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Research Article

Harnessing Satureja Mutica Extract for Green Synthesis of Zinc Oxide Nanoparticles: Antibacterial Efficacy and Catalytic Applications in Xanthene Synthesis

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ABSTRACT

In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized via a green chemistry approach using *Satureja mutica* extract as both a reducing and capping agent. The successful formation of ZnO NPs was confirmed through UV–visible spectroscopy, which revealed surface plasmon resonance at 356 nm. Fourier Transform Infrared Spectroscopy (FTIR) analysis identified phenolic compounds in the plant extract responsible for nanoparticle reduction and stabilization. Characterization of the ZnO NPs using Field Emission Scanning Electron Microscopy (FESEM), X-ray Diffraction (XRD), and Dynamic Light Scattering (DLS) showed that the nanoparticles are spherical with an average diameter of 42 nm and exhibit high stability. The catalytic potential of these biosynthesized ZnO NPs was evaluated in the synthesis of xanthene derivatives through a reaction of aromatic aldehydes with dimedone, yielding products with efficiencies ranging from 85% to 98%. Furthermore, antibacterial assays demonstrated that the ZnO NPs exhibit significant inhibitory effects against both Gram-positive and Gram-negative bacterial strains. These findings underscore the efficacy of *Satureja mutica* extract as a sustainable, non-toxic, and biocompatible method for ZnO NP synthesis, with substantial potential for applications in catalysis, bioecology, and biomedical fields.

Keywords: Zinc Oxide Nanoparticles, *Satureja mutica*, Green Synthesis, Catalysis, Antibacterial Activity, Xanthene Derivatives, Nanoparticle Characterization

1. Introduction

Nanotechnology is a foundational and rapidly advancing research domain within modern science, offering numerous applications across various disciplines and profoundly impacting multiple aspects of human life [1]. Nanoparticles, defined as particles with dimensions ranging from 1 to 100 nm, exhibit distinctive properties such as an exceptional surface-to-volume ratio and high surface energy [2]. Historically, nanoparticles were synthesized exclusively through physical and chemical methods [3-5]. These conventional approaches are typically costly, necessitate high temperatures and pressures, and generate toxic byproducts harmful to both the environment and living organisms [6-8].

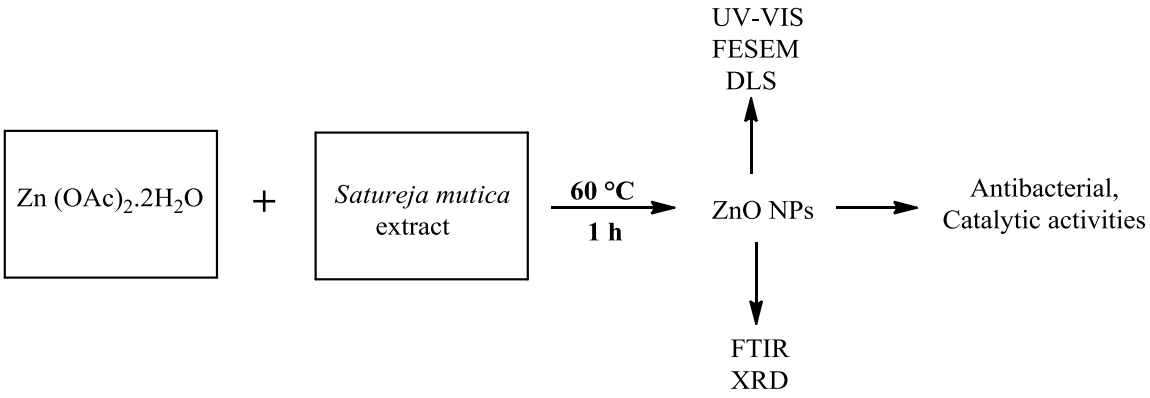
Recent advancements, however, underscore the pivotal role of biological systems (green methods) in the synthesis of metal nanoparticles, presenting a promising alternative to traditional techniques. These green methods are cost-effective, straightforward, environmentally friendly, and suitable for large-scale production [9-10]. Various biological entities, including bacteria, fungi, yeast, and plant extracts, are employed in the green synthesis of nanoparticles [11]. Microbial synthesis, which utilizes diverse microorganisms, finds applications in numerous fields, particularly medicine. Nonetheless, the initial phase of this method, involving the preparation and cultivation of microorganisms, is often more time-consuming and costly compared to the extraction of plant materials [12]. Consequently, plants are favored over microorganisms due to the absence of cell culture requirements and the less time-intensive preparation of raw materials for nanoparticle synthesis [13].

