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Free Radicals Scavenging Activity Of Lemon Grass, Mint Herbal Formulation Mediated Zinc Oxide Nanoparticles.

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ABSTRACT

BACKGROUND:The traditional synthesis of metal oxide nanoparticles frequently relies on physical and chemical methods that utilize toxic chemicals, which pose potential hazards to human health and the environment. To mitigate these risks, there is a growing shift toward "green chemistry," which utilizes herbal materials to provide eco-friendly, non-toxic alternatives for pharmaceutical and biomedical applications.

OBJECTIVE :To evaluate the in vitro antioxidant and free radical scavenging activity of the formulated biogenic nanoparticles at various concentrations using DPPH and hydroxyl radical (H2O2) scavenging assays.

MATERIALS AND METHOD:This study investigated the biosynthesis of zinc oxide nanoparticles (ZnO-NPs) utilizing a lemongrass and mint herbal formulation. This green synthesis method bypasses the need for toxic chemicals, intricate purification, and microbial cell cultures. The free radical scavenging activity of the synthesized nanoparticles was evaluated in vitro at various concentrations (10 µL to 50 µL) using

1. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay.
2. Hydroxyl radical (H2O2) scavenging assay.

RESULTS :The lemongrass and mint herbal formulation successfully and easily mediated the synthesis of ZnO-NPs. In both the DPPH and H2O2 assays, the biogenic nanoparticles demonstrated a strong, dose-dependent ability to neutralize free radicals. This free-radical scavenging activity closely competed with the standard antioxidant controls.

CONLUSION:The biosynthesis of zinc oxide nanoparticles using a lemongrass and mint formulation is a highly effective, non-toxic, and environmentally compatible alternative to conventional chemical synthesis. Due to their potent antioxidant capabilities and the simplicity of the plant-based procedure, these biogenic nanoparticles hold significant potential for scalable production and use in pharmaceutical and commercial medicinal applications to counteract oxidative stress .

Keywords: Zinc oxide nanoparticles, Biosynthesis, Herbal formulation, Antioxidant activity, Free radical scavenging, DPPH assay, Lemongrass, Mint...

INTRODUCTION

Recently, increasing public concern about hygiene has been driving many studies to investigate antimicrobial and antiviral agents. However, the use of any antimicrobial agents must be limited due to their possible toxic or harmful effects. In recent years, due to previous antibiotics' lesser side effects, the use of herbal materials instead of synthetic or chemical drugs is increasing. Herbal materials are found in medicines. Herbs can be used in the form of plant extracts or as their active components. Furthermore, most of the world's populations used herbal materials due to their strong antimicrobial properties and primary healthcare benefits. For example, herbs are an excellent material to replace nanosilver as an antibiotic and antiviral agent. Antimicrobial agents are biological balance with the human body and its living environments, but an uncontrolled and rapid used as antibiotic drugs to control infections in the human body, but they can cause many side effects.

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The growth of microbes can lead to some dangerous problems [1–4]. Antimicrobial agents are used as effects, especially increasing reactive oxygen species (ROS) in the human body.

Zinc oxide nanoparticles is a unique material that exhibits semiconducting, piezoelec - tric, and pyroelectric properties and has versatile applica- tions in its electric and optical properties, piezoelectric devices, chemical sensors, spin electronics, personal care products, and coating and paints (Wang 2004; Wahab et al. 2010; Akhtar et al. 2011). A number of physical and chemical methods have been employed for the synthesis of ZnO nanoparticles including use of toxic chemicals (Hu et al. 2004; Zhai et al. 2008). It is believed that broad usage of these metal oxide nanoparticles may lead to their potentially hazardous environment which could adversely affect human health (Patra et al. 2019).

