JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

BIOLOGICAL PROPERTIES OF ZnO PHYTONANOPARTICLES OBTAINED FROM *Annona muricata* L. FRUIT PULP FOR POSSIBLE CO-ADMINISTRATION WITH PROBIOTICS STRAINS

María Candelaria Morales-Ruiz[1], *Miguel Abud-Archila*[1], *Lucía María Cristina Ventura-Canseco*[1], *Federico Antonio Gutiérrez-Miceli*[1], *Benjamín Valdez-Salas*[2], *María Celina Luján-Hidalgo*[1]*

Address(es): Dra. María Celina Luján-Hidalgo,
[1] Tecnológico Nacional de México/Instituto Tecnológico de Tuxtla Gutiérrez, Carretera Panamericana, km. 1080. C.P. 29050, Tuxtla Gutiérrez, Chiapas, México.
[2] Universidad Autónoma de Baja California/Instituto de Ingeniería, Calle de la Normal s/n Colonia Insurgentes Este. C.P. 21280, Mexicali, Baja California, México.

*Corresponding author: maria.lh@tuxtla.tecnm.mx

https://doi.org/10.55251/jmbfs.9963

ARTICLE INFO

Received 8. 3. 2023
Revised 9. 10. 2024
Accepted 16. 10. 2024
Published xx.xx.201x

Regular article

OPEN ACCESS

ABSTRACT

Phytonanotechnology represents a new approach that uses plant extracts to synthesize metal oxide nanoparticles with higher biocompatibility. Recently, the production of ZnO phytonanoparticles (ZnO PTNPs) for food fortification has gained attention due to their lower risk and outstanding properties. Inclusion of ZnO PTNPs might have some effects as antimicrobial properties against pathogens strains, however, could result in challenges when intended to add probiotics in a nanofortified food matrix. For this reason, the co-administration of ZnO PTNPs with probiotics represents a new scenario for the development of modern functional food. This study aimed to evaluate the biological properties including the antioxidant activity, cytotoxicity, stability during gastrointestinal conditions, and the biological effect on the viability of a probiotic strain of ZnO phytonanoparticles obtained from soursop (*Annona muricata* L.) pulp for possible co-administration in food applications. The synthesis resulted in the formation of polydisperse ZnO nanowires with thicknesses ranging from 82 to167 nm and ζ potential values + 27.5 mV and +41.8, before and after sonication and stabilization protocol. ZnO PTNPs exhibited an improved antioxidant activity (IC_{50} = 6.14 mg/mL) compared with the ethanolic extract of *Annona muricata* L. Non-hemolytic activity in human erythrocytes was evidenced, whereas the interaction between ZnO PTNPs and *Lactiplantibacillus fabifermentans* BAL-27-ITTG allows the preservation of adequate viable counts of the probiotic microorganism (>1.2X10^9 UFC/mL), suggesting biocompatibility. Moreover, ZnO PTNPs were shown to be highly stable in stomachal and intestinal conditions with maximum rates of dissolution of 20% and 12%, respectively. Finally, it was confirmed a contribution of 45% of zinc for every dose of ZnO PTNPs used. The present study offers a promising path for the production of functional phytonanoparticles and suggests further investigation for future applications in food matrices.

Keywords: phytonanoparticles, ZnO, *Annona muricata* L., biological properties, *Lactiplantibacillus*

INTRODUCTION

Due to their improved chemical, physical, and biological properties and functionalities, nanoparticles (1-100 nm) are unique nanomaterials that have attracted attention in several fields of research which includes agriculture, medicine, and the food industry **(Bhuyar *et al.*, 2020)**. Nowadays, for the application of nanoparticles in food systems, studies focus on improving biocompatibility, as well as preserving nanoparticle stability in the food matrix when used as an antimicrobial agent or ensuring the controlled release of the constituents of the nanoparticle without affecting the sensory characteristics of the food when the main purpose is to enrich or biofortify with minerals. In this regard, phytonanotechnology is a promising field derived from nanotechnology that produces phytonanoparticles (PTNPs) from plant extracts. This method follows the principles of green synthesis by excluding the use of hazardous chemicals, providing an environmentally friendly synthesis, and representing an economic one-step process, that can be scaled up easily **(Bandeira *et al.*, 2020; Mahmood et al., 2022)**. Compared to nanoparticles obtained from physical and chemical methods, phytonanoparticles have proven to be reproducible, biocompatible, less toxic, and continuously released into systems so they can be used for the design of new products **(Sharma and Tripathi, 2022)**.

Soursop (*Annona muricata* L.) is the most cultivated and well-known species of the *Annonaceae* family, highly produced and consumed in Mexico **(Coelho de Lima and Alves, 2016)**. Soursop poses a high content of edible pulp in its fruit that can provide a more acceptable and easier way of delivering bioactive compounds that possess great antidiabetic, antiarthritic, antihypertensive, antimicrobial, anti-inflammatory, antioxidant, anticancer, and anticonvulsant properties **(Mahmood *et al.*, 2022)**. This bioactivity is conferred by the presence of components such as phenolic compounds like kaempferol 3-O-ruti-noside and myricetin; alkaloids like anonaine, asimilobine, and, nornuciferine, other compounds such as vitamin C, carotenes, tocopherol, among others, and mainly, acetogenins like muricin and montanacin that can also act as reducing and stabilization agents for the synthesis of PTNPs **(Gavamukulya *et al.*, 2019; Coria-Téllez *et al.*, 2018; Solorzano-Toalá *et al.*, 2020)**. Zinc oxide phytonanoparticles (ZnO PTNPs) are distinguished as the third most widely used based-metal nanomaterial **(Faizan *et al.*, 2020)**. In food field, these nanomaterials have shown several properties of interest like antimicrobial action against pathogenic bacteria, antioxidant, and anticancer capacity, as well as photocatalytic efficacy and biocompatibility as compared to their counterparts synthesized by chemical methods and are frequently incorporated into pharmaceutical creams and lotions for antibacterial applications **(Bandeira *et al.*, 2020; Mohammadi *et al.*, 2019)**. The exploitation, mainly, of antimicrobial properties of ZnO nanoparticles has elevated the interest in their incorporation into a food matrix. Previous studies have reported antimicrobial properties of ZnO NPs against popular foodborne Gram-negative and Gram-positive pathogen strains such as *E. coli*, *S. aureus*, *S. enterica*, *S. typhimurim* and *L. monocytogenes*, among others **(Kaushik *et al.*, 2019; Perveen *et al.*, 2020; Busi *et al.*, 2021; Fayhed *et al.*, 2022; Abd El-Nour *et al.*, 2023)**. Despite this, there is few information about the possible effect ZnO nanoparticles might have on beneficial microorganisms, such as probiotics, living non-pathogenic microorganisms that, when administered in sufficient amounts, can restrict the growth of pathogenic bacteria and promote the growth of other beneficial microorganisms **(Sanders *et al.*, 2018)**. Lactobacilli is an important group of microorganisms that are considered quintessential probiotics. Lately, novel species are being studied for possible application, including *Lactipantibacillus fabifermentans*, a Gram-positive lactic acid bacterium first described by **De Bruyne *et al.* (2009)**. This bacterium possesses a wide range of probiotic properties that can be especially interesting for food product manufacturing **(Korcari *et al.*, 2022; Ramírez-Pérez *et al.*, 2022)**. For this reason, it is of great interest to study the possibility of co-administering it with ZnO PTNPs for mineral fortification of food. In addition, the design, production, and application of PTNPs in food matrix should consider the resistance of nanomaterials to gastric fluids related to dissolution and aggregation events, which might impact the bioavailability of these **(Campos *et al.*, 2022)**. In consequence, this study aimed to evaluate the zinc content, antioxidant activity, hemocompatibility, and stability under simulated gastrointestinal conditions, as

well as the effect of the ZnO PTNPs on the viability of a potential probiotic strain to assess the possible co-administration of probiotics and phytonanoparticles for food applications.

MATERIAL AND METHODS

Synthesis of ZnO phytonanoparticles

Annona muricata L. fruit was harvested in Tapachula, Chiapas, México (14º54'00''N 92º16'00''O). After its reception, the fruit was carefully washed with soap and distilled water to remove traces of impurities. Subsequently, the peel, pulp, and seeds were manually separated, and the fresh pulp was frozen (-18 ºC) and lyophilized at -40 ºC and 0.03 mBar until complete drying (LABCONCO 2.5, USA). Extraction was performed by macerating 70 g of lyophilized fruit pulp in 350 mL of ethanol for three days (Gavamukulya *et al*., 2019). The mixture was filtered to remove all the solid matter and the obtained extract was used to quantify the total phenolic compounds (TPC) and then stored at 4 ºC until used **(Velázquez-Gamboa *et al.*, 2020)**.
The synthesis of the ZnO PTNPs was performed according to **Velázquez-Gamboa *et al.* (2020)**. 270 mL of the ethanolic extract was used to mixed with 630 mL of $ZnSO_4$ solution (0.2 M). The mixture was maintained with continuous agitation for 6 h after 900 mL of NaOH (2.0 M) was added. This solution was heated at 60 ºC for 12 h under magnetic agitation. Finally, the colloidal solution was centrifuged (Hermle, DE) at 4500 rpm for 20 min, and the precipitate was washed with ethanol (96%) and dried in a fume hood for 24 h. The ZnO PTNPs were stored in a sealed bag in darkness at 4°C.