In recent years, metal oxide nanoparticles have garnered significant interest from scientists due to their unique properties and versatile applications, spanning catalysis, optoelectronics, biological markers, and pharmaceutical and medical uses [14-15]. Among metal oxide nanoparticles, ZnO nanoparticles (NPs) have attracted considerable attention. Zinc oxide nanoparticles are noted for their lower toxicity and greater environmental compatibility compared to other metal nanoparticles, making them suitable for biomedical applications such as anticancer treatments, drug delivery, antibacterial agents, and diabetes management [16-19].

The genus *Satureja*, a member of the *Lamiaceae* family, is a well-known plant species predominantly found in Mediterranean regions [20]. In Iran, 16 species of savory grow mainly in the northern regions on calcareous soils and in moderate climates. *Satureja mutica* is a perennial, semi-woody plant with an average height of 40 cm, characterized by numerous flowering branches [21-22]. The plant's most significant phytochemical constituents include

phenols, thymol, carvacrol, and flavonoids [23-24]. Traditionally, this plant has been used to treat various ailments such as indigestion, muscle pain, diarrhea, and nausea [25-26].

There are numerous reports on the biosynthesis of zinc oxide nanoparticles using various plants, including *Glycosmis pentaphylla* [27], *Quassia indica* [28], *Rhododendron arboretum* [29], *Drynaria quercifolia tuber* [30], *Ziziphus spina-christi* [31], *Solanum lycopersicum* [32], and *Jatropha gossypifolia* [33]. However, many plants with potential for nanoparticle synthesis remain unexplored. Given the significance of green nanoparticle synthesis and its extensive applications in medicine, industry, and agriculture, and considering that *Satureja mutica* extract has not been previously utilized for zinc oxide nanoparticle production, this study reports the synthesis of zinc oxide nanoparticles using *Satureja mutica* extract (Scheme 1). Additionally, the antibacterial properties and catalytic activity of the synthesized nanoparticles are evaluated.



Scheme 1. The synthesis of ZnO NPs using *Satureja mutica* extract schematically

2. Experimental

2.1. Materials

The flowering branches of *Satureja mutica* were collected at the 50% flowering stage at the end of June from the field of Alborz Research Station (Alborz Province in Iran). Zn $(OAc)_2.2H_2O$ and Mueller Hinton broth medium were purchased from Merck Company. The microorganisms used in this experiment were Gram-negative bacteria *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853,* and Gram-positive bacteria *Staphylococcus aureus ATCC 25923* and *Bacillus subtilis ATCC 6633*. These strains of bacteria were obtained from Ira's National Center of Genetic and Biological Resources.

2.2. Preparation of Satureja mutica Extract

To obtain *Satureja mutica* extract, 4 g of the plant powder was heated in 200 mL of deionised water at 80 °C for 30 minutes. After cooling to room temperature, the solution was filtered twice through Whatman No. 1 paper to obtain a clear solution. The filtered solution was collected and stored in the refrigerator at four °C.

2.3. Green Synthesis of ZnO NPs using Satureja mutica Extract

20 mL of *Satureja mutica* extract was added to 100 mL of 10 mM Zinc acetate dihydrate solution, which was stirred at 60 °C. After 20 minutes, fine particles appeared in the solution, and the colour of the solution changed from yellow to green-yellow, which indicates the formation of zinc oxide nanoparticles. This solution was stirred under these conditions for one hour until the reaction was complete. The solution was applied directly for FESEM, DLS, and UV-vis spectroscopy. Then, centrifugation was performed at 10,000 rpm for 15 minutes. After washing several times with distilled water, sediment was dried at 60 °C for 10 h, followed by calcination at 400 °C for 3 h to obtain pure ZnO NPs. The pure nanoparticles were used for FTIR, XRD, antibacterial, and catalytic activities.

