

Green Synthesis Characterization and Biological Assessment of Nickle based Microparticles using *Justicia Adathoda L.* Leaf Extract

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Abstract

Nickel-based oxide/hydroxide microparticles (NiO/Ni(OH)_2 MPs) were synthesized via a green way utilizing *Justicia adathoda L.* leaf extract, serving as novel reducing and stabilizing agent. Plants phytochemicals such as alkaloids, flavonoids, and phenolics facilitated the eco-friendly formations of NiO/Ni(OH)_2 MPs without toxic chemicals. Structure analysis through X-ray diffraction (XRD) confirmed the crystalline structure, while FTIR confirmed the involvement of functional groups in reduction and stabilizations. Morphological characterization by Scanning Electron Microscopy (SEM) revealed a granular dense structure microparticle of size about $1\mu\text{m}$. Biological screening showed notable antibacterial and antifungal activities. NiO/Ni(OH)_2 MPs show 60.24% inhibition against *Staphylococcus aureus* and 70.24% inhibition against *Pseudomonas aeruginosa*, while antifungal activity reached 95% against *Aspergillus fumigatus*. Compared to standard pharmaceutical agents such as Ofloxacin and Miconazole, the NiO/Ni(OH)_2 MPs synthesized using *Justicia adathoda L.* leaf extract revealed potential antibacterial and antifungal activities. These results underline the value of *Justicia adathoda* as an eco-friendly and efficient biological resource for making functional NiO/Ni(OH)_2 MPs. The synthesized microparticles show strong applications in biomedical fields, including therapeutic formulations, antimicrobial coatings, and presenting a sustainable unconventional to chemically synthesized micromaterials.

Keywords: Green Synthesis, Ni Based Microparticle, SEM, FTIR, XRD, Antimicrobial Activity.

1. Introduction

Green synthesis has become a major topic of interest in the field of Micro-nanotechnology [1]. Developments in micro-nanotechnology have led to the production of innovative materials, particularly ecofriendly micro-based-nanostructure synthetic procedures used to synthesize metal and metal oxide micromaterials of any shape and size [2]. Most physical, chemical, hybrid methods tend to be costly and detrimental due to the presence of harmful substances like solvents, reducing agents, and precursors [3]. In contrast, employing green techniques for the preparation of microparticles (MPs) is new economical, ecofriendly, and simpler to utensils. Consequently, such technique is increasingly utilized

in the production of metallic based microparticles. A diverse span of natural deposit, inclusive of microorganisms, biomolecules, and plant extracts, was explored for green MP synthesis [4]. Although numerous studies have been conducted on the green synthesis of MPs utilizing biomolecules and microbes, plant extracts are often favored because they are cost-effective, efficient, and do not require the complex processes involved in isolating and maintaining microbial cultures [5]. Various plant species, their parts, and isolated compounds are effectively used to synthesize green MPs. Furthermore, the preparation of micromaterials can be made more efficiently by incorporating industrial and agricultural waste, in computation to being ecofriendly [6].

