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Antimicrobial Activity of Hydrocotyle umbellata L. Assisted Iron Oxide Nanoparticles against Urinary Tract Infection Pathogens

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Iron Oxide Nanoparticles have shown and proven promising effects in the field of biomedicals because of their special physico-chemical features. Hydrocotyle umbellata L, has the ability to metabolise both organic and inorganic substances proving to be beneficial. The main purpose of green chemistry is to develop products in a way that prevents the environment from getting polluted and to reduce the amount of waste products. The purpose of this study is to analyze the antimicrobial activity of Hydrocotyle umbellata mediated iron oxide nanoparticles. The time kill curve analysis was done to check the bactericidal properties of the nanoparticle against Escherichia coli, Enterococcus faecalis, Staphylococcus aureus and Streptococcus mutans. The plant solution was first prepared using the plant extract. Followed by the preparation of the nanoparticle solution. Antimicrobial activity and Time kill curve analysis was done to check the properties of the iron oxide nanoparticle which was synthesized by the plant Hydrocotyle umbellata against UTI pathogens. 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 UTI pathogens proving to be of various advantages.

Keywords: Antimicrobial property, Green synthesized, iron oxide nanoparticles, UTI pathogens.

1. Introduction

Nanotechnology is rapidly developing new therapeutic and diagnostic concepts in all fields of medicine. Mainly nanoparticles are used in Magnetic Drug Targeting, Magnetic Resonance Imaging for clinical diagnosis, gene therapy, cell tracking and so on Liu et al., (2007). 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 antimicrobial agent and so on Mubaraki et al., (2023). Drug delivery is one of the most important applications of nanoparticles. Antimicrobial resistance has become a major concern all over the world due to the misuse of antibiotics. Overuse of these antibiotics lead to the emergence of microbes that are resistant to antibiotics Kamble (2022). Recent studies have proved that silver, platinum, gold, and copper nanoparticles as well as metal oxides such as silver oxide, titanium oxide, magnesium oxide, zinc oxide, calcium oxide and iron oxide have the ability to kill bacteria that are resistant to multiple antibiotics. As the active compound of nanoparticles produces various therapeutic effects, these have been proved to be beneficial in many ways. They increase drug solubility, they promote rug penetration across biological barriers and they also manage drug release Sazib et al., (2023).

Iron oxide nanoparticles are one of the few particles that have a special morphology that enables it to be used as a microbial agent in treating and preventing infectious disease in both humans and animals Al-Rawi et al., (2023). The unique properties of iron oxide nanoparticles are its superparamagnetic properties, high surface to volume ratio, greater surface area and easy separation methodology that are used for magnetic refrigeration for MRI, cell sorting targeted drug therapy and hyperthermic cancer treatment Devi et al., (2019). Iron oxides present in nature include anti-ferromagnetic hematite, paramagnetic maghemite, and supermagnetic magnetite. Iron oxide nanoparticles show a minimum amount of toxicity, they are biocompatible, cost-effective and they have a higher surface to volume ratio than other nanoparticles kumar et al., (2021). Iron Oxide Nanoparticles are mostly synthesized by using organic solvents combined with thermal-decomposition of organo-metallic compounds Ajinkya et al., (2020). The properties of Iron Oxide Nanoparticles were examined under various methods. The optical, structural and morphological properties were examined using the FT-IR, SEM and XRD analysis. The XRD technique was used to understand the material and molecular structure of the iron oxide nanoparticle Saqib et al., (2019).

Hydrocotyle Umbellata L is an aquatic plant and a perennial herb from the family of Araliaceae that is mostly found in the Americas. This water plant is popularly known as Acaricoba and it is extremely useful for its anti-inflammatory uses Ali et al., (2022). It has a wide range of uses in folk medicine. This plant helps in many phytoremediation applications such as rhizofiltration, vegetative caps, hydraulic barriers, and Constructed wetlands Hamdy et al., (2018). The active compounds of Acaricoba are flavonoids, tannins, lignan, saponins and the essential oils that are present in the leaves and subterraneous parts. It was proved that lignan was the potential active compound that was majorly responsible for antinociceptive, and anxiolytic-like effects de Oliveira et al., (2019).

