the development of a new drug-delivery system using nanoparticles (NPs), advancing precision medicine. Advances in nanoparticle design can overcome delivery limitations and boost efficacy. Nanoparticles are divided into metal, ceramic, polymeric, and fullerene groups, with diameters ranging from 1-100 nm. Nanoparticles are architecturally complicated with three layers: a functional surface layer with metal ions, surfactants, or polymers, a shell layer, and the NP core (Alangari et al. 2022). Nanoparticle synthesis is a clean, simple, and environmentally friendly method using plant extracts, microbial cultures, mushrooms, and algae. Green synthesis offers advantages in cost, time, and complex steps. Nanotechnology improves the therapeutic delivery of antibiotics and antimicrobial agents, but size control is limited (Chester et al. 2017)**.** Metallic oxide NPs based on *Mentha piperita* are economically significant because they can be employed in biomedical therapies for diseases, such as antibiotics (Faisal et al. 2021). Mentha, a Lamiaceae plant, is used in traditional and modern herbal therapy to treat a variety of diseases including fever, common cold, sinusitis, bronchitis, nausea, cough, vomiting, indigestion, and intestinal colic. It also contains antibacterial and antioxidant effects and is used to flavor toothpaste, sweets, chewing gum, and pharmaceutical preparations. The essential oils, polyphenols, and flavonoids present in mint leaf extract are important for therapeutic purposes. The unusual combination of aromatic, hydrophobic compounds, and volatile essential oils derived from plants gives strong water vapor barrier properties (Shabir Ahmad et al. 2020). Iron-based nanoparticles with acceptable surface chemistry have been synthesized using several techniques, including physical, chemical, and biological procedures. Iron oxide nanoparticles ($Fe_2O_3$NPs) are increasingly being studied for antibacterial activities (Wang et al. 2018). Iron oxide nanoparticles possess unusual magnetic characteristics making them suitable for thermotherapy, photothermal therapy, and targeted drug delivery (Alphandéry 2019). Green synthesis of *Mentha piperita-based* iron oxide NPs is used for testing against bacterial infections such as *Staphylococcus aureus*, *Klebsiella pneumonia*, and *E. coli* are the leading cause of over 50,000 daily mortality. Vibrio cholera, *Staphylococcus aureus*,

*Salmonella spp., and Shigella spp*. are the most prevalent pathogens (Hussain et al. 2023). Because of their antibacterial capabilities, iron oxide nanoparticles are significant for medicinal applications. The current study focuses on the successful synthesis of $Fe_2O_3$ NP by *Mentha piperita* leaves extract and microscopic and spectroscopic techniques were applied to the synthesized NPs' characterization. By using the agar well diffusion method, the antibacterial ability of the $Fe_2O_3$ NPs was investigated against *E.coli, S. aureus, and P.aeruginosa*.

## Material and Methods:

## Materials

Ferric Chloride ($FeCl_3$) was purchased from Merck Millipore and was used without purification. *M. piperita* leaves were freshly collected from the fields of Rangeel Pur district Multan, Punjab. Bacterial strains of *E.coli, S. aureus, and P.aeruginosa* were purchased from Islamia University Bahawalpur. All the chemicals and reagents were used for analytical grades.

## Biogenic synthesis of Fe2O3 NPs using *M. piperita* leaves extract:

*M. piperita* (12g) leaves were washed with tap water and one time with distilled water, then finely chopped and mixed in 250ml d.$H_2O$ at 60°C for 1 hour. The mixture was cooled, filtered, and collected for extract filtration. The extracted filtrate was stored at 4°C for future use. The process of creating iron oxide nanoparticles involves dissolving black crystalline $FeCl_3$ in double distilled water (Natrayan et al. 2023). The production process of iron oxide nanoparticles involved the preparation of 0.1M aqueous solution of Iron Chloride ($FeCl_3$) and its subsequent applications. 20ml of leaf extract of *M. pierita* was added to 80ml of 0.1M $FeCl_3$ solution to make up the volume of 100ml and kept at room temperature the reaction mixture was stirred for 1 hour on a magnetic stirrer and pH adjustment was carried out by adding NaOH to bring pH up to 11. After stirring and pH adjustment the solution appears to be reddish brown in color indicating the formation of iron oxide nanoparticles. The mixture was centrifuged for 30 minutes at 9000 rpm, then the supernatant was discarded, and the pellet was dissolved in distilled water and centrifuged three times to remove contamination. The dark brown pellet was dried in a hot air oven for 3 hours (Freire et al. 2023).