2.4. Evaluation of Antibacterial Effects of ZnO NPs: Minimum Inhibitory Concentration (MIC)

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and *Bacillus subtilis* bacteria were grown in Mueller Hinton broth medium at concentrations of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL. The synthesised zinc oxide nanoparticles were used to evaluate the antibacterial effects on bacteria. Concentrations were determined by broth micro-dilution technique in a sterile 96-well plate. A volume of 100 μL of nanoparticles synthesised at a concentration of 2 mg/mL was placed into the plate microtiter's first well containing 100 μL MHB medium to obtain a 1 mg/mL concentration. Serial dilution was performed by pumping the contents of the first well, removing 100 μL from it, and adding it to the second well. This operation was done to the last well. Then, 100 μL of bacterial suspension, equivalent to 0.5 McFarland (1.5×10^6 CFU/ mL), was added to the wells. Each plate was prepared with a set of controls. Plates were placed in an incubator at 37 °C for all bacteria for 24 hours. The lowest concentration at which no visible bacterial growth could be found was taken as the MIC value [34].

2.5. General Procedure for the Synthesis of 1,8-Dioxo-octahydroxanthene Derivatives (3)

A mixture of aromatic aldehyde (1 mmol), dimedone (2 mmol), and the biosynthesised ZnO NPs (10 mol %) in EtOH (10 mL) was stirred in reflux condition. After completion of the reaction (as monitored by TLC), the catalyst was separated by filtration, and the solvent was concentrated in a rotary evaporator. The crude product was purified by recrystallisation from EtOH to obtain the pure compound. All the products were known compounds. They were characterised by their melting points and ^{1}H NMR spectral data.

2.6. Selected Spectral Data

2.6.1. 3,4,6,7-Tetrahydro-3,3,6,6-tetramethyl-9-phenyl-2H-xanthene-1,8(5H,9H)-dione (3a)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.02 (s, 6H), 1.12 (s, 6H), 2.22 (q, J = 16.2 Hz, 4H), 2.48 (s, 4H), 4.75 (s, 1H), 7.10-7.13 (m, 1H), 7.243-7.25 (m, 2H), 7.29-7.32 (m, 2H).

2.6.2. 3,4,6,7-Tetrahydro-3,3,6,6-tetramethyl-9-(4- Chlorophenyl)-2H-xanthene-1,8(5H,9H) -dione (3d)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.04 (s, 6H), 1.13 (s, 6H), 2.25 (q, J = 15.8 Hz, 4H), 2.56 (s, 4H), 4.87 (s, 1H), 7.03 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.0 Hz, 2H).

2.6.3. 3,4,6,7-Tetrahydro-3,3,6,6-tetramethyl-9-(2-nitrophenyl)-2H-xanthene-1,8(5H,9H) -dione (3e)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.04 (s, 6H), 1.17 (s, 6H), 2.21-2.54 (m, 8H), 4.90 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.49 (td, J = 7.6, 1.2 Hz, 1H), 7.56 (dd, J = 7.6, 1.2 Hz, 1H).

2.6.4. 3,4,6,7-Tetrahydro-3,3,6,6-tetramethyl-9-(3-methoxyphenyl)-2H-xanthene-1,8(5H,9H)-dione (3h)

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 1.03 (s, 6H), 1.12 (s, 6H), 2.23 (q, *J* = 16.6 Hz, 4H), 2.47 (s, 4H), 3.79 (s, 3H), 4.76 (s, 1H), 6.65-6.69 (m, 1H), 6.88-6.91 (m, 2H), 7.15 (t, *J* = 7.8 Hz, 1H).

