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# Biosynthesis of ZnO nanoparticles using the buttercup flower *(Tithonia diversifolia)* extract

# Biosíntesis de nanopartículas de ZnO empleando el extracto de la flor Botón de oro *(Tithonia diversifolia)*

*Borrás-Enríquez, A.J.* [1,2*], *González-Escobar, J.L.* [1], *Veana, F.* [1],

*De la Cruz-Martínez, A.* [2]

[1] *Tecnológico Nacional de México/IT de Ciudad Valles, Carretera Al Ingenio Plan de Ayala Km. 2, Col. Vista Hermosa, 79010 Ciudad Valles, San Luis Potosí, México.*
[2] *Tecnológico Nacional de México/ TES de San Felipe del Progreso. Av. Instituto Tecnológico S/N. ejido de San Felipe del Progreso, 50640, San Felipe del Progreso, Estado de México.*
[3] *Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava #6, Zona Universitaria, San Luis Potosí C.P. 78210, S.L.P., México.*

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## ABSTRACT

Zinc oxide (ZnO) nanoparticles excel in various technological applications, including gas sensors, ultraviolet and visible laser sensors, solar cells, photocatalytic cells, and catalysts, where their synthesis is crucial for each specific application. The use of biological components in nanoparticle synthesis, such as plant extracts, is of great interest since it is a sustainable and environmentally friendly method. *Tithonia diversifolia* is a forage plant that exhibits physiological adaptations to survive in harsh environments; its flowers currently have no practical use. This work focuses on producing zinc oxide via green chemistry, using extracts from buttercup flower (*Tithonia diversifolia*) as a reducing agent, and characterizes the material through X-Ray Diffraction, Diffuse Reflectance, and Scanning Electron Microscopy. The results show that the tree buttercup flower extract produces zinc oxide nanoparticles of about 21 nm in size, with a wurzite-like structure and a ratio of approximately 30:55 (Zn:O), due to impurities like magnesium, calcium, and potassium present in the extract. These impurities are advantageous for enhancing or modifying the nanoparticles for future technological developments.

**KEY WORDS:** Biosynthesis, Tihonia, flower, Zinc oxide, nanoparticles.

***Corresponding Author:**

**Borrás-Enríquez, A.J.** Tecnológico Nacional de México/IT de Ciudad Valles, Carretera Al Ingenio Plan de Ayala Km. 2, Col. Vista Hermosa, 79010 Ciudad Valles, San Luis Potosí, México. E-mail: anahi.borras@tecvalles.mx

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## RESUMEN

Las nanopartículas de óxido de Zinc (ZnO) destacan en aplicaciones tecnológicas como sensores de gas, sensores de láser ultravioleta y visible, celdas solares, celdas fotocatalíticas, catalizadores fotocatalíticos, donde su síntesis juega un rol importante para cada aplicación. La introducción de componentes biológicos en la síntesis de nanopartículas como extractos de plantas, es de gran interés, al ser un producto de fácil obtención y no contaminante al ambiente. *Tithonia diverisifolia* es una planta forrajera que muestra adaptaciones fisiológicas para hacer frente a entornos hostiles; dentro de sus componentes, su flor actualmente no presenta un uso, por lo que este trabajo se centra en la producción de óxido de zinc mediante química verde utilizando extractos de la flor de botón de oro (*Tithonia diversifolia*) como agente reductor y su caracterización mediante técnicas de Difracción de rayos X, Reflectancia Difusa y Microscopia Electrónica de Barrido. Los resultados mostraron que el extracto de Flor Botón de oro produce óxido de zinc en nanopartículas de tipo wurzuita de 21 nm con una proporción de 30:55 (Zn:O), debido a la presencia de impurezas (Magnesio, Calcio y Potasio), presentes en el extracto, las cuales resultan deseables para mejorar o modificar las nanopartículas para el desarrollo de nuevas tecnologías.

**PALABLAS CLAVE:** Biosíntesis, tithonia, flor, oxido de zinc, nanoparticulas

## Introduction

Currently, there is growing interest in developing nanoparticles through methods that enhance their physicochemical and optoelectronic properties, thereby expanding their applications as catalysts, support materials, and semiconductors. Among the most relevant nanoparticles is zinc oxide (ZnO), which is employed in gas sensors, ultraviolet and visible lasers, solar cells, photocatalytic cells, and photocatalytic catalysts. There are several methods for generating nanoparticles, among which biosynthesis is particularly notable. This process is defined as the production of nanoparticles using biological materials (Naiel *et al.,* 2022).

