



Green Synthesis of ZnO Nanoparticles Using *Myrcia Oblongata* DC: An Ecological Alternative for Biomedical Applications

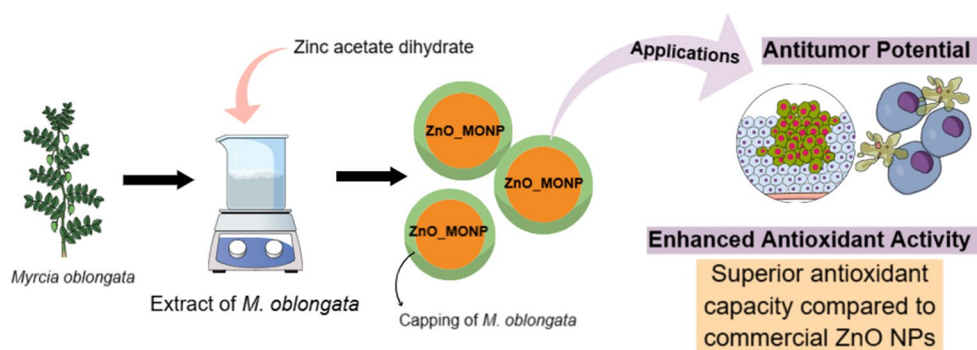
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Received: 2 December 2025 / Accepted: 17 February 2026 / Published online: 24 March 2026
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Abstract

The sustainable synthesis of metal oxide nanomaterials is a growing demand in nanobiotechnology, where ecological routes must deliver reproducible control over composition, crystallinity, and bioactivity. Here, we report the first green synthesis of zinc oxide nanoparticles (ZnO_MO NPs) using the aqueous extract of *Myrcia oblongata* DC, a native *Myrtaceae* species rich in polyphenols and terpenoids. Beyond introducing this novel plant-based route, the study provides insights into how specific phytochemical groups – especially phenolic and terpenoid compounds – can mediate Zn²⁺ complexation and hydrolysis, nucleation, and surface stabilization, contributing to a broader understanding of plant-assisted ZnO formation. The biosynthesized nanoparticles were systematically characterized by FTIR, XRD, DLS, HRTEM, UV–visible spectroscopy, photoluminescence (PL), and TGA. The ZnO_MO NPs exhibited a hexagonal zincite structure, high crystallinity, and an organic surface layer (≈45%) derived from plant metabolites, as confirmed by TGA and phenolic quantification (33.06 µg GAE mg⁻¹). UV–Vis and PL analyses revealed characteristic optical features of biofunctionalized ZnO nanostructures, indicating the presence of surface- and defect-related electronic states associated with the phytochemical capping. Compared to commercial ZnO NPs, ZnO_MO NPs displayed over twofold higher antioxidant capacity (690.6 vs. 311.2 µmol TE g⁻¹ in the ABTS assay) and a markedly lower polydispersity index (0.087±0.014), indicating enhanced colloidal homogeneity. Cytotoxicity assays revealed preferential toxicity toward glioblastoma (U87) cells while maintaining biocompatibility with non-tumoral keratinocytes (HaCaT), demonstrating a moderately selective and biocompatible therapeutic profile (SI₅₀ ≈ 1.45). These findings establish *M. oblongata* as a novel phytochemical platform for engineering multifunctional, biofunctionalized ZnO nanostructures and highlight the potential of green nanotechnology to generate biocompatible and selective materials for future biomedical applications.

Graphical Abstract



Keywords Green synthesis · Zinc oxide nanoparticles, *Myrcia oblongata* · Antioxidant activity · Antitumor potential

Introduction

In recent years, growing concerns about environmental sustainability and the harmful effects of chemical residues in biomedical and ecological systems have driven the development of biogenic and environmentally benign approaches to nanomaterial production. Among these, the green synthesis of metal oxide nanoparticles has emerged as a sustainable and non-toxic alternative to conventional chemical and physical routes. Zinc oxide nanoparticles (ZnO NPs) are particularly attractive due to their wide band gap, high catalytic activity, and intrinsic biocompatibility, which support applications ranging from nanomedicine and wound healing to biosensing and drug delivery. [1] Moreover, ZnO NPs have demonstrated antioxidant activities and selective cytotoxic effects, and when combined with conventional drugs, they may enhance therapeutic efficacy while minimizing systemic toxicity [2, 3].

However, conventional ZnO NPs syntheses often require toxic precipitating and complexing agents, organic solvents, and energy-intensive processes, leading to environmental and biomedical limitations. To overcome these drawbacks, biological synthesis has emerged as a viable alternative, employing extracts from plants, fungi, or microorganisms as complexing and stabilizing platforms. In these systems, biomolecules promote metal ion coordination, hydrolysis, nucleation, and nanoparticle stabilization while imparting natural surface biofunctionalization, which strongly influences colloidal behavior and biological interactions [4]. Plant-mediated synthesis stands out for its simplicity, cost-effectiveness, and scalability, as well as the inherent bioactivity of phytochemicals that can tune nanoparticle surface chemistry, aggregation, and cellular responses [5].

In parallel with advances in synthesis strategies, recent literature has increasingly emphasized the importance of correlating physicochemical features of green-synthesized ZnO NPs with their in vitro biological performance. Over the last five years, several studies have reported plant-derived ZnO NPs exhibiting antioxidant, antimicrobial, and selective anticancer activities, highlighting how particle size, surface chemistry, and organic capping govern cellular uptake, oxidative stress pathways, and cytotoxic profiles [6–9]. These works demonstrate that green synthesis is not merely an environmentally friendly alternative, but a powerful strategy to engineer surface-tailored ZnO nanostructures with tunable biological behavior. Nevertheless, most reports remain limited to a restricted number of botanical sources, and the influence of distinct phytochemical environments on ZnO nucleation, structural organization, and bioactivity remains insufficiently understood.

Within this context, species from the *Myrtaceae* family have drawn significant attention due to their abundance of

phenolics, terpenoids, and flavonoids—compounds known for antioxidant and antimicrobial properties. *Myrcia oblongata* DC, a native Brazilian species still underexplored in nanotechnology, represents a promising candidate for biogenic nanoparticle production. Although endemic to Brazil, *M. oblongata* shares phytochemical traits with many *Myrtaceae* species distributed worldwide, such as *Eucalyptus*, *Psidium* and *Eugenia*, reinforcing its relevance as a representative model for plant-mediated nanomaterial synthesis. Its extracts and essential oils contain caryophyllene oxide, α -pinene, β -myrcene, and other bioactive metabolites, which have demonstrated antioxidant, antifungal, and antimicrobial activities [10, 11]. These compounds may act simultaneously as complexing, stabilizing, and capping agents, suggesting that *M. oblongata* could provide a unique phytochemical environment for the formation of ZnO nanoparticles with tailored surface properties.

Despite the recognized potential of green synthesis, no prior study has systematically reported the use of *Myrcia oblongata* extract for ZnO nanoparticle synthesis, nor examined how its phytochemical profile influences the resulting physicochemical and biological features. While other *Myrtaceae*-based extracts have been used to produce ZnO NPs [12–14], the phytochemical complexity and endemic character of *M. oblongata* remain unexplored. This work therefore addresses a clear research gap by establishing the synthesis, detailed characterization, and biological evaluation of ZnO NPs mediated by *M. oblongata* extract.

Here, we hypothesize that the phytochemical environment of *M. oblongata* promotes efficient Zn^{2+} coordination, nucleation, and strong bio-organic capping, leading to the formation of ultrananocrystalline ZnO domains organized into biofunctionalized nanostructures. This study integrates green chemistry principles with nanobiotechnology, combining detailed physicochemical characterization with in vitro antioxidant and cytotoxicity assays, and positions *M. oblongata* as a novel phytochemical platform for engineering functional ZnO nanomaterials and for future investigations of formation mechanisms and structure–activity relationships.

Materials and Methods

Materials

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2 \text{H}_2\text{O}$), phosphate-buffered saline (PBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% (Trolox), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt $\geq 98\%$ (ABTS) and commercial zinc oxide nanoparticles (ZnO

NPs < 100 nm), were obtained from Sigma-Aldrich, MO, USA. Sodium hydroxide (NaOH) was obtained from Synth, Diadema, SP, Brazil. Folin-Ciocalteu 2 M, gallic acid P.A (GA), sodium carbonate P.A and potassium persulphate P.A was obtained from Scientific Exodus Fine Chemistry Industry and Commerce, Sumaré, SP, Brazil.

For cell culture and cytotoxicity assays, the following reagents were used: Dulbecco's Modified Eagle Medium (DMEM) obtained from Sigma-Aldrich; Fetal bovine serum (FBS) and Penicillin-streptomycin were obtained from Gibco Process Liquid and Buffer Solutions - Thermo Fisher Scientific; Resazurin reduction assay (AlamarBlue) and Hoechst 33,342 were obtained from Thermo Fisher Scientific; Phosphate-buffered saline (PBS) and Dimethyl sulfoxide (DMSO). Human immortalized keratinocytes (HaCat) were obtained from Cyton, and glioblastoma cells (U87) from ATCC.