The term UTI is used to refer to a wide range of medical conditions, from a life-threatening kidney infection to the presence of bacteria in urine without any symptoms. Some of the uropathogens are P.aeruginosa, K.pneumoniae, Enterococcus faecalis and P.mirabilis. The continuous use of antibiotics has led to the pathogens forming a resistance towards the antibiotics Wagenlehner et al., (2020). Urinary tract infection ranges from a simple self-limiting disease to severe sepsis. Above the age of 70 both sexes acquire UTIs, with a female to male ratio of 2:1. Hence, proving that females are more prone to UTIs LARCOMBE (2012). Urinary tract infections can be classified as Complicated UTIs, Uncomplicated UTIs and Catheter Associated UTIs. Complicated UTIs are found in individuals who have anatomical and functional abnormalities. Whereas, on the other hand, uncomplicated UTIs are found in individuals who do not have any functional and anatomical abnormalities. The most common Uro-pathogen is Escherichia coli, a gram-negative bacteria. This bacterium targets the uroepithelial cells of the bladder. In response the bladder has an ability to expel these bacteria with the help of the body's immune system. Kline et al., (2017). The most common bacteria that forms Urinary Tract Infections are E. coli and S. aureus. 80 – 90% of the UTIs caused by E. coli cannot be treated due to the widespread E. coli fluoroquinolone medication resistance Zagaglia et al., (2022).

In this study, iron oxide nanoparticles were prepared from the plant Hydrocotyle umbellata L. (Dollarweed), which is in turn used to treat urinary tract infections. The main objective of this research is to study the antimicrobial effect of iron oxide nanoparticles against urinary tract infections 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 no.1 Filter paper. The filtrate extracted was then stored 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 followed by the Rajeshkumar et al., (2024)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 121ºC. 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 Escherichia coli, Escherichia faecalis, Streptococcus mutans and Staphylococcus aureus 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 µg/mL of IONPs were added. H. umbellata, was used as a Control. 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 kinetic Analysis:

The time-kill kinetic assay was followed by Tharani et al., (2023). 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 resultant mixture was then distributed evenly in an ELISA plate that had 96 wells.

3. Results:

3.1 Preparation of iron oxide nanoparticles

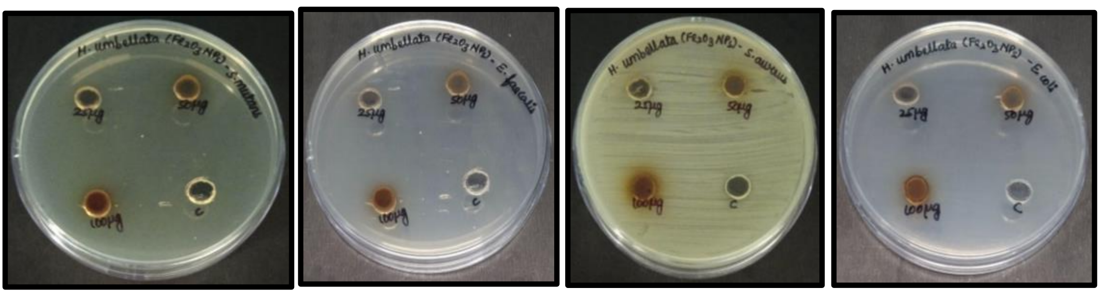
The visual observation of the green synthesis of iron oxide nanoparticles using H. umbellata was the first stage to observe the color changes of the solution (Figure 1). The reaction mixture solution contains plant extract and the nanoparticles precursor solution. The plant extract was used to the reducing agent of the metal oxide nanoparticles; iron chloride was reacted with the phytochemical substance of plant extract to convert into iron (ion). The iron oxide nanoparticles displayed the initial color in brown to darkish brown. It should confirm the synthesized nanoparticles.