The incorporation of biological components into nanoparticle synthesis, such as microorganisms, vitamins, plant and/or vegetable extracts, and their derived metabolites acting as reducing agents, has attracted considerable interest (Salinas-Estevané & Sánchez-Cervantes, 2012). The biological method, commonly referred to as biosynthesis, is considered environmentally friendly, as it does not generate toxic waste, does not require sophisticated equipment, and the synthesis conditions are relatively simple, typically taking place at room temperature (Nandhini *et al.,* 2024). The use of biological components in biosynthesis avoids the need for chemical reducing agents such as sodium borohydride ($NaBH_4$), sodium citrate ($Na_3C_6H_5O_7$), sodium ascorbate ($C_6H_7NaO_6$), and elemental hydrogen ($H_2$), whose use produces toxic by-products, thus, this places biosynthesis as a promising research area (Sundararajan & Muthukumar, 2020).

Gardea *et al.* (2002) were the first to report the potential of using plant extracts for the nanoscale synthesis of metals in their zero-valent state. Since then, plant extracts have been shown to act as effective stabilizers for nanoparticles, eliminating the need for commercial stabilizing agents and exhibiting excellent properties that prevent particle agglomeration. Similarly, Rodríguez *et al.* (2013) demonstrated that stevioside glycosides, major constituents of *Stevia rebaudiana*, function as biological templates for nanoparticle synthesis.

The synthesis of zinc oxide nanoparticles (ZnO NPs) has been reported by Lakshmeesha *et al.* (2014) through a mechanism in which phenolic compounds and tannins present in plants reduce zinc nitrate ($Zn(NO_3)_2$) ions. These compounds act as reducing agents, forming complexes with zinc ions which, after oxidation, are reduced to zinc oxide (ZnO) nanoparticles. The mechanisms proposed in this study involve redox processes and the participation of hydroxyl groups (-OH) found in phenolic compounds, which enable nanoparticle formation. On the other hand, Abdul *et al.* (2014) reported that the formation of zinc oxide nanoparticles can be generated from interactions with structures with functional groups (OH), such as ascorbic acid, glycosides, and gallic acid, leading to the redox reaction product to calcination.

Several studies have reported the synthesis of zinc oxide nanoparticles using *Moringa oleifera* extracts (Senthikumar *et al.,* 2014; Morales *et al.,* 2016; Ramesh *et al.,* 2015; Matinise *et al.,* 2017). FT-IR analyses of aqueous leaf extracts revealed the presence of phytoconstituents such as amino acids, alkaloids, flavonoids, and phenolic compounds, which contribute to the stabilization of nanoparticles by interacting with the surface of Zinc. However, these compounds are also important components of both human and animal nutrition, and their use for nanoparticle synthesis may lead to conflicts of interest within the food sector. Currently, no studies have been reported on the use of forage plants for the production of Zinc oxide nanoparticles. Therefore, forage species such as the buttercup plant (*Tithonia diversifolia*) represent a promising candidate as a reducing agent in the biosynthesis of ZnO for the technological development of new materials.

Buttercup is a forage plant similar to the Sunflower, but with smaller flowers, exhibiting remarkable physiological adaptability to withstand hostile environments, which enhances its proliferation and invasion of new areas. As a result, it is commonly found in vacant lots, along roadsides, near rivers, and around rural homes. Reports are indicating that both the stems and leaves are used for animal feed; however, the flowers are not employed for this purpose (Galindo *et al.,* 2017). Therefore, the present study focuses on the biosynthesis of Zinc oxide nanoparticles through green chemistry techniques (Biosynthesis), using the tree buttercup flower as the biological source for the reducing agent.

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## Material and Methods

### Collection and processing of plant material

The complete plant material was collected from the municipalities of San Felipe del Progreso, Atlacomulco, and Acambay. In the laboratory, the components of the plant were separated, and the flowers were subjected to a dehydration process through solar drying for 40 hours, with 8-hour exposure intervals per day. The dried sample was then ground using a mill (VEVOR, model X/2z) and sieved to obtain a mesh size of 40.

