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ZnO Nanorod-Mediated Metabolic Engineering of *Gardenia jasminoides* 'Variegata' for Enhanced Antimicrobial and Antioxidant Activities

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Abstract

NANOPARTICLES (NPs) are defined as particles with a size between one and one hundred nanometers. Recently, NPs have been employed as novel and effective elicitors to increase the yields of chemical compounds in plants. In the present study, zinc acetate and cetyltriammonium bromide (CTAB) were used as reactants to effectively synthesize ZnO-NPs using a straightforward hydrothermal process. Zinc oxide nanorods (ZnO-NPs) were evaluated using an X-ray diffraction pattern (XRD), and transmission electron microscope (TEM). Different Zinc oxide (ZnO) nanorod concentrations (5, 10, 25, 50, and 100 mg/l) were added to the liquid callus medium of *Gardenia jasminoides* Variegata, and the calli cultures were harvested after 8 days. The amounts of rosmarinic and cinnamic acids were quantified using HPLC. Antioxidant activities were examined by 2,2′- azino - bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS•+) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The total content of phenolics and flavonoids has been assessed. It was reported that adding 25 mg/l ZnO nanorods represents the highest fresh weight (6.5 ±0.3 g) compared to the control (5.14± 0.005 g). For accumulation of rosmarinic acid in callus culture of *G. jasminoides* Variegata using ZnO nanorods, 10 mg/l ZnO nanorods elevated the rosmarinic acid content fivefold compared with the control (250, 50 µg/GFW, respectively), in the same respect 10 mg/l ZnO nanorods recorded the highest antioxidant activity among the other concentrations with both methods ABTS as well as DPPH. The highest cinnamic acid (83 µg/GFW), considered twice its content in the control (39 µg/GFW), was recorded by adding 50 mg/l ZnO nanorods. The antimicrobial activity of the extract of each treatment was also examined against five microbial organisms. The results confirmed a potent activity against candida albicans for treatment No. 3, 5, and 6 extracts. While all the other extracts showed no activity against *Bacillus subtilis* and *Escherichia coli*. On the other hand, treatment No. 3 showed moderate activity against *Staphylococcus aureus* and *Shigella flexneri* due to the increased production of the active compounds.

Keywords: Antioxidant activity, Antimicrobial activity, *Gardenia jasminoides* 'Variegata', TEM, XRD, ZnO nanorods.

Introduction

Nanoparticles (NPs) are defined as particles with a size between one and one hundred nanometers. NPs accumulate mostly in water or soil. Plants are unable to avoid the effects of nano pollution because they are anchored to the two main environmental sinks where NPs accumulate: soil and water. One of the most crucial methods for processing advanced materials is the hydrothermal technique. The

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hydrothermal method has been employed to create ZnO nanomaterials featuring a one-dimensional structure, including nanowires and nanorods, on a large scale at a low cost. This is especially beneficial because of its advantage in handling nanostructured materials for various technological uses, such as electronics, optoelectronics, ceramics, magnetic data storage, biomedicine, and biophotonics. [1,2]. Zhai et al. 2003 [3] used CTAB as a surfactant to create ZnO nano and micromaterials through a solvothermal process. Zhang et al. 2008[4] used hydrothermal and reverse microemulsion techniques to create the ZnO nanowires.

According to recent research, NPs have a substantial impact on the physiology, development, and growth of plants. However, it is currently unclear how NPs affect the secondary metabolism of plants. It has been shown that all plant species exhibit an increase in reactive oxygen species (ROS) after interacting with nanoparticles. We believe that when plants interact with NPs, the ROS generated in the plants is likely to disrupt their secondary metabolism. This is due to the established connection between reactive oxygen species (ROS) and secondary signaling messengers, which results in the transcriptional control of secondary metabolism. [5].

Nanoparticles exhibit differences from bulk materials due to their size, such as altered chemical reactivity, enhanced energy absorption, and increased biological mobility [6].

In response to internal or external stimuli, plants normally provide bioactive metabolites; however, this production is restricted or inhibited [7]. A viable substitute for the labor-intensive process of obtaining valuable phyto-derived therapeutic compounds from cultivated or wild plants is required to fulfill the increasing demand. Many techniques and strategies have been developed as a result of industrial production [8]. The use of nanotechnology in plant tissue culture has been shown to have an unexpected impact on the growth and development of plants grown in vitro. Additionally, the biosynthesis of secondary metabolites has proven to be an effective substitute for plant tissue culture combined with nanoparticles. [9]. To increase the yields of chemical compounds in plants, for example, nanoparticles (NPs) have been employed as novel and effective abiotic elicitors [10,11]. It is well recognized that NPs can affect plants in both positive and adverse ways, depending on the concentration. It is known that NPs cause abiotic stress to plants at sub-toxic quantities and phytotoxicity at higher concentrations. [12]. Plants are known to enhance or initiate the biosynthesis of secondary metabolites in response to different biotic and abiotic stress [13,14]. It has been postulated that ROS produced by nanoparticles could serve as a stimulant for the development of secondary metabolism in plants [5]. According to De La Rosa et al.,2017 [15], it additionally improves agriculture by reducing diseases, promoting plant development as nano nutrients, and acting mostly as an insecticide.