M. oblongata leaves were collected on April 5, 2023, between 9:00 and 11:00 a.m., when the average temperature and humidity recorded were 22.5 °C and 65.6%, respectively (INMET), in the Paulo Gorski Ecological Park (24°57'34"S, 53°26'32"W), Cascavel, Paraná, Brazil. After collection, the leaves were washed with distilled water and dried in an air-circulating oven at 35 °C for 48 h, until reaching a residual moisture content of approximately 8–10%. The dried material was then ground in a Willey-type knife mill to obtain particles smaller than 0.42 mm, resulting in a fine, homogeneous powder. The powdered material was stored in airtight glass containers, protected from light and humidity. The botanical identification of the species was confirmed and deposited under the voucher number UNOP 11,462 in the Herbarium of the State University of Western Paraná (UNIOESTE).

All experiments were conducted using analytical grade water purified by reverse osmosis using the Elga DV25 purifier, Ontario, CA.

Synthesis of ZnO_MO NPs

The synthesis of ZnO_MO NPs was carried out through a biogenic approach using the plant *M. oblongata* as a complexing and capping agent. The synthesis was adapted from a previously established protocol for the green synthesis of ZnO NPs using *M. piperita* extract. [12] First, 1.0 g of *M. oblongata* was added to 100 mL of water heated to 75 °C and the mixture was stirred at this temperature for 30 min. After stirring, the extract was centrifuged at 4400 rpm for 10 min, and the resulting supernatant was collected and stored at room temperature. Subsequently, 50 mL of a solution of zinc acetate dihydrate at 0.050 mol L⁻¹ was prepared and mixed with the 100 mL *M. oblongata* extract obtained earlier. The pH of the mixture was adjusted to 9 using a

0.5 mol L⁻¹ NaOH solution and stirred for 1 h at 90 °C. The precipitated ZnO_MO NPs were separated by centrifugation at 4400 rpm for 5 min, washed three times with water, frozen at -20 °C and finally lyophilized for 48 h. The obtained ZnO_MO NPs were stored in a Falcon tube at room temperature and protected from light.

Characterization of ZnO_MO NPs

The characterization of ZnO_MO NPs was carried out using various techniques to investigate their physicochemical properties. Fourier transform infrared spectroscopy (FTIR) was performed using an Agilent Cary 630 spectrometer to identify the functional groups present on the surface of the biogenic ZnO_MO NPs and in the *M. oblongata* extract, with a resolution of 4 cm⁻¹ and a scanning range between 600 cm⁻¹ and 4000 cm⁻¹. X-ray diffraction (XRD) analysis was performed to determine the crystalline structure and phase purity of the synthesized ZnO_MO NPs. Powder samples were analyzed as obtained, using a Bruker D8 diffractometer operated with Cu K α radiation (λ = 1.5418 Å). The diffractograms were recorded over a 2 θ range of 10–110°, with a step size of 0.020°, under standard operating conditions. The experimental diffraction patterns were compared with the standard zincite structure available in the ICSD database (ICSD 179643) for phase identification and structural verification. Crystallite size was initially estimated using the Scherrer equation for comparison with literature data. For more detailed structural analysis, Williamson–Hall (W–H) plots were constructed to separate size-induced and strain-induced peak broadening. Individual diffraction peaks were fitted to extract full width at half maximum (FWHM) values, which were converted to radians. The $\beta\cos\theta$ versus $4\sin\theta$ plots were then generated, and linear fitting was applied to estimate the crystallite size and microstrain contributions. The average hydrodynamic size, polydispersity index (PDI), and zeta potential of the ZnO_MO NPs and commercial ZnO NPs were evaluated using the Zetasizer Nano ZS equipment (Malvern Instruments Co). The measurements took place in an aqueous medium at 20 °C, using a disposable capillary zeta cell with a fixed angle of 173°. High-resolution transmission electron microscopy (HRTEM) images were captured using Carl Zeiss LIBRA[®] 120 and JEOL 2010 h-TEM microscopes. The samples of ZnO_MO NPs were dispersed in water, bathed in ultrasound, and deposited on carbon grids to obtain the images. UV–visible spectroscopy was employed to investigate the optical absorption behavior of the biosynthesized ZnO_MO NPs and the *Myrcia oblongata* extract. The spectra were recorded using an Agilent 8454 UV–Vis spectrophotometer, operating in the range of 200–800 nm, using quartz cuvettes and deionized water as blank. For clarity of

analysis, the spectral region around the near-UV absorption edge (360–380 nm) was further examined and is presented in detail in the Results and Discussion section. Photoluminescence (PL) spectroscopy was used to probe the emission properties and electronic states associated with the biofunctionalized ZnO_MO NPs and *Myrcia oblongata* extract. PL measurements were carried out using a FluoroMax+ spectrofluorometer (HORIBA Scientific, Jobin Yvon Technology) equipped with a DeltaDiode™ excitation source and DeltaHub™ controller. For ZnO_MO NPs, emission spectra were recorded in the 375–600 nm range under excitation at 360 nm, whereas for the *M. oblongata* extract the emission was collected from 440 to 750 nm using an excitation wavelength of 430 nm. All measurements were performed at room temperature. Thermogravimetric analysis (TGA) was conducted to assess the thermal stability and organic content associated with the biosynthesized nanoparticles. Prior to analysis, the aqueous *Myrcia oblongata* extract was freeze-dried (lyophilized) to remove residual solvent and obtain a dry solid powder suitable for thermogravimetric measurements. The TGA curves were obtained using a TA Instruments Q600 thermogravimetric analyzer to evaluate the thermal decomposition profiles of both ZnO_MO NPs and the dried plant extract. Approximately 5.0 mg of each sample were placed in platinum pans and heated from 20 °C to 1000 °C at a controlled heating rate of 10 °C min⁻¹, under an inert argon atmosphere (100 mL min⁻¹).

Total Phenolic Compounds (TPC) of ZnO_MO NPs

The methodology for determining the phenolic compound content was based on the procedure described by Bobo-García et al. [15]. To perform the tests, Folin-Ciocalteu reagent was prepared as a 1:4 ratio solution (Folin: water). Standard solutions of Gallic Acid (GA) were prepared at concentrations ranging from 200 to 1.56 µg/mL. A 100 g/L solution of sodium carbonate was also prepared. The assays were performed in a 96-well microplate, utilizing three main control and samples groups: (i) Positive control: Gallic acid (GA) standard solutions, Folin-Ciocalteu reagent and sodium carbonate, (ii) Sample Groups: ZnO_MO NPs or commercial ZnO NPs, Folin-Ciocalteu reagent and sodium carbonate, (iii) Blank: ethanol, Folin-Ciocalteu and sodium carbonate. After adding the standard GA solutions, the ZnO_MO NPs or commercial ZnO NPs (or ethanol for the blank), and the Folin-Ciocalteu reagent, the plate was incubated in the dark for four minutes. Following the addition of sodium carbonate solution, the plate was protected from light for two hours. The absorbance readings were subsequently taken at 750 nm. The absorbance values were used to construct a standard curve from the GA concentrations and perform

the TPC calculations for the ZnO_MO NPs and commercial ZnO NPs samples.

Antioxidant Activity by the DPPH and ABTS Methods for ZnO_MO NPs

Antioxidant activity was assessed based on the methodologies described by Li et al. [16] To perform the DPPH test, it was necessary to prepare solutions of 0.3 mmol/L DPPH and Trolox standard solutions at concentrations ranging from 200 to 11.71 µmol/L. The DPPH assay was performed in a 96-well microplate. The reaction mixtures consisted of: (i) Negative Control: ethanol and DPPH solution, (ii) Positive control: Trolox standard solutions and DPPH solution, (iii) Sample Groups: ZnO_MO NPs, commercial ZnO NPs and DPPH solution, (iv) Blank: ethanol only. After keeping the plate away from light for 30 min, the absorbance was read at 515 nm.

To perform the ABTS test, it was necessary to prepare solutions of ABTS 7 mmol/L and potassium persulphate 2.45 mmol/L, and Trolox standard solutions (200–11.71.71.71 µmol/L). The ABTS assay was performed in a 96-well microplate. The reaction mixtures consisted of: (i) Negative control: ethanol and ABTS solution, (ii) Positive Control: Trolox standard solutions and ABTS solution, (iii) Sample Groups: ZnO_MO NPs, commercial ZnO NPs and ABTS solution, (iv) Blank: ethanol only. After keeping the plate protected from light for 10 min, the absorbance was read at 750 nm. The absorbance values from both assays were used to construct the graph from the Trolox calibration curve and perform the antioxidant calculations for the ZnO_MO NPs and commercial ZnO NPs.

Cell Culture

Human immortalized keratinocytes (HaCaT, Cytion) (non-cancerous cell line) and glioblastoma cells (U87, ATCC) (cancer cell line) were cultured in Dulbecco's Modified Eagle Medium with high glucose (DMEM; Sigma), supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin–streptomycin (Gibco). Cells were maintained at 37 °C in a humidified incubator containing 5% CO₂ and routinely subcultured upon reaching ~80% confluence. All cultures used in this study were screened for mycoplasma contamination by Hoechst 33,342 DNA staining prior to use in experiments and tested negative. Experiments were performed using cells at low passage number (≤6 passages).