Characterization of ZnO phytonanoparticles

The synthesis of ZnO PTNPs was confirmed through a spectral scan in a range of 200-800 nm in a UV-Vis spectrophotometer (Beckman Coulter DU-730, USA) and FTIR spectroscopy (Thermo Fisher Nicolet™ iS™ 10, USA) with a spectral scan from 4000 to 400 cm^{-1} **(Velázquez-Gamboa *et al*., 2020; Beltrán-Partida *et al*., 2019)**. Morphology and particle size were determined in a scanning electron microscope (SEM) at 10 kV (JEOL JSM 6010, JP). Zeta potential and particle size distribution were analyzed by dynamic light scattering technique (DLS) from a solution of ZnO PTNPs (200 ppm), before and after sonication (4 min, power: 30, pulse: 30) and stabilization protocol (1 drop of Tween 80).

Determination of Zinc content in ZnO phytonanoparticles

To determine the amount of Zinc contained in the ZnO PTNPs the methodology proposed by Fabricius *et al*. (2014) was followed with slight modifications. A solution of 100 mg/L of ZnO PTNPs was prepared and sonicated (Cole- Parmer, USA) for 30 min. Prior, 2 mL of the ZnO PTNPs solutions were mixed with 4.4 mL of HNO_3 (65%) and 1.2 mL of H_2O_2 (30%). To remove all the organic matter, the mixture was digested for 10 min at 240 ºC and 1600 W (CEM MARS 5, USA). The digested solution was diluted to 25 mL with a solution of HNO_3 (1% v/v) and analyzed by the ICP-OES technique (Thermo Fisher iCAP 6300, USA). Before the analysis, the calibration curve was built using a multi-element standard containing Zinc (µg/mL) (HIGH PURITY STANDARDS, USA). Yield of synthesis was calculated following Equation 1:

$$\text{Yield of synthesis} = \frac{quantity\ of\ Zn\ from\ ZnO\ PTNPs}{quantity\ of\ Zn\ from\ ZnSO_4} x100 \text{ (Equation 1)}$$

ZnO phytonanoparticles stability under *in vitro* gastrointestinal conditions

The stability of ZnO phytonanoparticles was evaluated based on the liberation of Zinc ions during an *in vitro* digestion test performed according to **Kaur Sidhu *et al*. (2020)**. Firstly, to simulate the gastric conditions, pepsin (Sigma-Aldrich, DE) was re-suspended in NaCl solution (0.5% w/v) to reach a concentration of 3 g/L, and then the pH was set to 2.0 with a solution of concentrated HCl. Small intestine conditions were simulated with a 1 g/L pancreatin (Sigma-Aldrich, DEU) solution prepared from the same saline solution previously described. Pancreatin solution was used with or without bile salt addition (0.3% w/v) and adjusted at pH 8.0 using NaOH solution (0.1 M).
ZnO PTNPs were re-suspended in deionized water to reach concentrations of 25, 50, and 100 mg/100 mL and then sonicated (Cole- Parmer, USA) for 30 min. 9 mL of intestinal or gastric simulated fluids and 1 mL of re-suspended ZnO PTNPs were placed in Falcon tubes, stirred in a vortex for 10 s, and incubated for 2 h at 37 ºC. To stop the digestion, an ice bath was prepared and the samples were placed there for 10 min. Subsequently, centrifugation (Hermle Z-236K, DE) was performed at 3500 g at 4 ºC for 1 h. The supernatant was used to determine the ions of ZnO PTNPs released upon digestion by the ICP-OES technique (Thermo Fisher iCAP 6300, USA). Finally, the percentage of dissolution was determined according to the subsequent relation:

$$\text{Percentage of dissolution (\%)} = \frac{(Zn^{+2}\ release * 100)}{(ZnO\ PTNPs\ dose * a)} \text{ (Equation 2)}$$

Where:
Zn^{+2} release = ionic portion of ZnO PTPs in the supernatant after *in vitro* digestion.
ZnO PTNPs dose = concentration of re-suspended ZnO PTNPs employed.
a = Zinc content for each mg of ZnO PTNPs employed.

Evaluation of the biological properties of ZnO phytonanoparticles

Antioxidant activity

The radical scavenging activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a free radical **(Zhang *et al*., 2013)**. ZnO PTNPs were re-suspended in deionized water to reach concentrations of 0.5, 1, 3, 5, 7, and 10 mg/mL and then sonicated (Cole- Parmer, USA) for 30 min. 1 mL of DPPH 0.05 mM (Sigma-Aldrich, DEU) was mixed with 2 mL of the ZnO PTNPs re-suspensions and incubated in darkness for 30 min. After this time, the absorbance of every sample was measured by UV-Vis spectrophotometry (Beckman Coulter DU-730, USA) at 517 nm. The blank solution consisted of a methanol-ZnO PTNPs solution with a 1:1 ratio (v/v), while as a control a methanol-DPPH solution with a 1:2 (v/v) ratio was used. The results were calculated with equation 2 and expressed as percent inhibition of DPPH absorbance.

$$DPPH\ inhibition\ (\%) = \frac{Abs\ sample - Abs\ blank}{Abs\ control}\ x\ 100 \text{ (Equation 3)}$$

The IC_{50} value was used to indicate the antioxidant capacity of the ZnO PTNPs and was calculated using linear regression analysis.

Hemolytic assay

Hemocompatibility of ZnO PTNPs was evaluated through the possible hemolytic effect in human blood cells **(Khan *et al*., 2018)**. The hemolytic assay was performed with the SPINREACT glycated hemoglobin (HbA_{1c}) detection kit. Samples of human blood were taken from healthy patients and treated with EDTA to prevent coagulation. Previous sonicated ZnO PTNPs (6, 15, and 30 mg/100mL) and zinc sulfate (30 mg/100mL) solutions were added to the blood samples (1:1 volume ratio) and incubated for 1 h at 37 ºC. Finally, a spectrophotometric analysis (Beckman Coulter DU-730, USA) at 660 nm was performed. A blood sample without the addition of ZnO PTNPs or metallic salt was also used as a negative control. The results were expressed as a percentage of hemolysis.

Effect of ZnO phytonanoparticles on the viability of *Lpb. fabifermentans* BAL-27-ITTG

To evaluate the effect of the ZnO PTNPs on the viability of an acid lactic bacteria the method described by Wang *et al.* (2020) was performed with slight modifications. Solutions of re-suspended ZnO PTNPs (7.5, 4.5, and 2.0 mg/100mL) were adjusted at different pH values (2.0, 4.5, and 7.0) with NaOH (1.0 M) and HCl (0.1 M), and sonicated for 30 min prior to use. Initially, 1 mL of *Lpb. fabifermentans* BAL-27-ITTG was inoculated in test tubes containing 9 mL of sterile MRS broth and incubated for 8 h at 37 ºC.
After, 1.1 mL aliquots of the ZnO PTNPs solutions were added to the tube with the bacterial culture, and the mixtures were incubated for 2 h at 37 ºC. Finally, cell viability was determined on Petri dishes of MRS agar (BD, USA) which were incubated at 37ºC for 36 h. The results were reported as colonies-forming units per milliliter (CFU/mL) and compared to a control solution without ZnO PTNPs addition. A control without ZnO PTNP addition was used as a negative control.

Analysis of results

Every analysis was performed in triplicate and the results were expressed as an average of each determination. The average of every variable response was evaluated through an Analysis of Variance (ANOVA) and compared with a Tukey analysis ($p \leq 0.05$) with Statgraphic Centurion V15.2 software. Evaluated doses of ZnO PTNPs were calculated based on the Mexican Recommended Dietary Allowance (15 mg/day).

RESULTS AND DISCUSSION

Synthesis of ZnO phytonanoparticles

Quantification of TPC of ethanolic extract of *Annona muricata* L. was expressed as mg of Gallic Acid Equivalents (GAE) for every 100 g of dry matter. TPC analysis evidenced a high quantity of these compounds (207.33 ± 0.06 mg GAE/100 dm) compared with the results previously reported by **Jiménez-Zurita *et al*. (2017)** and Siqueira *et al*. (2015). *Annona muricata* L. extract was used as a reducing agent of metallic precursor ($ZnSO_4$). This first one contains bioactive molecules including phenolic compounds that allow the synthesis through REDOX reactions **(Coria-Téllez *et al*., 2018)**. The formation of the ZnO PTNPs was evidenced by the shift in color solution, passing from a yellow-pale color to a light brown color, indicating the obtention of the ZnO PTNPs colloidal solution.