### Production of active flower extract

To obtain the active extract, 20 g of powdered, dehydrated flowers were dissolved in 100 mL of deionized water. The mixture was kept under stirring (450 rpm) at a temperature of 60-70 °C for 60 min. Then, the mix was then vacuum filtered using a Büchner funnel and a Whatman No. 40 filter paper. The resulting samples were stored at 4 °C until use.

### Phytochemical characterization of the flowers

The previously obtained flower extract was used to quantify total phenolics, flavonoids, and antioxidant activity (DPPH), using spectrophotometric techniques as reported by Borrás-Enríquez *et al.* (2021).

### Biosynthesis of Zinc oxide

Biosynthesis was performed in an open system using 20 mL of the flower extract and 2 g of $Zn(NO_3)_2 \cdot H_2O$ (Reactivos Meyer, Mexico). The mixture was stirred at 350 rpm at 80 °C until water evaporation was complete and a dry powder was obtained. The material was then subjected to calcination at 450 °C for 5 hours in a muffle furnace.

### Characterization of Zinc oxide nanoparticles

The Zinc oxide nanoparticles were characterized using powder X-Ray Diffraction (PXRD, INEL, Equinox 3000, Cu Kα), and Diffuse reflectance spectroscopy (UV-3600 Shimadzu UV-VIS-NIR Spectrometer), with a wavelength range of 200-300 nm. The morphology of the Zinc oxide nanoparticles was further analyzed by scanning electron microscopy (SEM).

### Calculation of lattice parameters from X-Ray Diffraction

The diffraction planes were analyzed using Bragg's Law (Equation 1), where $n$ is the diffraction order, $\lambda$ is the wavelength (nm), $d$ is the interplanar spacing (Å), and $\theta$ is the incidence angle of the radiation:

Equation 1. Bragg's Law

$$n\lambda = 2d.sin\theta$$

The interplanar spacing for hexagonal systems was calculated using Equation 2, where $h$, $k$, $l$ are the Miller indices, and $a$ and $c$ are the lattice constants:

Equation 2. Interplanar distance calculation

$$\frac{1}{d^2_{(h\,k\,l)}} = \frac{4}{3}\left(\frac{h^2 + hk + k^2}{a^2}\right) + \frac{l^2}{c^2}$$

The relationship between the incidence angle and the quadratic form of Bragg's equation is given in equation (3). The constant $A$ is defined in equation (4). The lattice parameters were calculated using equations (5) and (6).

Equation 3. Lattice parameter calculation

$$sin^2\theta = A\,(h^2 + hk + k^2)$$

$$A = \lambda^2/3a^2$$

$$a = \sqrt{\frac{\lambda^2}{3A}}$$

$$c = l.d$$

## Band gap calculation using Diffuse Reflectance

The energy band gap was calculated using the Kubelka–Munk function:

Equation 4. Kubelka–Munk Equation

$$\frac{k}{s} = \frac{1 - R\infty^2}{2R\infty}$$

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Where $k$ and $s$ are the absorption and scattering coefficients, respectively, and $R$ is the reflectance of an infinitely thick layer.

### Crystallite size estimation

The Average Crystallite Size ($D$) was calculated using the Scherrer equation:

Equation 5. Scherrer Equation

$$D = K.\lambda\beta.\cos\theta$$

Where $\lambda$ is the wavelength, $\beta$ is the full width at half maximum, $\theta$ is the diffraction angle, and $K$ is a shape factor (typically for spherical particles).

## Results and Discussion

The concentrated extract of *Tithonia diversifolia* flowers showed a total phenolic content of 1.64 ± 0.12 mg GAE/100 mL, a total flavonoid content of 160.08 ± 2.36 mg QE/100 g, and a free radical inhibition percentage of 73.86 ± 5.15%, indicating antioxidant activity (Table 1). Secondary metabolites such as polyphenols and flavonoids have been shown to act as reducing agents in biosynthesis reactions, providing stability during the reduction of chemical precursors and helping control the reaction rate, thereby facilitating the production of pure materials (Abdolhossien *et al.,* 2019). This effect may be attributed to the phenolic structures of flavonoids and polyphenols, which are capable of donating electrons to reduce metal precursors into nanoparticle forms (Rathod & Manukumar, 2016).