3. Results and Discussion

As previously documented, *Satureja mutica* contains bioactive phenolic and flavonoid compounds [23-24]. Given the potential reducing role of these compounds, this study is the first to utilize this plant for the synthesis of ZnO nanoparticles. Furthermore, the catalytic

activity and antibacterial effects of these nanoparticles against various bacteria were investigated.

3.1. Synthesis and Characterization of the Prepared ZnO NPs using Satureja mutica Extract

Adding an extract of *Satureja mutica* to zinc acetate dihydrate salt solution leads to the reduction of zinc ions and the formation of zinc oxide nanoparticles, accompanied by a change in the colour of the solution from yellow to yellow-green.

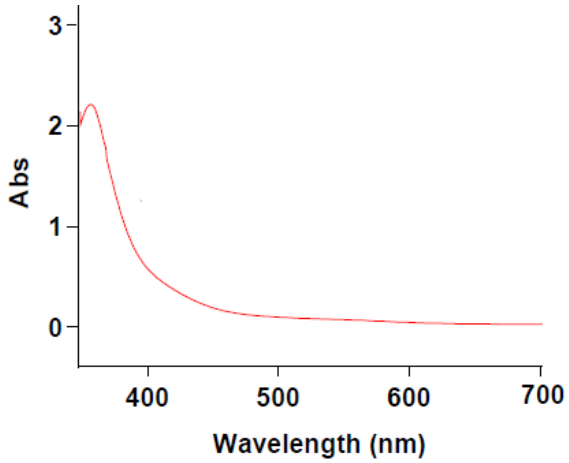


Figure 1. UV-vis spectra of the biosynthesised ZnO NPs using *Satureja mutica* extract

The formation and stability of the biosynthesized ZnO nanoparticles using *Satureja mutica* extract were examined through UV–vis spectroscopy (Figure 1). The absorption spectrum of the reaction solution displayed a maximum absorption at 356 nm [35], corresponding to the surface plasmon resonance due to the excitation of free electrons in the zinc oxide nanoparticles.

To elucidate the role of the plant extract in reducing $Zn^{2+}$to ZnO and stabilizing the nanoparticles, FTIR spectra of both the zinc oxide nanoparticles and *Satureja mutica* extract were recorded across wavelengths ranging from 400 to 4000 cm^{-1}. In the FTIR spectrum of the extract (Figure 2), a broad peak at 3317 cm^{-1} was attributed to the stretching vibration of the OH group. The band at 2923 cm^{-1} corresponded to aliphatic C-H stretching vibrations.

Absorption peaks at 1606, 1518, and 1414 cm^{-1} were associated with C=C stretching vibrations, while the band at 1050 cm^{-1} was due to C-O group stretching vibrations. Weak

absorption peaks in the 500-1000 cm^{-1} range were related to aromatic C-H out-of-plane bending vibrations, characteristic of polyphenols.

In the FTIR spectrum of ZnO nanoparticles, the peak at 423 cm^{-1} was attributed to the stretching vibration of the Zn-O bond (Figure 3), confirming the formation of ZnO nanoparticles [36]. Additionally, absorption bands corresponding to the plant extract were observed with slight shifts in peak absorption frequencies in the zinc oxide nanoparticle spectra, indicating the presence of the extract adsorbed on the nanoparticle surfaces.

