

# Anti-diabetic and anti-microbial activity of *aspalathus linearis* and *syzygium aromaticum* formulation mediated zinc oxide nanoparticles

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## ABSTRACT

**Introduction:** Zinc oxide nanoparticles (ZnO NPs) exhibit a wide range of biomedical applications majorly used as anti-inflammatory, anti-cancer, anti-diabetic, and anti-microbial activity and other biomedical applications because they show less toxicity and are very compatible. Zinc metal is an inorganic and essential element in the human body at the trace level. ZnO NPs are also GRAS substances (Generally Recognized As Safe). This study's main objective is synthesizing zinc oxide nanoparticles using red tea & clove mediated herbal formulation and screening for its anti-microbial, and anti-diabetic properties.

**Materials and Methods:** Red tea and clove-mediated ZnO NPs were synthesized using the green synthesis method. The anti-microbial activity was tested against oral pathogens using the agar well diffusion method, while the anti-diabetic activity was estimated using the alpha-amylase inhibitory assay method by using red tea and clove-mediated ZnO NPs.

**Results:** ZnO NPs were successfully synthesized using red tea and clove-formulated extract. The synthesized ZnO NPs using *Aspalathus linearis* (red tea) and *Syzygium aromaticum* (clove) mediated ZnO NPs were characterized using UV visible spectrophotometry and SEM (Scanning Electron Microscope) analysis. The green synthesized ZnO NPs show promising anti-microbial activity by exhibiting a 12 mm zone of inhibition against *S. aureus*, 11 mm in *E. faecalis*, 9 mm in *S. mutans*, and 11 mm in *C. albicans*. In anti-diabetic activity, the green synthesized ZnO NPs showed a maximum inhibition percentage of up to 80% at the maximum concentration of 50 µg/mL.

**Conclusion:** Green synthesized ZnO NPs using red tea and clove showed maximum efficacy in anti-microbial properties which can lead to huge potential use as antibacterial agents. Simultaneously, anti-diabetic activity showed an excellent inhibition percentage which can be a potent therapeutic agent in the field of nanomedicine in diabetes management.

## KEYWORDS:

Zinc oxide nanoparticles, anti-diabetic activity, green synthesis, biomedical applications.

## INTRODUCTION

Nanoparticles synthesized by physical and chemical methods have fewer uses in the field of clinical research because of their toxicity level.<sup>1</sup> Due to the physio-chemical properties of plant-based nanoparticles, this process also has the additional advantage of longer life span of nanoparticles that overcomes the restraint of conventional physical and chemical methods in the synthesis of nanoparticles.<sup>2</sup> Nanomaterials exhibit atom-like behavior when divided to nearly atomic size because of their enormous surface area. It is anticipated that the global nanotechnology revolution will have an impact on several biomedical research fields, including medication delivery, cancer therapy, and cell imaging.<sup>3</sup> Zinc Oxide nanoparticles (ZnO NPs) stand out among metal oxide nanoparticles due to their distinctive optical and chemical behaviors that can be easily controlled by altering their shape.<sup>4</sup> ZnO NPs are part of a group of metal oxides, which are distinguished by their ability for photocatalysis and photo-oxidation of chemical and biological species.<sup>5</sup> ZnO NPs were synthesized by the green synthesis method, which is eco-friendly because of their unique characteristics and adaptable nature, where they are widely used in sensors, biological labelling, bio-imaging, and biomedical applications owing to their anti-microbial, anti-inflammatory, anti-oxidant, and anti-diabetic activities.<sup>6</sup> Owing to their ease of use, environmental friendliness, and high level of biological activity, plant-mediated biological synthesis of nanoparticles is significant. ZnO NPs have demonstrated an encouraging future in biomedical research, particularly in the domains of anti-cancer and anti-microbial fields.<sup>7</sup> These fields are associated with their strong ability to induce insufficient oxygen species (ROS) formation, release zinc ions, and induce cell death. As more bacteria are becoming resistant to antibiotics and new strains are emerging, scientists have given much importance to metal and metal oxide NPs as antibacterial agents.<sup>8</sup> Zinc-based therapy is appealing because of the link between diabetes and an imbalance in zinc homeostasis. ZnO NPs were tested for anti-diabetic properties in many previous studies.<sup>9</sup> Silver, gold, and ZnO NPs have been synthesized using plants like *Withania somnifera*, *Amygdalus scoparia*, *Hibiscus subdariffa*, *Hibiscus rosa-sinensis*, *Murraya koenigii*, *Moringa oleifera*, *Tamarindus indica* L, *Ocimum sanctum*, *Cymbopogon citratus*, and it is also been stated that ZnO NPs have been made using *Aloe vera* leaf extract.<sup>10</sup>