Thus, there is a need for "green chemistry" that offers numerous benefits of ecofriendliness and compatibility for pharmaceutical and other biomedical applications, where toxic chemicals are not used for the synthesis protocol. (Don et al. 2024; Assaggaf et al. 2024) The use of agricultural wastes or plants and their parts has emerged as an alternative to chemical synthetic procedures because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures. However biosynthesis of zinc oxide nanoparticles (ZnO-NPs) by Aloe vera, Bacillus cereus, Fusarium spp, and Medicago sativa, as biological materials, is fully explored. (Don et al. 2024)

MATERIALS AND METHODS

ANTIOXIDANT ACTIVITY:

DPPH METHOD

Antioxidant activity

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of Justicia adathoda leaf extract interceded zinc oxide nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Ascorbic acid was used as standard.

The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

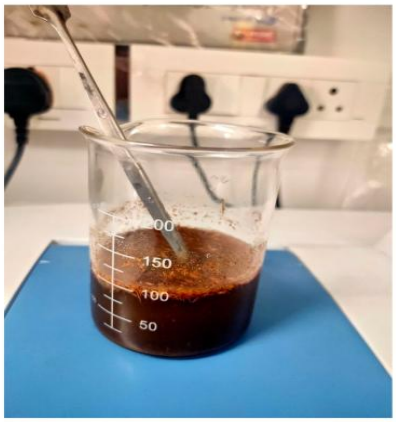
HYDROXYL RADICAL SCAVENGING ASSAY:

All solutions were prepared freshly.1.0mL of the reaction mixture contained 100µL of 28mM of 2-deoxy-2-ribose (dissolved in phosphate buffer,pH 7.4), 500µL solution of various concentrations of the Carcia papaya (10µL,20µL,30µL,40µL,50µL) 200µL of 200µM Fecl3 and 1.04mM EDTA (1:1 v/v),100µL H2O2(1.0mM) and 100µL ascorbic acid(1.0mM).After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution . Vitamin E was used as a positive control.

RESULTS AND DISCUSSION

Lemon grass mint herbal formulation mediated ZnNPs were biosynthesised with ease and showed good antioxidant activity compared to standard.

Stepwise procedure shown below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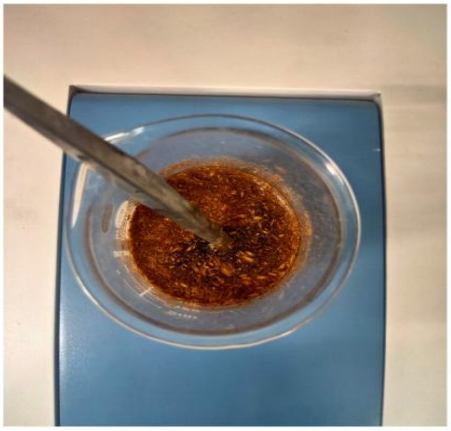

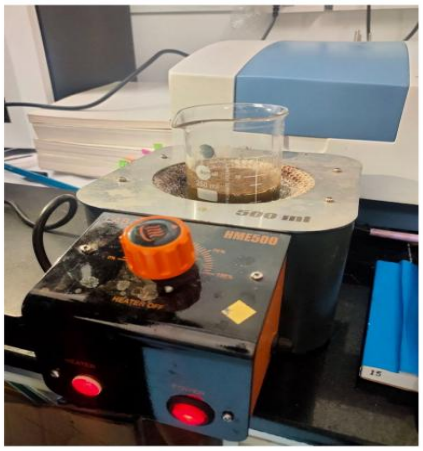
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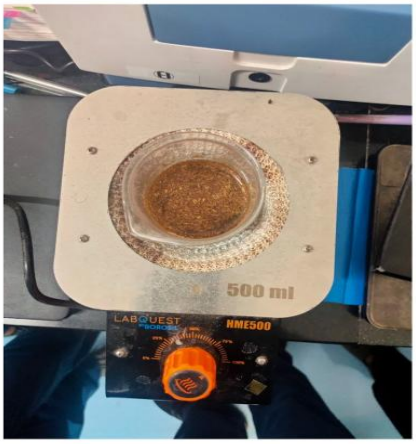
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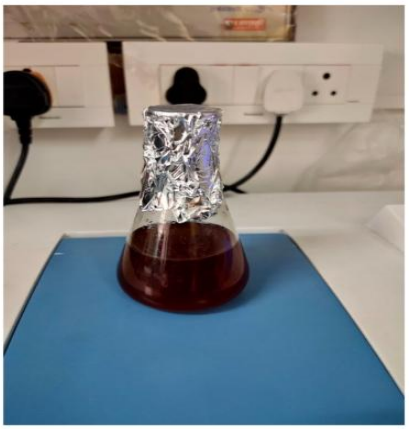

