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Development of a bio-synthesized zinc oxide nanoparticle sensor for the quantification of totarolone in *Tetraclinis articulata*

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Diterpenoids such as totarolone exhibit significant bioactivity, making their accurate quantification in plant extracts essential for pharmacological studies and quality control. Conventional analytical methods are often time-consuming, costly, or environmentally demanding, highlighting the need for rapid, sensitive, and eco-friendly alternatives. In this work, we report the electrochemical quantification of totarolone, a bioactive diterpenoid, in *Tetraclinis articulata* extract using a carbon paste electrode modified with green-synthesized zinc oxide (bio-ZnO) nanoparticles. Bio-ZnO was prepared *via* a plant-mediated route using *Calamintha nepeta* extract, providing a sustainable and eco-friendly alternative to conventional chemical synthesis. XRD analysis revealed that the bio-ZnO nanoparticles possess a hexagonal wurtzite structure with an average crystallite size of ~10 nm. The modified electrode exhibited enhanced sensitivity and stability, enabling the effective detection of totarolone by cyclic voltammetry (CV) and square wave voltammetry (SWV). A linear analytical response was obtained, with a LOD of 1.19 μM , a LOQ of 3.98 μM and a measured concentration of 0.133 mM in the plant extract. These findings highlight the potential of green nanomaterial-based electrochemical sensors for the reliable and sustainable analysis of bioactive compounds.

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Sustainability spotlight

The detection and quantification of bioactive molecules from medicinal plants are essential for ensuring their safe, effective, and standardized use in pharmaceutical and nutraceutical applications. However, traditional analytical approaches and nanoparticle synthesis methods often rely on toxic chemicals and energy-intensive processes. In this work, we present a sustainable alternative by using *Calamintha nepeta* extract to synthesize ZnO nanoparticles, which are then used to fabricate a sensitive electrochemical sensor for totarolone determination. This green methodology minimizes environmental impact, aligns with the principles of green chemistry, and supports responsible innovation. The study directly contributes to UN SDG 3 (good health and well-being) and SDG 12 (responsible consumption and production) by promoting eco-friendly sensor development and safe natural product analysis.

1. Introduction

Aromatic and medicinal plants have long served as valuable sources of bioactive compounds for traditional remedies and modern pharmacological applications.^{1,2} While significant attention has been devoted to widely used species, forest plants, particularly those from the Cupressaceae family, remain comparatively underexplored.³ Among them, *Tetraclinis*

articulata, an evergreen tree native to the Mediterranean region, stands out for its remarkable adaptability to harsh climatic conditions. Traditionally, this species has been widely utilized in herbal medicine for its therapeutic benefits.⁴ Scientific studies have confirmed the diverse biological activities of *T. articulata* extracts and essential oils, demonstrating antioxidant, antimicrobial, insecticidal, cytotoxic, and anti-inflammatory properties.^{5,6} These findings underscore its potential for various medicinal applications and highlight the need for further exploration of its bioactive constituents.

One such constituent, totarolone, is a naturally occurring diterpenoid that exhibits notable pharmacological activities, such as anti-lymphangiogenic effects, making it a candidate for therapeutic development in cancer research.^{7,8} Beyond its medicinal potential, its bioactive nature makes it an interesting subject for green chemistry approaches, where plant-derived molecules are explored as sustainable alternatives to synthetic

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Fig. 1 FTIR spectra of (A) *Calamintha nepeta* extract and (B) bio-synthesized ZnO nanoparticles.

the extraction, the crude residue was recovered by evaporation under reduced pressure. This extract, which contains the bioactive compounds, was stored at 4 °C in the dark for subsequent electrochemical analysis of totarolone content.²⁷

2.6. Electrochemical detection protocol

All experiments were conducted using a potentiostat OrigaStat 100 equipped with Origamaster5 software. A conventional three-electrode system was employed, comprising bio-ZnO/CPE as the working electrode, a platinum wire as the counter



Fig. 2 XRD analysis of bio-synthesized ZnO nanoparticles.