One well-known member of the Rubiaceae family is the medicinal and ornamental plant Gardenia jasminoides. This is an evergreen tree that grows throughout China [16]. In traditional Chinese medicine, it is frequently used to treat a variety of problems [17,18].

It has fragrant white flowers and grows in many temperate regions. In addition to its use for natural yellow dyes [19,20], it possesses a variety of biological activities, including the ability to lower blood sugar, reduce inflammation, treat depression, increase antioxidant capacity, and enhance sleep quality also it has anti-atherosclerotic, antithrombotic and anticancer activities [21,27]. Gardenia like most traditional herbs possesses a wide range of phytochemicals, several studies have identified their presence including flavonoids, natural pigments, and other compounds [21].

Researchers in a wide range of fields have been more interested in natural products in recent years due to the in-depth studies of plants. Figure (1, a.) shows rosmarinic acid (RA, C18H16O8), a fascinating and well-known example. This naturally occurring phenolic acid is classified as an ester according to its chemical structure. It is the esterification result of caffeic acid and 3,4-dihydroxyphenyl lactic acid. Numerous in vitro and in vivo studies have also examined its diverse pharmacological characteristics, such as its anti-oxidative, anti-inflammatory, antiviral, anti-diabetic, cancer-fighting, and neuroprotective actions.[28].

Cinnamic acid, a naturally occurring aromatic carboxylic acid, is one of the key compounds found in plants such as Panax ginseng and Cinnamomum cassia (Chinese cinnamon), as well as in fruits, whole grains, vegetables, and honey [29], Figure (1, b). Cinnamic acid has been shown in studies to have anti-inflammatory, neuroprotective, anti-microbial, anticancer, and antidiabetic characteristics [30]. Moreover, it is a flavoring and fragrance chemical found in detergents, cosmetics, and toiletries [31]. Phenylalanine can be chemically deaminated to produce cinnamic acid.

Because of its enormous influence on healthcare systems and service providers, the rising incidence of infectious diseases by resistant organisms poses a danger to global health and economic development. It is equally important to comprehend the possible risks that microbes provide for the health of both humans and animals. It represents a challenge to scientists. The absurd rise in morbidity and death trajectories due to resistant organisms motivates scientists to find and create new antimicrobial chemicals from innovative and safe sources to lessen the negative consequences of these organisms [32].

The current study aims to use nanobiotechnology's new application in the *in vitro* secondary metabolites field. It will use ZnO nanorods as an elicitor to maximize the accumulation of rosmarinic and cinnamic acids in the callus of *G. jasminoides* 'Variegata' and examine their antimicrobial effects.

Material and Methods

Plant material

Plant material was obtained from plantlets of *G. jasminoides* Variegata grown *in vitro*. For shoot multiplication, the plantlets were subcultured three times on MS medium [33] with 2 mg/l benzyl adenine (BA).

Establishment of callus culture:

In vitro G. jasminoides Variegata plantlets' leaves were separated into segments that were roughly 0.5 cm long. These segments were transferred to MS medium enhanced with 0.5 mg/l BA + 0.5 mg/l picloram for one subculture (three weeks) in the dark to induce callus. To promote calli growth, the inducted calli were transferred to MS medium enhanced with 4 mg/l Thidiazuron (TDZ).

Preparation of Zinc oxide nanorods (ZnO-NPs)

Cetyltrimethylammonium bromide (CTAB) (AR Grade, Sigma Aldrich, USA) and zinc acetate hydrated (Sigma Aldrich, USA) were utilized without additional purification. To create a transparent solution A under stirring, 0.4 g of CTAB and 10 g of sodium hydroxide (NaOH) were dissolved in 100 ml of distilled water using a CTAB-assisted low-temperature hydrothermal method. A translucent solution B was created by dissolving 12 g of zinc acetate in 100 ml of distilled water. After vigorous stirring, 50 ml of solution A was combined with 50 ml of solution B to create 100 ml of transparent solution C, which had $[Zn^{+}]$ = 0.2 M, $[OH^{-}]$ = 1.2 M, and [CTAB] = 0.0005 M. After an hour of continuous stirring at room temperature, the molar ratio of Zn^{2+}/OH− was 1:6.

X-ray diffraction (XRD)

The crystal structure of the prepared zinc oxide nanorods (ZnO-NPs) was recognized through a Philips X-ray diffractometer (PW 1930 generator, PW 1820 goniometer) delivered with Cu Kα radiation (45 kV, 40 mA, with λ = 0.15418 nm). The analysis's scans were completed via a step size of 0.02 and step time of 1s in a 2θ range of 5 to 80º.

Transmission electron microscopy (TEM)

The morphology of the prepared zinc oxide nanorods (ZnO-NPs) was identified via (TEM). The zinc oxide nanorods (ZnO-NPs) sample was kept in a vacuum desiccator and a TEM at 80 KV was used for the study (JEM-1200 EXII, JEOL, Japan).