Phenolic chemicals can operate as capping agents in microparticles manufacturing, whereas biological molecules offer unique uses in micro-nanotechnology, making metal microparticles durable and trustworthy [7]. Recent studies have proved the effectiveness of using micromaterials in different aspects of life, including environmental control, energy storage, solar cell, biomarking, bio probes, tissue engineering, cancer therapy, cancer diagnosis, and drug delivery [8]. The method of green synthesis consists in obtaining particles using safe materials as reducing agents and stabilizers, for example, products of biological origin. For example, the method can be carried out using extracts of plants or their individual parts, as well as microorganism (bacteria, yeast, fungi) [9]. A plant contains various biochemicals and metabolites that can be used for the green synthesis of microparticles as they possess reducing and stabilizing properties. Green synthesis technologies are environmentally friendly, cost effective stable and nontoxic than traditional biological, physical, and chemical approaches [10]. Biosynthesized metal and metal oxide MPs have rising applications in the medical sector including immunotherapy, dentistry, wound healing, diagnosis, regenerative medicines, bio toxicology and bio-sensing platform and its antiviral, fungicidal and bactericidal potentials [11]. Microparticles can be variable in dimension, shape, size and architectures [12]. In the environmental field, the green synthesis of MPs has been used to control environmental pollution, such as with the degradation of organic dyes and chlorinated organic pollutants, heavy metal removal, in addition to the treatment of wastewater [13]. *Justicia adathoda L.* also known as Malabar nut is a well-known and important medicinally important plant of family Acanthaceae growing in different Asian countries including Pakistan, Sirilanka, India and Nepal [14]. *Justicia adathoda L.* has diverse applications in traditionally used Ayurvedic and Unani medicines. The local populace uses the plants' leaves to treat coughs, colds, and asthma. *Justicia adathoda L.* plant has several key properties, including antibacterial, antioxidant, antiulcer, antidiabetic, anti-allergic, anti-genotoxic, and anti-phlogiston. *Adathoda vasica* leaves contain phytochemicals including flavonoids, saponins, tannins, alkaloids and phenolic compounds. *Justicia adathoda L.* leaf extract contains the two most significant quinazoline alkaloids, vasicine and vasicone [15]. Phytochemicals are bioactive compounds naturally found in plants, and their composition varies greatly among different plant species and even within different parts of the same plant. The most common classes of phytochemicals include polyphenols (like flavonoids and tannins), terpenoids, alkaloids, carotenoids, and saponins. These compounds are involved in plant defense mechanisms and can also offer various health benefits to humans. Antibacterial agents are utilized to inhibit the proliferation of harmful bacteria. They help prevent illness and eradicate microorganisms [16]. Numerous antibiotics are sourced from plants and various chemicals to combat diseases. Bacteria are the pathogens responsible for poisoning. For this assay, we used five different strains of pathogens [17]. A disc diffusion method was employed to assess bacterial activity. Bacteria are classified into two categories: Gram-negative and Gram-positive. *Staphylococcus aureus* (*S. aureus*) is typically recognized as a Gram-positive bacterium and is frequently used in antibacterial studies due to its

significant potential to cause infections in humans. *Escherichia coli* (*E. coli*), a Gram-negative bacterium, is established in contaminated water and along with as a pathogenic microbe that leads to human illness, as well as serving as an indicator of water quality. The antifungal test aims to eliminate microorganisms, especially fungi. Fungi pose significant risks and can lead to various illnesses. We utilize antifungal tests to identify infections caused by fungi. These organisms can damage fruits, vegetables, and plants [18]. Numerous plants are recognized for their antifungal properties. Unsanitary food and environments weaken the immune system, facilitating the spread of fungal infections. Antifungal medications are necessary to treat such infections [19]. Metallic nickel based microparticles are those that are used as the primary raw material without combining them with other metals, compounds or elements. The term nickel microparticle used in this review also refers to metallic nickel microparticle [20]. The successful production of Ni based MPs employing various plant extracts, as well as their enhanced biological potential, call for additional research into other medicinally essential plants for future enhancement [21]. Ni based MPs have gained much attention due to their unique magnetic, chemical, and physical properties as well as their potential applications in various technological fields such as catalysis, battery manufacture, incorporation in textile, and optical switches [22-25]. The current study aimed to synthesis Ni based MPs using *Justicia adathoda L.* leaf extract. The produced (NiO/Ni(OH))₂ MPs were examined by using modern analytical techniques, including X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM). Within inclusion, the antibacterial and antifungal potentials of the prepared Nickel based microparticles were measured.

2. Methodology

2.1. Chemicals and other Materials

The chemicals used were Distilled water and Nickel chloride. These chemicals were of analytical grade. Compounds with great clarity were purchased from Sigma-Aldrich and used without any further purification. Double distilled water was used throughout all the involved experiments as a solvent. The raw materials included NiCl₂·6H₂O (98%, Sigma-Aldrich), salvia plant extract that was collected from the natural habitats and methylene blue (MB; 98%, Sigma-Aldrich) dye.

