

Controlling of Wound Pathogens using Iron Oxide Nanoparticles Mediated Through Hydrocotyle Umbellata L.- An Invitro Study

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Novel nanomaterials have been discovered as a result of recent changes in nanoscience and nanotechnology, presenting the possibility of health and ecological hazards. Metallic nanoparticles that are affordable, energy-efficient, nontoxic, and biodegradable have been produced using biological resources like bacteria, fungi, algae, and plants. The purpose of this research is to learn about the antibacterial activity of Hydrocotyle umbellata mediated iron oxide nanoparticles and to see the antimicrobial activity against uropathogens. The time kill curve analysis was done to check the bactericidal properties of the nanoparticle against the uropathogens, Escherichia coli, Enterococcus faecalis, P. aeruginosa, and Streptococcus mutans. Preparation of the plant solution was done by using the plant powder. Followed by the preparation of the iron oxide nanoparticle solution. Antimicrobial activity and Time kill curve analysis against wound pathogens was done to check the properties of the iron oxide nanoparticle which was synthesized by the plant Hydrocotyle umbellata. The results have been graphically represented and it proves that the iron oxide nanoparticles are bactericidal, not only preventing the further growth of bacterias but also killing the remnants of the bacterias. This study concludes that iron oxide nanoparticles that were synthesized using the plant Hydrocotyle umbellata have an amazing antimicrobial property against wound pathogens, hence proving that it can be further modified for targeted drug therapy.

Keywords: Antimicrobial, Biological method, Nanotechnology, Iron oxide nanoparticles, Time kill curve assay, Wound pathogens.

1. Introduction

The applied application of science for controlling material at the molecular level is commonly referred to as nanotechnology (Senapati et al., 2005). The field of materials science and engineering has experienced tremendous growth in nanotechnology, leading to the development of new basic and applied fields such nanobiotechnology, bionanotechnology, quantum dots, surface-enhanced Raman scattering (SERS), and applied microbiology (Shanker et al., 2020). Initially the particles possess a large surface to volume ratio and are tiny in size, nanoparticles are very interesting because of the differences in their chemical and physical properties (e.g. physical characteristics, physiological and sterical properties, enzyme activity, the conductivity of electricity and heat, optical absorption, and melting point) (Khan et al., 2019). Nanotechnology is used not only for targeted drug therapy but also for wound dressing. The characterization of the nanoparticles was done using various methods such as Scanning Electron Microscopy, and X-ray Diffraction, Fourier Transform – Infrared Spectroscopy (Anghel et al., 2013). Nanoparticles are materials that have an overall dimension of 1 to 100 nanometers. These nanoparticles play a major and essential role in today's world of modern medicine Nanoparticles have many uses of which some are used as a catalyst, used as an antibacterial agent and so on (Mubarak et al., 2023).

Magnetite has been widely used in the fields of magnetic resonance imaging, hyperthermia, cancer treatment and inhibition of microbial colonization (Dinali et al., 2017). Iron Oxide Nanoparticles are considered as one of the best materials due to its intrinsic magnetic nature, enhanced physico-chemical properties, larger surface to mass ratio, higher reactivity and functionalized structure (Hasany et al., 2013). IONPs are found to have an immense effect on the different kinds of Gram-positive and Gram-negative bacteria thus, proving that IONPs are effective antibacterial agents. The magnetic response of iron oxide nanoparticles is confirmed via their migration in a few minutes towards a magnet placed near the solution (Abdulsada et al., 2021). *Hydrocotyle umbellata* L. is found in water bodies and has various other common names such as water pennywort and navelwort (Rodgers 1950). About 10 years ago, this plant was introduced in Thailand and now it is sold as an ornamental plant. *Hydrocotyle umbellata* L. has the ability to propagate through its vegetative parts (Lusk et al., 2007). An ideal bacterial agent should be non-toxic to human cells but harmful to the bacteria. Iron Oxide Nanoparticles has been approved by the U.S. Food and Drug Administration as an iron supplement for treatment of iron deficiency in patients (Armijo et al., 2020). Healing of wounds is a natural mechanism that occurs in response to an injury in the tissue. It is the process of restoring the tensile strength of injured skin. Response can be divided into four phases: hemostasis, inflammation, proliferation and maturation (Ziv-Polat et al., 2010). The development of such biomaterials plays an efficient role in the areas of health and even in hygiene. Skin diseases are of worldwide concern, not only to humans but also to animals. Superficial wounds tend to get infected leading to prolonged pain and discomfort (Agarwal et al., 2020). The aim of the research work, the green synthesis of iron oxide nanoparticles to determine the efficacy of antimicrobial activity against wound pathogens.