Polyphenols and flavonoids possess chemical structures rich in hydroxyl groups (-OH) and other features of the phenolic ring that can adsorb onto the surface of the formed nanoparticles. This adsorption avoids agglomeration, which could otherwise compromise their stability and desired properties. Phenolic compounds function as both physical and chemical stabilizers by forming a protective layer around the nanoparticles. The rate at which metal ions are reduced is influenced by the availability of electrons within phenolic compounds. By controlling the amount of flavonoids or polyphenols and the concentration of metal ions, it is possible to regulate the reduction rate, avoid uncontrolled nanoparticle formation, and produce a more homogeneous final product (Sundararajan & Muthukumar, 2020).

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**Table 1. Phytochemical characterization of buttercup flower (*Tithonia diversifolia*)**

| Total phenolics (mg de EAG/ml) | Total Flavonoids (mg EQ/ml) | Inhibition (%) |
|---|---|---|
| 1.64±0.12 | 30.43±3.46 | 71.24±3.12 |

The X-ray Diffraction (XRD) analysis of the ZnO nanoparticles is shown in Figure 1. The image reveals diffraction peaks at (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2), (2 0 1), (0 0 4), and (2 0 2). According to the study by Zagal-Padilla and Gamboa (2018), these 11 observed peaks correspond to the crystalline planes of the hexagonal wurtzite structure of ZnO.

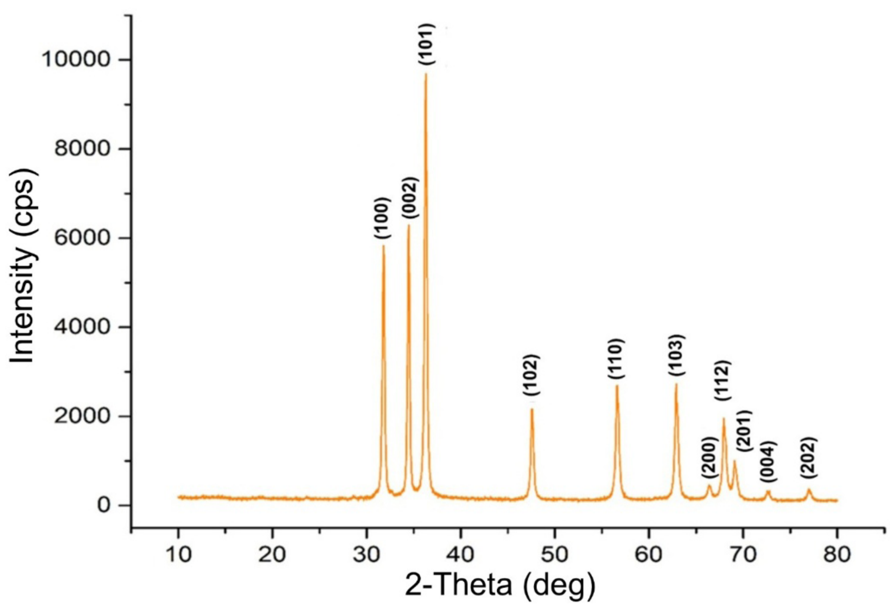


**Figure 1. XRD patterns of ZnO.**

The lattice parameters were calculated to obtain further information from the X-ray diffraction analysis (Table 2). The results show values similar to those reported in the literature for Zinc oxide compounds (Marin *et al.,* 2016). One of the main diffraction planes is (0 0 2), which represents the crystalline formation of the wurtzite-type structure and is associated with the lattice parameter $c$ (Das *et al.,* 2018). This peak exhibits a growth pattern that may be linked to variations in the $c$ parameter, which could indicate the formation of aggregates or secondary phases due to impurities. The (100) and (1 0 1) planes are also considered significant in the wurtzite ZnO structure, as these planes may undergo changes depending on the synthesis method used (Obeizi *et al.,* 2020). Nonetheless, the lattice parameters (0 0 1) and (0 0 2), with a=b (3.45 Å) and c (5.19 Å), are consistent with those reported by Zagal-Padilla and Gamboa (2018), who employed parsley extract as the reducing agent.

