doi: 10.4149/gpb_2025003

Crafting extract of Meniran-derived carbon decorated on zinc oxide as composite material for effective cancer treatment

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Abstract. The development of new composite materials derived from plant-based carbon for biomedical applications has posed a considerable challenge over the past decades. Herein, we successfully designed and synthesized ZnO/Meniran-C from Meniran-derived carbon (Meniran-C) decorated on zinc oxide for effective cancer treatment. These composites showed crystal microparticles and provided unique optical properties with high absorbance compared to pure Meniran and ZnO under an aqueous solution which has the potential for increased electron-hole. More importantly, *in vitro* analysis of MDA-MB-231 cells as breast cancer cells exhibited high toxicity after incubation with ZnO/Meniran-C for 24 h, in comparison with incubation with Meniran only. Hence, these composite materials provide a novel composite from bioactive compounds Meniran-derived carbon with excellent anticancer therapy and offer significant potential to contribute to the development of cost-effective, environmentally friendly, and widely applicable cancer therapies for future applications.

Key words: Meniran — ZnO/Meniran-C — Composites — Synthesized ZnO nanoparticles — Anticancer effect

Introduction

The incidence and mortality rates of cancer continue annually, making it a significant barrier to improving global life expectancy, as it remains the second leading cause of death worldwide (G et al. 2021; Łukasiewicz et al. 2021; Ryu et al. 2021; Sung et al. 2021). It includes a wide range of diseases characterized by the rapid division of abnormal cells, which subsequently spread to other areas of the body. Over the past several decades, substantial advancements have been achieved in improving our understanding and management of the various types of cancer. One of the primary factors contributing to cancer is the mutation of DNA within genes responsible for regulating cell growth. These DNA mutations can result from various causes, with one significant factor being exposure to free radicals (Martemucci et al. 2022; Chaudhary et al. 2023). Composite materials with a small size have been reported to bring significant advancements in the areas of cancer diagnostics and therapy (Steichen et al. 2013; Baranwal et al. 2023; Andoh et al. 2024). Moreover, various nanoparticles that exhibit responsiveness to the tumor microenvironment have been developed (Raju et al. 2022; Dong et al. 2023; Yang et al. 2023). These agents remain stable

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under normal physiological conditions but undergo physical and chemical transformations when exposed to specific conditions characteristic of cancer cells, such as pH (Chu et al. 2022), temperature (Ilhami et al. 2023), enzymes (Thornton et al. 2007), reducing environments (Loving et al. 2013), and reactive oxygen species (ROS) (Jomova et al. 2023) .

Zinc oxide (ZnO), an n-type semiconductor belonging to the II–VI group, is distinguished by its wide band gap of approximately 3.3 eV. It is considered a highly attractive material due to its effective performance as a photocatalyst, low cost, and ease of synthesis in both bulk and nanostructured forms (Kołodziejczak-Radzimska and Jesionowski 2014; Fatima et al. 2022; Gassim and Makkawi 2023). Numerous researchers have explored the potential of ZnO as a promising anticancer agent at both the nano- and micro-scale, owing to its ability to interact with the surface of cancer cells and/or their core, subsequently penetrating into the cellular core and inhibiting cancer growth (Bisht and Rayamajhi 2016; Perumal et al. 2024). Notably, ZnO has the ability to generate ROS on its surface, which can potentially induce cell death when ROS levels exceed the capacity antioxidant defenses of the cells (Raha and Ahmaruzzaman 2022). In addition, the possible utilization ZnO is the creation of new material with another two-dimensional (2D) material which potential provide unique physical and chemical properties.

The use of herbal medicine as a source of alternative medicine is often found in Indonesia. Traditionally, the use of plants as medicine has been widely used by the community for generations. Scientific research indicates that plants possess pharmacological properties due to the presence of active compounds, including flavonoids, tannins, phenols, and saponins. Meniran *(Phyllanthus niruri)*, commonly referred to as the insulin plant, is a perennial herbaceous species belonging to the Asteraceae family, indigenous to the Andes region of South America. In Indonesia, there is a growing trend in the widespread cultivation of Meniran, commonly utilized as both a food ingredient and an herbal remedy without further evaluating its bioactive compounds (Geethangili and Ding 2018; Kodoli et al. 2021; Nahrul et al. 2022; Rukmana et al. 2023). Furthermore, Meniran contains a significant quantity of carboxylic acids, which are readily soluble in aqueous solutions and are suitable for functionalization with other 2D materials. Despite its widespread use in the community as both a food ingredient and herbal medicine, but the development of herbal, e.g. Meniran, as potential for biomedical applications still limited due to the larger size of bioactive compounds within Meniran, making them difficult to absorb by the body (Pratiwi et al. 2019; Tolve et al. 2021; Wang et al. 2023). Thus, the utilization of Meniran as a carbon-based material can be functionalized with ZnO to form a composite material that may enhance the efficacy of the bioactive compounds for cancer therapy.