Characterization of ZnO phytonanoparticles

The synthesis was confirmed by the UV-vis analysis, following the absorption peak of ZnO which is around 385 nm (Fakhari *et al*., 2019). The absorption spectrum of the UV-vis analysis of the colloidal solution of ZnO PTNPs showed a characteristic peak at 370 nm (Figure 1). Moreover, it is known that in absorption spectroscopy the width of the band increases with the increase of the particle size, a fact that can suggest a significant variation in the particle size of the obtained ZnO PTNPs (Fakhari *et al*., 2019).

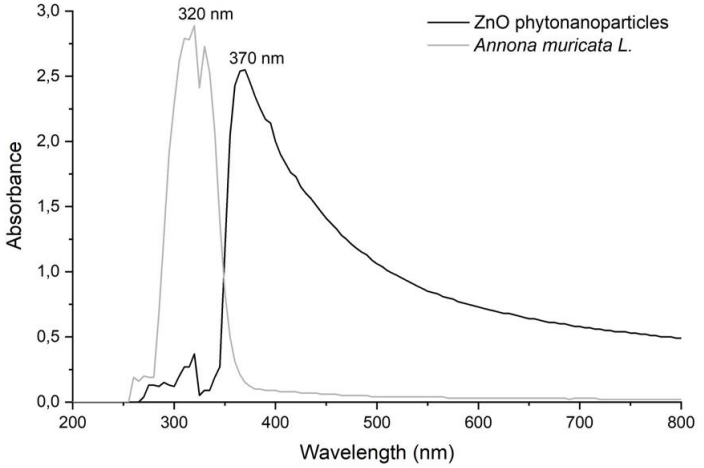

Figure 1 UV-Vis absorption spectrum of ZnO PTNPs and the ethanolic extract of *Annona muricata* L.

The FTIR spectrum was assessed to analyze the presence of phytochemicals in *Annona muricata* L. pulp that facilitate the formation of ZnO NPs. It has been reported that soursop pulp contains more than two hundred bioactive compounds, being a rich source of acetogenins, alkaloids, and phenolic compounds (Coria-Tellez *et al*., 2014), which contain numerous hydroxyl groups that reduce salt precursor to ZnO nanoparticles. Since the goal of the synthesis here reported is to maintain phytocomponents in the nanoparticles, the calcination process was not performed. The presence of main components of *Annona muricata* L. pulp was confirmed through four bands observed at 3382 cm^{-1},1550 cm^{-1}, 1436 cm^{-1}, and 1114 cm^{-1} and a signal in the fingerprint region between 400-600 cm^{-1} corresponding to the metal oxide formation as is shown in Figure 2. The signal at 3382 cm^{-1} could be related to the O-H stretching vibrations of hydroxyl groups, while the band at 1550 cm^{-1} could be attributed to the C=C stretching vibrations of alkene groups (Aziz *et al*., 2019). The appearance of a signal at 1437 cm^{-1} can be associated with C-C and C-O stretching vibrations of carbonyl, carboxyl, and aliphatic groups, and the band in 1114 cm^{-1} could be due to C-N bonds (Gavamukulya *et al*., 2019; Sabapati *et al*., 2019). According to Selvanathan *et al*. (2022) and González-Pedroza *et al*. (2021) signals correspond to the O-H bond of phenolic compounds, while C=C, C-C, C-O are characteristic bonds of the acetogenins, finally C–N bond could be originating from alkaloids in *Annona muricata* L. Also, it was possible to visualize the stretching vibrations of the Zn-O bonds by the appearance of an absorption band between 400-600 cm^{-1} (Ahmad y Kalra, 2020). It's important to notice that the absorption band of Zn-O bonds is smaller than expected, this could be due to the presence of the capping agents (phytocomponents) that can be hiding the signal. However, these results confirm the role of soursop as an active source of reducing and stabilizing agents responsible for the transformation of metallic salt into ZnO phytonanoparticles and evidence the presence of zinc oxide.

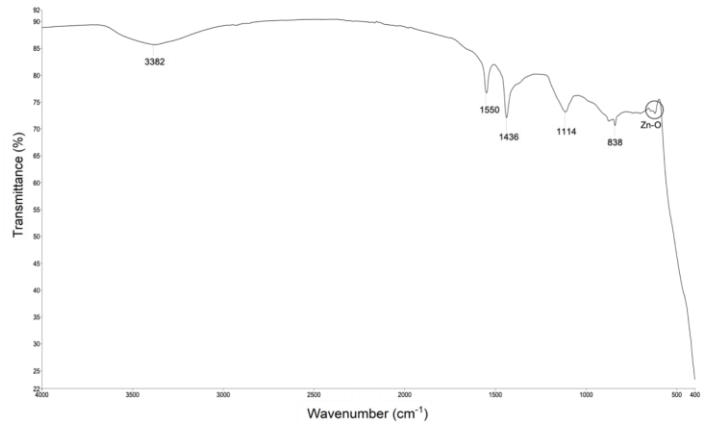

Figure 2 FTIR spectrum of the synthesized ZnO PTNPs. The signal detected at the fingerprint zone corresponds to the vibrations of the metal oxide.

SEM micrographs evidenced the obtention of polydisperse and agglomerated 1-D ZnO nanowires with an average width of 127 nm (Figure 3) and average length of 1.29 µm. The polydispersity observed could be related to the wide diversity of phytochemicals present in plant extracts, a fact that would be related to the broadband exhibited in the UV-Vis spectrum (Deshmukh *et al*., 2020).

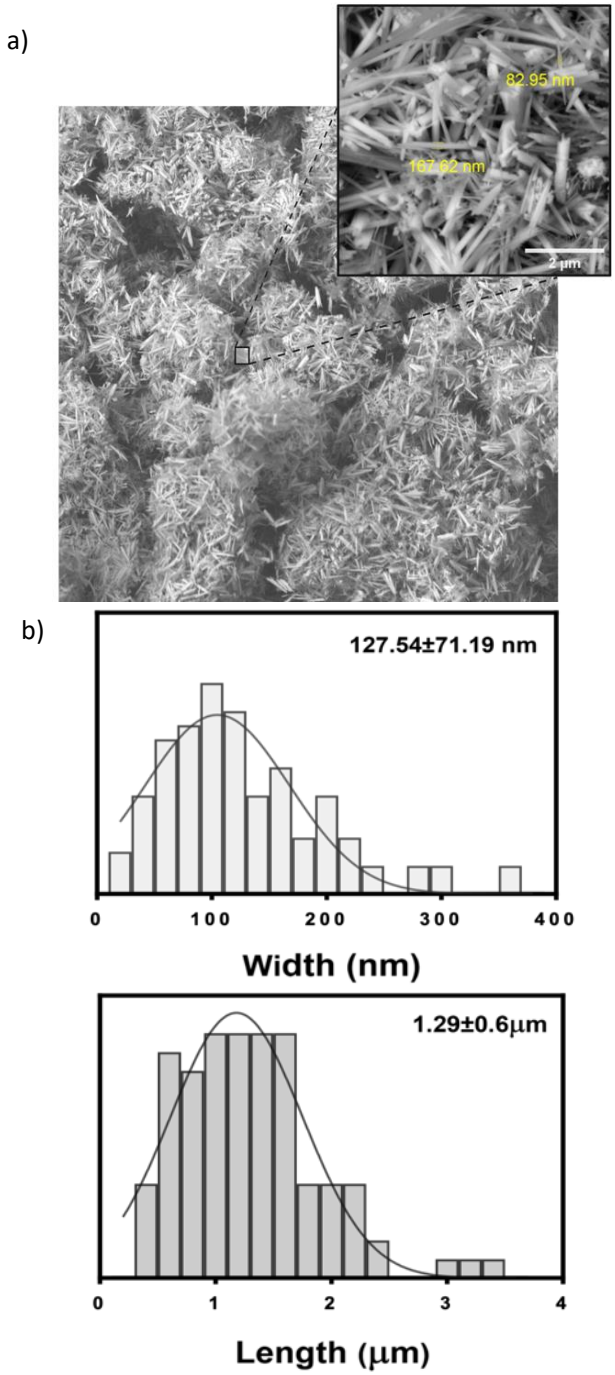

Figure 3 (a) SEM micrograph and (b) histograms of width and length particle size of ZnO nanowires.

