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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *JUSTICIA BETONICA* LEAVES AND IT'S ANTIOXIDANT ACTIVITY

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Abstract - *Justicia betonica*, also referred to as Chinese violet, is a perennial herbaceous plant in the Acanthaceae family. Several bioactive substances, including alkaloids, phenolic acids, and flavonoids, have been found in *Justicia betonica* leaves and have been linked to antioxidant effects. These substances have the ability to scavenge free radicals and stop oxidative cell damage, which is linked to a number of illnesses, including cancer, heart disease, and neurological disorders. Zinc oxide nanoparticles (ZnONPs) are tiny particles of zinc oxide that range in size from 1 to 100 nanometres. Due to their small size and unique properties, ZnONPs have many potential applications in various fields, such as electronics, optoelectronics, biomedicine, and environmental science. The ZnONPs can effectively scavenge various types of free radicals, including superoxide, hydroxyl, and DPPH radicals. These nanoparticles have also exhibited significant reducing power, which is a measure of their ability to donate electrons and neutralize free radicals.

Keywords - Inflammation; Zinc Oxide; Endothelium; Inflammatory Cytokines; Perennial Plant

I. INTRODUCTION

Rapidly advancing the realm of nanotechnology, the synthesis of nanoparticles has witnessed a paradigm shift towards sustainable and eco-friendly methods. Green synthesis, which harnesses the natural reducing and capping capabilities of plant extracts, has emerged as a promising avenue for nanoparticle production with reduced environmental impact. Among the plethora of plant candidates, *Justicia betonica*, with its rich bioactive composition and recognized medicinal attributes, has garnered attention as a potential source for nanoparticle synthesis [1-2]. This investigation explores the fascinating field of green synthesis, concentrating on the biosynthesis of zinc oxide nanoparticles (ZnONPs) from the leaves of *Justicia betonica*, and then reveals the synthesised nanoparticles' possible antioxidant properties. Conventional techniques for synthesising nanoparticles sometimes entail the use of dangerous chemicals and energy-intensive procedures, which exacerbate environmental damage. Green synthesis, on the other hand, eliminates the requirement for hazardous chemicals by utilising the natural ability of plant ingredients to promote reduction and stabilisation [3]. *Justicia betonica*, a perennial plant that has found utility in traditional medicine systems, boasts a diverse array of secondary metabolites such as flavonoids, alkaloids, and phenols. These bioactive compounds serve as excellent candidates for nanoparticle synthesis, offering a sustainable and biocompatible approach [4] Zinc oxide nanoparticles, renowned for their distinctive properties, have extensive applications in fields ranging from electronics to medicine. Their small size and high surface area-to-volume ratio grant them enhanced reactivity and unique optical and electrical properties. Using *Justicia betonica* leaves to synthesize ZnONPs underscores the sustainable principles of green synthesis and potentially imparts the nanoparticles with the therapeutic benefits associated with the plant's bioactive compounds. This amalgamation of plant-derived elements with nanotechnology paves the way for multifunctional nanoparticles with enhanced bioactivity [5]. One compelling facet of this synthesis approach is exploring the synthesized ZnONPs' antioxidant activity. Antioxidants play a pivotal role in mitigating oxidative stress, which is implicated in various chronic diseases. The incorporation of *Justicia betonica* leaves into the synthesis process introduces the intriguing possibility of infusing the nanoparticles with the plant's innate antioxidant potential. This, in turn, could potentially amplify the nanoparticles' radical scavenging abilities, rendering them potent candidates for combating oxidative stress-related ailments [6]. As the understanding of nanoparticle interactions with biological systems deepens, the assessment of their antioxidant activity gains significance. The synthesized ZnONPs could exhibit varied antioxidant effects depending on factors such as size, shape, and surface chemistry. Rigorous evaluation through in vitro and in vivo studies is necessary to ascertain the nanoparticles' potential as antioxidants and to comprehend their mechanisms of action [7]. In conclusion, the green synthesis of zinc oxide nanoparticles using *Justicia betonica* leaves exemplifies the convergence of nature and technology in the pursuit of sustainable nanoparticle production. This approach not only aligns with the global shift towards eco-friendly practices but also opens avenues for synergizing the bioactive potential of plants with the unique properties of nanoparticles [8,9].