Effect of different zinc oxide nanorods concentrations on calli cultures of Gardenia jasminoides Variegata fresh weight:

To study the effect of different zinc oxide nanorod concentrations on Gardenia calli cultures, about 5 g of friable calli were transferred to a liquid MS medium enhanced with 4 mg/ l TDZ (control) in a 250 ml conical flask. The treatments are summarized in Table 1**.**

Subsequently, the cultures were incubated on a shaker at 100 rpm in complete darkness. The experiment was conducted in triplicate and calli harvested after 8 days, fresh weight was recorded and samples were extracted for further analysis.

Sample extraction

The extraction process was conducted following the method outlined by Gabr *et al.* 2017[34].

The extraction of the samples was done using 0.5 g of friable calli from each treatment and extracted using 2.5 ml of 80% methanol.

Determination of rosmarinic and cinnamic acids content by High-Performance Liquid Chromatography (HPLC)

The methanol solution was concentrated through evaporation until a dry residue remained. The resulting extract was reconstituted in 1 ml of methanol and stored at 4°C in a dark environment. The phenolic compound content was analyzed using HPLC on a UNICAM CRYSTAL 200 Liquid Chromatograph (Column: Kromasil C18 5um 250*4.66 mm). The mobile phase consisted of methanol and water, both treated with 0.3% orthophosphoric acid p.a. - w/v. Phenolic compounds were eluted using a linear gradient from water to 50% methanol over 5 min, followed by isocratic elution with 50% methanol for 20 min. The flow rate was maintained at 1.4 ml/min. Detection of the substances was performed by measuring absorption at λ = 288 nm, and identification was achieved by comparing retention times and absorption spectra with standards of Rosmarinic acid and Cinnamic acid. The concentration of the sample was represented as µg/g of fresh weight, calculated utilizing the known concentration of the standard and the peak areas of both the standard and the sample [35].

Where Concentration of sample = [Area sample / Area of standard] * Concentration of Standard

The anti-oxidant activity

Several techniques based on different mechanisms are preferred to be used. In this study, the 2,2′-Asino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS•+) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were performed. Regarding the ABTS assay, the protocol established by Re *et al.*

1999 [36] was utilized. The scavenging activity was calculated using the following formula:

ABTS radical scavenging activity (%) = [(ABTS control – ABTS sample) / ABTS control] x 100

where ABTS represents the absorbance at 734 nm.

As for the DPPH, it was performed with some modifications according to Gabr *et al.*, 2012[37].

The scavenging activity was calculated using the following formula:

DPPH radical-scavenging activity (%) = (DPPH control - DPPH sample / DPPH control) × 100,

Where DPPH represents the absorbance at 515 nm.

The absorbance was measured using a Jenway 6715 UV/ Vis spectrophotometer.

Effect of ZnO nanorods on G. jasminoides Variegata total phenol and total flavonoid contents after eight days of treatment.

Total phenolic content

Total phenol content was assessed using the Folin–Ciocalteu micro-method **[**38, 39**].** Gallic acid served as the standard for constructing the calibration curve. The total phenolic content expressed as gallic acid equivalent was determined using the following equation:

A = 0.98C + 9.0925 × 10 -3 (R2= 0.9996)

Where A represents the absorbance of gallic acid and C denotes the concentration.

Total flavonoid content:

The total flavonoid content was assessed using the method outlined by Ordon *et al.*, 2006 [40]

The total flavonoid content, expressed as quercetin equivalent (QE), was calculated using the following equation derived from the calibration curve:

Y = 0.0255X (R^2 = 0.9812). Here, X represents the absorbance, and Y denotes the concentration (mg QE g−1 FW).

Estimation of antimicrobial activity of different plant extracts.

The susceptibility of reference organisms to different treatments of plant extracts was tested using the Kirby-Bauer disc diffusion agar technique **[**41, 42]. Five organisms were used: two gram-positive bacteria, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213; and two gram-negative bacteria, *Shigella flexneri* ATCC 12022 and *Escherichia coli* ATCC 25922, in addition to antifungal activity against *Candida albicans* ATCC 10231.

Inoculum and plant extracts preparations.

Freshly prepared agar slants of the reference organisms (Nutrient agar for bacterial strains and Potato dextrose agar for Candida organisms) were used to inoculate 5 ml of Tryptic Soy Broth medium. After 24 hours of incubation, a spectrophotometer adjusted the cultures to the 0.5 McFarland standard.

Antimicrobial assay

Sterile discs of Whatman No. 3 filter paper (6 mm) were impregnated with 20 µl of the prepared methanol extracts. The dried discs were located on the surface of plates inoculated with 100 µl of the cell suspension. The plates were stored at 4 °C for two hours to allow the diffusion of the extract compounds through the agar medium. Then, the plates were allowed to incubate at 32 °C for 24 hours, and the diameters of inhibition zones (DIZ) were measured. Discs loaded with the solvent used were also employed as a negative control. The assay was conducted in triplicate, and the mean (± SD) was calculated using Microsoft Excel.

Statistical analysis

All experiments were performed in triplicate, and the results are shown as Mean ± Standard Deviation (SD). A one-way ANOVA was carried out using GraphPad Prism version 5.01 to evaluate the p-value and significance. The chemical structures were generated using the King Draw program.