A B C D

Figure 1: Preparation of iron oxide nanoparticles using H. umbellata, A) H. umbellata solution, B) H. umbellata boiling using Heating mantle, C) iron chloride solution was mixed with H. umbellata extract, and D) H. umbellata mediated iron oxide nanoparticles.

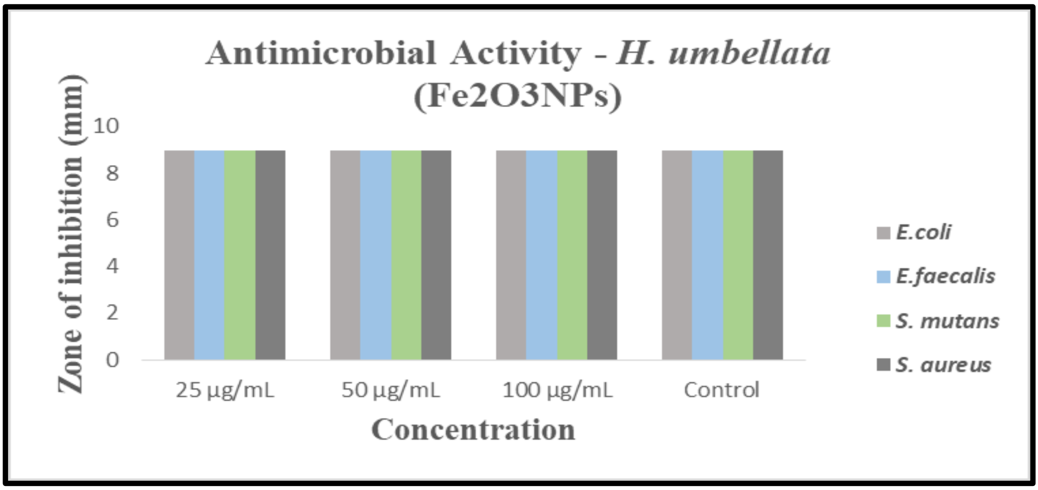
3.2 Antimicrobial activity

The antimicrobial activity of iron oxide nanoparticles using H. umbellata, was evaluated against UTI pathogens (E. coli, E. faecalis, S. mutans and S. aureus) was shown in Figure 2, respectively Graph 1, represented the antimicrobial efficacy of the iron oxide nanoparticles was revealed the concentration based on the inhibitory zone in the UTI pathogens. The various concentrations of the nanoparticles solution (25, 50, 100µg/mL) was compared with the control (Aqueous extract of H. umbellata). The green synthesized iron oxide nanoparticles revealed the minimum antimicrobial activity in the agar well plate technique against UTI pathogens.



A) S. mutans B) E. faecalis C) S. aureus D) E. coli

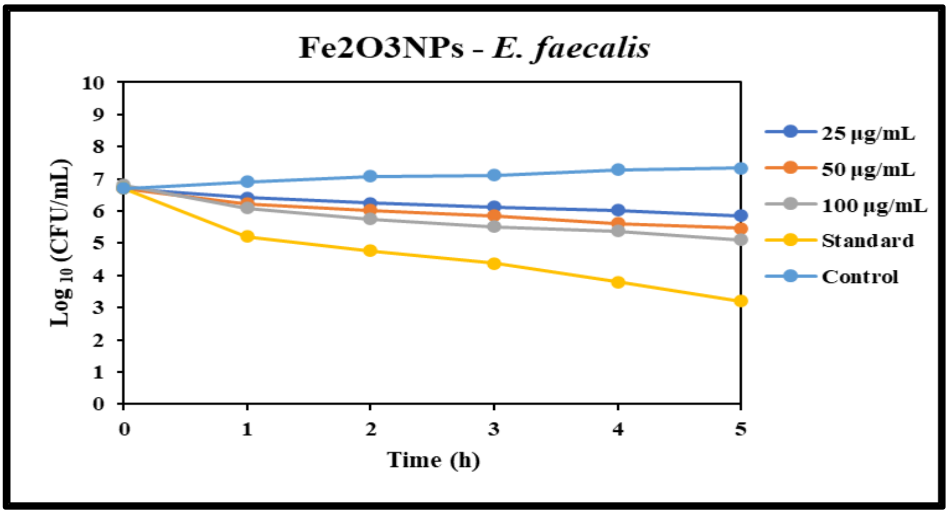
Figure 2: This agar well plate image represents the antimicrobial activity against UTI pathogens.