2.2. Synthesis of the Plant Extract

The leaves of *Justicia adathoda L.* were harvested in Khyber Pakhtunkhwa's Tehsil Timergara District Dir Lower. Following collection, the plant was identified and authenticated by the Flora of Pakistan. The collected plant leaves have been clean with filter water, shadow dried and grind within fine talc by utilizing pistil and motor. Approximately ten grams of powder were taken and 200 mL of distilled were added in a glass container and placed for 48 hours. The sample was then filtered through Whatman filter paper and leaf extract was obtained in a sterilized conical flask [26].

2.3. Green Synthesis of Ni based MPs

Ni based microparticles (NiMPs) were synthesized following

the standard plant-mediated green reduction method [27]. The extract from *Justicia adathoda L.* was utilized as a bio reductant in the production of nickel based microparticles. To form a NiCl solution (2M Nickel chloride solution), 0.95g of nickel chloride was dissolved in 150 ml of distilled water. Initially, the Nickel chloride solution was stirred at room temperature for one minute before incorporating the *Justicia adathoda L.* leaf extract. The mixture was then agitated for one hour. Following the addition of the leaf extract, the solution transitioned from colorless to a brownish-black hue, indicating the formation of Ni based MPs. Subsequently, the samples underwent centrifugation for 40 min at 14,000 rpm at 60°C. The resulting remnant from the centrifugation be desiccated at standard temperature 60°C prior to characterization and biological assessment.

2.4. Characterization of the Synthesized Ni based MPs

Characterization of microparticles stands as the most important factor in comprehending and controlling the synthesis and application of microparticles. This vital factor can be achieved by utilizing a variety of procedures including X-ray diffraction (XRD, D8-Advance Bruker) [28]. NiO/Ni(OH)₂ MPs was characterized by using X-ray powder diffraction using X-ray diffractometer [Model: Pert Pro (PAN Analytical)]. The XRD spectrum was taken over 2θ range of 5°-80°. Fourier transform infrared (FT-IR) analysis, using IR Prestige-21 Shimadzu 400-4000 cm⁻¹. Fourier transform infrared (FT-IR) analysis, using IR Prestige-21 Shimadzu, was implemented to characterize the functional groups on the surface of the Ni MP. The scanned spectra were in the range 400 - 4000cm⁻¹ at a resolution of 4cm⁻¹ [29]. UV-Vis analysis was performed using perkin Elmer UV Win lab 7.3.0.340 /220126 to study the optical properties as well as band gap of microparticles [30], field electron scanning electron microscopy (FESEM)/energy-dispersive X-ray spectroscopy (EDX, Tescan Brno-Mira3 Lmu). The purity of Ni MPs was confirmed by the means of EDX, which is capable of accurately measuring Ni along with displaying the Ni peaks without any impurities [31]. Elemental analysis was carried out by using EDX analysis to analyze the % amount of carbon, nickel and oxygen [32]. SEM was carried out to analyze the surface morphology of Ni based microparticles. The morphology of the prepared sample of Ni-MP was examined using a Scanning Electron Microscope SEM analysis (JEOL jsm-6480 LV) [33].

2.5. Antimicrobial Assay

The antimicrobial activity of the studied MPs was evaluated against 5 bacterial strains (*Escherichia coli ATCC 25922*, *B. subtilis*, *Staphylococcus aureus*, *P. auriginosa*, and *Salmonella Typhi*), belonging to Gram-positive and Gram-negative bacteria, and 7 yeast strains, *Trichophyton rubrum*, *Candida albicans*, *Aspergillus niger*, *Microsporium canis*, *Fusarium lini*, *Candida glabrata*, *Aspergillus fumigatus*. Were isolated from leaves extract of plant