In the present study, we successfully designed and synthesized of ZnO/Meniran-C from Meniran-derived carbon (Meniran-C) decorated on zinc oxide for effective cancer treatment. The extract of Meniran was produced under methanol, subsequently composited with ZnO by hydrothermal method. ZnO/Meniran-C composite has microparticle size and unique optical properties under aqueous solution. Interestingly, *in vitro* study ZnO/Meniran-C composite clearly exhibited highly toxicity towards cancer cells. Therefore, these composites newly developed from a traditional plant holds potential as an environmentally friendly of cancer treatment for a variety of biomedical applications.

Materials and Methods

Materials

Meniran *(Phyllanthus niruri)* was sourced from a local plant in East Java, Indonesia. Zinc acetate dihydrate [$Zn(CH_3COO)_2{\cdot}2H_2O$] (> 98%) and sodium hydroxide (NaOH) were obtained from Merck (Germany) in their highest available purity.

Meniran extraction process

Approximately 1 kg of dried Meniran leaf powder was extracted using the maceration method with methanol at a ratio of 1:3 (b/v). The extraction was performed for 24 h and repeated three times. The Meniran extract was then concentrated using a rotary vacuum evaporator at 45°C (Buchi Rotavapor R-100) (Tukiran et al. 2019).

Synthesis of ZnO/Meniran-C composite

The ZnO/Meniran-C composite was synthesized using the hydrothermal method (Nugroho et al. 2024). In this process, 0.2 g of $Zn(CH_3COO)_2$ was dissolved in 20 ml of purified water and directly mixed with 30 ml of a 70 mg Meniran-C solution under constant stirring. Subsequently, the pH of the mixture was adjusted to 10 by adding a NaOH solution, followed by agitation for 30 min at ambient temperature. The mixture was then transferred into a 50 ml Teflon-lined autoclave and heated at 180°C for 4 h through high-pressure about 1220 bar. The final product was collected through centrifugation and thoroughly rinsed multiple times with deionized water to remove any impurities. The synthesis procedure of ZnO/Meniran-C composite is depicted schematically in Figure 1.

Characterization

Fourier transform infrared (FTIR) spectroscopy was obtained by Shimadzu IRTracer-100 spectrophotometer to analyze the