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Common products such as rubber, cosmetics, and paint are produced using ZnO NPs, especially in biological applications that play a vital role because of their excellent biocompatibility and economically friendly nature.<sup>11</sup> ZnO NPs are well known for their anti-microbial activity because of their ability to cause cell death by membrane disruption. Anti-microbial compounds from herbal sources have good therapeutic potential and can be utilized.<sup>12</sup> Plant-derived nanoparticles play a vital role in the field of biomedicine due to their environmental friendliness, lack of harmful chemicals, elimination of the culture management procedure, and lack of physical and chemical parameters.<sup>13</sup> Many natural ingredients have been studied for their biomedical properties, such as anti-inflammatory, antioxidant, anti-microbial, anti-diabetic, anti-cancer, and so on. Clove (*Syzygium aromaticum*) and red tea (*Aspalathus linearis*) have been studied for their potential biomedical properties.<sup>14</sup> Clove has been found to have anti-microbial, antiseptic, anti-diabetic, and anti-carcinogenic properties. Red tea, also known as Rooibos tea, is rich in antioxidants and has been found to have anti-inflammatory and anti-cancer properties. Thus, herbal formulations can be developed as potent therapeutic agent.<sup>15</sup>

This study aimed to evaluate the anti-microbial properties of green synthesized ZnO NPs of *Aspalathus linearis* and *Syzygium aromaticum* against different oral pathogens. Anti-diabetic activity was assessed by alpha-amylase and beta-glucosidase enzyme inhibitory assays at various concentrations and compared to the standard tested.

## MATERIALS AND METHODS

### Preparation of Plant Extract

*Aspalathus linearis* and *Syzygium aromaticum* were purchased from a commercial store and used in further experiments. 1 g of clove and 1 g of red tea powder were added to 100 mL of distilled water and boiled in a heating mantle at 45-50 degrees Celsius for 15-20 mins. The boiled solution was filtered through Whatman No.1 filter paper, and the extracted solution was utilized to synthesize ZnO NPs.

### Synthesis of ZnO NPs

Zinc acetate (30 mM, millimolar) was dissolved in 50 mL of distilled water and stirred gently by adding 50 mL of the extracted plant solution. The solution was mixed for 24 hours using a magnetic stirrer, and the dark brown-yellow color confirmed the synthesis of ZnO NPs. After 24 hr of incubation the prepared solution was centrifuged at 8000 RPM (Rotation per minute) using a centrifuge and the pellet was collected.

### Characterization of Synthesized ZnO NPs

UV-Vis spectroscopy was also performed to further confirm the formation of ZnO NPs. The absorption spectrum of the synthesized ZnO NPs was analyzed using a UV-Vis spectrophotometer in the range of 350 to 750 nm. Scanning electron microscopy (SEM) was used to examine the surface features and particle shapes of the recently produced nanoparticle formulations.

### Anti-microbial Activity of Green Synthesized ZnO NPs

The anti-microbial activity of red tea- and clove-mediated ZnO NPs was investigated against *Candida albicans*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Staphylococcus aureus* strains. Mueller-Hinton Agar was used for this test to estimate the anti-microbial properties of the synthesized ZnO NPs. The medium was prepared and sterilized at 121°C for 15 minutes. A 9 mm sterile polystyrene tip was used to make wells in the sterilized plates, and the test organisms were spread on their respective plates. ZnO NPs at different concentrations (25, 50, and 100 µg/mL) were loaded, and in the respective wells, standard antibiotic Amoxyrite 20µL was added to the wells, which acted as a standard for bacteria, and fluconazole was used as a standard for fungi. The plates were maintained undisturbed for 24 h at 37 °C. After incubation, zones of inhibition were observed and measured in millimeters.