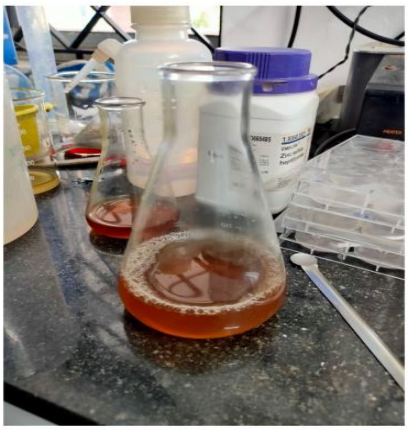

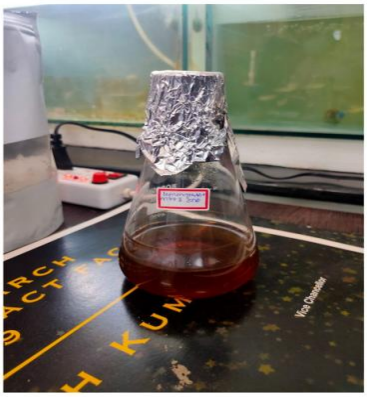

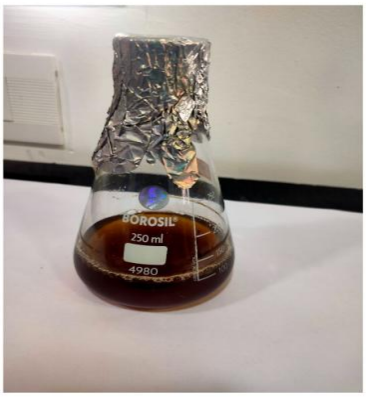
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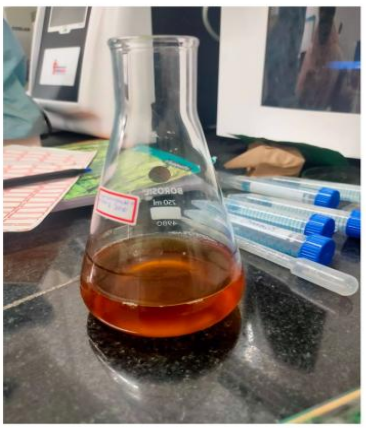