products [34]. Before use, bacterial strains were revived with subcultures in tryptone soya agar (TSA, Biokar, Beauvais, France) at 37°C for 24 h, while yeast was revived with subcultures in Sabouraud dextrose agar with chloramphenicol (Biokar, Beauvais, France) at 25°C for 48 h. Then, a microbial suspension equivalent to 0.5 McFarland (108 cfu/mL) was prepared in sterile physiological water (0.9% NiCL) and inoculated by swabbing on plates containing Mueller–Hinton agar (Biokar, Beauvais, France) [34]. On the surface of each plate, 10 µL of each MP solution at a concentration of 10 µg/mL was dropped on 6 mm diameter filter paper discs (Whatman Grade 4 Qualitative Filter Papers, Merck, Germany). Sterile distilled water (10 µL) was used as a negative control, while gentamicin (30 µg) was used as a positive control for bacteria and 10 µL of Canaflucan (fluconazole) Win® capsule 150 mg with a concentration of 0.6 µg/mL was used as a positive control for the yeast strain. The used plates were incubated at 37 °C for 18–24 h for bacteria and 25°C for 44–48°C for yeast. After incubation, the inhibition diameter was measured in millimeters (disk included). The antimicrobial activity was classified into three levels based on the produced inhibitory diameter: weak (inhibition zone ≤ 12.0 mm), intermediate/moderate (12.1 mm ≤ inhibition zone ≤ 20.0 mm), and strong (inhibition zone ≥ 20.1 mm).

2.6. Statistical Analysis

Origin software and Microsoft Excel were used for the generation of figures and statistical analysis. In this study, the experiments were carried out in triplicate, and the difference between groups was determined by using the student t-test (P < 0.05).

3. Results and Discussion

3.1. NiO/Ni(OH)₂ MPs Preparation and its Corroboration by SEM Spectroscopy

The SEM image of the resulting microparticles, as shown in the figure 1, displayed a crystalline, and slightly uneven shape, with a diameter 1 µm. Scanning electron microscopy became employed to scrutinize the physical dimensions with morphological characteristics of the biosynthesized Ni based microparticles [35]. The image shows that the microparticles have a highly crystalline as well as highly agglomerate and appear as cluster of microparticles with a size range of 1µm. The agglomeration of microparticles may be due to the exposure of microparticles to large volume of heat during calcination step or because of high surface tension of the ultrafine microparticles and high surface energy [36]. The SEM result is compatible with the result of who study the morphology of Ni based microparticles which are prepared by the sol-gel method using two different precursors nickel chloride hexahydrate and nickel nitrate hexahydrate. Our results are accurately similar to other studies in literature where it was revealed that Ni based MPs have important microparticles in other previous SEM analysis [37].