Results

Structural of the prepared of ZnO-NPs

The structure of the prepared ZnO-NPs was evaluated via X-ray diffraction pattern (XRD). Fig. 2 displays the acquired ZnO nanorod's XRD pattern. With strong crystallization, all diffraction peaks could be linked to hexagonal wurtzite ZnO (JCPDS Card No. 79-2205, a = 0.3249 nm, c = 0.5205 nm). All peaks related to ZnO- NRs appeared in the XRD pattern. The diffraction peaks of ZnO-NPs fashioned via the hydrothermal method were 32º, 34º, 38º, 47º, 58º, 63º, 67º, 68º, 69º, 73º and 78º of 2 theta degree. All the ZnO nanorods bands are associated with the crystal planes (100), (002), (101), (102), (110), (103), (200), (112), (004), and (202) respectively, as illustrated in (Fig.2).

The morphology of the prepared ZnO-NPs

Fig. 3 (a, b) displays typical TEM images of the ZnO nanorods before and after annealing. This method has generated ZnO-NPs with diameters between 10 and 40 nm appropriately. The ZnO nanorods are roughly 170-240 nm long. The materials remained morphologically intact even after being annealed for one hour at 550 ^{O}C in air.

Effect of different ZnO nanorods concentrations on calli cultures of Gardenia jasminoides Variegata fresh weight after eight days of treatment:

For studying the effect of ZnO nanorods on calli cultures fresh weight of *G. jasminoides* Variegata as represented in (Fig. 4), the control liquid medium was enhanced with (5, 10, 25, 50, and 100 mg/l) of ZnO nanorods and after eight days of incubation on a rotary shaker at 100 rpm in completely dark conditions, calli were harvested, and fresh weight was recorded. By taking a glum on the data in (Fig. 5). It could be reported that calli fresh weight increased with increasing ZnO nanorods till 25 mg/l ZnO nanorod then declined. The highest fresh weight (6.5 ± 0.3 g) was recorded by adding 25 mg/l ZnO nanorods while the lowest fresh weight (5.04± 0.04 g) was recorded by adding 100 mg/ l ZnO nanorods.

According to the one-way ANOVA test, using ZnO nanorods affect significantly the fresh weight of the calli cultures of *G. jasminoides* Variegata.

Determination of rosmarinic and cinnamic acids content by High-Performance Liquid Chromatography (HPLC)

To study the impact of ZnO nanorods on rosmarinic acid and cinnamic acid contents in callus cultures. The control liquid medium was enhanced with (5, 10, 25, 50, and 100 mg/l) of ZnO nanorods and after eight days calli were harvested and samples were extracted for further analysis. To evaluate the impact of ZnO nanorods on rosmarinic and cinnamic acids the HPLC was used. By taking a glum at the data represented in (Table 2), it was found that ZnO nanorods have a positive impact on both phenolic compounds. As for rosmarinic acid, the ZnO nanorods increased its content dramatically, since adding 10 mg/l ZnO nanorods to the medium, the rosmarinic acid recorded 250 µg/ GFW which represents five folds in its content in the control treatment (50 µg/GFW). Its impact on the cinnamic acid content was gradual to reach its highest content (83 µg / GFW) with adding 50 mg/l ZnO nanorods which represents about twice its content in the control treatment (30 µg/ GFW). With increasing the concentration of ZnO nanorods to 100mg/l the cinnamic acid content declined to reach (50 µg / GFW), while the rosmarinic acid was not detected at the higher concertation beginning from 10 mg/l ZnO (Table 2).

Effect of different ZnO nanorods concentrations on the anti-oxidant activity of Gardenia jasminoides Variegata after eight days of treatment.

No one procedure is appropriate for estimating the potency of antioxidants because numerous approaches may provide wildly inconsistent findings, as mentioned by Dorman *et al.,* 2003 [52]. Several techniques based on different mechanisms must be used. In this case, the ABTS and DPPH radical-scavenging activity assays were performed.

By analyzing data presented in Fig. (6, a), it could be noticed that the percentage of ABTS increased gradually with increasing the ZnO nanorods to reach its highest value (71.34 %) with adding 10 mg/ l ZnO nanorods compared with (64.865 %) with the control treatment then it began to decline to reach its lowest value (61.9 %) with adding 100 mg/ l ZnO nanorods.

Regarding the assessment of anti-oxidant activity using DPPH assay, by taking a look at Fig. (11, b), it could be observed that the scavenging activity of the samples using DPPH takes the same trend of ABTS analysis since it increased gradually with increasing ZnO nanorods to reach its highest value (21.02 %) with adding 10 mg/ l ZnO nanorods comparing with (10.54%) with the control treatment then it began to decline to reach its lowest value (3.78 %) with adding 100 mg/ l ZnO nanorods.

According to the one-way ANOVA test, there were no significant differences among the different treatments concerning the antioxidant activity using ABTS assay even though using 10 mg/ l ZnO gave a higher ABTS percentage than the other treatments. While among these treatments there was a significant difference using DPPH assay.

Effect of ZnO nanorods on G. jasminoides Variegata total phenol and total flavonoid contents after eight days of treatment.