According to the energy-dispersive X-ray spectroscopy spectrum (Figure 2), the sample contains impurities such as Magnesium, Potassium, and Calcium. These elements have also been reported in *Tithonia diversifolia* in other studies (Mabou *et al.,* 2018). The presence of these and other impurities within the ZnO matrix causes shifts in the main diffraction peaks. The incorporation of Mg into ZnO induces structural changes that depend on the concentration of this element, affecting the intensity of diffraction peaks, particularly the (0 0 2) plane. These results are attributed to the fact that Magnesium, due to its properties as an alkaline earth metal, is often used to modify the energy band gap of ZnO. The ionic radius of $Mg^{2+}$ (57 pm) is very similar to that of $Zn^{2+}$ (60 pm), allowing the formation of a stable phase and causing slight distortions in the lattice parameters. The crystallite size calculated for the zinc oxide biosynthesized using *Tithonia diversifolia* flower extract was 21.54 nm, which falls within the range reported by other authors (Rao & Rao, 2017).

**Table 2. Structural parameters of ZnO nanoparticles.**

| $2\theta$ | H | k | l | a | c |
|---|---|---|---|---|---|
| 31.83 | 1 | 0 | 0 | 3.45 | |
| 34.45 | 0 | 0 | 2 | | 5.19 |
| 36.29 | 1 | 0 | 1 | 3.58 | 2.47 |
| 47.58 | 1 | 0 | 2 | 3.93 | 3.82 |
| 56.66 | 1 | 1 | 0 | 4.61 | |
| 62.91 | 1 | 0 | 3 | 4.71 | 4.43 |
| 66.45 | 2 | 0 | 0 | 4.10 | |
| 68.02 | 1 | 1 | 2 | 4.75 | 2.75 |
| 69.16 | 2 | 0 | 1 | 5.36 | 1.36 |
| 72.62 | 0 | 0 | 4 | | 5.20 |
| 77.04 | 2 | 0 | 2 | 5.17 | 2.47 |

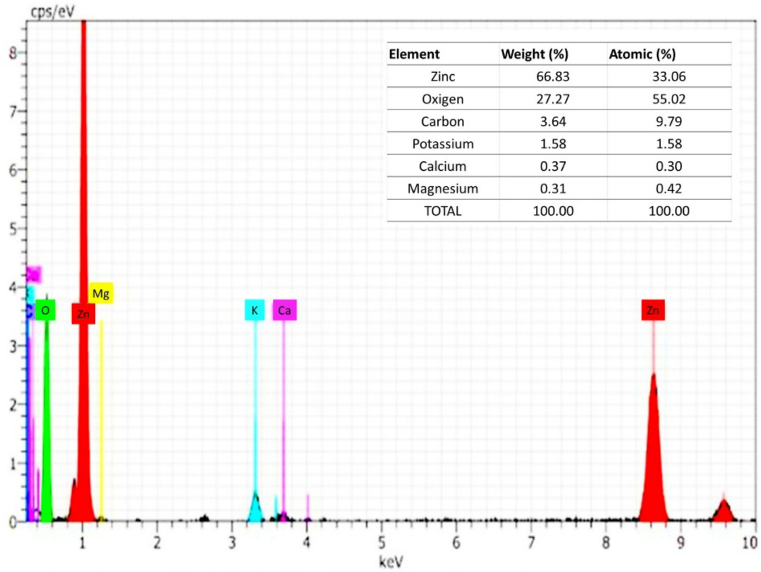


**Figure 2. EDX spectrum of ZnO NPs synthesis using Tithonia diversifolia flower extract.**

In Figure 3, the band gap value of the ZnO nanoparticles (ZnO NPs) is shown, represented by the intersection point of the line with the photon energy (*hv*) on the (*αhv*) versus energy (*cm*) plot. The obtained value was 3.16 eV, which is consistent with some values reported in the literature, such as 3.63 eV (Debanath & Karmakar, 2013).