The materials that are predicted are metal and metal oxide (MO) nanoparticles (NPs) because of their solubility, chemical stability, adhesiveness, and surface plasmon resonance. The antibacterial properties of ZnO, CuO, Ag2O, Fe2O3, CaO, NiO, and MgO NPs have garnered the most interest in recent studies. Zinc oxide (ZnO) is one of the NPs that may find use in biomedicine, especially in the areas of biosensing, cell imaging, antioxidant, antibacterial, α-amylase inhibitory action, anti-inflammatory, anti-cancer, and anti-diabetes. In underdeveloped nations, access to clean water and hygienic conditions are critical concerns. A catalyst made of ecologically benign, biosynthesised nanoparticles is one potential candidate material for waste water treatment [10].

They are an essential resource for the extraction of important bioactive ingredients that might lead to the development of new medications. The dimensions of nanoparticles, or NPs, range from 1 to 100 nm. NPs are well-known and essential parts of nanotechnology because of their many uses in fields including biology, geology, chemistry, optics, catalysis, electronics, and agriculture. Zinc (Zn) is a mineral that the human body absorbs in small amounts and is found in six different enzyme classes: hydrolases, ligases, oxidoreductases, transferases, and isomerases [38]. More precisely, substances containing zinc could be dangerous for both plants and animals [39]. ZnO in larger quantities has been deemed safe by the FDA, and ZnO nanoparticles are often more biocompatible [11,12].

The unique properties of nanomaterials drive the development of nanoscience and nanotechnology. Metal oxide nanoparticles (NPs) have garnered significant attention from the scientific community in this field. Their scalable properties bestow upon them a host of remarkable attributes, including high chemical stability, a high electrochemical coupling coefficient, a broad spectrum of radiation absorption, excellent photostability, easy accessibility, low cost, and non-toxicity. Worldwide, liver damage resulting from many types of environmental contaminants and compounds that are harmful to the liver is acknowledged as a serious medical problem. Worldwide, a large number of people suffer from hepatic impairment as a result of alcohol and drug abuse. The liver is the main organ that is impacted by chemicals initially because of its essential role in the metabolism of poisons and medications [13, 14].

A novel nanomaterial with improved and intriguing biological uses may be created by mixing metallic nanoparticles with plant extracts. Hepatotoxic substances such as CCl4 are well recognised for causing rapid liver damage. Steatosis, or the buildup of fat in the liver, is followed by centrilobular necrosis, or the death of liver cells in the centre of the liver lobule [15]. Long-term CCl4 infusion causes chronic liver damage, which is why it's a popular model for hepatic fibrosis development. Because of their link to high rates of disease, mortality, and increased treatment costs, the emergence of extensively drug-resistant (XDR) and multidrug-resistant (MDR) bacteria continues to be a serious public health concern. Since these resistant strains undermine the efficacy of currently available therapies, it is critical to investigate and develop novel approaches to fight infections and protect the public's health. To keep up with the constantly changing risks posed by bacteria, developing these new agents will require creative thinking and cooperation amongst scientists from different fields [16, 17].

The investigation into the antioxidant activity of the synthesized nanoparticles further amplifies their potential impact, hinting at their role in ameliorating oxidative stress-related disorders. As research advances in the realm of green nanotechnology, the synthesis of ZnONPs using *Justicia betonica* leaves serves as a testament to the innovative and promising prospects of this interdisciplinary endeavour.

II. MATERIALS AND METHODS

A. DPPH Assay:

In the previous literature [18], the antioxidant effect of zinc oxide nanoparticles using *Justicia betonica* leaves were evaluated using DPPH assay and the percentage of inhibition was calculated using the formula.

$$(\% \text{ of DPPH scavenging activity}) = [(A\,Control\; - Asample) \div Acontrol] \times 100$$

B. H_2O_2 Assay:

The H2O2 test was used in the prior literature [19] to assess the antioxidant impact of zinc oxide nanoparticles utilising *Justicia betonica* leaves, and the formula was used to determine the percentage of inhibition.

$$(\% \text{ of hydroxyl radical scavenging activity}) = [(A\,Control\; - Asample) \div Acontrol] \times 100$$

C. FRAP ASSAY:

Using *Justicia betonica* leaves, the FRAP test was employed in previous research [20] to evaluate the antioxidant effect of zinc oxide nanoparticles. The formula was then used to calculate the percentage of inhibition.