Total phenolic content

Phenolic acid accumulation was determined in different treatments after eight days Figure (12, a). By analyzing data in Fig (12, a), it could be observed that the control treatment recorded the highest total phenolic content (26.44 ±9.2 mg / GFW) followed by the treatment with 50 mg/l ZnO nanorods which recorded (18.27 ± 4.616 mg/GFW) which match to great extent with rosmarinic and cinnamic acid contents.

Total flavonoid content

Total flavonoid accumulation was determined in different treatments after eight days Figure (12, b). By analyzing data in Figure (12, b) it could be observed that there was a significant difference between the total flavonoid content of the control (0.0405 ± 0.0002 mg/GFW) and the different treatments except with adding 25mg/ l ZnO nanorods which recorded the lowest total flavonoid content (0.036± 0.0001 mg / GFW).

According to the one-way ANOVA test, there were no significant differences among the different treatments concerning the total phenolic content, while among these treatments there was a significant difference concerning the total flavonoid content. This matches to a great extent with the rosmarinic and cinnamic acid contents since using 10 mg/l ZnO nanorods boosted the rosmarinic acid content fivefold to reach 250 µg/GFW compared with the control, which recorded 50 µg/GFW. While, the cinnamic acid content was doubled using 50 mg/l

ZnO, comparing the control (83, 39 µg/GFW, respectively).

Antimicrobial assay.

The results illustrated in Table 3 show that no antibacterial activity was observed against *Bacillus subtilis* and *Escherichia coli*. This apparent resistance could be due to the lower concentrations of the active compounds. However, noteworthy activity was observed against *Staphylococcus aureus* and *Shigella flexneri* in treatment No. 3, attributing this activity to the synergistic effect between the higher concentration of produced rosmarinic acid and cinnamic acid, as well as the sensitivity of the two organisms to these compounds. On the other hand, significant anticandidal activity was observed in treatments No. 3, 5, and 6, which could be due to the elevated concentrations of rosmarinic acid in these samples.

Discussion

ZnO-NPs are extensively used in nanotechnology due to their small size, which ranges from 1 to 100 nm [43]. Plants may readily absorb these nanoparticles due to their huge specific surface area, high solubility, and diffusion rates [44]. Thus, the fast release of zinc dioxide (Zn 2+) can control physiological, biochemical, and molecular processes that eventually affect plant growth.

Our findings align with what has been reported by Wang *et al.*, 2023[45] who stated that, ZnO-NPs at a concentration of 25 mg/L increased *G. biloba's* fresh weight, whereas concentrations of 50 and 100 mg/L inhibited plant development. Also, our results follow what was reported by Predoi *et al.*, 2020[46], who noticed that low ZnO NP concentrations were necessary for plants to develop normally. Conversely, increased Zn concentrations in plants can have adverse impacts like inhibiting cell division and elongation, subsequently decreasing growth and biomass.

According to Fakruddin *et al.*, 2012 [47], nanomaterials may be a novel, efficient abiotic for promoting the creation of secondary metabolites. Numerous studies indicate that nanomaterials may enhance the expression of various genes that are responsible for producing secondary metabolites [48-49]. The profiles of secondary metabolites in plants are not static and can be modified in response to various stressors, including biotic factors (such as diseases and insect infestations) and abiotic factors (like drought, salinity, UV radiation, temperature variations, and exposure to heavy metals). Specifically, plant-nanomaterial interaction increases the production of different reactive oxygen species (ROS), which causes oxidative stress, membrane damage, changes in antioxidant activity, secondary metabolism, and hormonal pathways [50, 51]. Our findings are in line to a great extent with the earlier study by Wang *et al.*, 2023 [45] which found that 25 mg/L of ZnO-NPs considerably increased the amount of flavanol aglycones (such as isorhamnetin and quercetin) in *G. biloba* leaves. Furthermore, the flavonoid content of plants varies according to ZnO-NP concentration; in general, higher ZnO-NP concentrations prevent the accumulation of secondary metabolites.

Our results including the antioxidant activity match our results concerning the rosmarinic and cinnamic acids content. These results can be justified by taking into consideration that exposing plants to any stress either biotic or abiotic which includes heavy metals results in the overproduction of ROS and oxidative stress. According to Dat *et al.*, 2000 [53], ROS is a plant's rapid reactivity to any stress and has two purposes: it can either stimulate the plant's defensive mechanism or it can enhance cell damage or disrupt signal transduction according to its concentration. It was found that ZnO NPs (size 34 nm) increased total phenolic and flavonoid contents, antioxidant capacity, and the production of steviol glycosides (stevioside and rebaudioside A) in micro propagated shoots of *Stevia rebaudiana* Bert. The effect peaked at 1 mg L but decreased at higher concentrations [54].

Our results align with those reported by Chamani, *et al.*, 2015 [55], who declared that ZnO NP concentrations of 75 and 25 mg/ L, respectively, were shown to have the highest content of phenolics and flavonoids in the impact of ZnO NPs on Lilium ledebourii Bioss. Cultures. Also, it was found ZnO NPs which are <100nm in size with a concentration of 500 – 1500 mg/l were reported to boost phenolic and flavonoid synthesis in *Brassica nigra* L. (Black mustard) callus cultures [56].