## Characterization of $Fe_2O_3$ NPs:

## UV-visible spectroscopy

The optical properties of biologically synthesized $Fe_2O_3$ NPs were examined by UV-visible spectrophotometer (PerkinElmer, Japan) over 200-800nm wavelength (Campos et al. 2015).

## Fourier transform infrared spectroscopy (FTIR）

ATR-FTIR (Bruker IR, Japan) was used to record the FTIR spectra of *M. piperita* leaf extracts and produced $Fe_2O_3$ nanoparticles throughout a transmission range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ (Rajendran and Sengodan 2017).

## Scanning electron microscope (SEM)

SEM image of $Fe_2O_3$ NPs was obtained by scanning electron microscope (Hitachi High-Tech Flex SEM 1000 II). (Rajendran and Sengodan 2017) (Clarina et al. 2018)

## Energy dispersive X-ray spectroscopy (EDX)

EDX was used to scan the elemental alignments of the biogenically generated $Fe_2O_3$ NPs (Kamal et al. 2023).

## Antimicrobial activity

Using the agar well-diffusion method, the antibacterial activity of $Fe_3O_4$ nanoparticles produced using *Mentha piperita* against *E. Coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* was evaluated. On Muller Hinton agar, the pure

bacterial culture was sub-cultured. Using gel puncture, 8 mm diameter wells were created on nutritional agar. Using sterile cotton swabs, the strain was evenly swabbed onto each plate. 300ul of each strain's concentration, distributed across eight plates, was added to each well on the plates using a micropipette to measure the sample of nanoparticles. Following a 24-hour incubation period at 37°C, the bacteria's varying zone of inhibition was quantified (Jency Evanjelin et al. 2023).

## Results and Discussion

### Visual analysis

Aqueous extracts of different medicinal plants have been reported for the biogenic synthesis of $Fe_3O_4$. In the present work, $Fe_3O_4$ NPs were biosynthesized using an aqueous extract of *M. piperita* leaves. The synthesis was started by adding 1 mM FeCl3 solution to the *M. piperita* leaf aqueous extracts. This caused the extract's color to progressively change from dark green to reddish brown, indicating the production of iron nanoparticles (Kanagasubbulakshmi and Kadirvelu 2017).

## UV-vis spectroscopy analysis

UV-visible analysis was used to validate the formation of $Fe_2O_3$ nanoparticles a few hours after the reaction and color shift (dark green to reddish brown). According to Figure 1, the UV-visible validation showed an absorption peak at 250 nm, which is related to the surface plasmon resonance effect brought on by the creation of $Fe_2O_3$ nanoparticles. Our absorption peak falls within the range of $Fe_2O_3$ absorption, as research indicates that $Fe_2O_3$ NPs have an absorption peak in the 230–290 nm range (Campos et al. 2015).