2. Materials and Methods

2.1. Preparation of Plant Extract

The plant extract was prepared by adding 1g of plant powder mix to 100 ml of water and the solution boiled at 50 to 60°C for 15 to 20 mins using a heating mantle. The solution was then filtered using a Whatman Filter paper. The filtrate extracted was then preserved in the refrigerator.

2.2. Preparation of Nanoparticle Solution

The preparation of the nanoparticle solution was done by adding 0.486g of iron chloride to 50mL of distilled water along with 50 mL of the prepared plant extract. The final solution was then placed in an Orbital shaker for the following 48 hours. The solution was checked periodically for any obvious color changes, followed by UV reading to verify the synthesis of nanoparticles in various different intervals of time. The final solution was transferred into the sterile centrifuge tubes, the centrifugation process for 10 mins at 8000 rpm. The formed supernatant was removed and the pellet formed is collected for further biomedical research.

2.3. Antimicrobial Activity

The agar well diffusion technique was used to evaluate the antimicrobial activity of iron oxide nanoparticles. Mueller Hinton plates were prepared and sterilized with the help of an autoclave for 15 to 20 minutes while maintaining the temperature at 121°C. Following the sterilization, the medium was transferred to sterilized petri dishes and allowed to cool at room temperature. This was followed by the spreading of bacterial suspension consisting of *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis* was uniformly spread using sterile cotton swabs onto the agar plates. Then using polystyrene tip 9mm diameter wells were created in the agar plates. In each well, different concentrations such as 25, 50 and 100 100 µg/mL of IONPs were added. Amoxyrite, an antibiotic, was used as a standard. Post a pre-incubation period of four hours in a medium absent of any antimicrobial agents, the growth curves were drawn before the test to confirm that all pathogens had reached the early to mid-log phase. An inoculum composed of 0.5 McFarland of each individual pathogen was created in a sterile phosphate-buffered saline. The inoculum was prepared from cultures that had been cultivated on Mueller Hinton agar plates whose temperature was constantly maintained at 37 °C for the next 24 hours. The diameter of the inhibition zone that surrounded the wells was the main criteria for evaluating the antimicrobial activity. The diameter of the inhibition zone was measured using a ruler and the recording was done in millimeters (mm) to calculate the zone of inhibition.

2.4. Time Kill Curve Analysis

In order to assess the properties of the bacteria along with the concentration-dependent relationship between iron oxide nanoparticles synthesized from *Hydrocotyle umbellata*, a time-kill curve assay was done. The graph was drawn across the growth of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus mutans* and its zone of inhibition. Various concentrations such as 25, 50 and 100 µg/ml were used along with a control. 0.5 McFarland of each particular individual was used as an inoculum in order to create a sterile phosphate buffered saline. Preparation of the inoculum was done using the Mueller Hinton agar plates. The temperature of these plates was constantly kept at 37 °C for the following 24hrs. The prepared inoculum was then pre-heated to 37 °C, then 90µL of the

Nanotechnology Perceptions Vol. 20 No. S7 (2024)

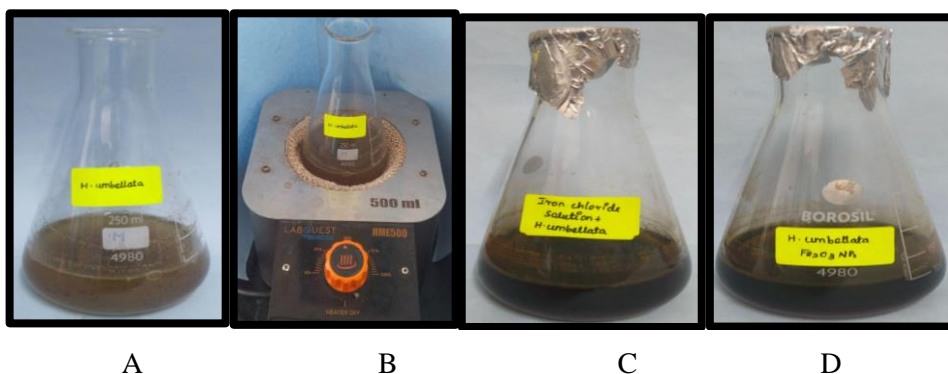
resultant mixture was then distributed evenly in an ELISA plate that had 96 wells.