### In-vitro Anti-diabetic Assay

#### Alpha-Amylase Inhibitory Assay

Alpha-amylase enzyme inhibition was obtained by estimating the quantity of maltose unfettered during the process, different levels of ZnO NPs (10, 20, 30, 40, and 50 µg/mL), and 100 µL of alpha-amylase solution for 30 min at room temperature. Next, starch solution (1% w/v) 100 µL was added to the solution and was incubated for 10 minutes. The DNSA reagent 100 µL was released to restrict the reaction by heating the water bath for 5 min. The control was maintained by replacing the sodium phosphate buffer, which was maintained at a pH of 6.9, with the same quantity of enzyme extract. At 540 nm, the observations were recorded, and the activity was performed in triplicate using acarbose as the standard.

% of inhibition =  $C-T/C \times 100$ , where C is the control and T is the test sample.

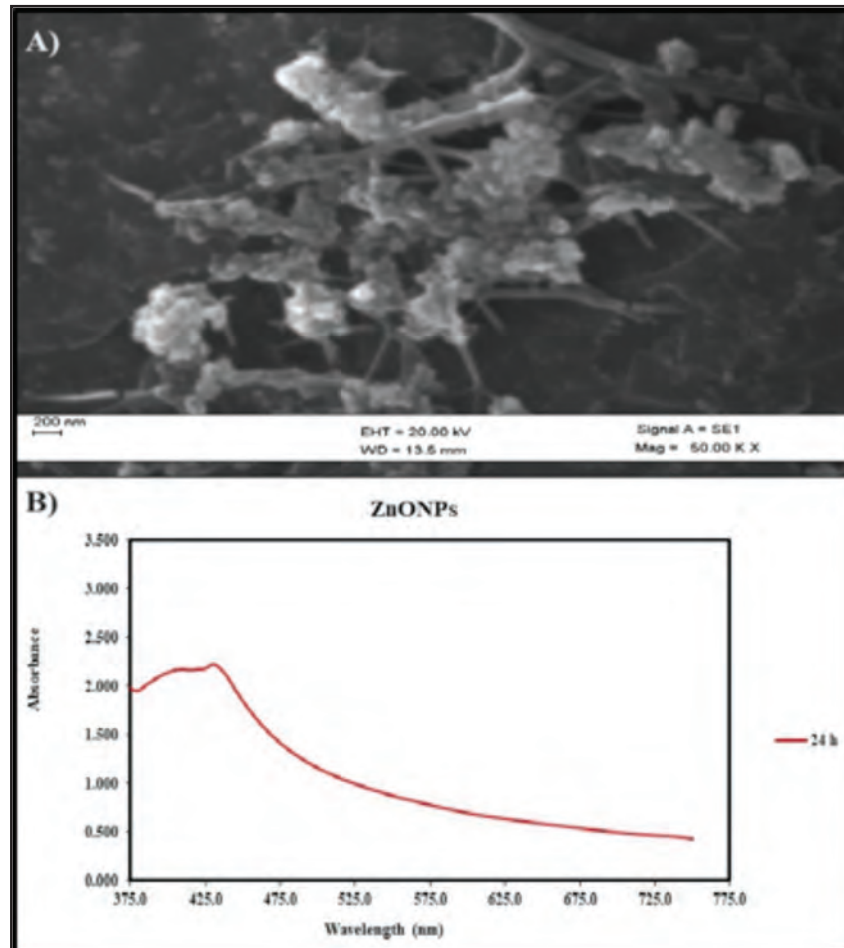
#### Beta-Glucosidase Inhibition Assay

The anti-diabetic effect of green-synthesized ZnO NPs was estimated using a β-glucosidase enzyme inhibitory assay. At different concentrations (10, 20, 30, 40, and 50 µg/mL), ZnO NPs were added to 1 mL of beta-glucosidase solution and starch solution in the presence of 0.2 M tris buffer at pH 8.0. The solution was incubated for approximately 40 min, and the reaction was terminated by adding 2 ml of 6N HCl as in previous literature.<sup>16</sup> In this study, acarbose was used as a positive control.