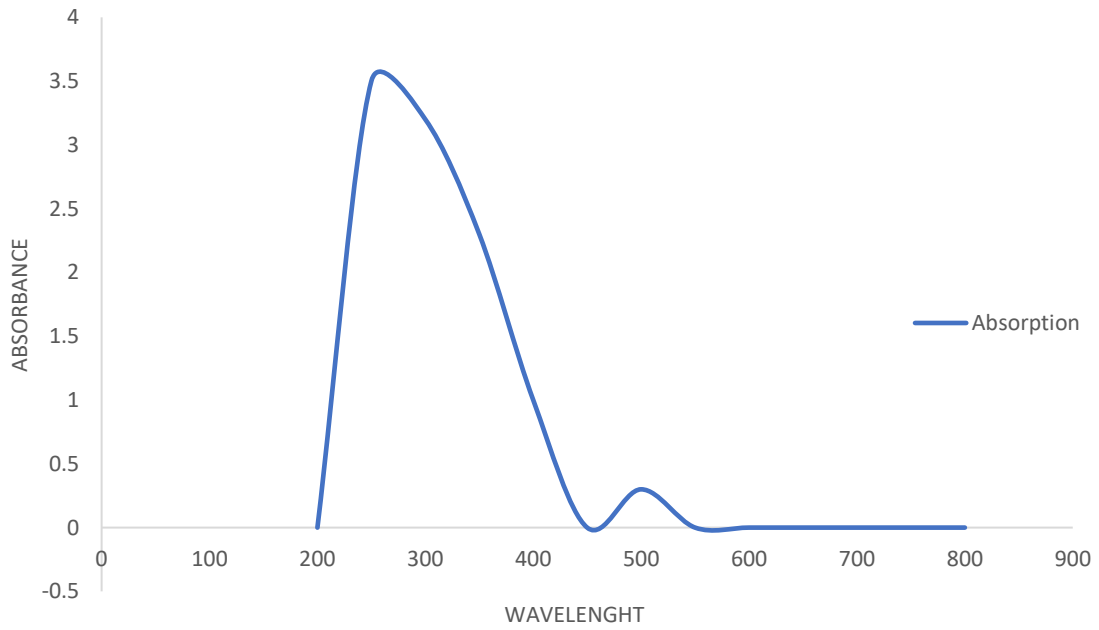


**Figure 1. UV-vis spectra of $Fe_2O_3$ NPs synthesized by *Mentha piperita***

## FTIR analysis

FTIR analysis was carried out to find out the important functional groups in the extract of *Mentha piperita* leaves and how they might help to reduce, cap, and stabilize $Fe_2O_3$. The FTIR spectra of *Mentha piperita* leaves extract showed a band around 3331.46$cm^{-1}$ indicating O-H stretching vibration of high concentration of alcoholic phytochemicals, a

peak at 2347.82cm$^{-1}$, 2029.58cm$^{-1}$, C=C suggesting amine and cyclic alkene, a peak at 1635.58cm$^{-1}$ suggesting alkene group and a peak at 958.23cm$^{-1}$ A broad band of FTIR of FeO-NPs occurs at 3647.98cm$^{-1}$, 3254.53cm$^{-1}$ indicating the stretching of the O-H alcoholic group, a peak observed at 2288.18cm$^{-1}$, 2178.88cm$^{-1}$, 2059.91cm$^{-1}$, 2001.97cm$^{-1}$ representing the association of C≡ C in alkyne. The peaks at 1923.70cm$^{-1}$, 1581.63cm$^{-1}$, and 1079.81cm$^{-1}$ indicate the stretching C=C, N=H of amine and cyclic alkenes. The peaks at 712.74cm$^{-1}$, and 631.33cm$^{-1}$ indicate the stretching of

C=C and aromatic rings of secondary alcoholic groups and the C-Cl bond of alkyl halides. Phytochemicals including flavonoids and phenols participate in the stabilization and capping of nanoparticles.(Rajendran and Sengodan 2017).