Nanowires are one of the most commonly synthesized 1D ZnO nanostructures and, consequently, are at the forefront of nanoscience and nanotechnology. Due to their crystalline structure, nanowires present a rapid growth in one particular direction, typically they have a hexagonal, cylindrical, square, or triangular cross-section, which is much smaller than the overall length (Galdámez-Martinez *et al*., 2020; Ding *et al.*, 2018). Some studies state that ZnO nanowires exhibit interesting properties because of their unique structural one-dimensionality which makes them ideal building blocks for many applications. Compared with other 1D nanomaterials, nanowires are highly biocompatible, so can be used for biological purposes (Ji, 2015; Ding *et al*., 2018). Recently, Sedefoglu (2023) reported the synthesis of green ZnO nanowires from *Myrtus communis* extract.

The ζ potential (ZP) is the electrical potential usually analyzed to measure the surface charge and stability of NPs in colloidal solutions (Sizochenko *et al*., 2021). The ZP of the ZnO PTNPs revealed a value of + 27.5 mV, while the hydrodynamic diameter showed the presence of ZnO PTNPs between 1-9,000 nm before the

sonication and stabilization protocol. On the contrary, sonicated and stabilized ZnO PTNPs showed a reduction the hydrodynamic diameter founding NPs between 1-50 nm and a ZP value of +41.8 mV. NPs with ZP values from -30 mV to +30 mV possess high stability. When higher values are observed NP are considered as strongly "cationic or anionic". According to the results previously reported, the ZnO PTNPs before the sonication and stabilization protocol showed good stability but tended to agglomerate increasing the particle size. After stabilization, the particle size reduction was notable and a cationic behavior of ZnO PTNPs was observed.

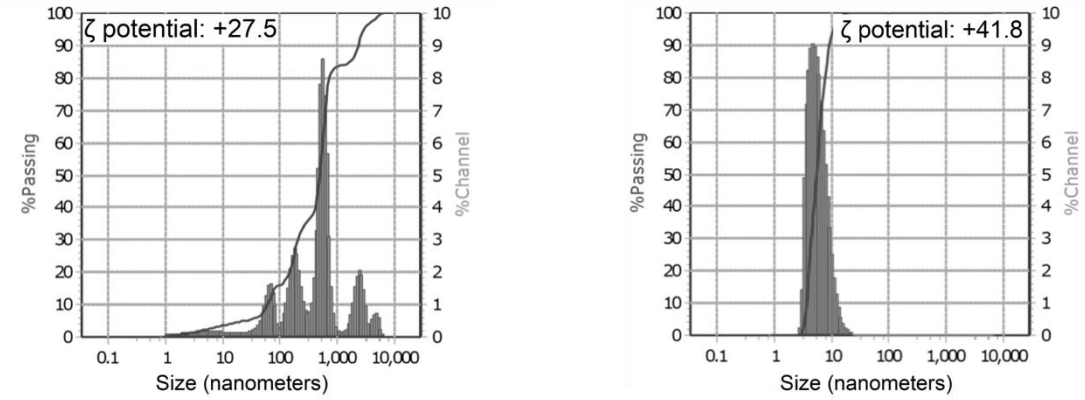

Figure 4 DLS plot and zeta potential values of ZnO phytonanoparticles. Before (right) and after (left) sonication and stabilization protocol.

Zinc content and yield of synthesis

The ICP-OES analysis quantified the zinc content present in the synthesized ZnO PTNPs. The resulting quantification evidence a relation of 0.45 mg of Zinc for every mg of ZnO PTNPs, with a yield of synthesis of 84.6%. Lately, Chinnasamy *et al.* (2018) reported an investigation for the green synthesis of ZnO NPs by evaluating the effect of time and temperature of synthesis, as well as the concentration of precursor salt and biological extract. They concluded that the higher temperatures and longer times, in combination with minimum concentration of salt precursor elevated the yield of synthesis at maximum. On the other hand, Singh *et al*. (2018) investigated the influence of temperature on the yield synthesis of green ZnO quantum dots, their results showed that higher temperature leads to a greater yield of ZnO NPs, but at the same time increases the size of particles. These results evidence that the yield of synthesis and the characteristics of ZnO nanoparticles depend on parameters such as the concentration of plant extract, the concentration of aqueous salt precursor, temperature, and time of synthesis. Even, though the yield is considered a good parameter, further experiments need to be performed to find optimal conditions to elevate the yield of synthesis.

ZnO phytonanoparticles stability under *in vitro* gastrointestinal conditions

Several investigations have evaluated the dissolution behavior of nanoparticles (NPs) in water. However, the need to evaluate the dissolution of NPs in fluids of physiological relevance like the environments during the digestive process has recently been exposed to know their possible biological effect (Sultana *et al*., 2020). Comprehension of the chemical and biological modifications of ZnO PTNPs that might occur throughout digestion should be considered essential since pH variations, enzymes and salts presence could lead to dissolution or agglomeration of PTNPs and might impact the bioavailability and absorption of the NPs, especially for those administrated in oral routes, as occurs in nanofoods.
To evaluate the stability of ZnO PTNPs the dissolution behavior was studied under acid and alkaline conditions, with simulated relevant biological fluids of the gastrointestinal tract (stomachal and intestinal stages). Table 1 illustrates the dissolution rate of different concentrations of ZnO PTNPs after the treatment in each test solution. The data analysis evidence the influence of the surrounding medium and the doses evaluated.

Table 1 Dissolution rate of ZnO PTNPs in the three stages of the gastrointestinal *in vitro* protocol

ZnO PTNPs (mg/100 mL)	Dissolution (%)		
	Pepsin	Pancreatin	Pancreatin + billiard salts
25	20.86±1.45[a]	12.19±0.52[a]	12.11±0.97[a]
50	16.85±0.47[c]	6.70±0.56[b]	6.44±0.74[b]
100	18.94±0.69[ab]	0.13±0.05[c]	1.34±0.46[c]
HSD	2.4190	1.1047	1.8921

Values with different letters in the same column are significantly different ($p \leq 0.05$).

Our results showed a higher dissolution percentage rate (16-20%) of ZnO PTNPs under acidic conditions (pH 2.0) in the pepsin simulant solution, while the alkaline (pH 8.0) conditions of the pancreatin simulant solution had a lower impact (0-12%) on the dissolution percentage rate of the ZnO PTNPs. It is important to notice that the dissolution rate of the ZnO PTNPs on the alkaline solutions showed a dependent behavior on the evaluation dose.
The presence of Van der Waals and repulsion forces govern the interactions between NPs (Voss *et al*., 2020). The loss in the balance of these forces has a strong influence on the dissociation behavior of NPs and is commonly related to the pH of the dispersion medium, one of the most important factors for controlling the stability of NPs (Marsalek, 2014). Fatehah *et al*. (2014) showed that the decrease in the pH of the medium leads to the loss of NPs stability, as a consequence of a greater presence of protons in the medium modifying the surface charge through protonation, causing NPs dissociation and the subsequent release of Zn^{+2} ions. On the other hand, a similar effect could be observed under alkaline conditions, Zhang *et al*. (2008) reported that alkaline environments, such as those involved in pancreatin solution, modify the surface charge by deprotonating the surface causing instability in the colloidal system and the subsequent dissociation of ZnO PTNP. Based on the results, is possible to infer that the behavior of ZnO PTNPS previously described is more prone to happen under acid conditions, causing a greater release of Zn^{+2} ions.
However, is important to notice that also, due to the proximity of the pH of the intestinal stage to the isoelectric point of ZnO NPs (6.9 to 9.8), the repulsion forces could also get weaker and lead to the aggregation of NPs (Domingos *et al*., 2013). Aggregation leads to the formation of various irregularly shaped unstable structures, which can slow down the dissociation rate and, in some cases, stop the process (Sizochenko *et al*., 2021). This could explain the behavior observed during this experiment. Voss *et al*. (2020) reported similar results when carrying out a three-step *in vitro* digestion protocol for commercial ZnO NPs, reporting greater dissociation during the gastric stage and lower results during the intestinal stage.
It is important to understand that the dissolution of PTNPs in the highly acidic environment of the stomach stage leads to a loss of the acquiree nanometric properties and can especially conduct to oxidative stress and cytotoxic effects due to the possible acid erosion of metallic ions (Rouhi *et al*., 2022).
These results evidenced the effect of the digestion process on the stability of NPs, however, to measure this property under a standard classification The Organization for Economic Cooperation and Development (2015) developed a scale to classify the solubility of NPs based on their dissociation behavior. The results show that the synthesized ZnO PTNPs are moderately soluble in highly acidic environments and poorly soluble in slightly alkaline environments which indicates great stability of ZnO PTNPs under these conditions and possible remarkable conservation of the ZnO PTNPs nanometric properties.