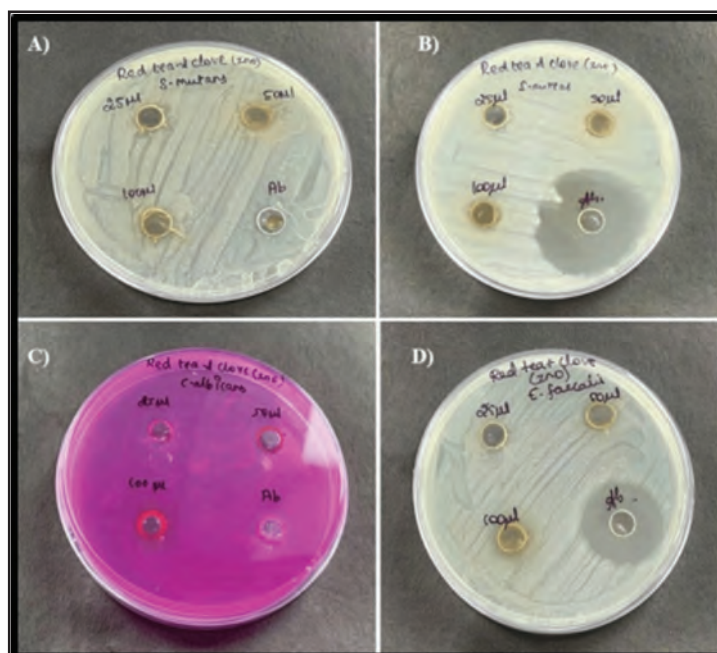
% of inhibition =  $C-T/C \times 100$ , where C is the control and T is the test sample.

### Statistical analysis:

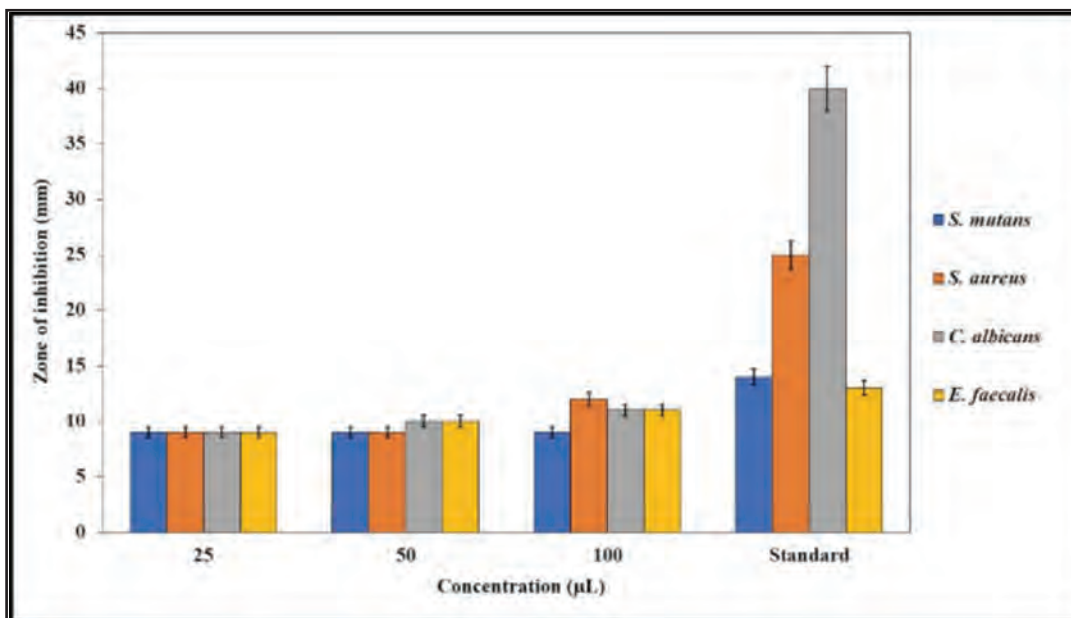
To ensure that the results were accurate, each experiment was performed three times. Statistical analysis of the measured anti-microbial and anti-diabetic activity data was performed using the standard error (SE) method. Standard error provides a measure of the variability of the sample means, allowing for an assessment of the importance and accuracy of the results.