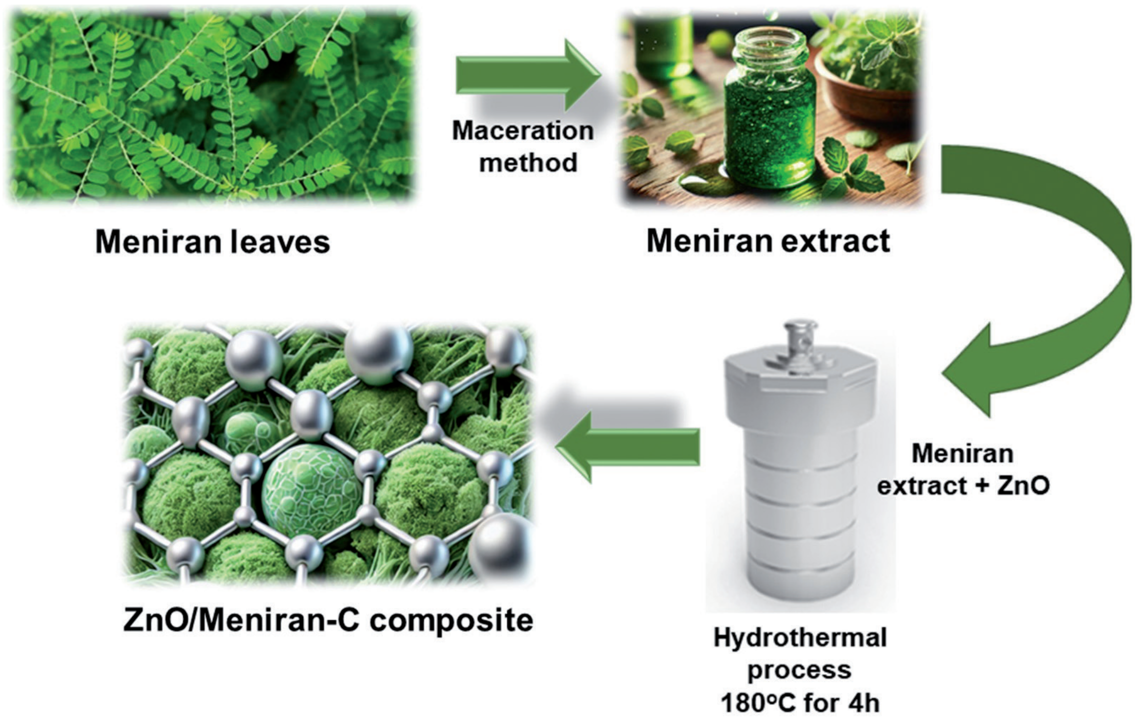

Figure 1. A graphical illustration depicting the synthesis process of Meniran-derived carbon decorated on ZnO (ZnO/Meniran-C) composite.

chemical composition. The spectra of Meniran, ZnO and ZnO/Meniran-C composite was then scanned over a range of 800 to 4000 cm^{-1} (Cheng et al. 2024).

X-ray diffraction (XRD) patterns were recorded using a Miniflex-600 X-ray diffractometer (Rigaku, Japan) within the range of 20–70°, utilizing Cu Kα radiation (λ = 1.5419 Å) at 30 kV and 10 mA (Cheng et al. 2024).

Ultraviolet-visible (UV-vis) spectrophotometry. The optical absorption of the samples was measured using a UV-1900 spectrophotometer (Shimadzu, Japan) within the wavelength range of 200–800 nm (Cheng et al. 2024).

Field Emission-Scanning Electron Microscopy (FE-SEM, JSM6500, JEOL, Tokyo, Japan), equipped with energy-dispersive X-ray spectroscopy (EDS), was used to examine the morphology and elemental composition of Meniran, ZnO and ZnO/Meniran-C composite (Cheng et al. 2024).

Cell culture

The Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia, provided the breast cancer cells line MDA-MB-231. The MDA-MB-231 cells were cultured in growth media that was composed of Alpha-MEM medium (Gibco) with added 1% penicillin-streptomycin (Gibco), 1% amphotericin-B (Gibco) and heat-inactivated 10% FBS (Gibco). Then, the cells were maintained in 5% carbon dioxide (CO_2) at 37°C. After confluence of cell, the cells were collected using trypsinization methods. The cells were used for treatment with the sample (Veterini et al. 2024).

Cell cytotoxicity assay

The cells were seeded into 96 wells at a density of approximately 5,000 cells/well, then incubated for 24 h. The next day, the growth media was replaced with the treatment medium. The treatment medium is growth media with Meniran, ZnO and ZnO/Meniran-C composite with concentration range from 12.5 μg/ml until 400 μg/ml. After incubation for 24 h using media treatment, the treatment media were replaced with MTT reagent. After incubation for 4 h, cells were observed under an inverted microscope with 100× magnification. Then stop reagent with DMSO was added, then their absorbance was measured using a 595 nm wavelength (Veterini et al. 2024).

Results and Discussion

Firstly, Meniran was extracted by maceration method subsequently, transferred into the hydrothermal process with zinc acetate in one-pot method (Fig. 1). The crystal structure of