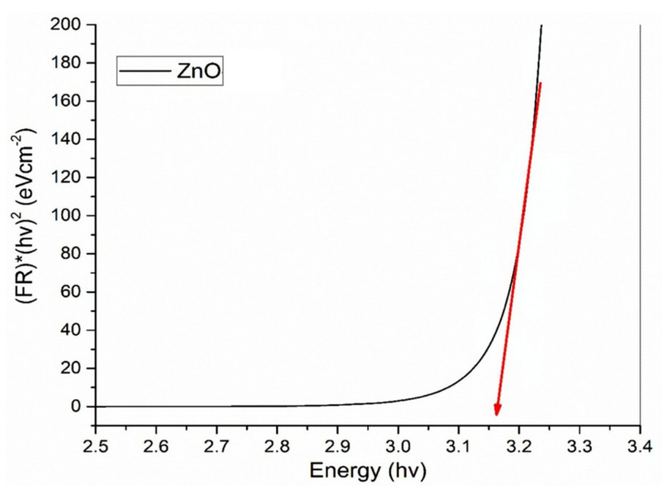


**Figure 3. Bandgap calculations for ZnO nanoparticles.**

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The SEM analysis of ZnO (Figure 4), revealed a nanoflake-like morphology, with particle sizes in the range of 20-25 nm. The ZnO micrographs show that the nanoparticles exhibit a spherical structure and tend to aggregate into larger formations. This morphology is consistent with the findings of Gendo *et al.* (2021), who reported similar structural features in the synthesis of Zinc oxide using leaf extracts of *Calpurnia aurea*.

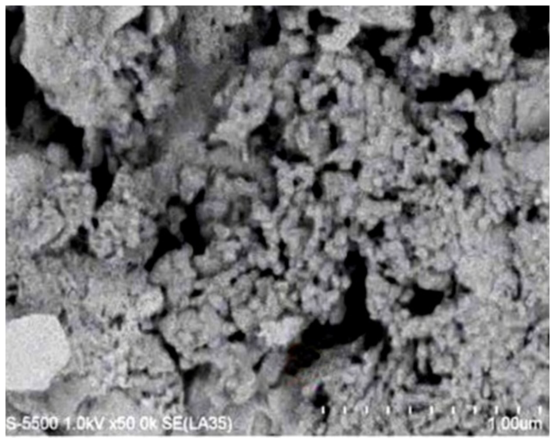


**Figure 4. Micrographs of ZnO nanoparticles.**

The stoichiometric ratio (Zn:O) was found to be 0.33:0.55 (Figure 2), validating the desirable presence of Magnesium during biosynthesis. According to Zagal-Padilla & Gamboa (2018), a stoichiometric ratio close to 1:1 is ideal for the production of Zinc oxide. However, when plant extracts are used, the presence of minerals, often regarded as impurities, is common. Nevertheless, as reported by Rao & Rao ,(2017) doping ZnO nanoparticles with minerals such as Magnesium, Calcium, and Potassium can enhance or modify their physical, optical, and electronic properties, opening new technological possibilities in fields such as photocatalysis, gas sensors, electronic devices, biomedical applications, and wastewater treatment.

## Conclusions

Extracts from the tree buttercup (*Tithonia diversifolia*) flower proved to be a viable source for the production of Zinc oxide through green chemistry. The resulting zinc oxide nanoparticles exhibited a wurtzite-type crystalline structure, with a spherical morphology and an average size of 21.54 nm. The presence of Magnesium, Calcium, and Potassium contributed to the enhancement of the physical, optical, and electronic properties of the ZnO nanoparticles, making them suitable for the development of new technologies.

## Author contributions

Methodology development: Borrás-Enríquez, A.J., and González-Escobar, J.L. Experimental validation: Veana, F., and De la Cruz-Martínez, A. Data analysis: Borrás-Enríquez, A.J., and Veana, F. Data management: González-Escobar, J.L., and De la Cruz-Martínez, A. Writing and manuscript preparation: Borrás-Enríquez, A.J., González-Escobar, J.L., Veana, F., and De la Cruz-Martínez, A.

Writing, review, and editing: Borrás-Enríquez, A.J., González-Escobar, J.L., Veana, F., and De la Cruz-Martínez, A. Project administration: Borrás-Enríquez, A.J.

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## Conflict of Interest

The authors declare no conflict of interest.

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