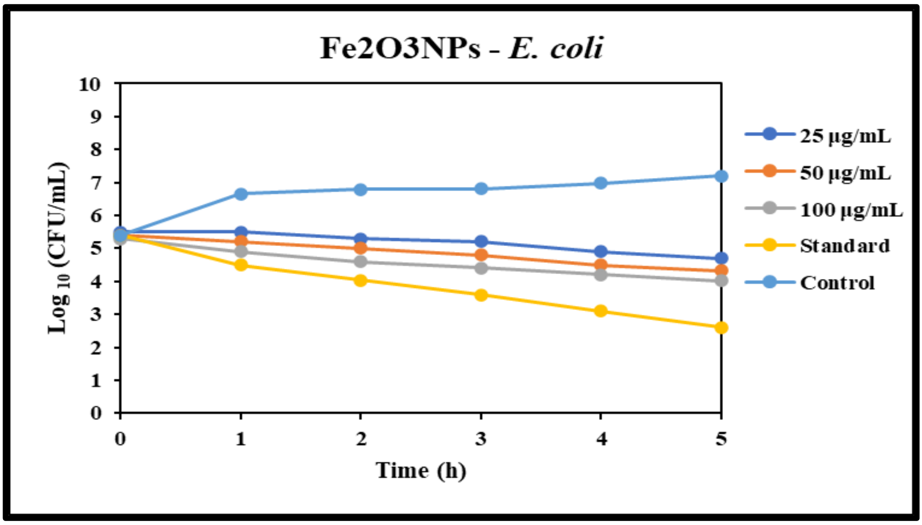
Graph 1: Antimicrobial Activity of Iron Oxide Nanoparticles

3.3 Time Kill Curve Analysis:

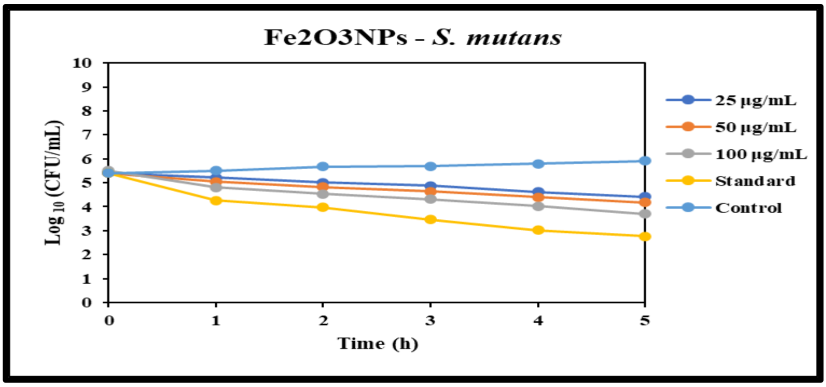
The time kill curve assay revealed the results and mentioned the reduction of viable microbial cells for the evaluation of UTI pathogens against iron oxide nanoparticles. In Graph (2a, 2b, 2c, & 2d) demonstrated the time kill curve assay, the iron oxide nanoparticles observed the bactericidal efficacy against UTI pathogens (E. coli, E. faecalis, S. mutans and S. aureus). On the S. mutans and S. aureus, the prepared nanoparticles determine the minimal bactericidal and bacteriostatic effects. The different concentration of nanoparticles solution (25, 50, 100µg/mL), the higher concentration of nanoparticles showed the higher growth reduction of E. coli and E. faecalis. The nanoparticles were compared with standard (amoxyrite) against UTI pathogens.