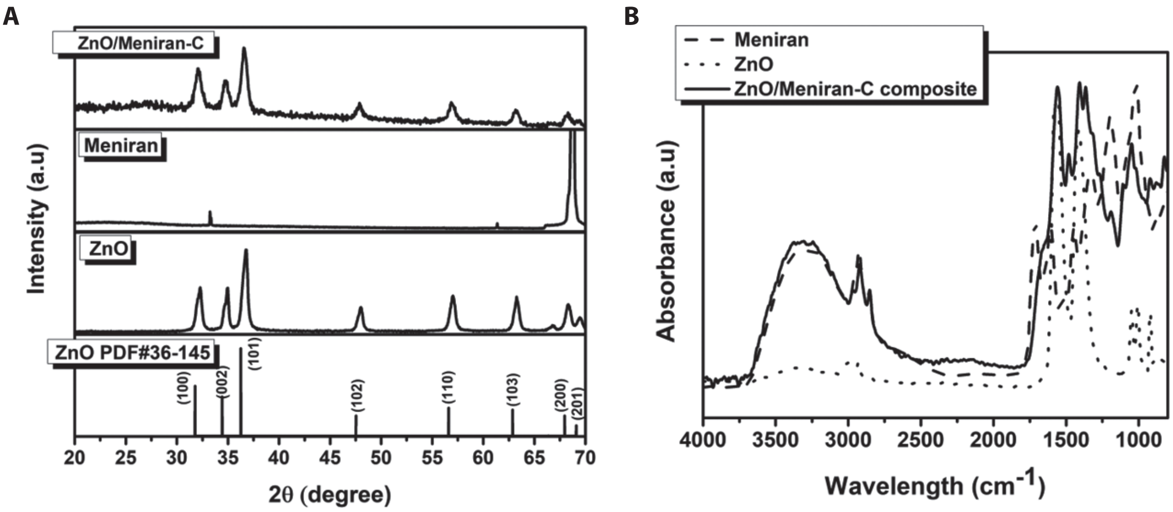

Figure 2. X-ray diffraction (XRD) patterns (**A**), and Fourier transform infrared (FTIR) spectra (**B**) of Meniran, ZnO, and ZnO/Meniran-C composite.

the sample was initially analyzed using XRD, as shown in Figure 2A. The diffractogram of Meniran reveals a partially amorphous structure, with the highest peak observed at 2θ = 69.6°, corresponding to the presence of highly disordered carbon atoms which confirms amorphous structure. This observation confirms the presence of carbon materials derived from the Meniran extract. The XRD pattern of pure ZnO clearly exhibits several high-intensity peaks at 2θ = 32.24°, 34.21°, 36.21°, 47.21°, 56.27°, 62.03°, 66.09°, 67.18°, and 68.2°, consistent with previous work (Elumalai and Velmurugan 2015). According to the standard PDF file #36–1451, these peaks indicate the successful formation of ZnO with a hexagonal/wurtzite crystal structure, corresponding to the (100), (002), (101), (102), (110), (103), (200), and (201) planes. Interestingly, XRD pattern of the ZnO/Meniran-C composite showed no significant difference compared to that of pure ZnO. Next, FTIR analysis in transmission mode was performed to further assess the presence of Meniran-C within the ZnO/Meniran-C composite heterostructures and to investigate the interaction between ZnO and Meniran-C. As illustrated in Figure 2B, the peak at 855 cm^{-1} corresponds to Zn–O vibrations, while the broad band at 3410 cm^{-1} is attributed to the stretching of –OH groups from surface-adsorbed water molecules (Zhang et al. 2009, 2015). In addition, bands at 1380 cm^{-1} and 1580 cm^{-1} are associated with residual acetate groups present within the material. Furthermore, the FTIR spectrum of Meniran-C exhibited a prominent and broad peak at 3460 cm^{-1}, corresponding to the stretching vibration mode of O–H bonds. The band observed at 1525 cm^{-1} is attributed to the stretching of C=C bonds in polycyclic aromatic hydrocarbons, while the peaks at 1050 cm^{-1} and 1430 cm^{-1} correspond to vibrations of C–O bonds in oxygen-containing groups, which contribute to excellent material in water solubility (De and Karak 2013). Conversely, the FTIR spectrum of the ZnO/Meniran-C composite exhibited peaks at 3460, 1519, 1411, and 1032 cm^{-1}, indicating a faint blue shift, which can be attributed to the interaction between ZnO and Meniran-C.

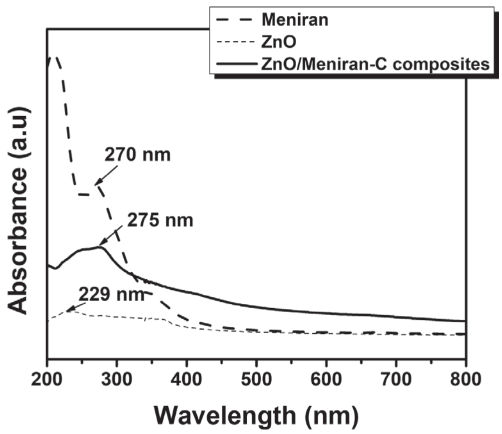

Figure 3. UV-vis spectra of Meniran, ZnO, and ZnO/Meniran-C composite.