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III. RESULTS

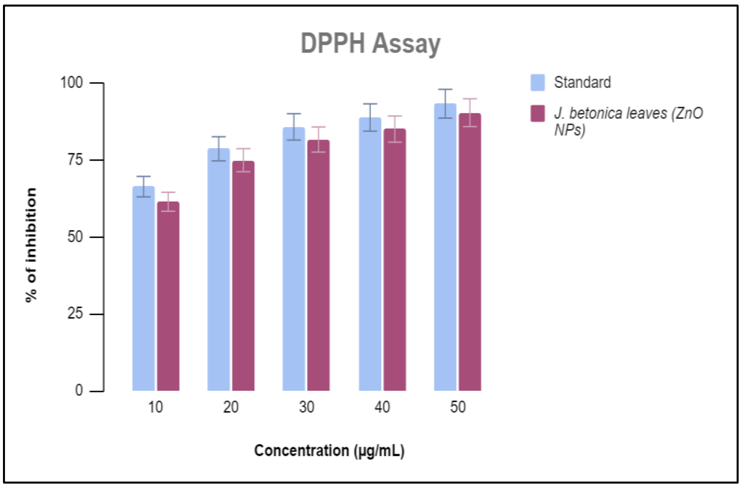

Fig.1. Employing the DPPH test to visually represent the zinc oxide nanoparticles' antioxidant activity, which is mediated by the plant extract of *Justicia betonica.* The error bars show the percentage of inhibition. The statistical analysis revealed that the percentage of inhibition differed between the treatment group and the standard group in a way that was statistically significant ($p<0.05$).

Fig.2. Using the H2O2 test, illustrates the antioxidant activity of zinc oxide nanoparticles mediated by plant extract from *Justicia betonica.* The proportion of inhibition is displayed by the error bars. The results of the statistical analysis showed that there were statistically significant ($p<0.05$) changes in the percentage of inhibition between the treatment group and the standard group.

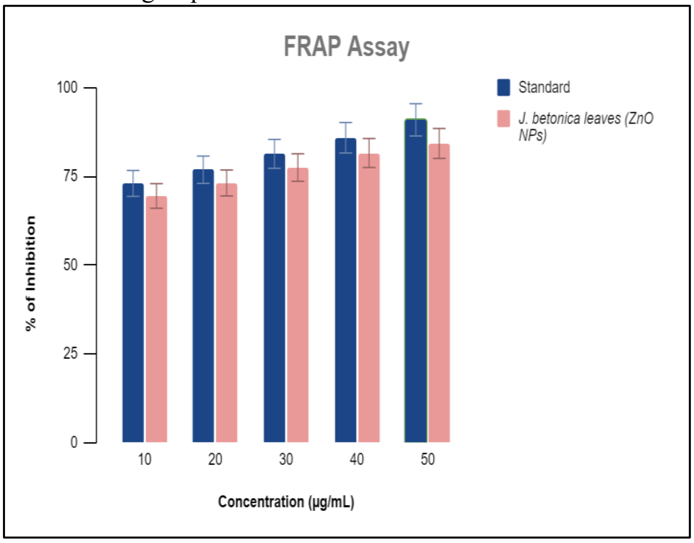

Fig.3. Illustrative depiction of the zinc oxide nanoparticles' antioxidant activity via the FRAP assay mediated by the plant extract of *Justicia betonica.* Percentage of inhibition is shown by the error bars. Following a statistical analysis, it was determined that there were statistically significant differences ($p<0.05$) in the percentage of inhibition between the treatment group and the standard group.

Due to its ecologically benign and sustainable approach, the green synthesis of nanoparticles has drawn a lot of interest. This is in contrast to standard approaches, which frequently use harsh chemicals and energy-intensive procedures. Plant extracts have become attractive options for nanoparticle production due to their ability to utilise the natural reducing and capping properties of their bioactive components. *Justicia betonica*, a plant with a variety of secondary metabolites and therapeutic uses, is a promising candidate for the environmentally friendly production of zinc oxide nanoparticles (ZnONPs) and the following investigation of their antioxidant capacity [21]. The findings of the DPPH test are displayed in Figure 1, the H2O2 assay results are displayed in Figure 2, and the FRAP assay results are displayed in Figure 3.