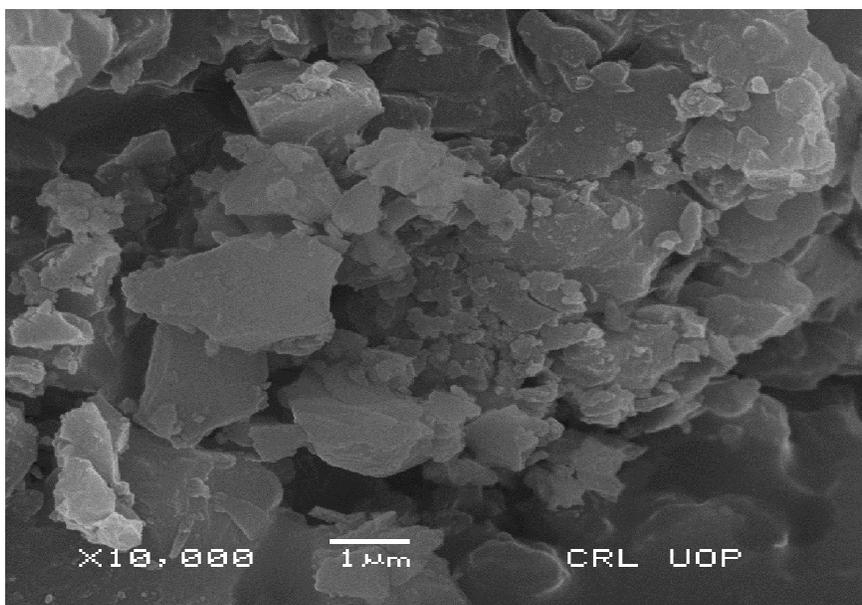


Figure 1: Image of Synthesized NiO/Ni(OH)₂ Microparticles

XRD Analysis

The XRD pattern was employed to characterize the structural features, crystalline information, and phase purity of the precursor NiCl₂·6H₂O and the synthesized Ni based MPs. The results match well with the standard cards of pure NiCl₂·6H₂O JCPDS card (No. 025-10440) [38]. To determine the known phase of the Ni based MPs, an X-ray diffraction analysis was conducted. The XRD pattern of green produced Ni based microparticles has been displaced. The spectra revealed five distinct independent

peaks at $2\theta = 10^\circ, 24^\circ, 27^\circ, 29^\circ$ and 32° corresponding to intensity planes (1900), (1200), (1200), (1700) and (1000) respectively. The highest intensity for the peak observed was at 2θ value 29° . The diffraction peak data calm coordinate with the FCC fabric report from the Joint Committee on Powder Diffraction Standards (JCPDS). Sprinkling small unexplained peaks were also seen, which could be due to bioorganic piece from the *Justicia adathoda L.* leaf extract crystallizing on the nickel surface [39].

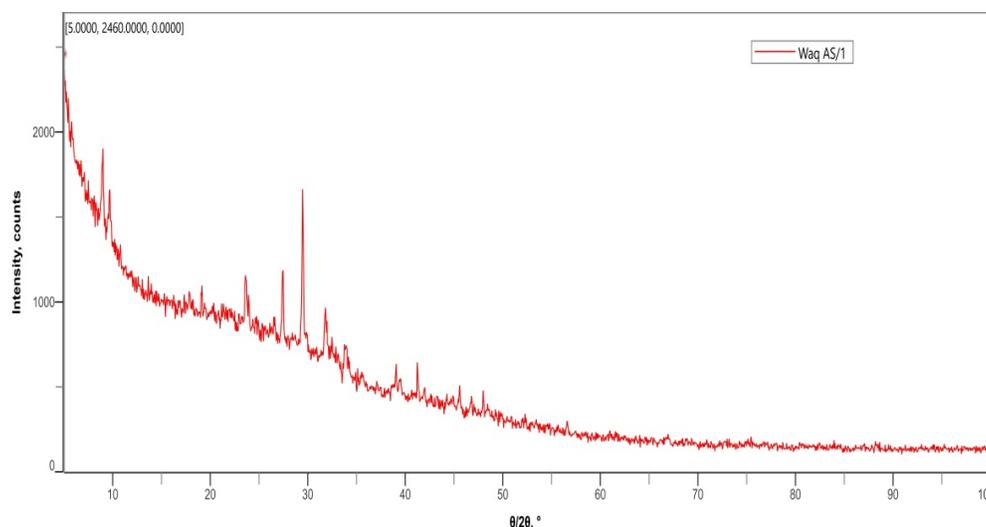


Figure 2: Analysis of NiO/Ni(OH)₂ Microparticles using XRD Spectroscopy

3.2. FTIR Analysis

FT-IR spectroscopy was used to study the functional groups present on the raw materials, NiCl, NiNO₃ and the product Ni based MPs.