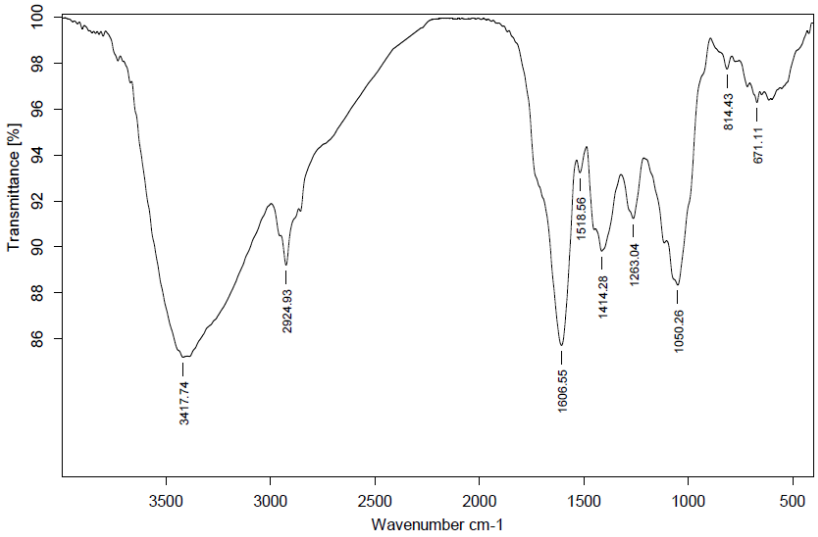
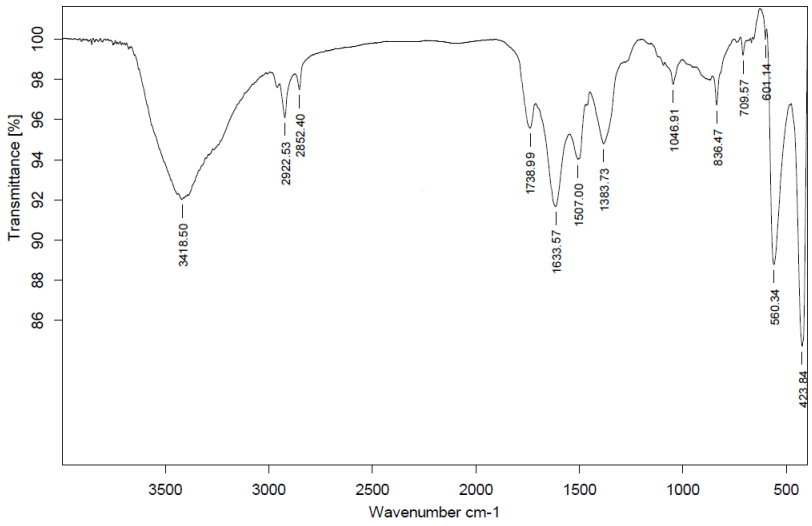


Figure 2. FTIR spectra of *Satureja mutica* extract



(b)

Figure 3. FTIR spectra of ZnO NPs

X-ray diffraction analysis was employed to examine the crystal structure of ZnO nanoparticles synthesized using *Satureja mutica* extract (Figure 4). The crystalline peaks observed at 2θ values of 31.82, 34.39, 36.36, 47.54, 56.60, 62.82, 66.46, 67.98, 69.09, 72.53, and 76.99 correspond to reflections from the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) crystal planes (JCPDF file no. 01-080-0075). The sharp peaks in the XRD pattern indicate a high degree of crystallinity in the nanoparticles. The (101) peak intensity is greater than that of the other peaks, indicating that the crystal planes of the ZnO

nanoparticles are primarily oriented in this direction. Using the Scherrer equation, the average size of the ZnO nanoparticles was estimated to be 25 nm.

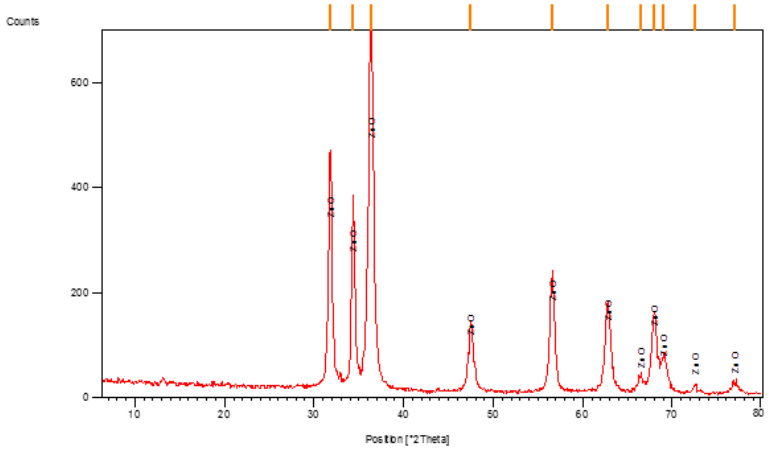


Figure 4. XRD pattern of the prepared ZnO NPs

The shape and size of the synthesized ZnO nanoparticles were examined using FESEM imaging (Figure 5). The FESEM image revealed that the nanoparticles are predominantly spherical, with sizes ranging from 33 to 51 nm.