3. Results

3.1. Preparation of iron oxide nanoparticles

The green production of iron oxide nanoparticles using *H. umbellata* was first used to visually evaluate the color changes in the solution (Figure 1). The reaction mixture contains both the plant extract and the nanoparticle precursor solution. The phytochemical component of the plant extract and iron chloride, which was used as a reducing agent for the metal oxide nanoparticles, reacted to form iron (ion). The iron oxide nanoparticles revealed their native shade, ranging from brown to dark brown. Evaluation of the produced nanoparticles is required.

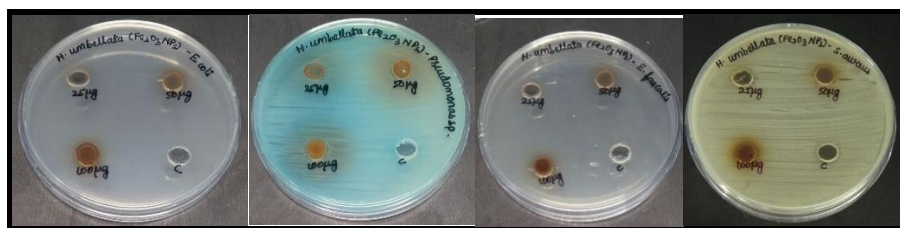
Figure 1: Green synthesis of iron oxide nanoparticles A) *H. umbellata* B) Boiling of Extract, C) Reaction mixture (Iron chloride solution + *H. umbellata* extract), and D) green synthesis of iron oxide nanoparticles.



3.2. Antimicrobial activity

Figure 2: showed the biosynthesized iron oxide nanoparticles had more efficacy of antimicrobial activity against wound pathogens (*E. coli*, *Pseudomonas* sp., *E. faecalis*, and *S. aureus*). *H. umbellata* mediated iron oxide nanoparticles have revealed the antimicrobial activity has enhanced the inhibitory zone. The agar well diffusion technique was used to determine the antimicrobial activity of biological synthesis of iron oxide nanoparticles against wound pathogens (Graph 1), whereas inhibitory zone of iron oxide nanoparticles at the various concentration of 25, 50, and 100 $\mu\text{g/mL}$ for 9 mm of all wound pathogens (*E. coli*, *Pseudomonas* sp., *E. faecalis*, and *S. aureus*), respectively. The Graph 1, shows that the zone of inhibition is the same in all concentrations. The iron oxide nanoparticle has managed to kill all the pathogens present even in the most minimum concentration.

Figure 2: Antimicrobial activity of iron oxide nanoparticles against wound pathogens, A) *E. coli*, B) *Pseudomonas* sp., C) *E. faecalis*, and D) *S. aureus*.