Fig. 3 SEM coupled with EDX analysis of CPE.

electrode, and a saturated calomel electrode (SCE) as the reference. Cyclic voltammetry (CV) was performed to detect pure totarolone in a 0.1 M NaCl supporting electrolyte (pH 4.5) at a concentration of 2 mM and a potential range from 1 V to 1 V



Fig. 4 SEM coupled with EDX analysis of bio-NPs-ZnO/CPE.



demonstrates strong potential as a sensitive electrochemical sensor for totarolone detection.⁴⁰

3.3. Optimization of experimental variable

3.3.1. Scan rate. To analyze the reaction kinetics of totarolone at the modified CPE, the effect of the scan rate (50–250 mV s⁻¹) on the oxidation peak current (I_{pa}) was investigated. As shown in Fig. 7A, I_{pa} increased steadily with increasing scan rate. Fig. 7B shows that the curve of the scan rate *vs.* anodic current was more linear than the plot of the square root of the scan rate *vs.* the anodic current (Fig. 7C), revealing that mass



Fig. 7 (A) Bio-NPs-ZnO/CPE CVs in 0.1 M NaCl solution (pH = 4.5) with 2 mM of totarolone at various SRs and a start potential of -1.0 V *vs.* Hg/Hg₂Cl₂, KCl sat. (B) Plot of I_{pa} *versus* v . (C) Plot of I_{pa} *versus* $v^{1/2}$.

transfer of totarolone on the bio-ZnO/CPE surface was mainly controlled by adsorption instead of diffusion.^{41,42}

3.3.2. pH effect. The influence of pH on the electrode response was investigated over a pH range from 3.5 to 6.5. The pH-dependent behavior (Fig. 8A) can be rationalized by a balance between adsorption and electron-transfer kinetics. From pH 3.5 to 4.5, hydrogen bonding between totarolone and hydroxylated bio-ZnO enhances adsorption, increasing the peak current. At pH 5.5, partial deprotonation to phenolate reduces hydrogen bonding and introduces electrostatic repulsion with the bio-ZnO surface, leading to a transient decrease. At pH 6.5, the predominance of the phenolate form of totarolone—more electroactive—improves electron-transfer kinetics enough to overcome the reduced adsorption, resulting in a slightly higher current.⁴³

Furthermore, information regarding the number of proton(s) and electron(s) involved in the redox process can be used to determine the structural evolution of totarolone after the electrooxidation reaction on bio-ZnO-NPs/CPE using eqn (1):⁴⁴

$$E_p = -2.303 \frac{mRT}{nF} \text{pH} \quad (1)$$

where E_p is the redox potential (mV), R is the gas constant ($\text{J mol}^{-1} \text{K}^{-1}$), T is the temperature (K), F is the Faraday constant



Fig. 8 (A) Influence of solution pH on the oxidation peak current of totarolone recorded at bio-NPs-ZnO/CPE in 0.1 M NaCl, at a scan rate of 100 mV s⁻¹ and a start potential of -1.0 V *vs.* Hg/Hg₂Cl₂, KCl sat, and (B) linear plot of E_{pa} *vs.* pH.





Fig. 9 Effect of accumulation time on the peak current of totarolone at the bio-NPs-ZnO/CPE electrode in 0.1 M NaCl solution (pH 4.5), at a scan rate of 100 mV s^{-1} and a start potential of $-1.0 \text{ V vs. Hg/Hg}_2\text{Cl}_2, \text{KCl sat.}$

(C mol^{-1}), and m/n is the slope, which represents the ratio of proton(s) and electron(s). The slope of the corresponding linear plot of E_{pa} vs. pH (Fig. 8B) is 28.2 mV pH^{-1} , which is close to the theoretical Nernstian value (29.50 mV) for a two-electron and one-proton transfer redox process.^{45,46} Therefore, a plausible redox reaction for totarolone oxidation can be proposed as



3.3.3. Accumulation time effect. The effect of accumulation time (AT) on the detection of totarolone was investigated over a range of 0 to 4 minutes in a 0.1 M NaCl solution (pH 4.5) containing 2 mM totarolone. As shown in Fig. 9, the peak current decreased with increasing accumulation time, indicating that the modified electrode enables rapid detection of totarolone.⁴⁷ The decrease in the oxidation peak current observed with increasing accumulation time can be explained by the saturation of active sites on the surface of bio-ZnO/CPE. During the accumulation step, a negative potential of -0.4 V was applied, which promotes the adsorption of totarolone onto the ZnO-modified electrode surface. Initially, the adsorption is rapid, leading to a measurable current increase; however, as the accumulation time extends, the electrode surface becomes progressively saturated. This saturation reduces the availability of free active sites for further electron transfer, resulting in a gradual decrease of the oxidation peak current over time.⁴⁸

Fig. 5 provides complementary evidence for this behavior. After 20 minutes of preconcentration, the SEM image shows the formation of small clusters on the bio-ZnO/CPE surface. These clusters correspond to complexes formed between bio-ZnO and totarolone. The presence of these clusters supports the notion that the decrease in current with longer accumulation times arises from the formation of an adsorbed layer, which limits the electron transfer efficiency.