Cytotoxicity Assay

Cell viability was determined using the resazurin reduction assay (AlamarBlue, Thermo Fisher). Experiments were

conducted in triplicate in three independent experiments. The treated groups were: (i) ZnO_MO NPs, (ii) commercial ZnO NPs, and (iii) the crude aqueous extract of *M. oblongata*. HaCaT or U87 cells were seeded in black 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h. Subsequently, cells were exposed for 24 h to ZnO_MO NPs or commercial ZnO NPs at final concentrations of 10, 20, 30, 40, and 60 $\mu\text{g/mL}$ (expressed as ZnO equivalent). For the *Myrcia* extract group, dilutions were prepared using the same dilution factors applied to the nanoparticle formulations, ensuring equivalent treatment conditions. Untreated cells served as negative controls, while 5% (v/v) dimethyl sulfoxide (DMSO) was used as a positive control.

After exposure, culture medium was carefully removed and cells were rinsed once with phosphate-buffered saline (PBS). Fresh medium containing 5% (v/v) AlamarBlue was then added, and plates were incubated for 1 h at 37 °C. Fluorescence was measured at 560 nm excitation and 595 nm emission using a Synergy H1 plate reader (Agilent). Viability was calculated relative to the mean fluorescence of the untreated control (set as 100%) and expressed as percentage viability. Data are shown as mean $\pm$ standard deviation (SD) from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test, comparing all treatments against the negative control.

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8.0 software. All experiments were performed using independent triplicate assays to ensure reproducibility and minimize analytical and biological variability. One-way ANOVA followed by Tukey's multiple comparisons test or two-way ANOVA followed by Sidak's post hoc test, as outlined in the Figure legends, were used to determine statistical significance. A *p* value of less than 0.05 was considered significant. All data are presented as mean $\pm$ SD from a minimum of three replicates.

Results and Discussion

Phytochemical Profile of *Myrcia Oblongata* DC

The selection of *M. oblongata* as a biomediating agent for ZnO NPs synthesis is underpinned by its well-documented rich phytochemical profile and established biological activities. Studies on *M. oblongata* extracts have revealed the presence of key secondary metabolites, including saponins, steroids, triterpenoids, tannins, and flavonoids. Specifically, the aqueous extract, akin to that employed in the current

green synthesis, has been shown to contain saponins and tannins. These diverse bioactive compounds are known for their significant biological properties, such as antimicrobial and antioxidant effects. For instance, a study by Santana et al. [11] highlighted the antimicrobial capacity of *M. oblongata* extracts against various microorganisms, emphasizing their potential as natural alternatives to synthetic compounds in controlling pathogens. [10, 17]

Furthermore, while comprehensive studies focusing specifically on the aqueous extract for nanoparticle synthesis are limited, research on the essential oil of *M. oblongata* has identified major compounds like caryophyllene oxide (22.03%) and trans-verbenol (11.94%) [17]. Trans-verbenol is a compound resulting from the bioconversion of β -pinene. Other significant compounds identified in the essential oil include α -pinene (36.81%), β -pinene (9.74%), β -myrcene (13.99%), and sylvestrene (11.28%). These terpenes and their derivatives, which are also found in many other *Myrtaceae* species, have been linked to antioxidant activities. For example, the antioxidant potential of *M. oblongata* is often associated with the presence of caryophyllenes, verbenols, and phenolic compounds. These compounds act through mechanisms such as hydrogen transfer and electron donation to neutralize free radicals. The functional groups, particularly hydroxyl (-OH) groups (found in compounds like pinocarveol and α -terpineol in *M. tenella*, as well as acetosyringone in *E. uniflora*, which share chemical similarities with *M. oblongata* compounds) and aldehyde groups found in these phytochemicals, play a crucial role in conferring biological effects by inducing structural and functional changes in cell membranes and inhibiting enzymatic activities [10]. This intrinsic bioactivity of *M. oblongata*'s phytochemicals is hypothesized to not only facilitate the coordination and stabilization of ZnO NPs but also to impart enhanced biocompatibility and therapeutic properties to the resulting nanomaterial, positioning it as an ecological and functional candidate for biomedical applications.

Characterization of ZnO NPs from *Myrcia Oblongata* Extract

The green synthesis of ZnO NPs using *M. oblongata* extract (ZnO_MO NPs) was confirmed through various physicochemical characterization techniques. The FTIR analysis was crucial for identifying the main functional groups involved in the complexation and stabilization of the nanoparticles. By comparing the spectra of the pure plant extract and the synthesized ZnO_MO NPs (Fig. 1), significant changes were observed. Specifically, the disappearance or shifting of bands associated with hydroxyl (O-H) and carbonyl (C=O) groups, typically found in the 3200–3500 cm^{-1} and 1600–1700 cm^{-1} regions respectively, were

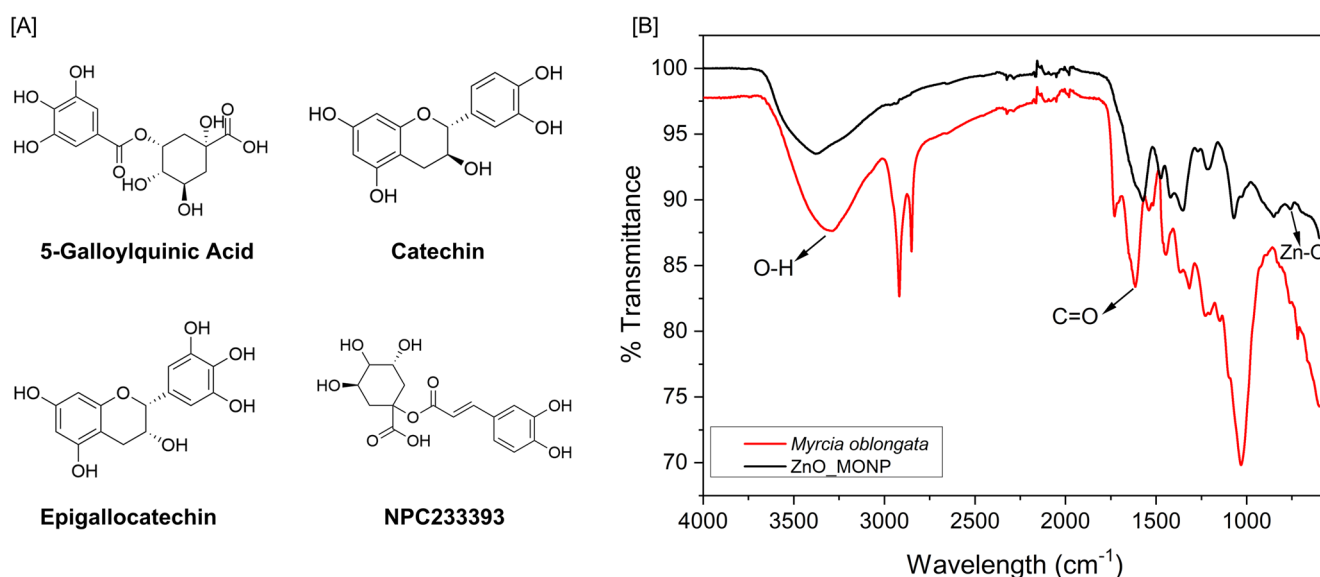


Fig. 1 (A) Representative phenolic compounds identified in *Myrcia oblongata* by high-resolution MS/MS analysis with confidence level 1, adapted from [Food Research International, 2025], highlighting functional groups involved in Zn²⁺ coordination. (B) FTIR spectra of

M. oblongata aqueous extract (red line) and ZnO_MONP nanoparticles (black line), showing the persistence of O–H and C=O bands after nanoparticle formation and the characteristic Zn–O vibration region

noted. These alterations suggest the direct involvement of bioactive compounds present in the *M. oblongata* extract, such as flavonoids, phenolic compounds, and terpenoids, in the complexation and coordination of Zn²⁺ ions to facilitate the formation of ZnO and in stabilizing the newly formed nanoparticles [11].

Similar spectral changes have been reported in the green synthesis of ZnO NPs using other plant extracts, where compounds like polyphenols and amines act as both complexing and capping agents, ensuring nanoparticle stability and preventing aggregation. For instance, ZnO Bio NPs synthesized using *Mentha piperita* extract also displayed similar organic functional groups (O–H at 3299 cm⁻¹ and C–H at 2922 cm⁻¹) acting as capping agents [12]. This is a feature often absent or minimal in chemically synthesized ZnO NPs, which primarily show inorganic ZnO bands and some surface hydroxyls. Furthermore, the ZnO_MONP NPs exhibit a phytochemical signature akin to that seen in ZnO NPs synthesized with *Terminalia catappa* fruit pericarp extract, which present a range of O–H (3387 cm⁻¹, 3015 cm⁻¹), C–H (2925 cm⁻¹, 2855 cm⁻¹), C=C (1626 cm⁻¹, 1591 cm⁻¹), C–O (1254 cm⁻¹), and C–N (1088 cm⁻¹, 1032 cm⁻¹) vibrational modes [18].

Recent high-resolution MS/MS analysis of *Myrcia oblongata* revealed a predominance of phenolic derivatives identified with confidence level 1, including 5-galloylquinic acid, catechin, epigallocatechin, and related polyphenolic compounds [19]. These metabolites are characterized by multiple hydroxyl and carbonyl functionalities, which are

known to coordinate metal ions and adsorb onto inorganic surfaces.