Evaluation of the biological properties of ZnO phytonanoparticles

Antioxidant activity of ZnO phytonanoparticles

Antioxidants are compounds that stabilize and prevent the damage caused by free radicals that transfer electrons to the damaged cells. The antioxidant activity (AA) of ZnO PTNPs was demonstrated by evaluating the radical scavenging activity. The results obtained during this analysis confirmed the antioxidant properties of the ZnO PTNPs, which show an increase in the AA with dose-dependent behavior. The values found in this study were within a range of 17.9% (0.5 mg/mL) to 70.5% (10 mg/mL). The concentration to achieve the IC_{50} value was reported at 6.14 mg/mL of ZnO PTNPs. Soren *et al*. (2018) attributed the antioxidant property of ZnO NPs to the transfer of electrons from oxygen atoms to nitrogen atoms containing the odd electrons in the DPPH molecule. More recently, Hasnain *et al*. (2021) postulated that phytomolecules with antioxidant capacity involved in the

reduction of metal ions and stabilization of PTNPs during the synthesis process could also be responsible for imparting the antioxidant capacity of PTNPs. The inclusion of natural antioxidants obtained from natural sources can play a role in preventing auto-oxidation of fats and oils in food matrices, so the presence of phytonanoparticles is important for antioxidant activity (Ramli *et al*., 2021). Also, is important to notice, that according to our results, to achieve 70% of DPPH inhibition using the ethanolic extract of *Annona muricata* L. 2 g/mL is required (data not shown), while only 10 mg/mL of ZnO PTNPs was needed to reach this value, which suggests that the inclusion of a zinc oxide core capped with phytonanoparticles may act all together to reduce the effect in front of free radicals. Nevertheless, previous reports have revealed that phytonanoparticles exhibit major antioxidant potential compared with chemical nanoparticles. Ashraf *et al.* (2023), recently reported a comparative study between green and chemical ZnO nanoparticles, revealing that even though both types of nanoparticles showed excellent antioxidant activity, a greater percentage of radical scavenging activity (80.1% ± 1.3%) was noticed on phytonanoparticles.

All data previously described confirm that PTNPs possess excellent antioxidant activity, taking into consideration that antioxidant activity of *Lactobacillus* species has been proven (Riane *et al*., 2021; Kim *et al*., 2022), the combination of NPs and probiotics in food could represent a good source of the antioxidant agents.

Hemolytic assay

Another outstanding issue that has been emerging recently in the nanoparticles (NPs) consumption area is related to the passage of PTNPs from the intestine to the bloodstream, and the future interaction with blood cells. Due to this condition, hemolytic activity of ZnO PTNPs was evaluated with a hemolytic assay through a quantitative determination of glycated hemoglobin (HbA_{1c}). The quantification of the HbA_{1c} showed significant differences ($p \leq 0.05$) between the control and the evaluated treatment due to an increment in the values of HBA_{1c} of the blood samples with ZnO NPs and $ZnSO_4$ addition due to an increase in HbA_{1c} (Table 2).

Table 2 Glycated hemoglobin quantification in samples treated with ZnO PTNPs and zinc sulfate

Treatment	Concentration (mg/100 mL)	HbA_{1c} (%)	Hemolysis (%)
Negative control	0	3.3 ± 0.3^{a}	
$ZnSO_4$	30	3.83 ± 0.06^{b}	Non hemolytic
ZnO PTNPs	6	3.97 ± 0.15^{b}	Non hemolytic
ZnO PTNPs	15	3.87 ± 0.23^{b}	Non hemolytic
ZnO PTNPs	30	3.76 ± 0.21^{b}	Non hemolytic

**HSD: 0.5551.* Negative control: Blood sample without ZnO PTNPs or zinc sulfate addition. *Values with different letters are significantly different ($p \leq 0.05$)*

Although the increase in the values of the samples treated with ZnO PTNPs is evident, some reports indicate the decrease in glycated hemoglobin values when hemolytic processes occur due to the release of hemoglobin to the medium (Balen *et al*., 2012). This information along with the results suggests a non-hemolytic effect of the evaluated doses of ZnO PTNPs and the obtention of biocompatible PTNPs. On the other hand, the observed increase in values could be attributed to a possible absorption of light by the zinc present in the metal salt and PTNPs. Furthermore, Aziz *et al*. (2019) carried out a comparison study of the biological activity of green nanoparticles obtained from *Annona muricata* L. pulp and their chemical counterparts, chemically ZnO nanoparticles showed lethality towards human erythrocytes, contrary to phytonanoparticles. The same behavior was observed by Mahalakshmi *et al*. (2019) and confirmed the biosafety and hemocompatibility of green nanoparticles. Within this framework, works such as those carried out by Jan *et al.* (2020) have consistently confirmed the biocompatibility and biosafety of ZnO PTNPs which could be related to the coating of biological molecules present on NPs surfaces.

With all this information is possible to postulate that ZnO PTNPs dissociation occurring in the gastrointestinal tract may lead to an absorption of Zn^{+2}, an important component for enzymatic activity, proper cellular function, and synthesis and transcription of proteins, RNA, and DNA (Chasapis *et al*., 2020). This phenomenon could also allow the release the phytochemicals on the surface of ZnO PTNPs that might have important biological properties as antioxidant, anticancer, hypoglycemic, anti-inflammatory, hepatoprotective, and gastroprotective, as well as anxiolytic and anti-stress activity (Coria-Tellez *et al*., 2018). On the opposite side, the absorption of nano-zinc could be safely absorbed into the bloodstream and distributed to target organs and tissues where they can provide several positive effects.

Effect of ZnO phytonanoparticles on the viability of *Lpb. fabifermentans* BAL-27-ITTG

Until now, few studies have reported the effect of nano-zinc on probiotic strains due to consistent reports about loss of viability of microorganisms in the presence of several oxide metal nanoparticles. In this investigation, we evaluated the possible biological effect of the ZnO PTNPs on the viability of lactic acid bacteria (LAB) such as *Lpb. fabifermentans* BAL-27-ITTG. Two factors of interest were evaluated: the pH of the re-suspension medium and the concentration of the ZnO PTNPs. The results of cell concentration after the treatment of the bacterial culture with the ZnO PTNPs are shown in Figure 4.

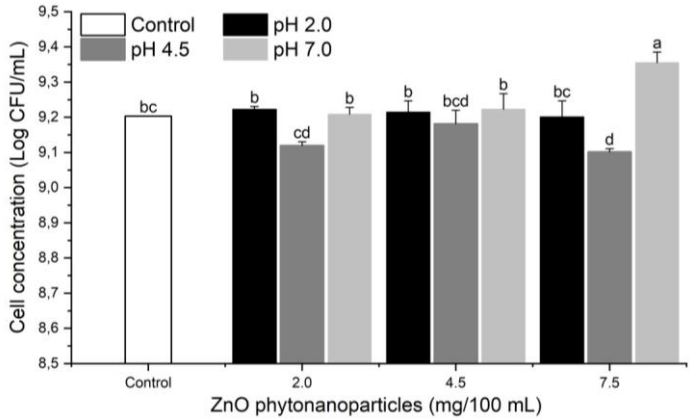

Figure 4 Effect of ZnO PTNPs on the growth of *Lpb. fabifermentans* BAL-27-ITTG**.** *Values with different letters are significantly different ($p \leq 0.05$).*

As is shown, the presence of the ZnO PTNPs had a significant effect on the viability of Gram-positive *Lpb. fabifermentans* BAL-27-ITTG. Significant differences ($p \leq 0.05$) were found in the treatments with the highest evaluated dose of ZnO PTNPs (7.5 mg/100mL) combined with pH values of 7.0 and 4.5. The interaction between the highest concentration of ZnO PTNPs and neutral pH value significantly increases the viability of the LAB, probably due to an agglomeration process of ZnO PTNPs that causes an increase in the particle size and reduces the interaction and internalization of ZnO PTNP into the microorganism. Opposite to this, the treatment added with 7.5 mg/100 mL of ZnO PTNPs and a re-suspension medium adjusted to pH 4.5 showed a significant decrease in the viability of *L. fabifermentans* BAL-27-ITTG equal to ~20%, according to these results only the of higher doses and certain pH conditions causes a decrease in the viable counts.

In acidic environments (pH <3.4), the surface charge of nanoparticles is modified as a consequence of protons concentration, causing the dissociation of ZnO nanoparticles into Zn^{+2} ions, however, when the concentration of protons lower and sufficient to increase the repulsion forces between nanoparticles, as happens at pH 4.5, the dispersion of colloidal systems is promoted, preventing aggregation, and facilitating PTNP-cell interaction. (Yang *et al*., 2008; Fatehah *et al*., 2014). Bhuyar *et al*. (2020) have reported that mechanism of antimicrobial has reported that smaller particles possess higher bactericidal effect compared to larger particles as a consequence of their larger surface area.

The results obtained during this assay, suggest an excellent survival of *Lpb. fabifermentans* BAL-27-ITTG when exposed to ZnO PTNPs. Contrary to this, Fayed *et al*. (2023) and Abd El-Nour *et al*. (2022) have reported that Gram-positive bacteria are particularly sensitive to zinc oxide nanoparticles, as a consequence of their cell wall composition which contains high amounts of peptidoglycan and acidic components that are prone to bind strongly to the positively charged ZnO NPs, increasing their antibacterial action.