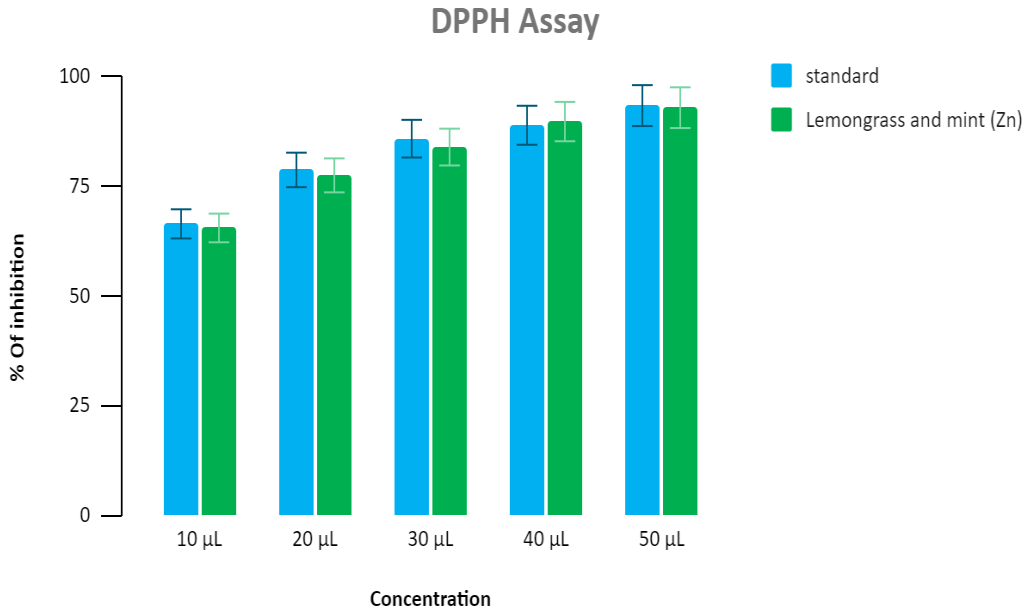

Figure 1: In vitro antioxidant evaluation using the DPPH assay. The bar graph illustrates the dose-dependent antioxidant activity of the lemongrass and mint herbal formulation mediated zinc nanoparticles (green) compared to the standard (blue) at concentrations ranging from 10 µL to 50 µL. Error bars denote standard deviation.

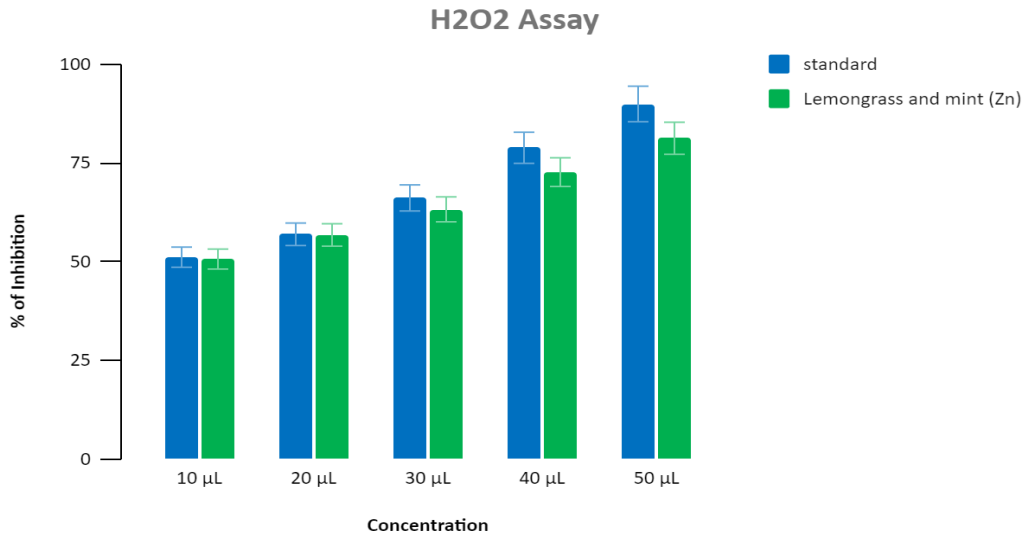

Figure 2: In vitro antioxidant evaluation using the hydrogen peroxide (H2O2) scavenging assay. The bar graph depicts the dose-dependent increase in the percentage of inhibition for both the standard (blue) and the biogenically synthesized lemongrass and mint zinc oxide nanoparticles (green) at concentrations ranging from 10 µL to 50 µL. Error bars represent standard deviation.