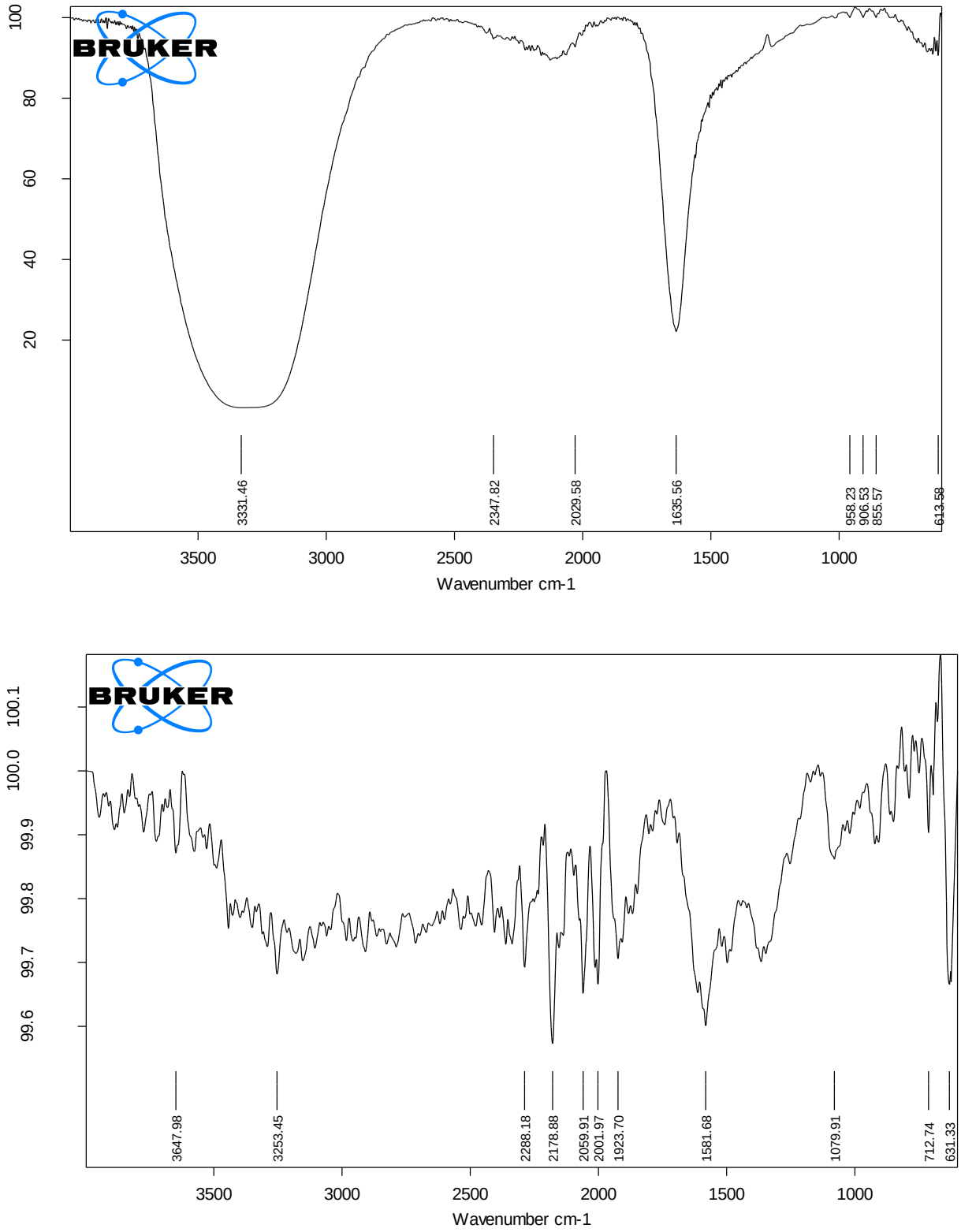


**Figure 2. FTIR spectra of Mentha piperita extract and $Fe_2O_3$**

## SEM analysis:

Scanning Electron Microscope was used to look at the shape of the $Fe_2O_3$ NPs and their composites. The SEM pictures showed that the shape of $Fe_2O_3$ NPs was round and mostly irregular in shape due to slight agglomeration at low and high magnification (Figure 4). The phytochemicals in the *M. piperita* extract may be the cause of this. Understanding the function of nanoparticles (NPs) depends in large part on their surface shape, and earlier research has shown that plant extract contributes to the reduction of NPs' size (Rajendran and Sengodan 2017) (Clarina et al. 2018).