Nevertheless, results here showed could be attributed to the diverse defense mechanisms responsible for conferring resistance to this kind of microorganisms (Gram-positive) making them capable of surviving over these conditions. Amaro *et al*. (2021) have reported that these strategies include overexpression of genes encoding metal ion efflux pumps and reactive oxygen species (ROS) scavenging systems, as well as reduced NPs uptake or adsorption due to the production of exopolysaccharides (EPS), which acts as a physical barrier that difficult the penetration of NPs.

Mu *et al.* (2016) demonstrated that *Bacillus subtilis* (Gram-positive) has several responses to stress when exposed to aluminum oxide nanoparticles. This study revealed that *B. subtilis* is capable of over-expressing genes related to ROS scavenging systems, in addition to inducing changes in the fatty acid profile of the membrane and the formation of biofilms. This information would support the findings of this research. Based on these results, the effect of the ZnO PTNPs over *Lpb. fabifermentans* BAL-27-ITTG does not reduce drastically viability, allowing to obtain enough viable counts that can meet the specifications required in regulations for probiotic microorganisms in food ($1x10^6$ UFC).

CONCLUSION

The synthesis of ZnO PTNPs was successfully performed using soursop (*Annona muricata* L.) pulp extract as biological material and is an excellent alternative for chemical synthesis due to its easy fabrication process and low cost. The formation of ZnO was confirmed by a color change in the solution from yellowish-brown to brown after the complete reaction. UV-vis confirmed the synthesis of nanoparticles and FTIR analysis revealed that the capping ligand for the formation and stabilization of Zn-O nanoparticles might be phenolic compounds, alkaloids, and acetogenins. SEM micrographs demonstrated the obtention poly-dispersed 1-D nanowires, whereas zeta potential displayed a positive polarity. Radical scavenging activity suggests that ZnO PTNPs might be an excellent exogen antioxidant and a source of zinc, properties that can be interesting in diverse food matrices. The stability of ZnO PTNPs under simulated gastrointestinal conditions evidenced good stability under acid and alkaline conditions which along with the hemocompatibility might have interesting effects on the human body when ingested. Biological activity of ZnO nanoparticles on *Lpb. fabifermentans* BAL-27-ITTG were found to be dependent on doses and pH values, where high concentrations and pH values >4.5 showed the best survival rate. Even if, the results are promising further investigations of cytotoxicity on intestinal cell lines should be performed, while, on the other hand, a deeper investigation of stability needs to be performed to ensure bioavailability and absorption of zinc. As probiotics can survive under the presence of ZnO PTNO, all findings suggest the possible co-administration of ZnO PTNPs and probiotics strains, two of the most prominent and employed technologies in the food market that represent a novel and attractive approach to developing functional products.

Acknowledgments: This study was funded by the Consejo Nacional de Ciencia y Tecnología (CONACYT) and the Tecnológico Nacional de México (10666.21-P).

REFERENCES

Abd El-Nour, A., Abou-Dobara, M., El-Sayed, A., & El-Zahed, M. (2023). Extracellular biosynthesis and antimicrobial activity of Bacillus subtilis ATCC 6633 zinc oxide nanoparticles. *Scientific Journal for Damietta Faculty of Science*, 0(0), 0–0. http://dx.doi.org/10.21608/sjdfs.2023.173178.1064

Ahmad, W., & Kalra, D. (2020). Green synthesis, characterization and antimicrobial activities of ZnO nanoparticles using *Euphorbia hirta* leaf extract. *Journal of King Saud University-Science*, 32(4), 2358-2364. http://dx.doi.org/10.1016/j.jksus.2020.03.014

Amaro, F., Morón, Á., Díaz, S., Martín-González, A., & Gutiérrez, J. C. (2021). Metallic nanoparticles—friends or foes in the battle against antibiotic-resistant bacteria? *Microorganisms*, 9(2), 364. http://dx.doi.org/10.3390/microorganisms9020364

Ashraf, H., Meer, B., Iqbal, J., Ali, J. S., Andleeb, A., Butt, H., Zia, M., Mehmood, A., Nadeem, M., Drouet, S., Blondeau, J.-P., Giglioli-Guivarc'h, N., Liu, C., Hano, C., & Abbasi, B. H. (2022). Comparative evaluation of chemically and green synthesized zinc oxide nanoparticles: their *in vitro* antioxidant, antimicrobial, cytotoxic and anticancer potential towards HepG2 cell line. *Journal of Nanostructure in Chemistry*, 13(2), 243–261. http://dx.doi.org/10.1007/s40097-021-00460-3

Aziz, A. A., Shaniba, V. S., Jayasree, P. R., & Kumar, P. M. (2019). Physico-chemical, photocatalytic and cytotoxicity evaluation of *Annona muricata* L. fruit extract derived zinc oxide nanoparticles in comparison to the commercial chemical version. *Current Science*, 117(9), 1492-1504. http://dx.doi.org/10.18520/cs/v117/i9/1492-1504

Badmus, J. A., Oyemomi, S. A., Adedosu, O. T., Yekeen, T. A., Azeez, M. A., Adebayo, E. A., Lateef, A., Badeggi, U. M., Botha, S., Hussein, A. A., & Marnewick, J. L. (2020). Photo-assisted bio-fabrication of silver nanoparticles using *Annona muricata* leaf extract: exploring the antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities. *Heliyon*, 6(11), e05413. http://dx.doi.org/10.1016/j.heliyon.2020.e05413

Balen, M. J., Lukenda, V., Jandrić, I., Raguž, A., Zukanović, S., & Miškić, B. (2012). HbA1C--overall glycemia marker and hemolytic anemia indicator. *Medicinski Glasnik*, 9(2): 406-408.

Bandeira, M., Giovanela, M., Roesch-Ely, M., Devine, D.M., & da Silva Crespo, J. (2020). Green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation. *Sustainable Chemistry and Pharmacy*, 15, 100223. http://dx.doi.org/10.1016/j.scp.2020.100223

Beltrán-Partida, E., Valdez-Salas, B., Valdez-Salas, E., Pérez-Cortéz, G., & Nedev, N. (2019). Synthesis, characterization, and *in situ* antifungal and cytotoxicity evaluation of ascorbic acid-capped copper nanoparticles. *Journal of Nanomaterials*, 2019:1-10. http://dx.doi.org/10.1155/2019/5287632

Busi, S., Rajkumari, J., Pattnaik, S., Parasuraman, P., & Hnamte, S. (2021). Extracellular synthesis of zinc oxide nanoparticles using *Acinetobacter schindleri* SIZ7 and its antimicrobial property against foodborne pathogens. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 407-411. http://dx.doi.org/10.15414/jmbfs.2016.5.5.407-411

Bhuyar, P., Rahim, M. H. Ab., Sundararaju, S., Ramaraj, R., Maniam, G. P., & Govindan, N. (2020). Synthesis of silver nanoparticles using marine macroalgae *Padina* sp. and its antibacterial activity towards pathogenic bacteria. *Beni-Suef University Journal of Basic and Applied Sciences*, 9(1). http://dx.doi.org/10.1186/s43088-019-0031-y

Campos, D., Goméz-García, R., Oliveira, D., & Madureira, A. (2022). Intake of nanoparticles and impact on gut microbiota: *In vitro* and animal models available for testing. *Gut Microbiome*, 3: E1. http://dx.doi.org/10.1017/gmb.2021.5

Chasapis, C. T., Ntoupa, P. S. A., Spiliopoulou, C. A., & Stefanidou, M. E. (2020). Recent aspects of the effects of zinc on human health. *Archives of toxicology*, 94, 1443-1460. http://dx.doi.org/10.1007/s00204-020-02702-9

hinnasamy, C., Tamilselvam, P., Karthick, B., Sidharth, B., & Senthilnathan, M. (2018). Green Synthesis, Characterization and Optimization Studies of Zinc Oxide Nano Particles Using Costusigneus Leaf Extract. *Materials Today: Proceedings*, 5(2), 6728–6735. http://dx.doi.org/10.1016/j.matpr.2017.11.331

Coria-Téllez, A. V., Montalvo-Gónzalez, E., Yahia, E. M., & Obledo-Vázquez, E. N. (2018). *Annona muricata*: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*, 11(5), 662-69. http://dx.doi.org/10.1016/j.arabjc.2016.01.004

Coelho de Lima, M. A., & Alves, R. E. (2011). Soursop (*Annona muricata* L.). In *Postharvest biology and technology of tropical and subtropical fruits. Mangosteen to white sapote*, 4th edn. Woodhead Publishing Limited, Sawton, pp 363-391. http://dx.doi.org/10.1533/9780857092618.363