The FT-IR of the raw materials and products are summarized in figure [31]. FTIR spectroscopy in the spectral range of 400 cm⁻¹ to 4000 cm⁻¹ was performed to know about the possible involvement of

different functional groups of plant extract in the reduction process of the Nickel based microparticles. The FTIR profile of NiMPs displayed vibration at 3309.75 cm⁻¹ for -OH groups, at 2943.78 and 2831.77 cm⁻¹ indicating the C-H stretching, while other multiple peaks at 1559.94, 1559.94, 1449.04, 1415.19, 1114.62 and 1021.53 cm⁻¹ were related to C=C and C-O stretching for aromatic ring and polyphenols. The peak at 616.36 cm⁻¹ displayed information about NiO in NiNO₃. The presence of Ni(OH)₂ and NiO in the FT-IR spectra indirectly indicates the synthesis of Ni based MPs. Our results are meticulously similar to other studies in literature where it was revealed that Ni based MPs have important microparticles and

related with other previous FTIR analysis [40]. The FTIR spectra displays functional groups exist in diverse locations. Figure shows displaced FTIR spectra of Ni based MPs and control leaf extract. *Justicia adathoda L.* leaf extract exhibits similar transmittance peaks at 3309 and 616.36 cm⁻¹. The extensive and secure bands at 3309 cm⁻¹ were caused by the leaf extract's bounded hydroxyl (-OH) or amine group (-NH). The C-H group can be examined by the peak screen at 2943cm⁻¹. The tensile vibration of the C-O, C=C group caused the peaks at 1559.94 cm⁻¹, 1449.04 cm⁻¹, 1415.19 cm⁻¹, 1114.62 cm⁻¹, 1021.53 cm⁻¹, and Ni based peak at 616.36 cm⁻¹ [41].

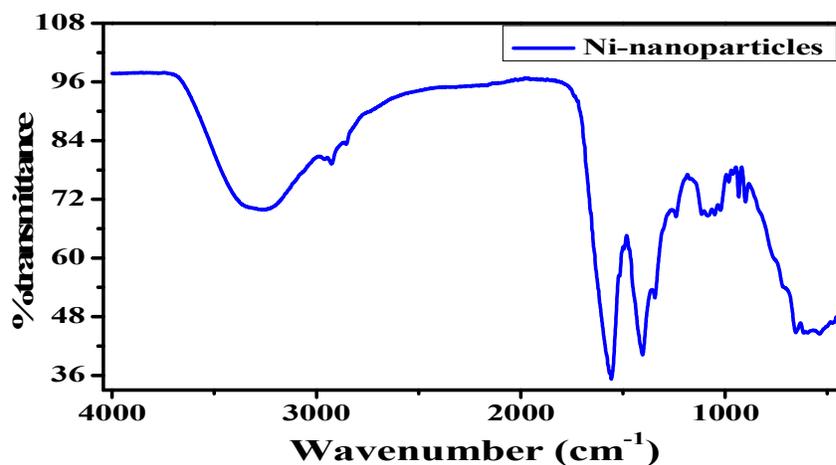


Figure 3: Analysis of NiO/Ni(OH)₂ Microparticles

3.3. Antibacterial Activities of *Justicia Adathoda L.* Leaf Extract and NiO/(OH)₂ MPs

We used the disc diffusion method to examine the antibacterial activity of Ni based microparticles biosynthesized from *Justicia adathoda L.* leaf extract. Six exertions in all were used to examine the biological activity. Three are gram negative (*Escherichia. Coli*), (*Salmonella. Typhi*) and (*P. Aeruginosa*) and three are gram positive (*S. Aureus*), (*M. Letus*) and (*B. Subtilis*). The finding was described in table. The consequences opposed five exertions were conflict with those prevail utilize streptomycin as the positive control. A region of hindering was seen after a day. Vernier callipers were used to measure the inhibition zone [42]. The concentration of Ni based MPs increased in tandem with the inhibition of bacterial growth. The sample's inhibition of

gram-negative bacteria is 90.2%. 84.25% inhibition against gram-positive *S. aureus* bacteria and *E. coli* [42]. A previous study found that biosynthetic Ni based MPs derived from *solanum nigrum* leaf extract showed a correlation between the particle's size and antibacterial activity, significantly more antibacterial action found in smaller particles. The precise mechanism by which Ni based MPs inhibit bacterial growth is unknown, based on studies in the literature [43]. Sondi and Sondi, though, confirmed that the antibacterial activity of Ni based MPs against gram-negative bacteria depended on the Ni based MPs concentration and was directly associated to the establishment of pits in bacterial cell walls. Our results are meticulously similar to other studies in literature where it was revealed that Ni based MPs have important antibacterial activities against the verified bacterial strains [44].