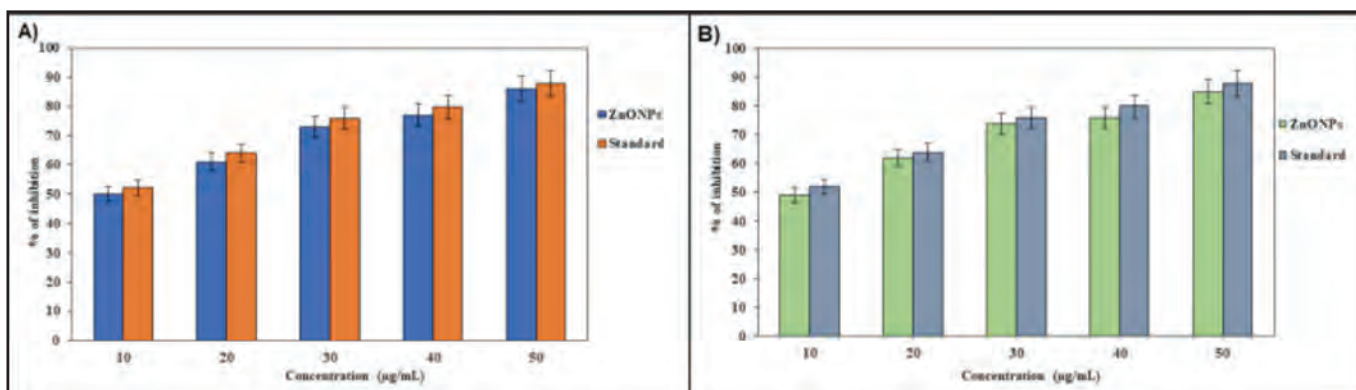
**Fig. 1:** A) UV-visible spectra of *A. linearis* and *S. aromaticum*-mediated ZnO NPs formulation  
 B) SEM analysis of green synthesized ZnO NPs  
 ZnO NPs: zinc oxide nanoparticles; *A. linearis*– *Aspalanthus linearis*; *S. aromaticum*– *Syzygium aromaticum*



**Fig. 2:** Image depicting the anti-microbial activity of ZnO NPs against oral pathogens A) *S. mutans*, B) *S. aureus*, C) *C. albicans* D) *E. faecalis*  
 ZnO NPs - Zinc oxide nanoparticles



**Fig. 3:** Graph representing the antibacterial activity of *A. linearis* and *S. aromaticum*-mediated ZnO NPs against oral pathogens ZnO NPs: zinc oxide nanoparticles, *A. linearis*-*Aspalathus linearis*, *S. aromaticum*-*Syzygium aromaticum*. Error bars in the graph represent standard error. Two-way ANOVA was used to evaluate the significance. The P-value ( $P < 0.0005$ ) was statistically significant.



**Fig. 4:** Anti-diabetic activity of ZnO NPs synthesized from *A. linearis* and *S. aromaticum* formulation A) α-amylase enzyme inhibition assay, and B) β-glucosidase enzyme inhibition assay. The error bar in the graph represents standard error. Two-way ANOVA is used to evaluate the significance. The P value ( $P < 0.0005$ ) is statistically significant.

**RESULTS**

*Visual Confirmation & Its Characterization Techniques*

Green-synthesized red tea and clove-mediated ZnO NPs were successfully synthesized. The visible color change from light brown to dark brown indicated the reducing and capping ability of the red tea and clove-mediated herbal formulation. The shape and size distribution of the synthesized ZnO NPs were determined using SEM. The production of ZnO NPs with a consistent and irregular shape, strong crystallinity, and well-defined size distribution was verified by SEM analysis, as shown in Figure 1A. Understanding the characteristics and possible uses of ZnO NPs in a variety of industries, such as photocatalysis, sensors, and biological applications, depends on these findings. The synthesized ZnO NPs prepared using red tea and clove exhibited a maximum peak at 410 nm, as

measured using a UV-visible spectrophotometer, as shown in Figure 1B. After 24 h, the UV readings were noted and centrifuged at 8000 rpm (rotation per minute). The supernatant was discarded and the pellet was collected and used for further studies.