This research successfully demonstrated the straightforward biosynthesis of zinc oxide nanoparticles using a lemongrass and mint herbal formulation. When evaluated in vitro using both DPPH and hydroxyl radical (H2O2) scavenging assays, these biogenic nanoparticles displayed potent antioxidant capabilities. Specifically, across concentrations ranging from 10 µL to

50 µL, the nanoparticles exhibited a strong, dose-dependent ability to inhibit and neutralize free radicals. Furthermore, the study noted that the free-radical scavenging activity of this eco-friendly formulation closely competed with the standard antioxidant controls used during the testing(Figueirinha et al. 2010). Overall, the findings indicate that this herbal formulation effectively mediates the production of nanoparticles with excellent antioxidant properties.

DISCUSSION

The current study highlights the critical shift toward eco-friendly and sustainable practices in nanotechnology, specifically through the "green chemistry" synthesis of zinc oxide nanoparticles (ZnO-NPs)(Figueirinha et al. 2010; Cheel et al. 2005). Traditionally, the production of these metal oxide nanoparticles relies heavily on physical and chemical methods that often utilize toxic chemicals. Such conventional processes raise significant environmental and human health concerns due to the potentially hazardous nature of the resultant materials(Lopes et al. 2022; Luís et al. 2017). By employing an alternative biosynthesis route using a lemongrass and mint herbal formulation, this study eliminates the need for toxic chemicals, intricate purification steps, and the maintenance of complex microbial cell cultures (Ahmed et al. 2024).

The transition toward utilizing herbal materials over synthetic or chemical drugs is largely driven by the goal of minimizing adverse side effects while leveraging their strong, inherent therapeutic properties and primary healthcare benefits (Ahmed et al. 2024; Paul et al. 2021). The unmoderated use of traditional antimicrobial agents and antibiotic drugs to manage infections can lead to a dangerous biological imbalance, heavily contributing to the increased generation of reactive oxygen species (ROS) within the human body. Therefore, developing biogenic materials that can safely counteract these free radicals is of immense clinical value(Ahmed et al. 2024; Paul et al. 2021; Shah et al. 2021).

The in vitro antioxidant evaluations conducted in this study specifically the DPPH and hydroxyl radical (H_2O_2) scavenging assays demonstrate the robust efficacy of the formulated nanoparticles (Olejnik et al. 2021). The results indicate that the lemongrass and mint-mediated ZnO-NPs exhibit an impressive, dose-dependent ability to neutralize free radicals, closely competing with standard antioxidant controls. This strong free-radical scavenging activity proves that plant-mediated nanoparticles can successfully mitigate oxidative stress without the severe toxicological profiles associated with synthetically derived counterparts(Olejnik et al. 2021; Yusefi-Tanha et al. 2020).

CONCLUSION

In conclusion, this original research successfully demonstrates that zinc oxide nanoparticles can be biosynthesized with remarkable ease using a lemongrass and mint herbal formulation. The green synthesis approach utilized in this study proved to be a highly effective, environmentally compatible, and non-toxic alternative to standard chemical procedures (Radić Brkanac et al. 2024).

The resulting biogenic ZnO nanoparticles exhibited potent antioxidant capabilities, acting as a highly effective free radical scavenger across multiple biochemical assays. These findings strongly support the viability of herbal-mediated nanomedicines in counteracting dangerous oxidative stress and reactive oxygen species in biological systems (Saravanan et al. 2018).

Because this method relies on easily accessible plant materials and simple procedures, it holds significant potential for scalability. The study concludes that this lemongrass and mint formulation may be reliably employed in large-scale production. Ultimately, these biogenic nanoparticles offer a highly promising, eco-friendly solution for various pharmaceutical, biomedical, and commercial medicinal applications where safe, effective antioxidants are critically required(Sánchez-Argüello et al. 2023).

Lemon grass mint herbal formulation mediated ZnNPs were biosynthesised with ease and showed good antioxidant activity compared to standard. This study concludes that lemon grass mint herbal formulation ZnO nanoparticles have the potential to be used as an effective antioxidant. Hence, it may be employed in large scale production and may be used in many medicinal applications where there is a need for antioxidants (Banupriya et al. 2022)..

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