These findings align with those reported by Ruwizhi and Aderibigbe, 2020 [57], who stated that cinnamic acid has minimal antibacterial effectiveness against certain gram-positive and gram-negative organisms at lower concentrations. Similarly, Ivanov et al., 2022[58] reported that rosmarinic acid has promising anticandidal activity, with MIC values between 0.1 and 0.2 mg/ml. This finding also correlates with what was reported by Sova, 2012 [59], who stated that cinnamic acid has good anticandidal activity.

Conclusion

Currently, the applications of nano-biotechnology significantly influence the domain of plant secondary metabolites, enhancing the production of target compounds. To our knowledge, this is the first study to apply ZnO nanorods as an elicitor to elevate the rosmarinic and cinnamic acid contents in the calli cultures of *G. jasminoides* Variegata. Data indicates that using 10 mg/l ZnO nanorods boosted the rosmarinc acid content fivefold to reach 250 µg/GFW compared with the control, which recorded

50 µg/GFW, additionally has the highest antioxidant capacity compared with the control as well as other ZnO nanorods concentrations. The cinnamic acid content was doubled using 50 mg/l ZnO, comparing the control (83, 39 µg/GFW, respectively). This area of interest requires further research to assess the concentrations and duration of elicitation. On the other hand, the elevated production yield of cinnamic and rosmarinic at treatment No. 3 increases the potency against *Staphylococcus aureus*, *Shigella flexneri*, and *Candida albicans* while the extracts of all treatments showed no activity against the five organisms. Furthermore, treatments No. 5 and 6 showed obvious activity against *Candida albicans* which related to the produced cinnamic acid.

Ethics approval and consent to participate

Not applicable

Funding statement

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Conflicts of interest

The authors declare there are no conflicts of interest.

TABLE 1. Different culture treatments with ZnO nanorods

Treatment Number	ZnO NPs Concentration
Treatment No.1	Control without ZnO nanorods
Treatment No.2	Control +5 mg/l ZnO nanorods
Treatment No.3	Control +10 mg/l ZnO nanorods
Treatment No.4	Control +25 mg/l ZnO nanorods
Treatment No.5	Control +50 mg/l ZnO nanorods
Treatment No.6	Control +100 mg/l ZnO nanorods

TABLE 2. The impact of ZnO nanorods on Rosmarinus and cinnamic acids content in the G. jasminoides Variegata callus extracts after eight days of treatment:

Treatment	Phenolic compound in µg /g fresh weight	
	Rosmarinic acid	Cinnamic acid
Control	50	39
5 mg /l ZnO nanorods	ND*	47
10 mg /l ZnO nanorods	250	65
25mg /l ZnO nanorods	ND	ND
50 mg /l ZnO nanorods	ND	83
100 mg /l ZnO nanorods	ND	50

* ND= Not Detected

TABLE 3. Antimicrobial activity of different plant extracts.

Treatment	Diameter of inhibition zone (DIZ) in mm				
	Bacillus subtilis	***Staphylococcus aureus***	***Shigella flexneri***	***Escherichia coli***	***Candida albicans***
Control	ND*	ND	ND	ND	ND
5 mg /l ZnO nanorods	ND	ND	ND	ND	ND
10 mg /l ZnO nanorods	ND	8 ± 0.4	8 ± 0.1	ND	8 ± 0.1
25mg /l ZnO nanorods	ND	ND	ND	ND	ND
50 mg /l ZnO nanorods	ND	ND	ND	ND	8 ± 0.3
100 mg /l ZnO nanorods	ND	ND	ND	ND	7 ± 0.2

* ND = Not detected

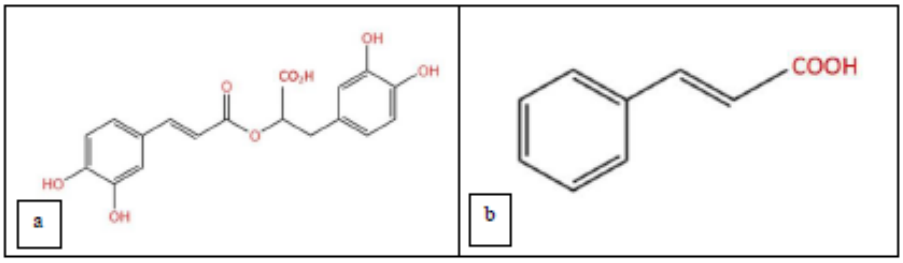

Fig. 1. Chemical structure of a. rosmarinic acid and b. cinnamic acid.