Figure 1A presents representative molecular structures reported for *M. oblongata*, highlighting functional groups relevant to Zn²⁺ complexation. Figure 1B shows the FTIR spectra of the aqueous extract and ZnO_MONP NPs. The broad absorption band in the 3200–3500 cm⁻¹ region corresponds to O–H stretching vibrations, consistent with the abundance of hydroxylated phenolic compounds. The bands observed around 1600–1700 cm⁻¹ are attributed to C=O stretching vibrations from phenolic acids and flavonoid structures.

After nanoparticle formation, these bands persist with noticeable shifts and intensity changes, indicating that the phytochemicals remain associated with the ZnO surface. The prominent absorption below 600 cm⁻¹ corresponds to Zn–O stretching vibrations, confirming the presence of zinc oxide. Together, these results indicate that phenolic constituents from *M. oblongata* are strongly involved in Zn²⁺ coordination and remain bound as a bio-organic corona on the nanoparticle surface.

Rather than a single compound, the green synthesis of ZnO_MONP NPs is therefore better described as a cooperative multicomponent process, in which polyphenols and related metabolites collectively act as complexing, stabilizing, and capping agents. This bio-organic coating is not merely residual but plays a decisive role in governing nanoparticle assembly, colloidal behavior, and biological interactions discussed in subsequent sections.

This collective evidence from FTIR underscores the efficacy of *M. oblongata* phytochemicals in mediating the

synthesis and highlights the crucial role of this bio-organic corona in enhancing stability, biocompatibility, and potential therapeutic properties of the resulting nanoparticles for biomedical applications. Recent studies on green-synthesized ZnO nanomaterials have highlighted the wide diversity of plant-mediated routes and demonstrated that variations in botanical source, extract composition, and synthesis conditions strongly influence nucleation pathways, surface chemistry, aggregation behavior, and the resulting physicochemical and functional properties of the nanoparticles [20].

In addition to phytochemical composition, experimental parameters such as extract concentration, reaction temperature, and reaction time play a decisive role in plant-mediated ZnO synthesis, directly influencing Zn^{2+} complexation, hydrolysis, nucleation rate, crystal growth, and surface functionalization [21, 22]. In the present work, all syntheses were carried out using extract prepared from the same plant batch and under identical experimental conditions in order to minimize variability and ensure internal reproducibility. The selected parameters were based on preliminary optimization experiments and literature precedents, aiming to promote controlled ZnO formation while maximizing phytochemical adsorption on the nanoparticle surface.

The high organic surface coverage detected by TGA (~42–47%), the narrow size distribution obtained by DLS ($\text{PDI}=0.087\pm0.014$), and the consistent crystallinity observed by XRD indicate that the selected synthesis conditions favored efficient phytochemical coordination, regulated nucleation, and the formation of stable biofunctionalized nanostructures. Variations in extract concentration

or reaction temperature are known to strongly affect particle aggregation, crystallite size, and surface chemistry in green synthesis systems; therefore, the controlled conditions employed here were essential to obtain reproducible ZnO_MO nanoparticles with consistent physicochemical properties [8, 9]. In agreement with this interpretation, recent studies have demonstrated that the phytochemical composition of plant extracts and synthesis parameters play a decisive role in controlling the nucleation, growth behavior, and final physicochemical features of green-synthesized ZnO-based nanomaterials, reinforcing the strong structure-directing function of botanical media during nanoparticle formation [23].

X-ray diffraction (XRD) analysis of ZnO_MO NPs (Fig. 2) confirmed their crystalline nature, showing characteristic diffraction peaks that match the zincite phase of zinc oxide (ZnO). The experimental diffractogram exhibits well-defined and intense diffraction peaks at $2\theta\approx 31.8^\circ$, 34.4° , 36.3° , 47.5° , 56.6° , 62.9° , 66.4° , 68.0° , 69.1° , and 72.6° , which are indexed to the crystallographic planes (100), (002), (101), (102), (110), (103), (200), (112), (201), and (004), respectively. These reflections are in excellent agreement with the standard zincite structure reported in the ICSD database (ICSD 179643), confirming the phase identity of the synthesized nanoparticles.

The experimental pattern shows a high degree of superposition with the ICSD reference profile (Fig. 2), indicating the high crystallinity and structural purity of the ZnO_MO NPs. The absence of additional peaks associated with secondary phases, metallic zinc, zinc hydroxide, or other zinc-containing byproducts were detected, demonstrating that the green synthesis mediated by *Myrcia oblongata* extract yields phase-pure ZnO nanoparticles. Furthermore, the relative peak intensities closely match those of the reference pattern, suggesting the absence of preferential orientation or pronounced texture effects, and indicating isotropic growth without a defined anisotropic particle shape.

Similar XRD profiles have been reported for ZnO NPs synthesized using plant extracts, which supports the effectiveness of the green synthesis approach adopted in this work. For instance, Shanavas et al. [24] obtained ZnO NPs from *Artabotrys hexapetalus* and *Bambusa vulgaris* extracts, while another study using *Mentha piperita* also reported the zincite phase as the dominant crystalline pattern, in agreement with the ICSD-44,477 reference [12]. It is worth noting that the presence of phytochemicals from the extract can act as capping agents, often leading to the partial formation of amorphous phases; however, the well-defined peaks in our diffractogram suggest that such effect was minimal in this synthesis.

For quantitative structural analysis, the average crystallite size was first estimated using the Scherrer equation

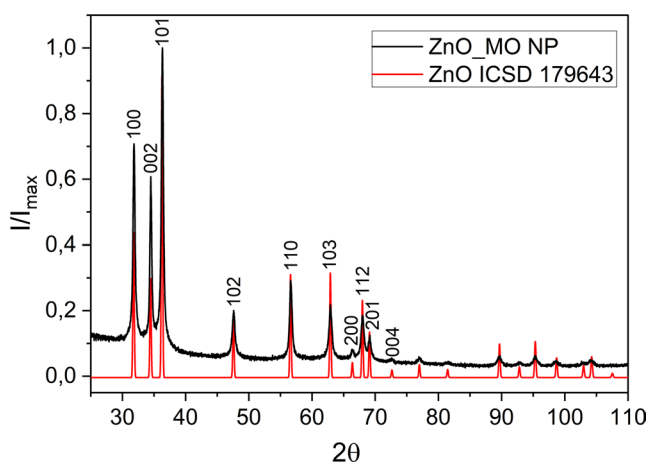


Fig. 2 X-ray diffraction (XRD) pattern of ZnO_MO nanoparticles (black line) compared with the standard zincite ZnO reference from the ICSD database (ICSD 179643, red line). Diffraction data were collected using Cu K α radiation ($\lambda=1.5418$ Å) over the 2θ range of 10 – 110° . The well-defined reflections indexed to the hexagonal ZnO phase confirm the high crystallinity and phase purity of the biosynthesized nanoparticles. Individual peaks were fitted and used for crystallite size and microstrain evaluation by the Scherrer and Williamson–Hall methods

to allow comparison with literature data. To obtain deeper insight, crystallite size and lattice distortion were further evaluated using the Williamson–Hall (W–H) method, which separates size-induced and strain-induced contributions to peak broadening. Individual diffraction peaks were fitted to extract reliable full width at half maximum (FWHM) values. The W–H analysis revealed ultrananometric coherent domains of approximately 2–3 nm, associated with a low microstrain ($\epsilon \approx 1.9 \times 10^{-3}$). In contrast, the average crystallite size estimated by the Scherrer equation from the (100) and (101) reflections was approximately 23 nm. This larger value is expected, since Scherrer's method does not decouple strain and defect contributions, which are significant in strongly capped biogenic nanomaterials. These results indicate that the ZnO_MO NPs consist of ultrasmall coherent ZnO domains embedded within larger nanostructures, highlighting the hierarchical organization of the biogenic nanoparticles.

Importantly, the crystallite size obtained by XRD is markedly smaller than the particle size measured by dynamic light scattering (DLS), which reflects the hydrodynamic diameter of solvated and partially aggregated nanoparticles. This discrepancy is expected and indicates that the ZnO_MO NPs consist of ZnO nanocrystallites embedded in a bio-organic matrix and assembled into larger biofunctionalized aggregates in aqueous suspension, in agreement with the HRTEM observations.

Dynamic light scattering (DLS) analysis revealed that ZnO_MO NPs exhibit an average hydrodynamic diameter of 212.6 ± 7.7 nm. It is important to note that DLS measures the hydrodynamic diameter in suspension, which includes not only the inorganic ZnO domains but also the associated bio-organic capping layer and hydration shell. Therefore, larger size values compared with XRD-derived crystallite dimensions are expected for biofunctionalized systems. [25]

The polydispersity index (PDI) of 0.087 ± 0.014 indicates a narrow size distribution and good colloidal homogeneity, suggesting effective control of the green synthesis process. This low PDI, together with the HRTEM observations, supports the formation of relatively uniform biofunctionalized aggregates rather than a highly heterogeneous dispersion.

Regarding colloidal stability, the zeta potential of -27.2 ± 0.7 mV suggests satisfactory electrostatic stabilization, as absolute values above approximately ± 25 mV are commonly associated with stable colloidal systems. This negative surface charge indicates the effective adsorption of phytochemicals from the *Myrcia oblongata* extract onto the nanoparticle surface, likely involving phenolic and carboxyl-containing compounds, which contribute to electrostatic repulsion and dispersion stability.