Deshmukh, A. R., Aloui, H., & Kim, B. S. (2020). *In situ* growth of gold and silver nanoparticles onto phyto-functionalized boron nitride nanosheets: Catalytic, peroxidase mimicking, and antimicrobial activity. *Journal of Cleaner Production*, 270, 122339. http://dx.doi.org/10.1016/j.jclepro.2020.122339

Ding, M., Guo, Z., Zhou, L., Fang, X., Zhang, L., Zeng, L., Xie, L., & Zhao, H. (2018). One-Dimensional Zinc Oxide Nanomaterials for Application in High-Performance Advanced Optoelectronic Devices. *Crystals*, 8(5), 223. http://dx.doi.org/10.3390/cryst8050223

Domingos, R. F., Rafiei, Z., Monteiro, C. E., Khan, M. A., & Wilkinson, K. J. (2013). Agglomeration and dissolution of zinc oxide nanoparticles: role of pH, ionic strength and fulvic acid. *Environmental Chemistry*, 10(4), 306-312. http://dx.doi.org/10.1071/en12202

Fakhari, S., Jamzad, M., & Kabiri Fard, H. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. *Green chemistry letters and reviews*, 12(1), 19-24. http://dx.doi.org/10.1080/17518253.2018.1547925

Fatehah, M. O., Aziz, H. A., & Stoll, S. (2014). Stability of ZnO nanoparticles in solution. Influence of pH, dissolution, aggregation and disaggregation effects. *Journal of Colloid Science and Biotechnology*, 3(1), 75-84. http://dx.doi.org/10.1166/jcsb.2014.1072

Fabricius, A. L., Duester, L., Meermann, B., & Ternes, T. A. (2014). ICP-MS-based characterization of inorganic nanoparticles—sample preparation and off-line fractionation strategies. *Analytical and bioanalytical chemistry*, 406, 467-479. http://dx.doi.org/10.1007/s00216-013-7480-2

Faizan, M., Hayat, S., & Pichtel, J. (2020). Effects of zinc oxide nanoparticles on crop plants: A perspective analysis. Sustainable Agriculture *Reviews 41: Nanotechnology for Plant Growth and Development*, 83-99. http://dx.doi.org/10.1007/978-3-030-33996-8_4

Fayed, R., Elnemr, A.-M., & El-Zahed, M. M. (2023). SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND ELECTROCHEMICAL STUDIES OF BIOSYNTHESIZED ZINC OXIDE NANOPARTICLES USING THE PROBIOTIC BACILLUS COAGULANS (ATCC 7050). *Journal of Microbiology, Biotechnology and Food Sciences*, e9962. http://dx.doi.org/10.55251/jmbfs.9962

Galdámez-Martinez, A., Santana, G., Güell, F., Martínez-Alanis, P. R., & Dutt, A. (2020). Photoluminescence of ZnO Nanowires: A Review. *Nanomaterials*, 10(5), 857. http://dx.doi.org/10.3390/nano10050857

Gavamukulya, Y., Maina, E. N., Meroka, A. M., Madivoli, E. S., El-Shemy, H. A., Wamunyokoli, F., & Magoma, G. (2020). Green synthesis and characterization of highly stable silver nanoparticles from ethanolic extracts of fruits of *Annona*

muricata. *Journal of Inorganic and Organometallic Polymers and Materials*, 30(4), 1231-1242. http://dx.doi.org/10.1007/s10904-019-01262-5

Greene, L. E., Yuhas, B. D., Law, M., Zitoun, D., & Yang, P. (2006). Solution-Grown Zinc Oxide Nanowires. ChemInform, 37(48). *Portico*. http://dx.doi.org/10.1002/chin.200648227

González-Pedroza, M.G.; Argueta-Figueroa, L.; García-Contreras, R.; Jiménez-Martínez, Y.; Martínez-Martínez, E.; Navarro-Marchal, S.A.; Marchal, J.A.; Morales-Luckie, R.A.; Boulaiz & H. Silver. (2021). Nanoparticles from *Annona muricata* Peel and Leaf Extracts as a Potential Potent, Biocompatible and Low-Cost Antitumor Tool. *Nanomaterials*,11, 1273. http://dx.doi.org/10.3390/nano11051273

Hasnain, J., Muzamil, S., Anisa, A., Shah, F., Aishma, K., Muhammad, R., Samantha, D., Christophe, H., & Bilal, H. A. (2021). Plant-based synthesis of zinc oxide nanoparticles (ZnO-NPs) using aqueous leaf extract of *Aquilegia pubiflora*: their antiproliferative activity against HepG2 cells inducing reactive oxygen species and other *in vitro* properties. *Oxidative medicine and cellular longevity*, 2021:1-14. http://dx.doi.org/10.1155/2021/4786227

Jan, H., Shah, M., Usman, H., Khan, M. A., Zia, M., Hano, C., & Abbasi, B. H. (2020). Biogenic synthesis and characterization of antimicrobial and antiparasitic zinc oxide (ZnO) nanoparticles using aqueous extracts of the Himalayan Columbine (*Aquilegia pubiflora*). *Frontiers in Materials*, 7, 249. http://dx.doi.org/10.3389/fmats.2020.00249

Ji, Y. (2015). One-step method for growing of large scale ZnO nanowires on zinc foil. *Materials Letters*, 138, 92–95. http://dx.doi.org/10.1016/j.matlet.2014.09.095

Kaushik, M., Niranjan, R., Thangam, R., Madhan, B., Pandiyarasan, V., Ramachandran, C., Deog-Hwan, O. G., & Venkatasubbu, G. D. (2019). Investigations on the antimicrobial activity and wound healing potential of ZnO nanoparticles. *Applied Surface Science*, 479, 1169-1177. http://dx.doi.org/10.1016/j.apsusc.2019.02.189

Kaur Sidhu, M., Lyu, F., Sharkie, T. P., Ajlouni, S., & Ranadheera, C. S. (2020). Probiotic yogurt fortified with chickpea flour: physico-chemical properties and probiotic survival during storage and simulated gastrointestinal transit. *Foods*, 9(9), 1144. https://doi.org/10.3390/foods9091144

Khan, R., Inam, M. A., Zam, S. Z., Park, D. R., & Yeom, I. T. (2018). Assessment of key environmental factors influencing the sedimentation and aggregation behavior of zinc oxide nanoparticles in aquatic environment. *Water*, 10(5), 660. http://dx.doi.org/10.1016/j.apsusc.2019.02.189

Kim, S., Lee, J. Y., Jeong, Y., & Kang, C.-H. (2022). Antioxidant Activity and Probiotic Properties of Lactic Acid Bacteria. *Fermentation*, 8(1), 29. http://dx.doi.org/10.3390/fermentation8010029

Korcari, D., Ricci, G., Fanton, A., Emide, D., Barbiroli, A., & Fortina, M. G. (2022). Exploration of *Lactiplantibacillus fabifermentans* and *Furfurilactobacillus rossiae* as potential cocoa fermentation starters. *Journal of Applied Microbiology*, 133(3), 1769-1780. http://dx.doi.org/10.1111/jam.15687

Jiménez-Zurita, J. O., Balois-Morales, R., Alia-Tejacal, I., Sánchez Herrera, L. M., Jiménez-Ruiz, E. I., Bello-Lara, J. E., García-Paredes, J.D., & Juárez-López, P. (2017). Cold storage of two selections of soursop (*Annona muricata* L.) in Nayarit, Mexico. *Journal of Food Quality*, 2017. http://dx.doi.org/10.1155/2017/4517469

Mahalakshmi, S., Hema, N., & Vijaya P. P. (2019). *In Vitro* Biocompatibility and Antimicrobial activities of Zinc Oxide Nanoparticles (ZnO NPs) Prepared by Chemical and Green Synthetic Route— A Comparative Study. *BioNanoScience*, 10(1), 112–121. https://doi.org/10.1007/s12668-019-00698-w

ahmood, R. I., Kadhim, A. A., Ibraheem, S., Albukhaty, S., Mohammed-Salih, H. S., Abbas, R. H., Jabir, M. S., Mohammed, M. K. A., Nayef, U. M., AlMalki, F. A., Sulaiman, G. M., & Al-Karagoly, H. (2022). Biosynthesis of copper oxide nanoparticles mediated *Annona muricata* as cytotoxic and apoptosis inducer factor in breast cancer cell lines. *Scientific Reports*. 28;12(1). http://dx.doi.org/10.1038/s41598-022-20360-y

Marsalek, R. (2014). Particle size and zeta potential of ZnO. *APCBEE Procedia*, 9:13–17. https://doi.org/10.1016/j.apcbee.2014.01.003