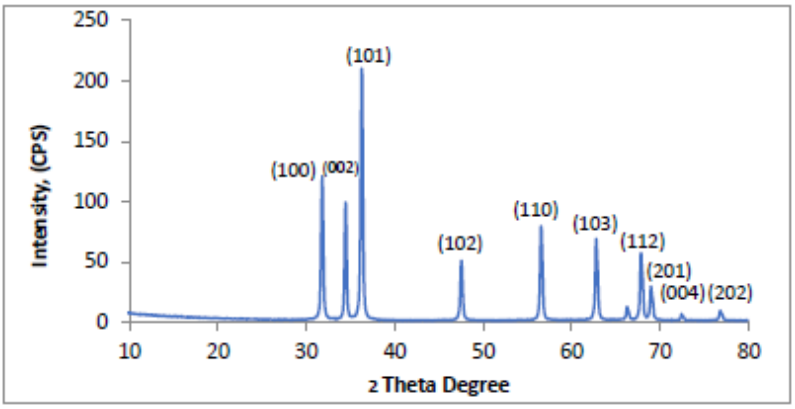

Fig. 2. The XRD of the prepared ZnO-NPs

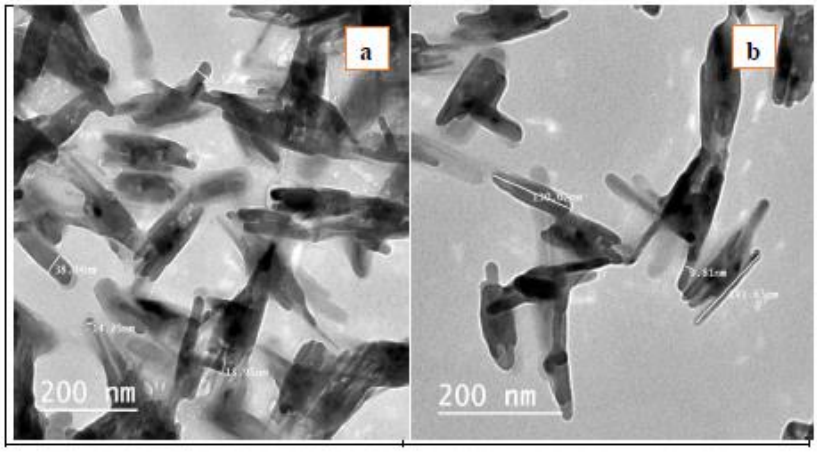

Fig. 3. TEM image of the prepared ZnO nanorods

Fig. 4. *G. jasminoides* Variegata calli cultures used as plant material.

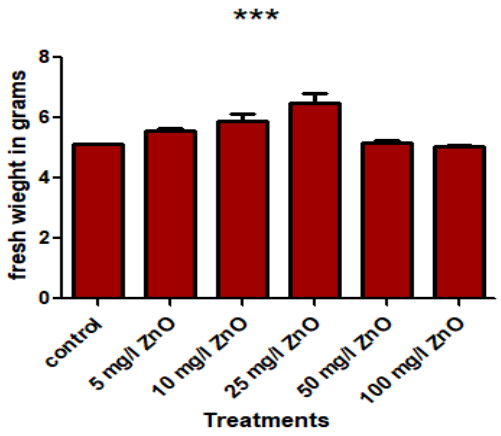

Fig. 5. Effect of ZnO nanorods on fresh weight of *G. jasminoides* Variegata after 8 days of treatment.

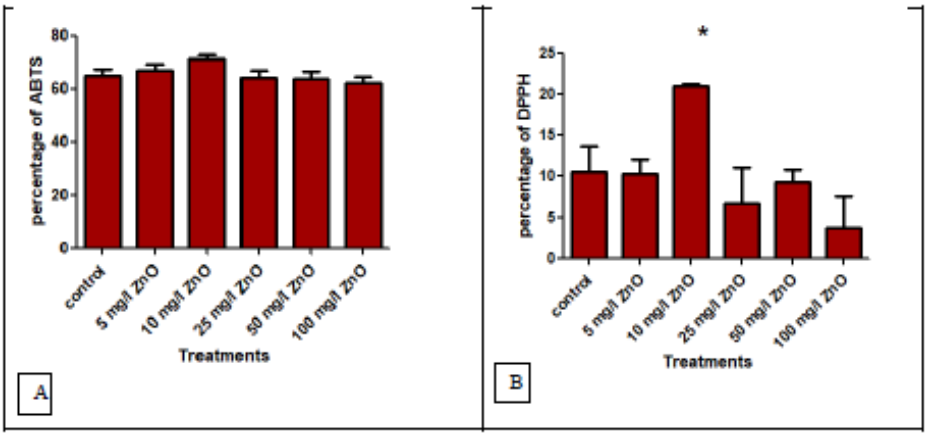

Fig. 6. Effect of ZnO nanorods on antioxidant activity of *G. jasminoides* Variegata after eight days of treatment.