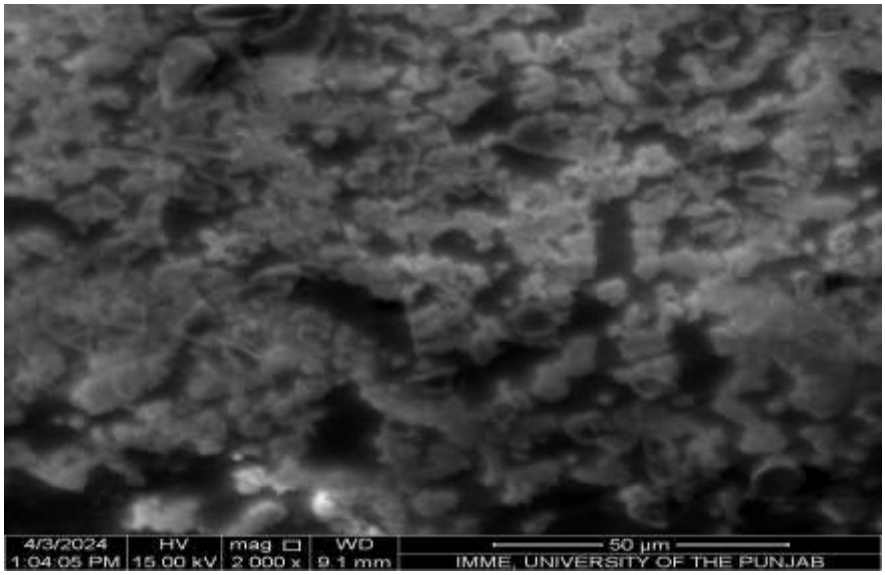


**Figure 3. SEM image of synthesized Fe2O3 NPs**

## EDX analysis

As shown in Figure, EDX was used to scan the elemental alignments of the biogenically generated Fe2O3 NPs. Iron was detected by the EDX examination together with silicon, oxygen, calcium, magnesium, chlorine, and sodium which may have been caused by the introduction of contaminants as the material was being ground. At 0.05, 6.5, and 7 keV, the three prominent peaks of Fe indicate the presence of iron. As seen in Figure, these are the characteristic absorption peaks of iron nanoparticles with the percentage of Fe at 53.82% and oxygen having 15.47% which supports the

previous report of Fe having a percentage of 52.6% and oxygen having a percentage of 30.5% presented by (Kamal et al. 2023).