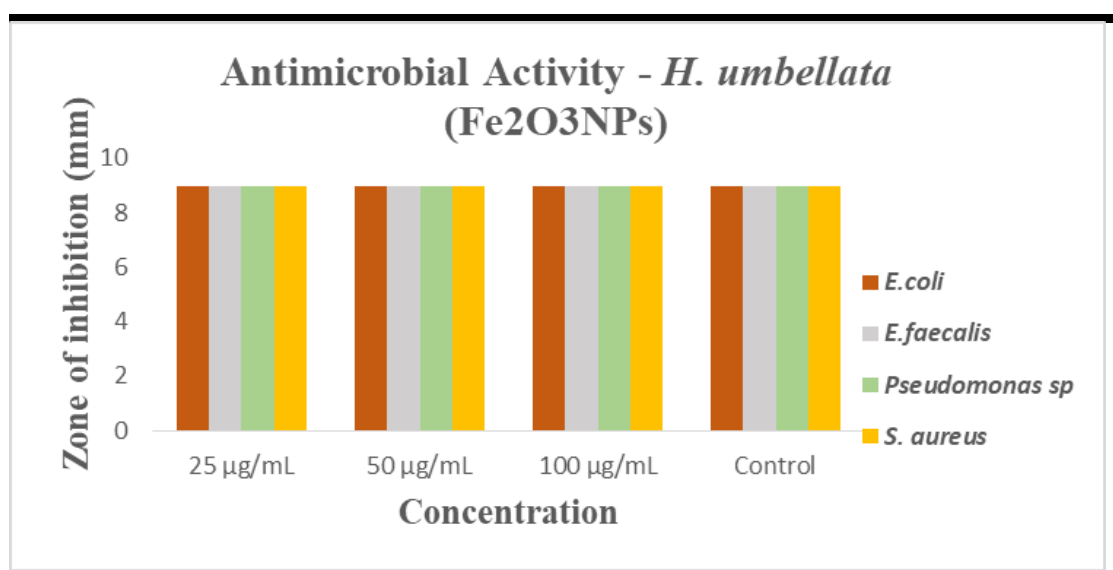
A

B

C

D

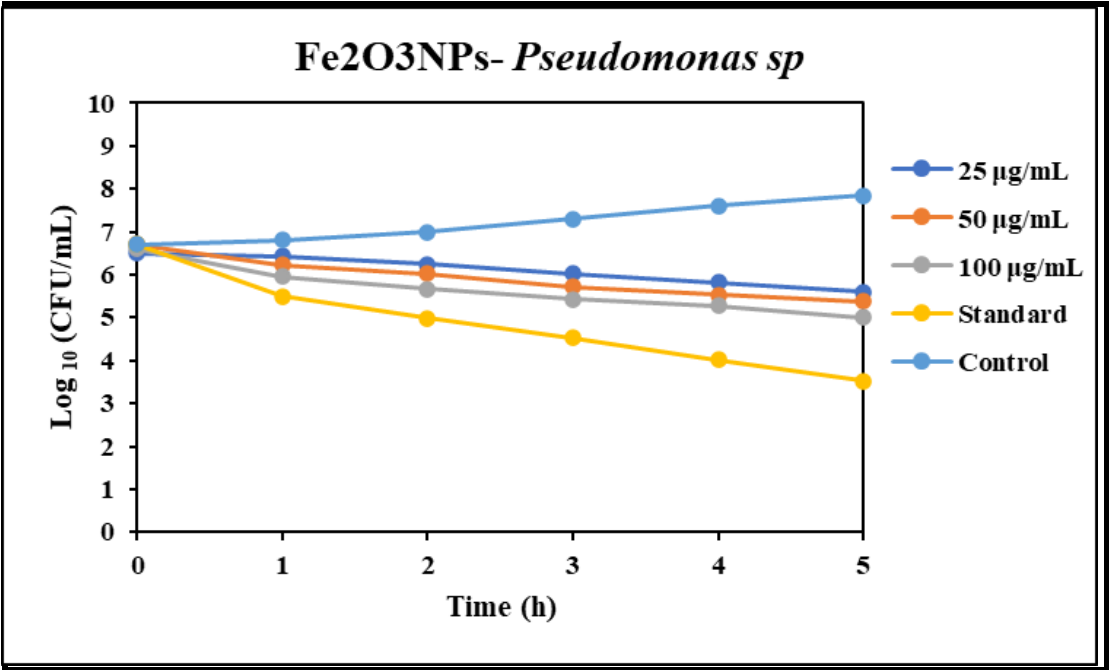
Graph 1: Antimicrobial activity of iron oxide nanoparticles against wound pathogens.



3.3. Time Kill Curve Analysis:

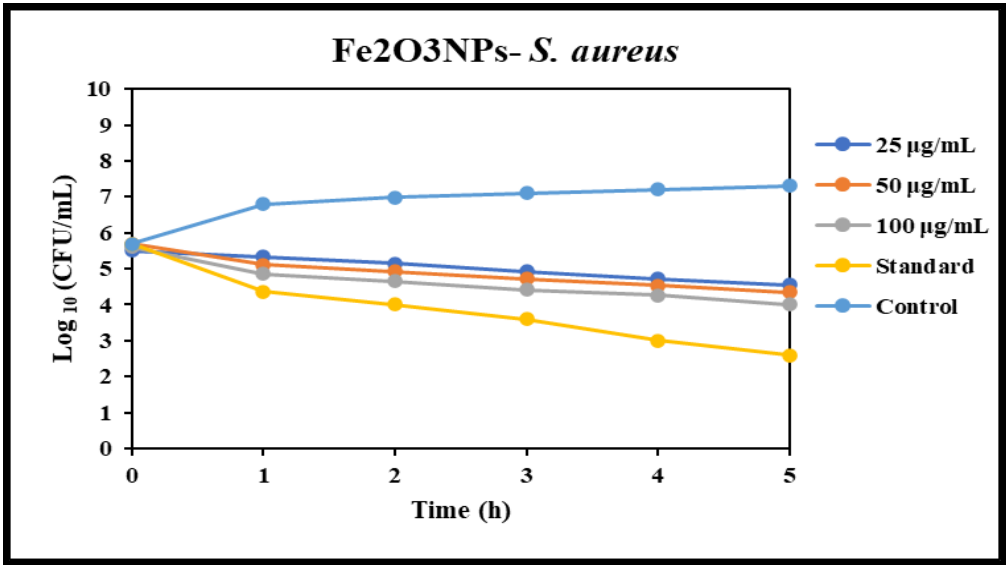
The time-kill kinetic assay revealed that the iron oxide nanoparticles using *H. umbellata* exhibited the antimicrobial efficacy as compared with the standard (amoxycillin) and the control (only microbial broth culture). At the iron oxide nanoparticles have various concentrations (25, 50, and 100 µg/mL) and similarly reduce the bactericidal growth. Graph 1a, 1b, 1c, and 1d represent the bacterial growth reduction as denoted the iron oxide nanoparticles. Especially at the 100 µg/mL concentration, has reduced the bacterial growth and the bacteriostatic. The *H. umbellata* mediated iron oxide evaluation of time kill curve assay against wound pathogens, as various concentrations.