IV. DISCUSSION

This discussion delves into the multifaceted aspects of this synthesis approach, emphasizing both its eco-friendly nature and the potential antioxidant benefits of the synthesized nanoparticles. Green synthesis encapsulates the principles of sustainability, aligning with the global pursuit of eco-conscious practices. Traditional methods of nanoparticle synthesis can lead to toxic byproducts and excessive energy consumption. In contrast, green synthesis methods utilize plant extracts as reducing and capping agents, avoiding the use of harmful reagents. The utilization of *Justicia betonica* leaves in this context capitalizes on the plant's natural compounds, such as flavonoids, alkaloids, and phenols, which possess inherent reducing properties [22].

By using this method, the environmental impact of creating nanoparticles is lessened and the advantageous characteristics of the plant are incorporated into the nanoparticles themselves. Because of its special physicochemical characteristics, zinc oxide nanoparticles are used in a wide range of industries. They are renowned for their small size, large surface area, and optical and electrical characteristics. The synthesis of ZnONPs using *Justicia betonica* leaves introduces an intriguing dimension by potentially imparting the nanoparticles with the plant's bioactivity. This marriage of nanotechnology and plant biology opens avenues for multifunctional nanoparticles that can harness the synergistic effects of both components. Furthermore, the incorporation of plant-derived elements might contribute to improved biocompatibility and reduced toxicity, enhancing the nanoparticles' potential for biomedical applications [23].

The exploration of antioxidant activity becomes particularly significant in the context of green-synthesized ZnONPs. Antioxidants are pivotal in neutralizing reactive oxygen species and oxidative stress, which are implicated in various chronic diseases, including cancer, neurodegenerative disorders, and cardiovascular conditions. *Justicia betonica,* known for its antioxidant-rich composition, offers an ideal platform to infuse these qualities into the synthesized nanoparticles. The unique combination of nanoparticle properties and plant-derived antioxidants could potentially yield nanoparticles with enhanced radical scavenging capabilities. However, assessing the antioxidant activity of nanoparticles is complex, as it involves intricate interactions between the nanoparticles and biological systems.

Factors such as nanoparticle size, shape, surface chemistry, and concentration can influence their antioxidant effects. In vitro studies involving cell cultures and in vivo experiments on animal models are crucial to comprehensively evaluate the nanoparticles' potential as antioxidants. Additionally, understanding the underlying mechanisms by which nanoparticles exert antioxidant effects is essential for optimizing their applications and ensuring their safety. Furthermore, the variability in plant composition due to factors like geographic location and seasonal changes can impact the nanoparticle synthesis process [24].

Standardization of extraction protocols and characterization techniques is imperative to ensure reproducibility and consistency in nanoparticle synthesis. Moreover, thorough toxicity assessments are necessary to ascertain the nanoparticles' safety for potential therapeutic applications. This includes studying their potential interactions with cells, tissues, and organs, as well as their long-term effects. In conclusion, the green synthesis of zinc oxide nanoparticles using *Justicia betonica* leaves epitomizes the convergence of sustainable practices and advanced technology.

This approach not only reduces the ecological footprint of nanoparticle synthesis but also explores the integration of plant-derived bioactivity into the nanoparticles themselves. The investigation into the antioxidant activity of these nanoparticles adds a layer of complexity, underscoring their potential to combat oxidative stress-related disorders [25]. As research advances, it is imperative to elucidate the mechanisms underlying the nanoparticles' antioxidant effects, standardize synthesis and characterization protocols, and conduct rigorous toxicity assessments. The synergy between nanotechnology and plant biology in the context of green synthesis holds tremendous promise, offering novel avenues for sustainable technology and biomedicine [26].

In comparison to the positive control BHA, the radical quenching activity (DPPH) of the nanoparticles (Ts-ZnONPs, 79.67%) was found to be rather good in the preceding study. Furthermore, it has been observed that ZnO NPs' ability to prevent DNA damage improves with concentration. The highest dose at which the DNA damage inhibitory effect was seen was 100 mg/L. According to the thorough findings, it was concluded that a wide range of applications might benefit from the application of zinc oxide nanoparticles made using the green synthesis approach. the structure of zinc oxide nanoparticles (ZnO NPs) produced in different ways employing the green synthesis technique from Thymbra Spicata L. plant [27].