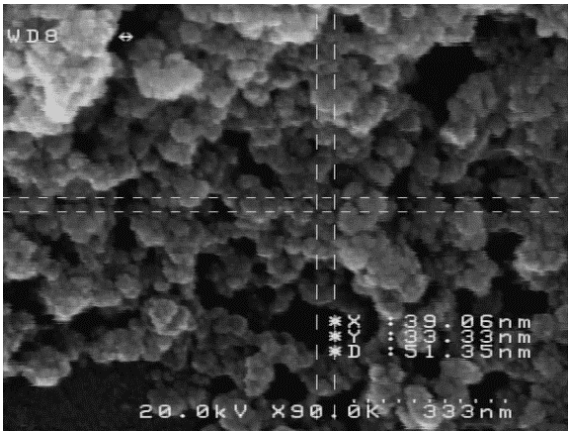


Figure 5. FESEM image of the prepared ZnO NPs

DLS analysis was utilized to determine the size distribution of ZnO nanoparticles. The average size of the ZnO nanoparticles, as estimated by DLS, ranges from 30 to 60 nm, with a mean size of approximately 45 nm (Figure 6).

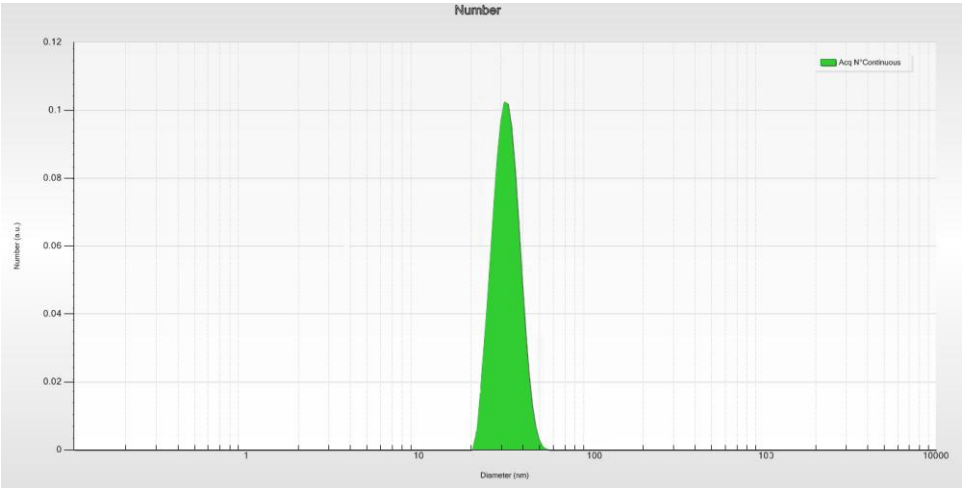


Figure 6. Size distribution of the prepared ZnO NPs

Raut et al. synthesized ZnO nanoparticles using *Ocimum tenuiflorum* extract, achieving sizes of approximately 11-25 nm and a hexagonal shape [37]. In this study, the synthesized zinc oxide nanoparticles had a size range of 33-51 nm and were spherical. Similarly, Yedurkar et al. used zinc acetate and *Ixora coccinea* leaf extract to produce spherical zinc oxide nanoparticles with sizes ranging from 80 to 130 nm [38]. Although the particle sizes differed from those in this study, the spherical shape was consistent. Bala et al. also synthesized spherical nanoparticles using zinc acetate and *Hibiscus subdariffa* extract, with sizes ranging from 12 to 46 nm [39]. Additionally, Bhumi et al. employed *Catharanthus roseus* leaf extract to produce zinc oxide nanoparticles, resulting in sizes between 23 and 57 nm, which were similar in both shape and size to the nanoparticles prepared in our research [40].