Graph 2a: Time kill curve analysis of iron oxide nanoparticles against *Pseudomonas* sp.



The time kill curve analysis of *Pseudomonas* sp depicts that iron oxide nanoparticles against this bacteria are bactericidal. It not only prevents the further growth of bacteria but also kills the already existing bacteria. This proves that the iron oxide nanoparticles synthesized from *Hydrocotyle umbellata* is very much effective against the wound pathogen, *Pseudomonas* sp.

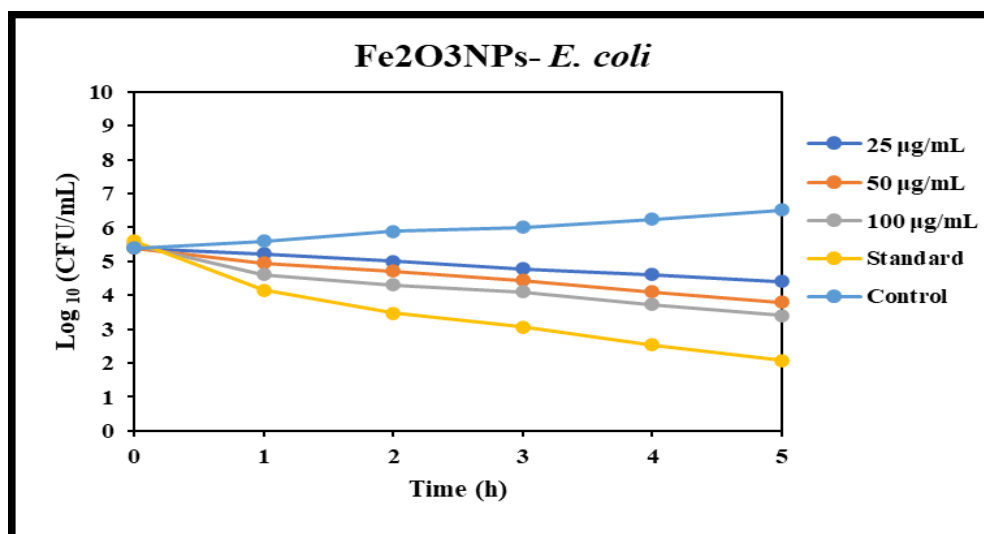
Graph 2b: Time kill curve analysis of iron oxide nanoparticles against *S. aureus*.



The time-kill kinetic analysis of *Staphylococcus aureus* depicts that iron oxide nanoparticles
Nanotechnology Perceptions Vol. 20 No. S7 (2024)