*Anti-microbial Activity*

As shown in figure 2 and 3, the green-synthesized ZnO NPs nanoparticles were tested for antimicrobial activity against oral pathogens: A) *S. mutans*, B) *S. aureus*, C) *C. albicans*, and D) *E. faecalis*. The synthesized ZnO NPs have exhibited a maximum zone of inhibition of 9 mm in *S. mutans*, 12 mm in *S. aureus*, 11 mm in *E. faecalis*, and 11 mm in *C. albicans*. The synthesized nanoparticles were compared with standards for both fungi and bacteria. The maximum zone of inhibition



**Table. 1: Anti-diabetic activity of ZnO NPs synthesized from *A. linearis* and *S. aromaticum* formulation A)  $\alpha$ -amylase enzyme inhibition assay, and B)  $\beta$ -glucosidase enzyme inhibition assay.**

A)					
Concentration ( $\mu\text{g/mL}$ )	10	20	30	40	50
Standard (%)	53.34	60.43	64.56	73.76	81.54
ZnONPs (%)	52.67	64.43	76.53	80.35	88.53

B)					
Concentration ( $\mu\text{g/mL}$ )	10	20	30	40	50
Standard (%)	52.36	64.76	76.53	80.43	88.28
ZnONPs (%)	50.54	61.25	72.38	79.65	83.92

was found to be 12 mm in *S. aureus* and 11 mm in *C. albicans*, which represents the efficacy of the synthesized ZnO NPs.

#### *In vitro* Anti-diabetic Activity

##### *Alpha-Amylase Enzyme Inhibition*

The enzyme amylase breaks down or transforms carbohydrates into glucose, and as a result, it is crucial for the regulation of blood glucose levels. There must be a technique to manage glucose levels, as high glucose levels might cause major clinical problems. To assess the anti-diabetic activity of ZnO NPs, various quantities of nanoparticles (10-50  $\mu\text{g/mL}$ ) have been examined against the enzyme, and the results showed maximum inhibition of 50, 61, 73, 77, 86 % at the various concentrations of 10, 20, 30, 40, 50  $\mu\text{g/mL}$ . The standard readings of anti-diabetic activity were observed as 52, 64, 76, 80, and 84% at the concentration of 10-50  $\mu\text{g/mL}$ . Overall, the anti-diabetic assay showed similar variations, with dose-dependent inhibition, as shown in Figure 4A.

##### *Beta-Glucosidase Enzyme Inhibition*

As shown in Figure 4B, ZnO NPs exhibited a similar percentage to the standard, which was concentration-dependent in the  $\beta$ -glucosidase enzyme assay. At concentrations of 10  $\mu\text{L}$ , 20  $\mu\text{L}$ , 30  $\mu\text{L}$ , 40  $\mu\text{L}$ , and 50  $\mu\text{L}$ , the corresponding percentages of inhibition were 49%, 62%, 74%, 76%, and 85%. The percentage of inhibition increased in concentration-dependent ranging from 52% to 88% for the standard. The results indicate not only the efficacy of ZnO NPs, but also how the herbal formulation enhances the anti-diabetic activity of these nanoparticles. These findings suggest the possible ways for novel therapeutic strategies for green-synthesized ZnO NPs in diabetes management. The values for both alpha-amylase and beta-glucosidase enzyme inhibition are shown in Table I.

#### DISCUSSION

Overall, this study defines the potential of *Aspalathus linearis* (red tea) and *Syzygium aromaticum* (clove)-mediated ZnO NPs in the eradication of oral pathogens and their anti-diabetic effects using alpha-amylase and beta-glucosidase enzyme inhibition assays. In terms of anti-microbial activity, *S. aureus* and *E. faecalis* showed a higher zone of inhibition of approximately 12 mm at a concentration of 100  $\mu\text{g/mL}$ , whereas *S. mutans* and *C. albicans* were less resistant to the synthesized ZnO NPs. In anti-diabetic activity, the inhibition percentage of synthesized ZnO NPs using alpha-amylase inhibition assay ranges from 50%, 61%, 73%, 77%, and 86% whereas the standard value ranges up to 52, 64, 76, 80, and 88% of inhibition using alpha-amylase inhibition assay. Similarly, the  $\beta$ -glucosidase enzyme inhibition method demonstrated a concentration-dependent inhibition pattern, with increasing percentages from 49%, 62%, 74%, 76%, and 85%, whereas the standard ranged from 52 to 88%. Overall, the synthesized ZnO NPs exhibited potential anti-microbial and anti-diabetic activities, which could be further developed into beneficial therapeutic agents.