The large hydrodynamic diameter measured by DLS, when compared with the ultrasmall crystallite size obtained

by Williamson–Hall analysis, confirms that ZnO_MO NPs exist in aqueous suspension as biofunctionalized aggregates composed of ultrafine ZnO domains embedded within an organic matrix. This interpretation is fully consistent with the HRTEM observations, which revealed dense agglomerates and diffuse contrast attributed to strong bio-organic capping.

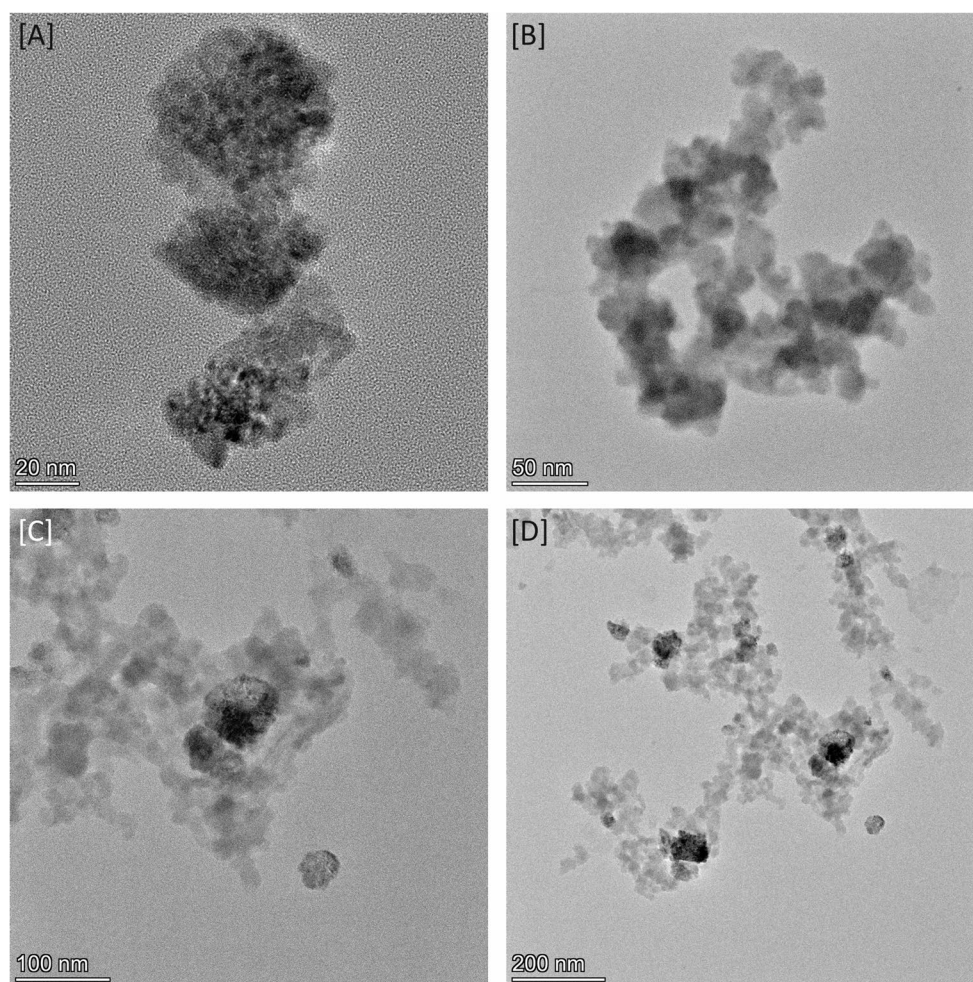
Comparison with the literature reinforces the variability of the physicochemical properties of ZnO NPs synthesized with plant extracts. While ZnO_MO NPs exhibited a hydrodynamic size of 212.6 ± 7.7 nm, studies such as that by Rani et al. [26], using *Azadirachta indica* leaf extract, observed nanoparticles with significantly larger hydrodynamic sizes, 580.41 nm and 356.2 nm. These high values were attributed to the interaction of phytochemicals on the surface of nanoparticles in aqueous suspension. Interestingly, the zeta potential of these nanoparticles was even more negative when compared to ZnO_MO NPs (-47.17 mV and -51.70 mV), indicating excellent colloidal stability.

This comparative analysis of DLS and zeta potential data demonstrates that the specific nature of *Myrcia oblongata* extract is crucial for the production of nanoparticles with unique characteristics. When compared to commercial ZnO NPs from Sigma-Aldrich, which have an average hydrodynamic size of 165 ± 24 nm, a PDI of 0.285 ± 0.010 , and a zeta potential of -22 ± 0.5 mV, ZnO_MO NPs show a slightly larger size but a significantly lower PDI, indicating a more uniform size distribution. In addition, the zeta potential of ZnO_MO NPs is more negative than commercial nanoparticles (-27.2 mV), suggesting potentially superior colloidal stability. This analysis reinforces the effectiveness of *Myrcia oblongata* extract in producing a nanobiomaterial with competitive colloidal characteristics relative to commercially available alternatives.

High-resolution transmission electron microscopy (HRTEM) images (Fig. 3) provided direct visualization of the morphology and aggregation state of the biosynthesized ZnO_MO NPs. Low- and high-magnification micrographs reveal the formation of nanoscale structures organized in irregular, strongly aggregated assemblies. Individual nanoparticles are not clearly resolved as isolated entities, but rather appear embedded within larger clusters surrounded by diffuse contrast, consistent with the presence of a bio-organic capping layer derived from the *Myrcia oblongata* extract.

The pronounced aggregation observed likely arises from strong interparticle interactions mediated by phytochemicals adsorbed on the nanoparticle surface, as supported by the presence of a significant organic fraction evidenced by TGA analysis. Although partial agglomeration may occur during solvent evaporation in TEM sample preparation, the persistence of large hydrodynamic diameters in aqueous

Fig. 3 High-resolution transmission electron microscopy (HRTEM) images of ZnO_MO nanoparticles at different magnifications (scale bars: 200 nm, 100 nm, 50 nm, and 20 nm). The micrographs reveal nanoscale structures organized in irregular and strongly aggregated assemblies, with diffuse contrast surrounding the inorganic domains, consistent with the presence of a dense bio-organic capping layer derived from the *Myrcia oblongata* extract. The absence of well-isolated particles supports the interpretation that ZnO_MO NPs are present as biofunctionalized aggregates composed of ultrafine crystalline domains, in agreement with XRD and DLS analyses



suspension (DLS) indicates that aggregation is an inherent feature of the biofunctionalized system rather than solely an imaging artifact.

Due to the strong aggregation and low contrast between adjacent inorganic domains and the surrounding organic matrix, well-defined lattice fringes suitable for reliable interplanar spacing measurements could not be consistently distinguished. Similarly, the acquisition of localized selected area electron diffraction (SAED) patterns attributable to individual ZnO nanocrystals was not feasible. Instead, the HRTEM images predominantly reveal hierarchical biofunctionalized aggregates composed of ultrafine inorganic domains embedded within a continuous organic environment.

Although direct lattice-resolved analysis was limited, the HRTEM observations provide important complementary information to the XRD and DLS results. The formation of dense agglomerates supports the Williamson–Hall analysis, which indicated the presence of ultrasmall coherent crystalline domains, and explains the significantly larger hydrodynamic diameter obtained by DLS. Together, these findings demonstrate that the ZnO_MO NPs are not present

as isolated nanocrystals, but rather as strongly capped, biofunctionalized nanostructures formed by the assembly of ultrafine ZnO domains.

The irregular morphology and absence of defined anisotropic structures observed by HRTEM are consistent with isotropic growth behavior, in agreement with the XRD pattern, which showed no evidence of preferential orientation. Compared with ZnO nanoparticles synthesized using other plant extracts that often exhibit rod-like or petal-like morphologies [12, 27], the structures obtained with *Myrcia oblongata* highlight the strong influence of the phytochemical environment on nanoparticle assembly, surface organization, and final nanostructure.