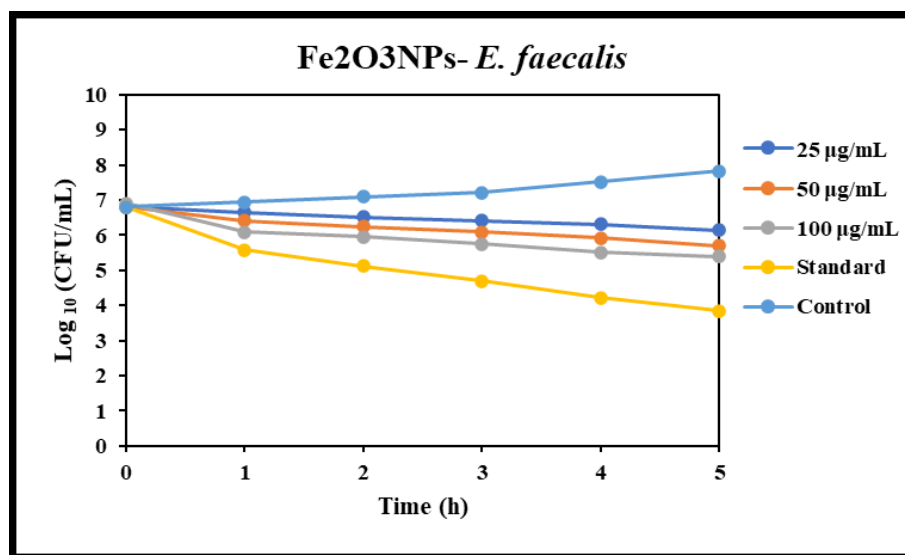
against this bacteria are bactericidal. It not only prevents the further growth of bacteria but also kills the already existing bacteria. This proves that the iron oxide nanoparticles synthesized from *Hydrocotyle umbellata* is very much effective against the wound pathogen, *Staphylococcus aureus*.

Graph 2c: Time kill curve analysis of iron oxide nanoparticles against *E. coli*.



The time-kill kinetic analysis of *E. coli* depicts that iron oxide nanoparticles against this bacteria are bactericidal. It not only prevents the further growth of bacteria but also kills the already existing bacteria. This proves that the iron oxide nanoparticles synthesized from *Hydrocotyle umbellata* is very much effective against the wound pathogen, *Escherichia coli*.

Graph 2d: Time kill curve analysis of iron oxide nanoparticles against *E. faecalis*.



The time kill curve analysis of *Enterococcus faecalis* depicts that iron oxide nanoparticles against this bacteria are bactericidal. It not only prevents the further growth of bacteria but also kills the already existing bacteria. This proves that the iron oxide nanoparticles synthesized from *Hydrocotyle umbellata* is very much effective against the wound pathogen, *Enterococcus faecalis*

4. Discussion

The present research work, the green synthesized iron oxide nanoparticles confirmed the initial stage as color changes of the reaction mixture. The iron oxide nanoparticles have a yellowish brown color to the dark shade of the nanoparticles solution. In a previous study, the black seed cumin mediated iron oxide nanoparticles from brown color to a darker shade of brown (Shanmugam et al., 2024). In other hands, *Lagenaria siceraria* mediated iron oxide nanoparticles show the colour change Yellowish brown to brownish black (Kanagasubbulakshmi et al., 2017). The antimicrobial activity of the iron oxide nanoparticles showed the inhibitory zone against wound pathogens. Similarly, Henna and Gardenia Leaves Extract mediated iron oxide nanoparticles The ZOI of iron nanoparticles from *Lawsonia inermis* and *Gardenia jasminoides* was effective against *Escherichia coli*, *Salmonella enterica*, and *Proteus mirabilis*. The leaf extract measurements were 14 mm and 15 mm, 9 mm and 12 mm, 11 mm and 13 mm, in that frequency (Naseem et al., 2015).

Previous studies have proved that Iron oxide nanoparticles do have an antimicrobial activity against *E.coli*. The iron oxide nanoparticles synthesized using *Lactobacillus fermentum* was proved to have an inhibition activity against pathogenic activity hence proving that it is effective. The iron oxide nanoparticles synthesized from *Hydrocotyle umbellata* showed maximum effect in killing the strain of all four bacterias that were used (Shehabeldine et al., 2023).

The time-kill kinetic analysis showed a reduction of 56% on the *E. coli* strains and 55% when used on the *Klebsiella oxytoca* (Qureshi et al., 2022). The time kill curve analysis performed in this study proved to terminate all the bacteria and above all, it also prevented the further growth of this bacteria. Iron Oxide nanoparticles have proved to kill the bacteria's to a maximum amount and stop the further growth of bacteria. With studies showing a minimal antimicrobial effect, the natural synthesis of iron oxide nanoparticles have proved to be a major advantage and hence have been proved to play an essential role in targeted drug therapy.

5. Conclusion

The biological synthesis mediated iron oxide nanoparticles using the plant extract from *H. umbellata* exhibited antimicrobial activity and time kill curve assay against wound pathogens (*E. coli*, *E. faecalis*, *S. aureus*, and *Pseudomonas sp.*). This method of synthesized nanoparticles has both eco-friendly, cost efficacy, and biocompatibility. This can further be modified and utilized in targeted drug therapy against wound pathogens.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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