Mendez-Díaz, D. P., Gutiérrez-Miceli, F. A., Ventura-Canseco, L. M. C., Abud-Archila, M., Valdéz Salas, B., Morales-Ruiz, M. C., & Luján-Hidalgo, M. C. (2023). Caracterización estructural y funcional de nanopartículas de ZnO-EPS sintetizadas a partir de exopolisacáridos producidos por *Lactiplantibacillus fabifermentans* BAL-27-ITTG. *Biotecnia*, *25*(3), 55–64. https://doi.org/10.18633/biotecnia.v25i3.1950

Mohammadi, P., Hesari, M., Samadian, H., Hajialyani, M., Bayrami, Z., Farzaei, M. H., & Abdollahi, M. (2019). Recent advancements and new perspectives of phytonanotechnology. In *Comprehensive analytical chemistry* (Vol. 84, pp. 1-22). Elsevier. http://dx.doi.org/10.1016/bs.coac.2019.04.011

Mu, D., Yu, X., Xu, Z., Du, Z., & Chen, G. (2016). Physiological and transcriptomic analyses reveal mechanistic insight into the adaption of marine *Bacillus subtilis* C01 to alumina nanoparticles. *Scientific Reports*, 6(1), 29953. http://dx.doi.org/10.1038/srep29953

Organization for Economic Cooperation and Development (OECD). (2015). Considerations for using dissolution as a function of surface chemistry to evaluate the environmental behavior of nanomaterials in risk assessments. A preliminary case study using silver nanoparticles; Series on the safety of manufactured Nanomaterials, no. 62, ENV/JM/MONO(2015)44, Paris, France. https://one.oecd.org/document/ENV/JM/MONO(2015)44/en/pdf. Accessed 10 April 2022.

Perveen, R., Shujaat, S., Qureshi, Z., Nawaz, S., Khan, M. I., & Iqbal, M. (2020). Green versus sol-gel synthesis of ZnO nanoparticles and antimicrobial activity evaluation against panel of pathogens. *Journal of Materials Research and Technology*, 9(4), 7817-7827. http://dx.doi.org/10.1016/j.jmrt.2020.05.004

Ramírez-Pérez, J. I., Álvarez-Gutiérrez, P. E., Luján-Hidalgo, M. C., Ovando-Chacón, S. L., Soria-Guerra, R. E., Ruiz-Cabrera, M. Á., & Abud-Archila, M. (2022). Effect of linear and branched fructans on growth and probiotic characteristics of seven *Lactobacillus* spp. isolated from an autochthonous beverage from Chiapas, Mexico. *Archives of Microbiology*, 204(7), 364. http://dx.doi.org/10.1007/s00203-022-02984-w

Ramli, A. N. M., Sukri, N. A. M., Azelee, N. I. W., & Bhuyar, P. (2021). Exploration of antibacterial and antioxidative activity of seed/peel extracts of Southeast Asian fruit Durian (*Durio zibethinus*) for effective shelf-life enhancement of preserved meat. *Journal of Food Processing and Preservation*, 45(9). *http://dx.doi.org/10.1111/jfpp.15662*

Riane, K., Sifour, M., Ouled-Haddar, H., Idoui, T., Bounar, S., & Boussebt, S. (2021). Probiotic properties and antioxidant efficiency of *Lactobacillus plantarum* 15 isolated from milk. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 516-520. http://dx.doi.org/10.15414/jmbfs.2019/20.9.3.516-520

Rouhi, M., Nateghi, L., Aghamirzaei, M., Yousefi, S., Garavand, F., Feyzi, S., Nooshkam, M., & Jafari, S. M. (2022). Safety, Standards and Regulations of Nanotechnology in Food. In *Encapsulation in Food Processing and Fermentation* (pp. 335-358). CRC Press. http://dx.doi.org/10.1201/9780429324918-13

Sabapati, M., Palei, N. N., CK, A. K., & Molakpogu, R. B. (2019). Solid lipid nanoparticles of Annona muricata fruit extract: formulation, optimization and *in vitro* cytotoxicity studies. *Drug development and industrial pharmacy*, 45(4), 577-586. http://dx.doi.org/10.1080/03639045.2019.1569027

Sedefoglu, N. (2023). Characterization and photocatalytic activity of ZnO nanoparticles by green synthesis method. *Optik*, 288, 171217. http://dx.doi.org/10.1016/j.ijleo.2023.171217

Selvanathan, V., Aminuzzaman, M., Tan, L. X., Win, Y. F., Guan Cheah, E. S., Heng, M. H., Tey, L.-H., Arullappan, S., Algethami, N., Alharthi, S. S., Sultana, S., Shahiduzzaman, M., Abdullah, H., & Aktharuzzaman, M. (2022). Synthesis, characterization, and preliminary in vitro antibacterial evaluation of ZnO nanoparticles derived from soursop (*Annona muricata L.)* leaf extract as a green reducing agent. *Journal of Materials Research and Technology*, 20, 2931–2941. http://dx.doi.org/10.1016/j.jmrt.2022.08.028

Singh, A. K., Pal, P., Gupta, V., Yadav, T. P., Gupta, V., & Singh, S. P. (2018). Green synthesis, characterization and antimicrobial activity of zinc oxide quantum dots using Eclipta alba. *Materials Chemistry and Physics*, 203, 40–48. http://dx.doi.org/10.1016/j.matchemphys.2017.09.049

Siqueira, A. D. M. O., Moreira, A. C. C. G., Melo, E. D. A., Stamford, T. C. M., & Stamford, T. L. M. (2015). Dietary fibre content, phenolic compounds and antioxidant activity in soursops (*Annona muricata* L.). *Revista Brasileira de Fruticultura*, 37, 1020-1026. http://dx.doi.org/10.1590/0100-2945-211/14

Sizochenko, N., Mikolajczyk, A., Syzochenko, M., Puzyn, T., & Leszczynski, J. (2021). Zeta potentials (ζ) of metal oxide nanoparticles: A meta-analysis of experimental data and a predictive neural networks modeling. *NanoImpact*, 22, 100317. http://dx.doi.org/10.1016/j.impact.2021.100317

Solorzano-Toalá, R., Gonzalez-Mendoza, D., Valdez-Salas, B., Mendez-Trujillo, V., Gutierrz-Miceli, F., Beltran-Partida, E., & Tzintzun-Camacho, O. (2020). Green Synthesis of Silver Nanoparticles Using *Annona diversifolia* Leaf Extract and Their Antimicrobial Application. *Journal of Renewable Materials*, 8(9), 1129-1137. http://dx.doi.org/10.32604/jrm.2020.09845

Soren, S., Kumar, S., Mishra, S., Jena, P. K., Verma, S. K., & Parhi, P. (2018). Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. *Microbial pathogenesis*, 119, 145-151. http://dx.doi.org/10.1016/j.micpath.2018.03.048

Sultana, S., Alzahrani, N., Alzahrani, R., Alshamrani, W., Aloufi, W., Ali, A., Najib, S., & Siddiqui, N. A. (2020). Stability issues and approaches to stabilised nanoparticles-based drug delivery system. *Journal of Drug Targeting*, 28(5), 468-486. http://dx.doi.org/10.1080/1061186x.2020.1722137

Velázquez-Gamboa, M. C., Rodríguez-Hernández, L., Abud-Archila, M., Gutiérrez-Miceli, F. A., González-Mendoza, D., Valdez-Salas, B., González-Terreros, E., & Luján-Hidalgo, M. C. (2021). Agronomic biofortification of *Stevia rebaudiana* with zinc oxide (ZnO) phytonanoparticles and antioxidant compounds. *Sugar Tech*, 23(2), 453-460. http://dx.doi.org/10.1007/s12355-020-00897-w

Voss, L., Saloga, P. E., Stock, V., Böhmert, L., Braeuning, A., Thünemann, A. F., Lampen, A. & Sieg, H. (2019). Environmental impact of ZnO nanoparticles evaluated by *in vitro* simulated digestion. *ACS Applied Nano Materials*, 3(1), 724-733. http://dx.doi.org/10.1021/acsanm.9b02236

Yang, Z., Xie, C., Xia, X. y Shuizhou, C. (2008). Zn^{2+} release behavior and surface characteristics of Zn/LDPE nanocomposites and ZnO/LDPE nanocomposites in simulated uterine solution. *J Mater Sci: Mater Med*, 9, 3319–3326. https://doi.org/10.1007/s10856-008-3467-5.

Zhang, L., Liu, C., Li, D., Zhao, Y., Zhang, X., Zeng, X., Yang, Z., & Li, S. (2013). Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus*

plantarum C88. *International Journal of Biological Macromolecules*, 54, 270-275. http://dx.doi.org/10.1016/j.ijbiomac.2012.12.037
Zhang, Y., Chen, Y., Westerhoff, P., Hristovski, K., & Crittenden, J. C. (2008). Stability of commercial metal oxide nanoparticles in water. *Water Research*, 42(8-9), 2204-2212. http://dx.doi.org/10.1016/j.watres.2007.11.036