Next, the optical characteristics of Meniran, ZnO, and ZnO/Meniran-C composite were analyzed by UV-vis spectrophotometer. As shown in Figure 3, the characteristic absorption of ZnO is observed in the UV region, with peak absorption at a wavelength of 229 nm. In contrast, the absorbance spectrum of Meniran displayed a broader range around 270 nm with exhibited significant absorption in the near-infrared spectrum. Notably, ZnO/Meniran-C compos-

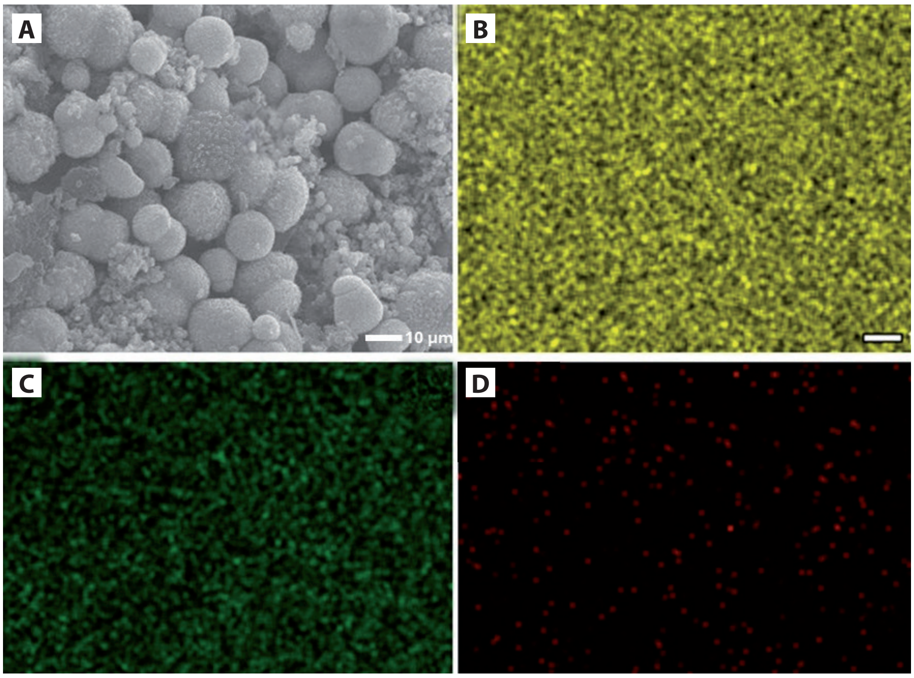

Figure 4. ZnO/Meniran-C composite. SEM image (**A**), and elemental mapping of ZnO/Meniran-C composite for zinc (**B**), oxygen (**C**), and carbon (**D**).

ite exhibited broad optical absorbance with a slight redshift to 275 nm, attributed to the highly planar π-conjugated structure, which enhanced the delocalization of π-electrons within the 2D framework (Pati et al. 2017). Another important information, the absorption of ZnO/Meniran-C composite sample is significantly higher than Meniran and ZnO, indicating its enhanced ability to absorb a greater intensity of light compared to pure Meniran and ZnO.

To investigate the surface morphology and microstructure of the samples, FE-SEM equipped with EDS was performed at a working voltage of 15 kV. As depicted in Figure 4A, the morphology of ZnO showed crystal structure, while Meniran extract displayed with layers morphology. More importantly, the ZnO/Meniran-C composites exhibited a microparticle-like structure with larger pores on the Meniran surface, attributed to the interaction between ZnO and the Meniran-C layer. These results also indicate that interaction led to the encapsulation of ZnO by the 2D Meniran-C layer. To support SEM result, we also performed elemental analysis by X-ray spectroscopy (EDS) analysis.

The elemental composition of each sample, expressed in atomic percentages, was determined through EDS analysis and is presented in Table 1. The pure Meniran sample exhibits a carbon content exceeding 65% and an oxygen content of approximately 35%. The pristine ZnO is composed of zinc and oxygen, with atomic percentages of 37% and 63%, respectively. The composite sample contains carbon, zinc, and oxygen with atomic percentages of 18.04%, 40.03%, and 32.92%, respectively, confirming the successful formation of the ZnO/Meniran-C composite. To further confirm the elemental distribution, EDS mapping analysis was performed on the ZnO/Meniran-C composite sample. As shown in Figure 4, the elements within the ZnO/Meniran-C composite, namely Zn, O, and C, are uniformly and consistently distributed across the entire mapping acquisition area. As a prediction, all elemental components including Zn, O and C were found in ZnO/Meniran-C composite which is consistent with XRD and FTIR analysis.