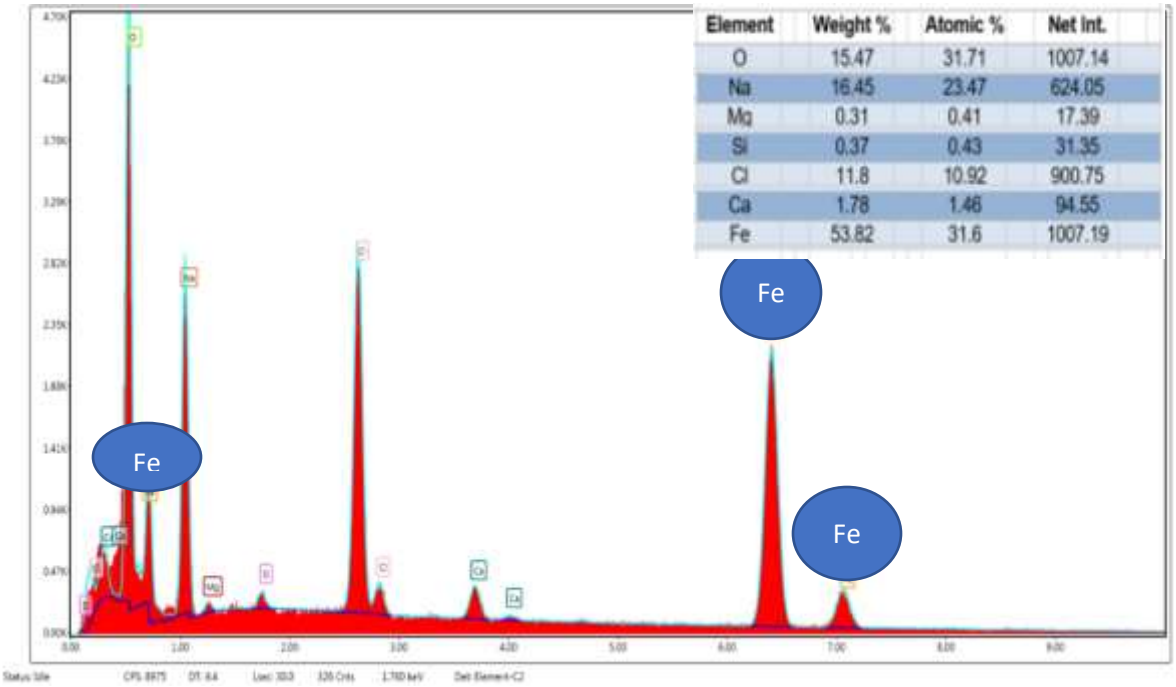


**Figure 4. EDX image of synthesized $Fe_2O_3$ NPs**

## Antibacterial activity of $Fe_2O_3$ NPs

Iron nanoparticles can firmly bind with the negatively charged bacterial membrane, which leads to a change in the permeability of the membrane and induces oxidative stress that eventually kills the bacterial cells. According to our research, $Fe_2O_3$ significantly inhibits the growth of all tested bacterial strains; it is most effective against *S. aureus* and least effective against *E. coli* and *P. aeruginosa*. The positive control showed 16mm ZOI against E.coli, 17mm ZOI against S. aureus, and 19mm ZOI against P. aeruginosa. To ascertain the impact of the solvent in the test solution on the growth of the tested strains, distilled water was utilized as a negative control; no ZOI was found in every instance, suggesting that the antibacterial action of Fe nanoparticles is independent of the solvent. The $Fe_2O_3$ NPs have shown ZOI against E.coli was 6mm in diameter and 8mm ZOI shown by S. aureus and P. aeruginosa have ZOI of 6mm in diameter. Plant extract showed ZOI of 3mm and 4mm against E.coli and S. aureus but no ZOI against P. aeruginosa (Umar, Aliyu, and Ozsahin 2024), (Packialakshmi et al. 2021) (Abbas, Krishnan, and Kotakonda 2020)