3.3.4. Concentration effect. The SWV voltammograms at different totarolone concentrations (Fig. 10A) show that the current intensity increased with the increase of the concentration. This demonstrates the linear relationship ($R^2 = 0.989$) from 2 mM to 12.3 mM illustrated in Fig. 10B. The sensor's sensitivity was validated by the calculation of the detection limit ($\text{LOD} = 3S_b/S$) and the quantification limit ($\text{LOQ} = 10S_b/S$). S_b is the standard deviation of seven measurements and S is the slope of the calibration plot.^{49,50} The findings obtained are illustrated in Table 1.

A calibration curve equation ($I = 0.03 [C] + 0.878$) was obtained from the standard curve of current as a function of concentration. This equation is then used to quantify totarolone in the extract.

3.3.5. Quantification of totarolone in the extract. To quantify the concentration of totarolone in the extract, a solution was prepared by dissolving 1 g of the extract in 25 mL of a 0.1 M NaCl solution, ensuring optimal solubilization of totarolone. The analysis was performed using square wave voltammetry (SWV) over a potential range from -1.5 V to 1.5 V . Fig. 11 shows



Fig. 10 (A) SW voltammograms of bio-NPs-ZnO/CPE for the determination of totarolone in 0.1 M NaCl solution (pH 4.5); amplitude: 20 mV; potential step: 5 mV; start potential: $-1.5 \text{ V vs. Hg/Hg}_2\text{Cl}_2, \text{KCl sat.}$ (B) Calibration graph corresponding to the peak of totarolone obtained from various concentrations in the range of 2 to 12.3 mM.

Table 1 LOD and LOQ values for totarolone at bio-NPs-ZnO/CPE

Concentration range	LOD	LOQ
2–12.3 mM	1.19 μM	3.98 μM





Fig. 11 SW voltammograms of bio-NPs-ZnO/CPE for the quantification of the totarolone in 1 g of extract dissolved in 25 mL of 0.1 M NaCl solution; amplitude: 20 mV; potential step: 5 mV; start potential: -1.5 V vs. Hg/Hg₂Cl₂, KCl sat.



Fig. 12 Evaluation of the reproducibility of bio-NPs-ZnO/CPE; amplitude: 20 mV; potential step: 5 mV; start potential: -1.5 V vs. Hg/Hg₂Cl₂, KCl sat.

a current peak of 0.882 mA at a potential of -0.5 V, attributed to totarolone, confirming its presence in the sample. Using the previously obtained calibration curve equation, the concentration of totarolone in 1 g of extract was determined to be 0.133 mM. This value provides an estimate of totarolone's content in the extract and helps assess the efficiency of the extraction process.

3.3.6. Reproducibility. To evaluate the repeatability of bio-ZnO-NPs/CPE in the quantification of totarolone, three consecutive measurements were performed. As shown in Fig. 12, the results demonstrate consistent sensor performance, confirming the method's reliability and reproducibility in detecting the molecule within the extract solution.⁵¹

3.3.7. Stability. The stability of bio-NPs-ZnO/CPE was evaluated after two months of storage. The electrode retained approximately 98% of its original response, as shown in Fig. 13, demonstrating its excellent long-term stability for the quantification of totarolone.



Fig. 13 Bio-NPs-ZnO/CPE stability; amplitude: 20 mV; potential step: 5 mV; start potential: -1.5 V vs. Hg/Hg₂Cl₂, KCl sat.



Fig. 14 Proposed mechanism of totarolone detection on bio-NPs-ZnO/CPE.

3.4. Proposed electrochemical sensing mechanism

The surface of biosynthesized ZnO (Fig. 14), enriched with hydroxyl groups (OH), along with residual organic groups from the plant extract (C=O, C=C, and C-N), provides abundant active sites for the adsorption of totarolone. The molecule interacts primarily *via* hydrogen bonding with the hydroxyl groups of ZnO, while the residual organic groups contribute secondary polar interactions, stabilizing the molecule on the surface. This adsorption facilitates a proton-coupled electron-transfer (PCET) process, allowing efficient transfer of electrons from totarolone to the electrode. The ZnO nanoparticles not only provide a high surface area for adsorption but also promote electron transfer between totarolone and the electrode surface, thereby enhancing the oxidation current. This mechanism explains the well-defined anodic peak observed during voltammetric measurements.

4. Conclusions

This study reports the first quantification of totarolone using bio-ZnO nanoparticles synthesized *via* *Calamintha nepeta* extract. FTIR analyses confirmed the successful formation of ZnO nanoparticles, while XRD revealed a hexagonal wurtzite structure with an average crystallite size of ~ 10 nm. SEM-EDX validated the incorporation of ZnO into the CPE matrix and