As we know Meniran demonstrates excellent anticancer agent due to several bioactive compounds within natural products (Kodoli et al. 2021; Puspitarini and Sapti 2022; Puspitarini et al. 2024). Additionally, pristine ZnO have been reported in preclinical and biomedical research, including drug delivery and cellular imaging for cancer treatment. We further assessed

Table 1. Atomic percentages of each Zn, O, and C in Meniran, ZnO and ZnO/Meniran-C composite

Samples	Zn (%)	O (%)	C (%)
Meniran	–	35.45 ± 0.11	64.55 ± 0.07
ZnO	37.22 ± 0.09	62.78 ± 0.12	–
ZnO/Meniran-C	32.92 ± 0.10	49.03 ± 0.14	18.04 ± 0.11

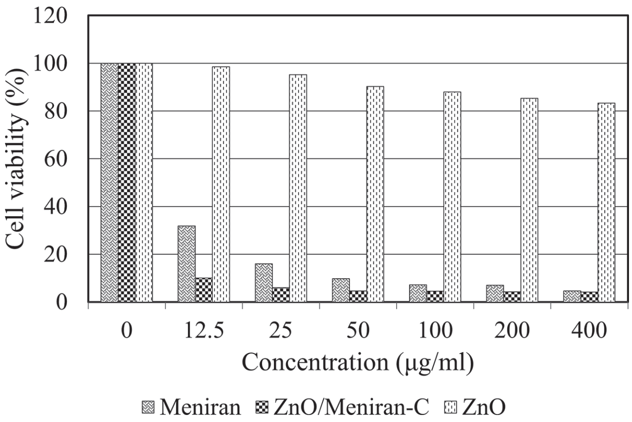

Figure 5. MTT analysis of ZnO/Meniran-C composite towards MDA-MB-231 cell line for 24 h.

the potential cancer treatment by quantitative cytotoxicity assays of ZnO/Meniran-C composite toward MDA-MB-231 cell line as breast cancer cells model. As shown in Figure 5, after 24-h incubation treament against MDA-MB-231 cell line, ZnO/Meniran-C composite has high cytotoxicity compared ZnO nor Meniran extract only. Additionally, in the smallest concentration, i.e. 12.5 µg/ml, ZnO/Meniran-C composite could inhibit around 90% the MDA-MB-231 cells line growth. Thus, pristine ZnO enhance efficacy of chemotherapeutic for in MDA-MB-231 cells line. This is the first study which reported about the successfully designed and synthesized of ZnO/Meniran-C using hydrothermal method which has anticancer effect, especially MDA-MB-231 cells line. Other studies also reported in ZnO green synthesis toward cancer cells, such as ZnO with precipitation method against MDA-MB 231 breast cancer cells (Kumar Janakiraman et al. 2023), ZnO with colchicine against MDA-MB 231 cells (Cheng et al. 2024), and ZnO with *Nigella sativa* against HepG2 cancer cells (Fakhar-e-Alam et al. 2024).

Conclusion

We successfully report the hydrothermal synthesis of a ZnO/Meniran-C composite derived from the natural Meniran plant. These composites showed crystal microparticles and provide unique optical properties with highly absorbance compared to pure Meniran and ZnO under an aqueous solution. More importantly, cytotoxicity study of ZnO/Meniran-C composite clearly exhibited highly toxicity towards MDA-MB-231 cell as model cancer. Therefore, these composite materials produced through green synthesis from a natural plant can provide potential to contribute to the development of cost-effective and wide cancer therapy for future applications.

Funding. Ministry of Research, Technology, and Higher Education Republic of Indonesia (DRTPM) (contract no. B/61627/UN38.III.1/LK.04.00/2024).

Conflict of interest. The authors declare no competing financial interest.

Acknowledgments. This study was supported financially by the Ministry of Research, Technology, and Higher Education Republic of Indonesia (DRTPM) (contract no. B/61627/UN38.III.1/LK.04.00/2024).

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Received: November 12, 2024
Final version accepted: February 11, 2025