3.2. MICs of the Biosynthetized ZnO NPs

Antibiotic resistance is one of the most critical challenges facing the global healthcare system, with the rise of multidrug-resistant infections severely impacting current antibacterial treatments. Numerous efforts have been made to develop new antimicrobial materials, such as plant-mediated nanomaterials, which contain diverse bioactive compounds with proven

therapeutic properties [41-43]. Plant extracts have emerged as a promising approach for nanoparticle synthesis due to their dual role as reducing and capping agents.

In this study, the antibacterial activity of the ZnO nanoparticles synthesized using plant extract was evaluated. The minimum inhibitory concentration (MIC) of the biosynthesized ZnO nanoparticles was tested against several bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. The MIC results were obtained after incubation at 37 °C. Among the tested strains, *S. aureus* exhibited higher sensitivity to the zinc oxide nanoparticles at all concentrations. The biosynthesized ZnO nanoparticles demonstrated an average inhibiting effect on bacteria, with an MIC of 0.5 mg/mL for *E. coli, B. subtilis, and P. aeruginosa*, and 0.25 mg/mL for *S. aureus* (Table 1).

Bacterial strains	*P. auerogínosa*	*E. coli*	*B. subtilis*	*S. aureus*
MIC (mg/mL)	0.5	0.5	0.5	0.25

Table 1. MIC values of the biosynthesised ZnO NPs by *Satureja mutica* extract

The results obtained in this study are comparable to those reported in several recent papers in the literature [44-45]. Also, the antibacterial activity of zinc oxide nanoparticles was compared with the aqueous extract of *Satureja mutica*. The extract did not show antibacterial activity at 1 mg/mL, the highest concentration of the samples examined in this study.

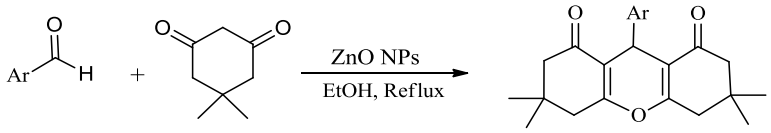
The exact mechanism of ZnO's antibacterial effect is still under discussion. A probable mechanism is based on electrostatic forces, which lead to the direct killing of bacteria due to the binding of the abrasive surface texture of ZnO nanoparticles to the bacterial surface [46].

3.3. Catalytic Activity of the Biosynthesised ZnO NPs by Satureja mutica Extract

Xanthenes represent a vital class of heterocyclic compounds with notable properties, including various biological [47] and pharmacological activities such as antibacterial [48], antiviral [49], anticancer [50], and anti-inflammatory [51] effects. Given the absence of reports on using ZnO nanoparticles produced by plant extracts for synthesizing xanthene derivatives, we were motivated to investigate the catalytic effect of ZnO nanoparticles generated using Satureja mutica extract in the preparation of 1,8-dioxooctahydroxanthenes.

The reaction between benzaldehyde and 5,5-dimethylcyclohexane-1,3-dione (dimedone) in refluxing ethanol was chosen as a model to optimize the catalyst amount. This reaction was conducted using 2, 5, 8, and 10 mol% of ZnO nanoparticles (Table 2, entries 1-4). It was determined that using 10 mol% of the biosynthesized ZnO nanoparticles in ethanol at 78 °C was sufficient to complete the reaction with maximum yield (Table 2, entry 1). Additionally, the model reaction was tested without zinc oxide nanoparticles, resulting in only a slight amount of the desired product (Table 2, entry 5). These findings demonstrate the catalytic activity of the biosynthesized ZnO nanoparticles in synthesizing 1,8-dioxooctahydroxanthenes.