S.no	Bacterial species	Percentage inhibition of sample	Percentage inhibition of drug) ofloxacin)
1	<i>E. Coli</i>	55.3%	90.2%
2	<i>B. subtilis</i>	No inhibition	85.21%
3	<i>S. aureus</i>	60.24%	84.25%
4	<i>P. auriginosa</i>	70.24%	92.61%
5	<i>S. typhi</i>	No inhibition	88.31%

Table 1: Percent Inhibition of NiO/(OH)₂ Microparticles

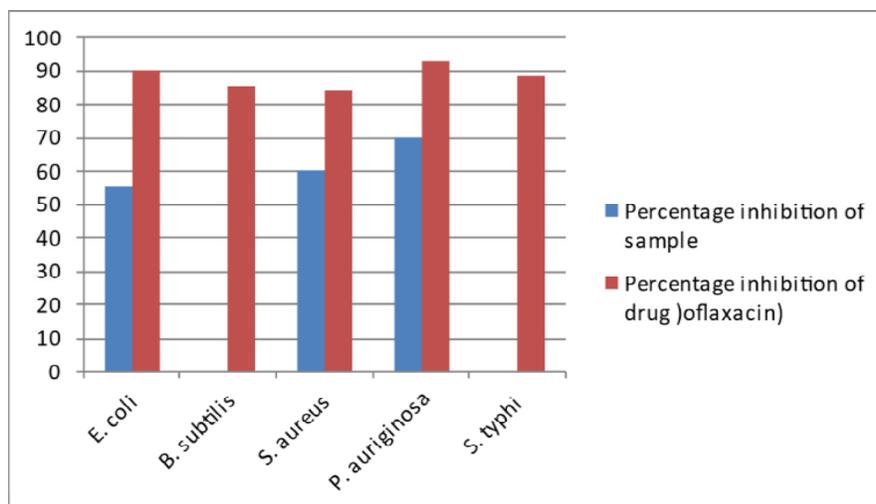


Figure 4: Anti-Bacterial Activity Of NiO/(OH)₂ Microparticle

3.4. Antifungal Activity

Justicia adathoda L. leaf extract was utilized in antifungal research to assess the antifungal efficiency of biologically formed Ni based MPs against numerous fungus strains. The experiment was run, Table comprises the outcomes. Seven fungi such as *Candida albicans*, *Microsporium canis*, *Trichophyton rubrum*, *Fusarium lini*, *Aspergillus fumigatus* and *Candida glabirata* were used. In this experiment, diverse fungal strains were exposed to the antifungal possessions of green produced Ni based microparticles from *Justicia adathoda L.* leaf extract. The results showed hopeful antifungal activities, *Aspergillus* was strongly inhibited by Ni based MPs, which resulted in 95%. This indicates that Ni based MPs have a great deal of potential for managing this pathogenic fungus. 85% inhibition was shown by *Trichophyton*

rubrum and 82% *Candida albicans*, while the remaining fungal species exhibits i-e *Candida glabritus*, *Aspergillus niger*, *Fusarium lini* 75% inhibition [45]. Overall, the result demonstrates the antifungal properties of Ni based microparticles mediated from *Justicia adathoda L.* leaf extract and exhibit significant potential in fighting *Candida albicans*, *Trichophyton rubrum*, *Aspergillus Niger*, *Fusarium lini*, *Microsporium canis*, *Aspergillus fumigatus*, and *Candida Glabirata*. Important repressive effects were also seen when associated to the other three strains. Miconazole and Amphotericin-B were utilized as encouraging control and distilled water was used as an adverse control [38]. A previous examination detected the antifungal features of Ni based microparticles made with extract from *Camellia sinensis*, or green tea [22].