Plants and microorganisms have drawn a lot of interest as possible biological sources for environmentally benign nanoparticle synthesis. In this work, stable spherical zinc oxide nanoparticles (ZnO-NPs) were synthesised using zinc nitrate and Aspergillus niger. The synthesised nanoparticles' structure, shape, and optical characteristics were assessed using the FTIR, DLS, SEM, TEM, and XRD techniques. Electron microscopy pictures showed that the nanoparticles were polydisperse and ranged in length from 30 to 70 nm. The bulk of the particles had spherical shape. In terms of antioxidant ability, the produced ZnO-NPs demonstrated an IC50 of around 1000 µg ml−1. In neoplastic MCF-7 cells, the synthesised ZnO-NPs also caused apoptosis and slowed down cellular proliferation [28].

AgNPs mediated by cumin oil were easily biosynthesised and had strong antioxidant efficacy in comparison to standard. According to the findings of this study, cumin seed oil-mediated silver nanoparticles may find application as a powerful antioxidant. As a result, it may be utilised in large-scale manufacturing as well as several medical applications where antioxidants are required [29].

The production of chitosan in the presence of silver nanoparticles in TEM verified the nanocomposite's creation. The antibacterial capabilities of nanocomposites against Escherichia coli, Rhizobium radiobacter, Streptococcus, and Klebsiella pneumoniae were evaluated using agar well diffusion. The results showed that silver nanoparticles with chitosan coating had exceptional antioxidant and antibacterial capabilities, indicating that they may be applied in the future to enhanced biomedicine [30].

V. CONCLUSION

In the era of sustainable technology and eco-conscious practices, the green synthesis of zinc oxide nanoparticles (ZnONPs) through *Justicia betonica* leaves emerges as a pioneering approach that marries the realms of nanotechnology and natural resources. This process encapsulates the essence of green chemistry by employing plant extracts to orchestrate the reduction and capping of nanoparticles, sidestepping the ecological pitfalls of traditional synthesis methods. Throughout this exploration, the fusion of nature's bioactive compounds with cutting-edge nanotechnology holds the promise of yielding nanoparticles that are not only environmentally benign but also inherently bioactive.

The utilization of *Justicia betonica* leaves as a conduit for nanoparticle synthesis taps into the plant's rich repository of secondary metabolites. These compounds, such as flavonoids, phenols, and alkaloids, infuse the nanoparticles with their intrinsic reducing properties, intertwining the botanical essence with the nanoparticles' structure. This intertwining grants the synthesized ZnONPs the potential to exhibit not only the unique properties typical of nanoparticles but also the bioactivity attributed to the plant's components. This convergence fuels the exploration of these nanoparticles' antioxidant activity, which holds great significance in combating the escalating burden of oxidative stress-related disorders.

The antioxidant activity of nanoparticles, harnessed through their interaction with reactive oxygen species, presents a promising avenue for innovative therapeutic interventions. The integration of *Justicia betonica* leaves into the synthesis process introduces the intriguing potential to amplify the nanoparticles' radical scavenging capabilities. While challenging, elucidating the mechanisms underlying the nanoparticles' antioxidant effects is vital for translating these findings into tangible clinical applications. However, the journey towards harnessing the potential of green-synthesized ZnONPs is not devoid of challenges. Ensuring reproducibility and standardization of synthesis protocols, characterizing the nanoparticles comprehensively, and rigorously assessing their safety are crucial steps. Toxicity evaluations need to span various biological models to ascertain the nanoparticles' effects on both healthy and diseased systems. Furthermore, the understanding of nanoparticle-cell interactions and long-term effects is pivotal for their successful integration into therapeutic regimes.

In the broader context, the synthesis of ZnONPs through *Justicia betonica* leaves epitomizes the harmonious convergence of nature's wisdom and technological innovation. This approach extends beyond the boundaries of nanoparticle synthesis, sparking interdisciplinary collaborations that explore the intersections of nanotechnology, plant biology, and medicine. By delving into the realm of green synthesis and deciphering the potential of these nanoparticles as antioxidants, we unveil a world of possibilities that could reshape healthcare strategies and environmental stewardship. As we navigate the uncharted territories of green nanotechnology, the synthesis of ZnONPs using *Justicia betonica* leaves serves as a beacon, guiding us toward a sustainable, bioactive, and harmonious future.

CONFLICT OF INTEREST:

The authors did not have any conflict of interest.

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