To further correlate the hierarchical structural organization of ZnO_MO NPs with their electronic and surface states, UV–vis absorption and photoluminescence analyses were subsequently performed. UV–vis spectroscopy was employed to investigate the optical response of the biosynthesized ZnO_MO NPs and to correlate it with the structural features revealed by XRD and HRTEM. As shown in Fig. 4A, ZnO_MO NPs exhibit an absorption feature in the near-UV region (≈ 360 – 380 nm) that is absent in the

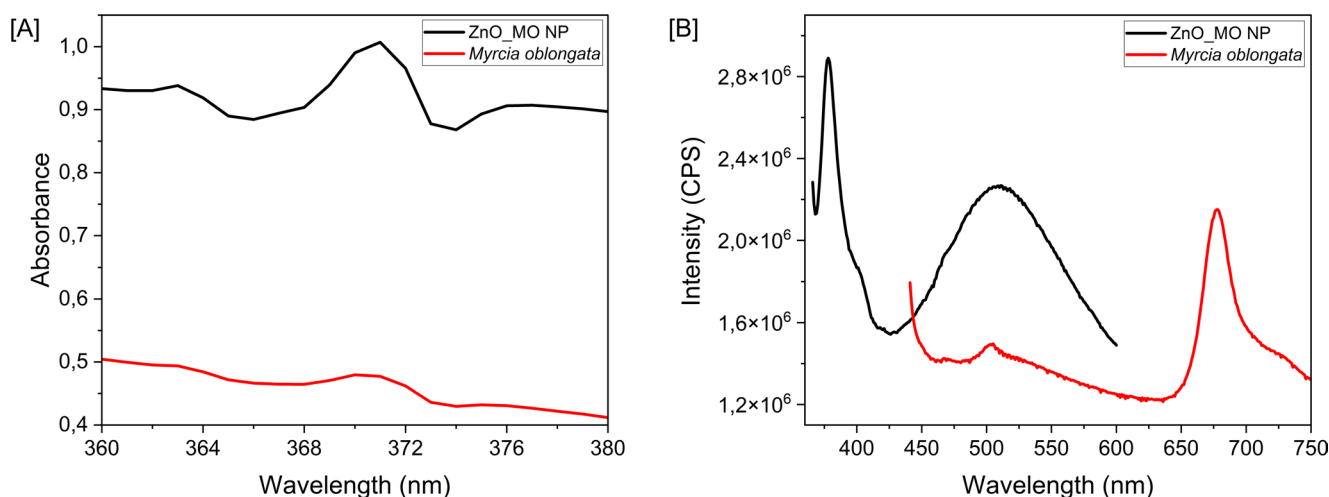


Fig. 4 (A) UV–visible absorption spectra of ZnO_MO nanoparticles (black line) and *Myrcia oblongata* aqueous extract (red line) in the 360–380 nm region. (B) Photoluminescence (PL) emission spectra of

ZnO_MO nanoparticles (black line) and *Myrcia oblongata* extract (red line), showing a broad visible emission for ZnO_MO NPs associated with surface and defect-related states

Myrcia oblongata extract, indicating the emergence of inorganic domains with optical behavior distinct from the plant matrix. The absorption profile is relatively broad and does not display a sharp absorption edge, which is consistent with the presence of ultrasmall coherent crystalline domains (~2–3 nm) identified by Williamson–Hall analysis and with the strong aggregation and bio-organic coating observed by HRTEM. In nanostructured ZnO systems, such broadening is commonly associated with size dispersion, surface states, and lattice defects, rather than bulk-like optical transitions. Similar green-synthesis approaches have reported tunable optical properties and band gap values of biosynthesized ZnO nanoparticles, highlighting the influence of size and surface capping on electronic transitions and photocatalytic behavior [28].

This behavior agrees with literature reports on ZnO nanoparticles, where absorption in the 350–380 nm region is attributed to electronic transitions involving ZnO band states, while spectral broadening reflects the high surface-to-volume ratio and defect-rich nature of nanoscale domains. Sahai and Goswami reported broadened UV absorption features for ZnO nanoparticles, which were linked to discrete electronic states arising from nanoscale dimensions and surface defects [29]. Similarly, comprehensive analyses of green-synthesized ZnO systems emphasize that strong surface functionalization and aggregation typically lead to diffuse absorption profiles rather than well-defined band edges [30]. The UV–vis response of ZnO_MO NPs is therefore consistent with the hierarchical organization inferred from XRD and HRTEM, namely ultrasmall ZnO crystalline domains embedded within biofunctionalized aggregates.

Photoluminescence (PL) spectroscopy was further used to probe the electronic states associated with ZnO_MO

NPs and to evaluate how nanoparticle formation modifies the emissive behavior of the plant extract (Fig. 4B). While the *M. oblongata* extract exhibits a dominant red emission (~650–700 nm) associated with organic fluorophores, ZnO_MO NPs present a broad visible emission centered in the blue–green region (~480–540 nm). This marked spectral shift indicates the generation of new emissive centers following nanoparticle formation.

In ZnO nanostructures, visible PL bands are widely attributed to deep-level defects such as oxygen vacancies, zinc interstitials, and surface-related states. Sahai and Goswami demonstrated that broad blue–green emissions in ZnO nanoparticles originate from defect-mediated recombination pathways, whose intensity and spectral position are strongly influenced by crystallite size and surface chemistry. Broader optical studies further report that nanostructured and surface-modified ZnO is dominated by defect-related luminescence rather than near-band-edge emission [29].

In the present system, the PL profile of ZnO_MO NPs is fully consistent with the ultrasmall coherent domains revealed by XRD and the diffuse, strongly capped aggregates observed by HRTEM. The broad visible emission suggests a high density of defect and surface states, which is expected for ZnO domains embedded within a bio-organic matrix. Moreover, the strong organic fraction evidenced by TGA and the functional groups detected by FTIR likely contribute to interfacial states that further modulate radiative recombination. The clear difference between the PL spectra of ZnO_MO NPs and the plant extract demonstrates that the optical response of the nanomaterial does not simply arise from residual phytochemicals, but reflects the establishment of new electronic states associated with the biofunctionalized ZnO nanostructure.

To investigate the thermal stability and quantify the organic content adsorbed on the surface of biosynthesized ZnO_MO NPs, a comparative thermogravimetric analysis (TGA) was performed using the *M. oblongata* extract as reference (Fig. 5). The joint analysis of the ZnO_MO NP curves (black line) and the *M. oblongata* extract (red line) is crucial to understanding the synthesis process and the final composition of the nanomaterial.

The ZnO_MO NP curve exhibits a decomposition profile that diverges from that of the pure extract, indicating enhanced thermal stability of organic components after biosynthesis. The profile displays a gradual multistep degradation behavior rather than well-defined discrete stages. The first mass loss, approximately 7–8%, occurs up to about 150–200 °C and is attributed to the evaporation of physically adsorbed water and residual solvents. A gentle mass loss, corresponding to approximately 20% of the total mass, is observed in the temperature range between 200 °C and 800 °C, associated with the gradual loss of adsorbed organic material and low molecular weight components. The most intense degradation event, involving a significant mass loss of approximately 38%, occurs above 800 °C. This behavior at high temperatures, which contrasts with the more distributed decomposition of the extract, suggests that the organic capping agents (flavonoids, phenolic compounds, and terpenes) gained significant thermal stability upon binding to the inorganic surface. The residue should consist mainly of ash and uncoated zinc oxide.

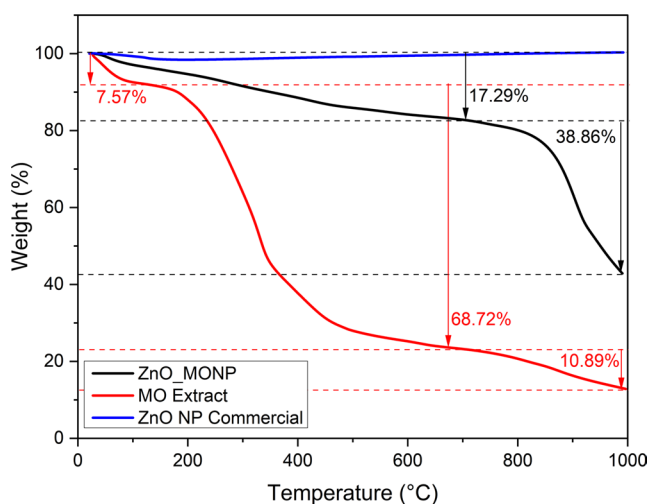


Fig. 5 Thermogravimetric analysis (TGA) curves of ZnO_MO nanoparticles (black line), *Myrcia oblongata* extract (red line) and ZnO NP Commercial (blue line) recorded from room temperature to 900 °C. The comparison reveals a markedly different thermal decomposition profile after nanoparticle formation, with ZnO_MO NPs exhibiting a high organic mass fraction (~42–47%) associated with phytochemical capping agents bound to the ZnO surface. The enhanced thermal stability of the organic components after biosynthesis supports the formation of a strongly biofunctionalized organic–inorganic nanostructure

The behavior of the pure *M. oblongata* extract is markedly different. The range of 20–200 °C corresponds to the initial loss of mass mainly due to the evaporation of water and other volatiles present in the extract matrix, a value slightly higher than that observed in the nanoparticles, which is within expectations due to the greater amount of water bound in the pure extract. The extract then undergoes complex and substantial thermal degradation, accounting for approximately 78–81% of total mass loss between 200 °C and 800 °C. The final inorganic residue of the pure extract is therefore very low, at approximately 10–12% ash and metal oxides.

The thermogravimetric profiles revealed that ZnO_MO NPs contain approximately 42–47% organic matter, confirming the strong association of *M. oblongata*-derived compounds on the nanoparticle surface. This result provides clear evidence of biofunctionalization and effectively distinguishes the biosynthesized nanomaterial from the pure plant extract and from chemically synthesized or commercial ZnO NPs, which typically exhibit negligible organic mass loss. The pronounced organic fraction is a hallmark of plant-mediated green syntheses, where phytochemicals act as both complexing and stabilizing agents. Comparative data from previous studies corroborate this variability: ZnO NPs synthesized using *Mentha piperita* extract displayed an organic content near 53%, while those obtained from *Lavandula pubescens* extract (LP-ZnO NPs) showed markedly lower values, between 6.0% and 14.5%, depending on extract concentration [31]. Such discrepancies reflect differences in phytochemical composition, extraction method, and metal–ligand affinity, all of which govern the degree of surface coverage and thermal decomposition profile. The high organic fraction observed in ZnO_MO NPs, in agreement with the FTIR bands of phenolic and terpenoid groups, confirms the formation of a stable bio-organic coating. This surface layer is not merely residual—it plays a decisive role in the nanoparticles' physicochemical behavior, providing enhanced dispersion stability and forming the basis for their superior antioxidant and biocompatible performance discussed in subsequent sections. Earlier reports on biosynthesized ZnO nanomaterials emphasized the fundamental role of phytochemical constituents in governing nucleation and growth processes, as well as in defining surface chemistry and final nanoparticle properties [32]. These studies established that plant-derived metabolites do not merely act as passive stabilizers, but actively participate in the physicochemical pathways leading to ZnO formation.