A. Using ABTS radical-scavenging activity assay

B. Using DPPH radical-scavenging activity assay

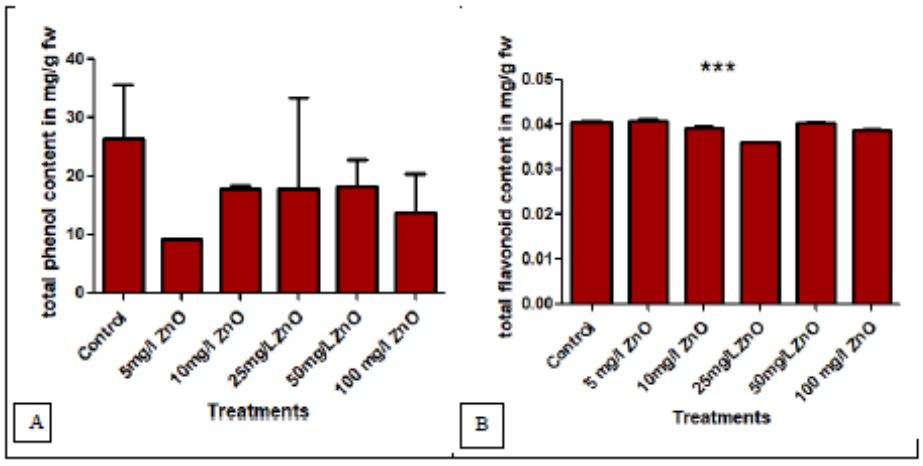

Fig. 7. Effect of ZnO nanorods on *G. jasminoides* Variegata;

A. Total phenol content after eight days of treatment,
B. Total flavonoid content after eight days of treatment

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الهندسة الأيضية باستخدام قضيب أكسيد الزنك النانوي لنبات الجاردينيا الياسمينية المبرقشة لتعزيز الأنشطة المضادة للميكروبات ومضادات الأكسدة

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الملخص

تعرف الجسيمات النانوية (**NPs**) بأنها جسيمات يتراوح حجمها بين واحد ومائة نانومتر. في الآونة الأخيرة ، تم استخدام **NPs** كمحفزات جديدة وفعالة لزيادة كمية المركبات الكيميائية في النباتات. في هذه الدراسة ، تم استخدام أسيتات الزنك وبروميد سيتيل تريامونيوم (**CTAB**) كمتفاعلات لتخليق **ZnO-NPs** بشكل فعال من خلال عملية حرارية مائية مباشرة. تم تقييم قضبان أكسيد الزنك النانوية (**ZnO-NPs**) باستخدام نمط حيود الأشعة السينية (**XRD**) ، والمجهر الإلكتروني النافذ (**TEM**). تمت إضافة تركيزات مختلفة من قضبان أكسيد الزنك النانوية (5 و 10 و 25 و 50 و 100 مجم / لتر) إلى البيئة المغذية السائلة لكالس نبات الجاردينيا الياسمينية المبرقشة لمدة ثمانية ايام ، وتم حصاد مزارع الكاللس بعد 8 أيام. تم تحديد كميات احماض الروزمارينيك والسينامیك باستخدام الكروماتوغرافيا السائلة عالية الأداء(**HPLC**). تم دراسة مضادات الأكسدة بواسطة تكنيك 2،2′- أزينو - مكرر (3-إيثيل بنزوثيازولين-6-سلفونات) الكاتيون الجذري (**ABTS** • +) و كذلك تكنيك 2،2-ثنائي فينيل -1-بيكريل هيدرازيل (**DPPH**). و كذلك تم تقييم المحتوى الكلي للفينولات والفلافونويد. وجد أن إضافة 25 مجم / لتر من قضبان اكسيد الزنك النانوية يعطي أعلى وزن طازج (6.5 ±0.3 جرام) مقارنة بمعاملة المقارنة (5.14± 0.005 جرام). بالنسبة لتراكم حمض الروزمارينيك في مزارع الكالس لنبات الجاردينيا الياسمينية المبرقشة باستخدام قضبان اكسيد الزنك النانوية ، فقد وجد ان استخدام قضبان اكسيد الزنك النانوية بتركيز 10 مجم / لتر رفعت محتوى حمض الروزمارينيك خمسة أضعاف مقارنة بمعاملة المقارنة (250 ، 50 ميكروغرام / جرام وزن طازج على التوالي) ، في نفس الصدد ، سجلت نفس المعاملة 10 ملجم / لتر من قضبان اكسيد الزنك النانوية أعلى نشاط مضاد للأكسدة من التركيزات الأخرى باستخدام كلتا الطريقتين **ABTS** وكذلك **DPPH**. تم تسجيل أعلى معدل من حمض السينامیك (83 ميكروغرام / جرام وزن طازج) والذي يعتبر ضعف محتواه في معاملة المقارنة (39 ميكروغرام / جرام وزن طازج) بإضافة 50 ملجم / لتر من قضبان اكسيد الزنك النانوية. كما تم اختبار النشاط المضاد للميكروبات لمستخلص كل معاملة ضد خمسة كائنات ميكروبية. أكدت النتائج وجود نشاط قوي ضد ***candida albicans*** للمعاملات رقم 3 و 5 و 6. في حين أن جميع المستخلصات لم تظهر أي نشاط ضد ***Bacillus subtilis*** و ***Escherichia coli***. من ناحية أخرى ، أظهرت المعاملة رقم 3 نشاطا معتدلا ضد ***Staphylococcus aureus*** و ***Shigella flexneri*** بسبب زيادة إنتاج المركبات النشطة.

الكلمات الدالة: نشاط مضاد للأكسدة ، نشاط مضاد للميكروبات ، جاردينيا ياسمينة مبرقشة ، المجهر الإلكتروني النافذ **TEM**، نمط حيود الأشعة السينية **XRD**، قضبان اكسيد الزنك النانوية .