S.no	Fungal species	Linear growth in sample in mm		Percentage Inhibition	Drug
		Sample	Control		
1.	<i>Trichophyton rubrum</i>	15mm	100mm	85%	Miconazole
2.	<i>Candida albicans</i>	18mm	100mm	82%	Miconazole
3.	<i>Aspergillus niger</i>	20mm	100mm	80%	Amphotericin-B
4.	<i>Microsporium canis</i>	25mm	100mm	75%	Miconazole
5.	<i>Fusarium lini</i>	40mm	100mm	60%	Miconazole
6.	<i>Candida glabirata</i>	35mm	100mm	65%	Miconazole
7.	<i>Aspergillus fumigatus</i>	5mm	100mm	95%	Miconazole

Table 2: Percent Inhibition of NiO/(OH)₂ MPs

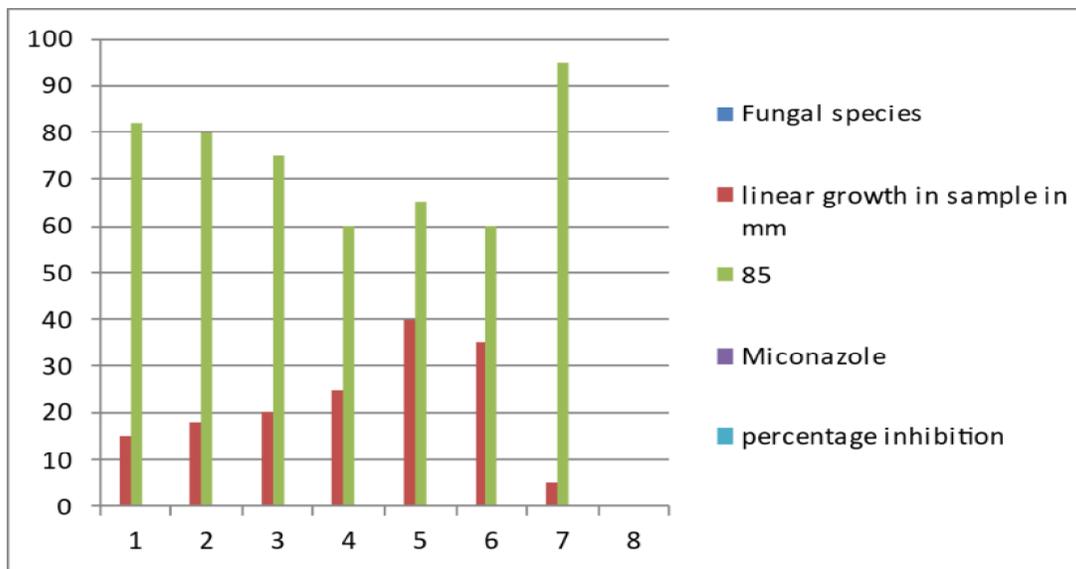


Figure 5: Antifungal Activity of NiO/Ni(OH)₂ MPs

4. Conclusion

Recent study has focused on the green production of MPs utilize plant extracts. Plant metabolites promote the environmentally benign production of microparticles. Plant-produced MPs have been used in numerous medicinal and industrial submissions. In the recent study, Ni based MPs were formed biologically with *Justicia adathoda L.* extract as a capping agent, which is a maintainable and biologically friendly method that is a cheap alternate to other traditional procedures. The Ni based MPs and leaf extract derived from *Justicia adathoda L.* were also tested for antibacterial and antifungal activities. The aqueous extract of *Justicia Aadathoda L.* was shown to be less effective than Ni based MPs in terms of antibacterial and antifungal properties. The supplied plant extracts and Ni based MPs remained tested for MIC and MBC. Both the Ni based MPs, and the extract revealed probable antibacterial and antifungal properties when tested against the DPPH and ABTS radicals. The current study concluded that *Justicia adathoda L.*-based NiO/Ni(OH)₂ MPs should be used as alternate treatments due to their antibacterial and antifungal capabilities. Though, additional vivo testing determination is required to expand their applications to biotic systems [46-50].

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