In a previous study, ZnO-NPs were effectively synthesized by optimizing the extraction and synthesis process using aqueous *Allium cepa* L. waste peel extract. Using a conventional methodology, we examined the antioxidant and antibacterial activities of green-synthesized ZnO NPs to demonstrate their potential bioactivity. The growth of inhibition was observed to be exclusively greater in the synthesized ZnO NPs than in the usual antibacterial product.<sup>17</sup> Similarly, the antibacterial potential of red tea and clove-mediated ZnO NPs showed a maximum zone of inhibition which displays the impact of ZnO NPs at various concentrations on pathological bacterial strains and the bacterial growth was substantially suppressed by high quantities of nanoparticles (5 mg/mL). ZnO NPs derived from leaf and flower extracts consistently inhibited all bacterial strains utilized in the analysis, as indicated by the zones of

inhibition. It is hypothesized that the generation of reactive oxygen species (ROS) following bacterial cell membrane attachment, which results in membrane damage and protein malfunction, is the mechanism of action responsible for suppressing bacterial growth.<sup>18</sup> The potential reason for the antibacterial activity of green-synthesized ZnO NPs could be attributed to the presence of leaf extract, which functions as a capping agent inside the NPs, reducing particle size and augmenting anti-microbial activities. This could be explained simply by noting that smaller particles often have a higher surface-to-volume ratio, meaning that their antibacterial activities are more effective overall.<sup>19</sup> Plant extracts of red tomato fruit (*Lycopersicon esculentum* M.), chamomile flowers (*Matricaria chamomilla* L.), and olive leaves (*Olea europaea*) were successfully used in this study to biosynthesize ZnO NPs. ZnO NPs synthesized from olive leaves (*Olea europaea*) at 16.0 µg mL<sup>-1</sup> showed the maximum zone of inhibition in antibacterial activity. The study showed that the antibacterial efficacy of the synthesized ZnO NPs was size-dependent.<sup>20</sup>

The potential medicinal uses of biosynthesized NPs as anti-diabetic medications have been investigated. Compared to the standard medication, Zn-doped *Catharanthus roseus* NPs demonstrated good α-amylase inhibitory activity. Overall, this research showed that Zn-doped *C. roseus* NPs can have strong anti-diabetic effects. Furthermore, when compared to the standard sample, these green NPs exhibited zero adverse impacts.<sup>21</sup> In a previous study, the solution combustion approach was used to synthesize ZnO NPs for the first time using extracts from Areca catechu leaves. Several bioprocesses, including glucose metabolism, are facilitated by Zn. The effects of biosynthesized ZnO NPs on anti-diabetic and anti-cancer activities were investigated in this study. ZnO NPs demonstrated excellent efficacy in treating diabetic problems, as well as potent anti-cancer activity against cancer cell types. These results provide different ways and prospective uses of plant-mediated nanoparticles in many biological issues.<sup>22</sup> A biological synthesis approach was used to successfully synthesize Pro-ZnO NPs from methanol propolis extract. Pro-ZnO NPs were identified as nanoparticles with a hexagonal quartzite structure. Pro-ZnO NPs demonstrated substantial inhibition rates against α-amylase, and α-glucosidase-ZnO NPs can thus be used as a natural anti-diabetic drug.<sup>23</sup> Similarly, the anti-diabetic effects of red tea and clove-mediated ZnO NPs were evaluated using alpha-amylase and beta-glucosidase enzyme inhibition. The alpha-amylase inhibitory assay showed a great potential of 85% at a concentration of 50 µL, and 86% in beta-glucosidase enzyme inhibitory assay, which showed the concentration-dependent inhibition. Thus, the synthesized ZnO NPs have great potential as novel drugs for diabetes management.

## CONCLUSION

The era of modern medicine is an important field that can utilize the green synthesis of nanoparticles assisted by plants and herbs, which may be a replacement for future medicine. The use of medicinal and herbal plants has increased

because of fewer adverse effects and because they are significantly effective against infectious pathogens. Based on previous studies, it is concluded that herbal-assisted ZnO NPs show potentially effective anti-diabetic and anti-microbial effects. Based on these findings, green synthesis of ZnO NPs can be used in additional research, such as toxicological testing and investigations involving animals, for further improvements. It has been implicated in the healing of wounds in diabetic patients, antifungal and antibacterial lotions for infections, and nanoparticle-coated dental implants.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest would prejudice the impartiality of this scientific work.

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