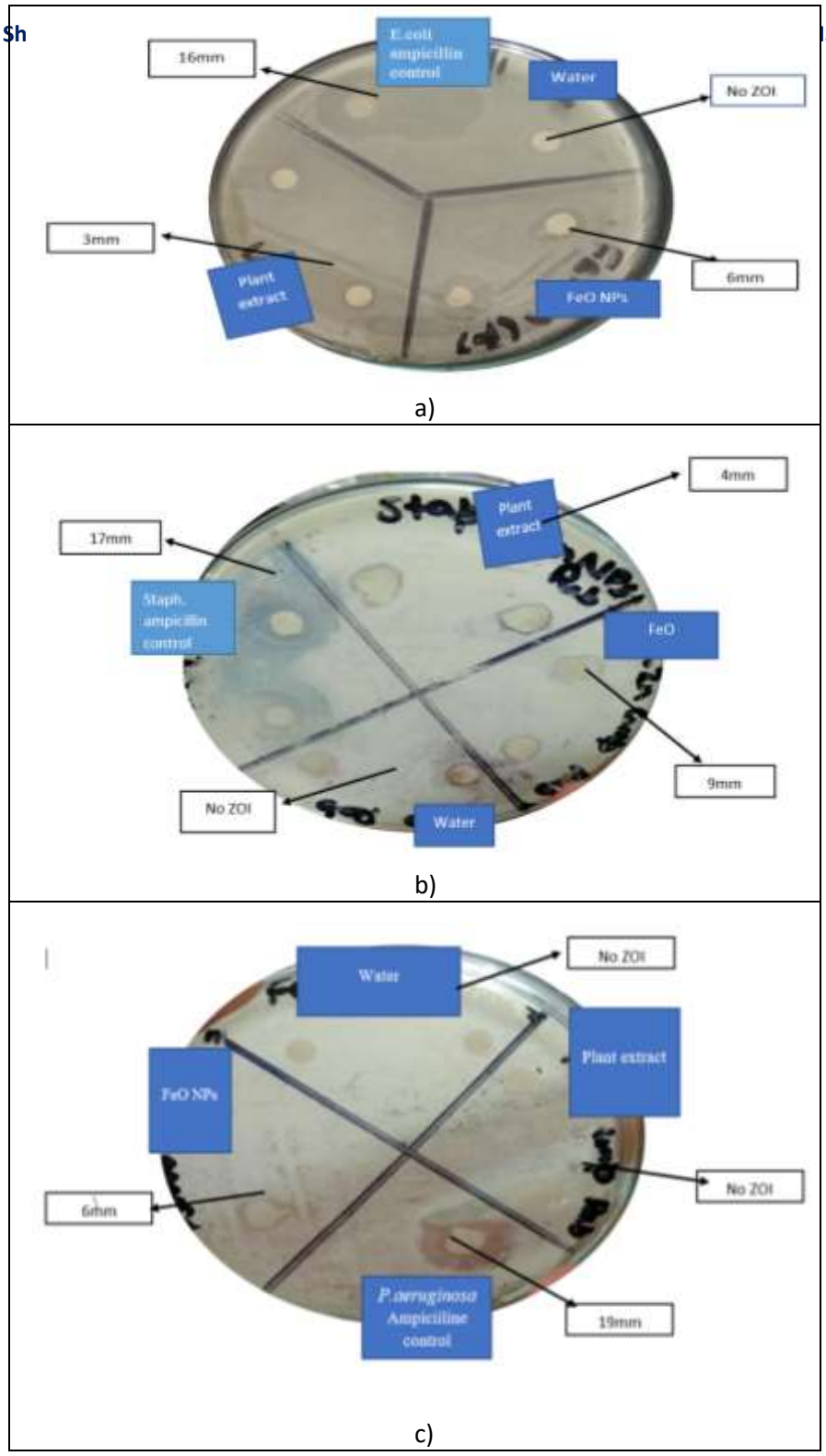


**Figure 5. Antibacterial activity of $Fe_2O_3$ NPs, Plant extract, water as a negative control, and ampicillin as a positive control against a) *E.coli* b) *S. aureus* c) *P. aeruginosa***

**Table 1. Zone of inhibition (mm) of *Mentha piperita-mediated* $Fe_3O_4$ nanoparticles**

| Test organism | Antibiotic disc ZOI | Fe-NPs ZOI | Plant extract | Water |
|---|---|---|---|---|
| *E. Coli* | 16mm±0.50 | 6mm ± 0.5 | 3mm±1 | NO ZOI |
| *Pseudomonas aeruginosa* | 19mm±1.5 | 6mm±0.5 | NO ZOI | NO ZOI |
| *Staphylococcus aureus* | 17mm±0.50 | 9mm±0.50 | 4mm±0.50 | NO ZOI |

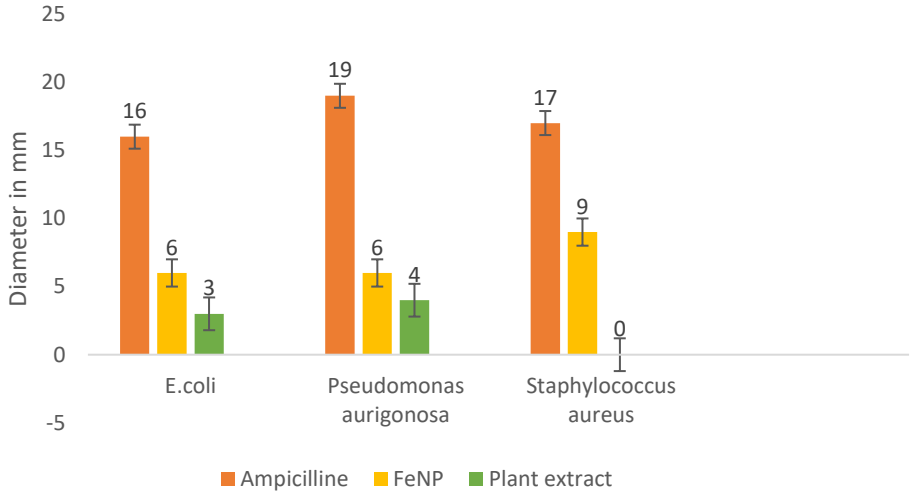


**Figure 6 Graph representing antibacterial activity against ampicillin, FeNPs, and Plant Extract**

## Conclusion

Aqueous extract of fresh leaves of *M. piperita* can be used as cost-effective, safer, and eco-friendly reducing and capping agents to produce iron nanoparticles. $Fe_2O_3$ showed efficient antibacterial activities against *P. aeruginosa*, *E. coli*, and *S. aureus*.

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