In agreement with the TGA data, FTIR spectra of ZnO_MO NPs revealed characteristic bands associated with phenolic, hydroxyl, and terpenoid functional groups, indicating the presence of phytochemicals coordinated to the ZnO surface. Although the specific molecular-level reaction

pathways remain to be fully elucidated, the complex and synergistic nature of plant extracts makes it experimentally unfeasible to isolate the contribution of individual metabolites. Instead, the green synthesis of ZnO_MO NPs is better described as a cooperative multicomponent process, in which polyphenols, flavonoids, and terpenoids collectively act as complexing, reducing, and stabilizing agents. This bio-organic coating is not merely residual, but plays a decisive role in governing nanoparticle assembly, stability, and biological behavior, as discussed in subsequent sections.

Antioxidant Activity and Determination of Total Phenolic Compounds (TPC) of ZnO_MO NPs

The tests performed with ZnO_MO NPs and commercial ZnO NPs at concentrations of 50 µg/mL for antioxidant activity and 200 µg/mL for total phenolic compounds allowed the results of the nanoparticles to be quantified based on the curve constructed using the reference standards. Table 1 shows the antioxidant values, expressed in micromoles of Trolox equivalent per gram of sample, and the TPC values, expressed in micrograms of gallic acid equivalent per milligram of sample.

The antioxidant activity of ZnO_MO NPs showed superior performance compared to ZnO NPs, as evidenced by the DPPH and ABTS radical assays (Table 1). In the DPPH assay, the antioxidant capacity of ZnO_MO NPs was 532.75 µmol TE/g, while commercial ZnO NPs showed 256.57 µmol TE/g. Similar results were observed in the ABTS assay, in which ZnO_MO NPs exhibited 690.59 µmol Trolox/g, in contrast to the 311.18 µmol TE/g obtained with commercial ZnO NPs. The higher antioxidant efficacy observed in the ABTS method can be attributed to its ability to react with hydrophilic and lipophilic compounds, unlike the DPPH radical, which has greater selectivity for lipophilic antioxidants and whose response strongly depends on the concentration and polarity of the compounds evaluated [33].

The superior antioxidant performance of ZnO_MO NPs compared with commercial ZnO NPs can be directly associated with the high surface concentration of phenolic and terpenoid compounds derived from *M. oblongata*. However, this enhancement should be interpreted with

caution, as differences in the specific surface area between the samples represent a potential confounding variable [12]. Commercial ZnO NPs typically possess larger particle sizes and lower surface-to-volume ratios, which naturally limit the number of reactive sites available for radical scavenging [12]. In contrast, the biosynthesized ZnO_MO NPs, despite exhibiting a larger hydrodynamic diameter due to the organic corona, likely have a nanocrystalline core with a high effective surface area accessible to redox-active phytochemicals [34]. Consequently, the observed antioxidant superiority may result not only from the intrinsic activity of the adsorbed biomolecules but also from morphological and surface effects that increase the density of functional sites capable of donating electrons or hydrogen atoms to stabilize free radicals. This interplay between surface chemistry and specific surface area should therefore be considered when attributing antioxidant improvements solely to phytochemical capping.

In the literature, different studies with ZnO NPs synthesized by green routes have demonstrated variable antioxidant capacities, depending on both the synthesis conditions and the plant extract used. For example, ZnO NPs obtained from *Aquilegia pubiflora* showed antioxidant potential between 45.73 and 178.45 TEAC in the ABTS assay (Jan et al.) [35], while nanoparticles synthesized with *Verbascum × calcicolum* revealed 185.56±0.15 mg TE/g in the DPPH assay [36]. These data corroborate the influence of the method and conditions of synthesis on the antioxidant activity of nanoparticles, especially when plant extracts are used, whose phytochemicals, such as phenolic compounds and flavonoids, can be adsorbed on the surface of the nanoparticles, giving them enhanced antioxidant properties [37–39].

In the present study, the TPC content of ZnO_MO NPs was estimated at 33.06 µg GAE/mg, a value consistent with other green synthesis studies, such as those reported for ZnO NPs from *Pediastrum boryanum*, *Verbena officinalis*, and *Verbena tenuisecta*, which presented 7.92, 4.7, and 0.7 mg GAE/g, respectively. [40, 41] Although the quantification of TPCs of ZnO NPs was not possible at the concentrations evaluated, the result obtained for ZnO_MO NPs reinforces its superiority in terms of surface functionalization and antioxidant potential. In comparison, higher TCA values were observed in ZnO NPs from *Eriobotrya japonica*, ranging from 90.51 to 315.86 µg GAE/g [42], which demonstrates the influence of the specific phytochemical characteristics of each plant species used in the synthesis.

The high total phenolic content quantified for ZnO_MO NPs not only confirms the strong phytochemical contribution of *M. oblongata* to nanoparticle formation but also provides a plausible explanation for the observed colloidal stability. Phenolic compounds are known to adsorb onto ZnO surfaces through coordination and hydrogen

Table 1 Total Phenolic Compounds (TPC) expressed in micrograms of gallic acid equivalents (GAE) per milligram of sample and Antioxidant Activity (DPPH and ABTS methods) of zinc oxide nanoparticles (Commercial ZnO NPs and ZnO_MO NPs)

Samples	DPPH (µmol TE/g NPs)	ABTS (µmol TE/g NPs)	TPC (µg GAE/ mg NPs)
ZnO_MO NPs	532.75	690.59	33.06
Commercial ZnO NPs	256.57	311.18	-

(-) not detected

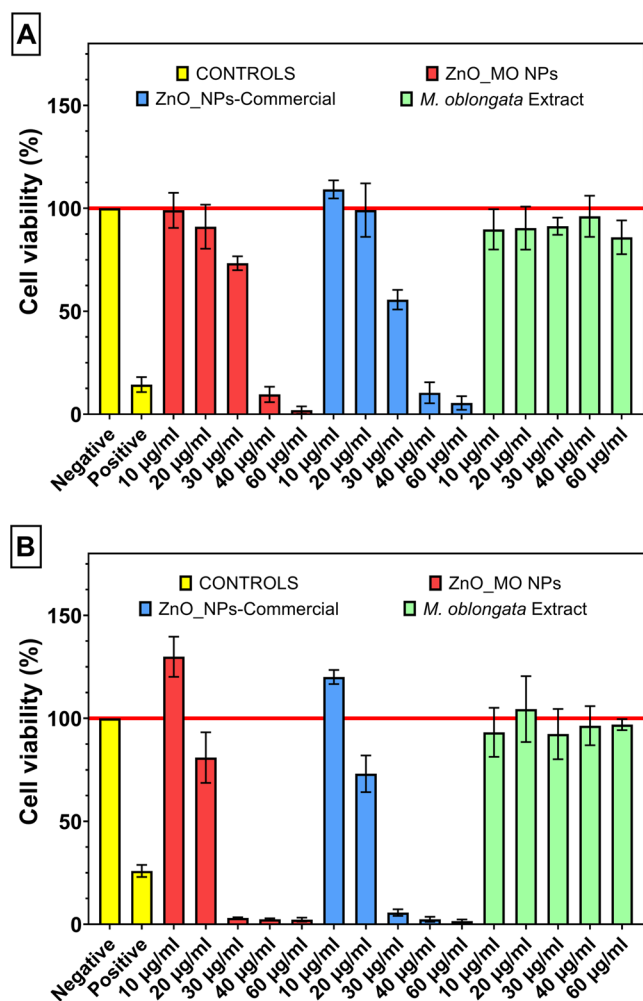


Fig. 6 Cell viability of HaCaT keratinocytes (A) and U87 glioblastoma cells (B) after 24 h exposure to treatments (ZnO_MO NPs (red columns), commercial ZnO NPs (blue columns), and *M. oblongata* extract (green columns). Concentrations are expressed relative to ZnO content; for the extract group, equivalent dilutions were applied to match the nanoparticle treatments. Negative control corresponds to untreated cells (100% viability), and positive control was 5% v/v DMSO. Data are expressed as mean $\pm$ standard deviation (SD) of three independent experiments ($n=3$), normalized to the negative control. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparisons test against the negative control ($p<0.05$)

bonding, generating a negatively charged organic corona that enhances electrostatic repulsion between particles and prevents aggregation. This organic interface, in turn, supports the long-term dispersion of the nanomaterial in aqueous media and likely contributes to the moderate zeta potential value (-27.2 mV) reported, reinforcing the role of surface-bound antioxidants as dual agents of stabilization and bioactivity.