To showcase the general applicability of this method, various aromatic aldehydes were reacted with dimedone to synthesize xanthene derivatives in the presence of 10 mol% of the biosynthesized ZnO nanoparticles (Table 2). When dimedone reacted with aromatic aldehydes containing halogen and electron-withdrawing groups on the phenyl ring, the corresponding products were formed with yields of 94-98% (Table 2, entries 6–11). Benzaldehydes with electron-donating groups also produced high yields of the desired products (Table 2, entries 12–15), although these reactions required longer times and resulted in lower yields compared to benzaldehydes containing electron-withdrawing groups.



1a-k **2** **3a-k**

Entry	Ar	Product [b]	Amount of catalyst (mol %)	Time (min)	Yield [c]	M.p (°C) [Ref]
1	C_6H_5 (1a)	3a	10	90	95	203–205[52]
2	C_6H_5 (1a)	3a	8	90	65	203–205[52]
3	C_6H_5 (1a)	3a	5	90	50	203–205[52]
4	C_6H_5 (1a)	3a	2	90	40	203–205[52]
5	C_6H_5 (1a)	3a	-	12	5	203–205[52]

6	2-Br-C_6H_4 (1b)	3b	10	80	94	225–227[53]
7	3-Cl-C_6H_4 (1c)	3c	10	65	96	182–185[52]
8	4-Cl-C_6H_4 (1d)	3d	10	35	98	226–229[52]
9	2-NO_2-C_6H_4 (1e)	3e	10	40	95	245–247[52]
10	3-NO_2-C_6H_4 (1f)	3f	10	35	96	169–170[52]
11	4-NO_2-C_6H_4 (1g)	3g	10	35	97	226–227[52]
12	3-MeO-C_6H_4 (1h)	3h	10	120	90	161–163[54]
13	4-MeO-C_6H_4 (1i)	3i	10	125	88	241–242[52]
14	4-Me-C_6H_4 (1j)	3j	10	120	88	216–217[55]
15	4-HO-C_6H_4 (1k)	3k	10	125	85	245–247[52]

Table 2. Synthesis of 1,8-dioxo-octahydroxanthene derivatives (3) using the biosynthesised ZnO NPs as a catalyst [a]

[a] Reaction conditions: reactants 1 (1.0 mmol) and 2 (2.0 mmol) in refluxing ethanol. [b] All compounds are known, and their structures were established from their ^{1}H NMR spectral data and melting points compared to literature values.

[c] Yields refer to isolated products.

In our previous work, ZnO nanoparticles synthesized via the uniform precipitation method were employed as catalysts for the preparation of 1,8-dioxooctahydroxanthene derivatives [56]. The results obtained from the synthesis of xanthenes using the biosynthesized ZnO nanoparticles are comparable to those reported in our earlier study. This comparison demonstrates that the biosynthesized ZnO nanoparticles are equally effective as catalysts for organic reactions, underscoring their efficiency in catalyzing the formation of xanthene derivatives.

4. Conclusions

In this study, zinc oxide nanoparticles were synthesized through a biological method utilizing *Satureja mutica* extract, achieving nanoparticle production without the use of harmful chemicals or environmental damage. UV–vis spectroscopy confirmed the successful formation of the zinc oxide nanoparticles. Characterization techniques, including FESEM, XRD, and DLS, revealed that the nanoparticles are predominantly spherical with an average particle size of 42 nm. The ZnO nanoparticles synthesized with *Satureja mutica* extract exhibited effective antibacterial activity against several bacterial strains, with MIC values ranging from 0.25 to 0.5 mg/mL. Additionally, these biosynthesized ZnO nanoparticles demonstrated excellent catalytic performance in the synthesis of 1,8-dioxooctahydroxanthene derivatives in ethanol.

Compared to nanoparticles synthesized from other plant sources, the ZnO nanoparticles produced using *Satureja mutica* were smaller in size and were prepared using a relatively low concentration of plant extract. This highlights a notable advantage of this approach over similar methods reported in the literature. Overall, the findings of this study affirm that *Satureja mutica* serves as a low-cost, non-toxic, and environmentally friendly natural resource for the synthesis of ZnO nanoparticles. This method holds potential for the development of new antibacterial agents for biomedical applications as well as for the creation of inexpensive, green catalysts for a variety of chemical transformations.

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