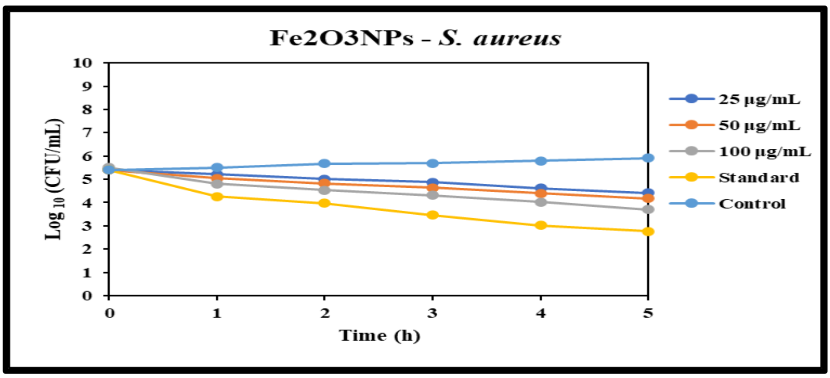
Graph 2a: Time Kill Curve Analysis of iron oxide nanoparticles against Enterococcus faecalis.



Graph 2b: Time Kill Curve Analysis of iron oxide nanoparticles against Escherichia coli



Graph 2c: Time Kill Curve Analysis of iron oxide nanoparticles against Streptococcus mutans



Graph 2d: Time Kill Curve Analysis of iron oxide nanoparticles against Staphylococcus aureus

4. Discussion

Aditi et al., (2023), described the green synthesized iron oxide nanoparticles and confirmed the visual observation as a first stage of the synthesized nanoparticles. In present research work, the iron oxide nanoparticles were changed from yellowish brown to darkish brown. In a previous study, black cumin seed mediated iron oxide nanoparticles showed a light brown to dark shade of brown color Shanmugam et al., (2024).

Previous studies have proved that Mansoa alliacea mediated Iron oxide nanoparticles have shown moderate antimicrobial activity when tested with 6 gram positive and 2 gram negative bacteria. In this study it has been proven that the nanoparticles of iron oxide have shown immensely effective results against the four bacterias that were tested Prasad et al., (2016). Another study showed the antibacterial assay against E. coli (5+ 0.09), S. dysentery, and S. aureus (8 + 0.11), proving that a very minimum amount of antimicrobial activity was found against the bacterias Ogunniran (2009). The plant synthesis iron oxide nanoparticle showed maximum effect against the different strains of bacterias used in this study. In a previous study, in metal oxide nanoparticles showed the efficacy of time kill curve assay, the time-kill kinetic analysis showed a reduction of 56% on the E. coli strains and 55% when used on the Klebsiella oxytoca Shehabeldine et al., (2023). 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 bacterium. At a concentration of 400 µg/mL, the inhibition percentages observed for Escherichia coli, Salmonella typhimurium and Klebsiella pneumoniae were respectively 81, 80 and 58% Paulo et al., (2010). Iron Oxide nanoparticles have proved to kill the bacterias 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

This study concludes that Hydrocotyle umbellata L mediated iron oxide nanoparticles is an effective agent to eradicate wound pathogens completely. Giving a maximum in the termination of these bacterias have been successful using the naturally produced iron oxide nanoparticles from the plant Hydrocotyle umbellata. These nanoparticles have shown a greater effect than other studies proving to be useful in targeted drug therapy.

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