Evaluation of Antitumor Potential and Biocompatibility

Green-synthesized ZnO nanoparticles have been widely reported to exhibit relevant biological activities, including antioxidant effects and selective cytotoxicity toward tumor cells. These effects have been associated with the combined influence of particle size, surface chemistry, defect states, and the presence of bio-organic capping layers derived from plant extracts, which collectively modulate nanoparticle–cell interactions and biological responses [43]. Within this context, evaluating the cytotoxic profile and selectivity of phytomediated ZnO nanostructures is essential to assess their potential for biomedical applications.

Cytotoxicity was investigated in HaCaT keratinocytes (non-tumoral) and U87 glioblastoma (tumoral) cells after 24 h exposure to ZnO_MO NPs, commercial ZnO NPs and the free *M. oblongata* extract. In HaCaT cells (Fig. 6A), both nanoparticle formulations were tolerated at 10–20 $\mu\text{g/mL}$, but a significant reduction in viability was observed from 30 $\mu\text{g/mL}$ onwards. At this concentration, ZnO_MO NPs reduced viability to 73% ($p=0.0006$), while ZnO NPs decreased it more sharply to 56% ($p<0.0001$). At higher doses (40–60 $\mu\text{g/mL}$), both formulations caused drastic cytotoxicity, with viability falling below 10% ($p<0.0001$). In contrast, the free *M. oblongata* extract did not produce significant effects at any concentration. Detailed statistical outputs for HaCaT assays are provided in Supplementary Tables S4–S6.

In U87 cells (Fig. 6B), both nanoparticle formulations displayed a biphasic response. At 10 $\mu\text{g/mL}$, they significantly increased viability (ZnO_MO NPs 130%, $p=0.0003$; ZnO NP 120%, $p=0.0259$). However, at 20 $\mu\text{g/mL}$ the effect reversed, with viability reduced to 81% ($p=0.0388$) for ZnO_MO NPs and 73% ($p=0.0043$) for ZnO NPs. From 30 $\mu\text{g/mL}$ upwards, both formulations caused near-total loss of viability, remaining below 6% ($p<0.0001$). In these cells, the free *M. oblongata* extract also did not produce significant effects at any concentration. Complete statistical analyses for U87 cells are reported in Supplementary Tables S1–S3.

The calculated IC_{50} values were 32.83 ± 0.8 $\mu\text{g/mL}$ (ZnO_MO NPs) and 33.38 ± 5.8 $\mu\text{g/mL}$ (commercial ZnO NPs) in HaCaT, and 22.66 ± 1.2 $\mu\text{g/mL}$ and 23.14 ± 1.6 $\mu\text{g/mL}$ in U87, resulting in a selectivity index (SI_{50}) of 1.45 and 1.44, respectively. These results indicate that ZnO NPs were more toxic to glioblastoma cells than to non-tumoral keratinocytes, evidencing a preferential effect on tumor cells. Although the presence of *M. oblongata* extract did not markedly shift IC_{50} values compared with uncoated ZnO, its intrinsic biocompatibility indicates that it can be safely used as a natural capping agent. Importantly, the extract did

not interfere with the antitumor activity of ZnO against U87 cells, suggesting that it may provide a stable and biocompatible coating without compromising therapeutic potential.

Our findings are consistent with previous reports showing that ZnO NPs exert dose-dependent cytotoxicity in tumor and non-tumoral cells. Pieretti et al. demonstrated IC_{50} values of approximately 27 $\mu\text{g/mL}$ in SNU449 hepatoblastoma cells and 42 $\mu\text{g/mL}$ in HepG2 liver cancer cells after 24 h exposure [1], which are in close agreement with the values obtained here for U87 glioblastoma and HaCaT keratinocytes. These comparisons indicate that ZnO-based systems display a reproducible cytotoxic profile across different cellular models, supporting their potential as antitumor agents while also emphasizing the importance of dose in balancing efficacy and biocompatibility.

Conclusion

This study reports, for the first time, the biogenic synthesis of zinc oxide nanoparticles (ZnO_MO NPs) using the aqueous extract of *Myrcia oblongata* DC as a natural complexing and stabilizing agent. Comprehensive physicochemical analyses confirmed that the process yielded highly crystalline, hexagonal ZnO nanoparticles with a substantial organic surface layer (~45%), as verified by TGA and FTIR. The phytochemical corona, rich in phenolic and terpenoid compounds, directly enhanced the antioxidant capacity and colloidal stability of the nanomaterial, resulting in over twice the antioxidant performance compared to commercial ZnO NPs. While the exact molecular mechanism underlying nanoparticle formation could not be dissected due to the intrinsic complexity of the *M. oblongata* extract, this limitation is common to plant-mediated syntheses and does not diminish the mechanistic consistency observed through FTIR and TGA evidence. The reaction likely proceeds through a synergistic network of redox-active phytochemicals, which collectively govern nucleation, growth, and surface stabilization. Future studies employing metabolomic and computational approaches may help clarify these cooperative interactions and provide deeper insight into the biogenic synthesis pathway.

Biologically, ZnO_MO NPs demonstrated moderately selective cytotoxic profile against glioblastoma (U87) cells while preserving compatibility with non-tumoral keratinocytes (HaCaT), highlighting their promise as targeted and sustainable nanoplateforms for biomedical use. Although a direct molecular link between surface chemistry and biological effects was not established, the high phenolic content (33.06 $\mu\text{g GAE mg}^{-1}$) and strong antioxidant activity indicate the presence of a stabilizing organic corona that modulates Zn^{2+} release and oxidative stress. Ongoing

metabolomic studies aim to clarify this connection. Despite the zeta potential values supporting short-term stability, additional testing under physiological ionic and serum conditions is necessary to evaluate colloidal persistence and aggregation risk, currently being investigated to advance biomedical translation.

Future directions should include a mechanistic investigation of the interaction between the phytochemical capping layer and cellular targets, as well as in-depth evaluation of reactive oxygen species (ROS) modulation, genotoxicity, and cellular uptake pathways. Further studies may also explore the scaling of the synthesis process, incorporation of ZnO_MO NPs into polymeric or hydrogel matrices, and in vivo assessments to validate their therapeutic efficacy and biosafety. Collectively, these advances position *Myrcia oblongata*-mediated ZnO nanoparticles as a sustainable and versatile nanomaterial platform bridging green chemistry and biomedicine, paving the way for next-generation antioxidant and antitumor nanotherapeutics.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10876-026-03000-7>.

Acknowledgements The authors are thankful for the financial support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) 001 (all authors) and (88881.711920/2022-01) (ABS). We are also thankful for financial support from CNPq (313117/2019-5 (ABS), and from São Paulo Research Foundation (FAPESP, grants 2022/14645-2 (ABS), 2024/12112-2 (ABS), 2023/16363-7 (RAR), 2024/20778-0 (MH), 2024/04079-5 (RSN), 2022/04262-9 (RAGS). This study was supported in part by the INCT Nanotechnology for Sustainable Agriculture, National Council for Scientific and Technological Development (MCTI-CNPq - INCTNanoAgro #405924/2022-4) (ABS), by MCTI/FINEP/FNDCT - process number TRANSFORMAT 1005/24, and by the Araucária Foundation through the Novos Arranjos em Pesquisa e Inovação (NAPI) Biodiversidade: Recursos Genéticos e Biotecnologia program.

Author contributions MH: methodology, investigation, data curation, writing-original, draft, validation. JR: methodology, investigation, data curation, validation. VDPC: methodology, investigation, data curation, draft, validation. GCB: methodology, data curation, validation. CBA: methodology, data curation, validation. KFM: methodology, data curation, validation. RSN: methodology, investigation, data curation, draft, validation. RAGS: methodology, data curation, validation. FGSP: conceptualization, writing-review & editing, supervision, funding. ABS: conceptualization, writing-review & editing, supervision, funding. RAR: conceptualization, methodology, investigation, data curation, writing-original, draft, validation. All authors have given approval to the final version of the manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES (ROR identifier: 00x0ma614). For open access purposes, the authors have assigned a Creative Commons CC BY license to any accepted version of the article. This work was

supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Grants 2022/14645-2, 2024/12112-2, 2023/16363-7, 2024/20778-0, 2024/04079-5, 2022/04262-9. By the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Grants 001 and 88881.711920/2022–01). By the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant 313117/2019–5 and Project Nanotechnology for Sustainable Agriculture, Grant 405924/2022–4). By MCTI/FINEP/FNDCT - process number TRANSFORMAT 1005/24. By the Araucária Foundation through the Novos Arranjos em Pesquisa e Inovação (NAPI) Biodiversidade: Recursos Genéticos e Biotecnologia program. Morgana Halfeld, Jéssica Rosset, Victor D. P. Cinelli, Cecília B. Aragão, Kelli F. Mariano, Renan S. Nunes, Gabriela C. Battistini, Ricardo A.G. da Silva, Fabiana G.S. Pinto, Amedea B. Seabra, Roberta A. dos Reis acknowledge individual or institutional support from these agencies.

Data Availability The data supporting this article have been included as part of the ESI.

Declarations

Ethical approval and consent to participate Since animals or humans cannot be used in this study, no ethical approval is necessary.

Competing interests The authors declare no competing interests.

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