Pharmacogn J. 2024; 16(6) Suppl: 1222-1233
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Citrus Extract-Mediated Zinc Oxide Nanoparticles and Their Capacity to Attenuate Free Radicals and Inflammation

Tabisa Diniso[1], Ayodeji O. Oriola[1]*, Gugulethu M. Miya[1], Simon K. Kuria[2], Yiseyon S. Hosu[3], Opeoluwa O. Oyedeji[4], Mike O. Ojemaye[4], Ludwe Majiza[5], Jerry O. Adeyemi[1], Adebola O. Oyedeji[1]*

ABSTRACT

Introduction: The exploitation of plant materials for the green synthesis of nanoparticles (NPs) for biological applications, is considered an eco-friendly technology because it does not involve the use of toxic chemicals. **Objective:** The study was carried out to synthesize citrus extract-mediated ZnO NPs and evaluate their free radical scavenging and anti-inflammatory capacity. **Materials and Methods**: ZnO NPs were green synthesized, using the peel and leaf aqueous extracts of three citrus plants: Nova mandarin, Satsuma mandarin and Eureka lemon. The citrus extract based ZnO NPs were characterized by UV-Vis and FTIR spectroscopy, microscopy (SEM and TEM), EDX and XRD analyses. They were screened against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) free radicals. Finally, their inhibitory effects against egg albumin denaturation (EAD) were determined spectrophotometrically. **Results:** The six afforded biogenic NPs consistently exhibited FTIR vibrational band around 500 cm^{-1}, which is characteristics of a metal oxide (Zn-O) band. They also showed UV-Vis absorption peaks at 387 and 415 nm, suggesting the formation of ZnO NPs. Nova mandarin peel (NMP) ZnO NPs exhibited the best DPPH and NO radical scavenging activities, with 50% inhibitory concentration (IC_{50}) of 7.61±0.69 and 19.93±0.40 µg/mL, respectively. It also gave the best inhibition against EAD, with an IC_{50} of 14.80±1.29 µg/mL. Morphological assessment of NMP extract-based ZnO NPs revealed rod-shaped particles at 35-50 nm range. **Conclusion:** It has been shown through this study that citrus extract based ZnO NPs, especially those prepared with NMP, may have the capacity to attenuate free radical release and inflammation in biological systems.

Keywords: Citrus plants, Nova mandarin, Zinc oxide nanoparticle green synthesis, Free radical scavenging activity, Anti-inflammatory capacity.

Tabisa Diniso[1], Ayodeji O. Oriola[1]*, Gugulethu M. Miya[1], Simon K. Kuria[2], Yiseyon S. Hosu[3], Opeoluwa O. Oyedeji[4], Mike O. Ojemaye[4], Ludwe Majiza[5], Jerry O. Adeyemi[1], Adebola O. Oyedeji[1]*

[1]Department of Chemical and Physical Sciences, Walter Sisulu University, Mthatha, 5117, SOUTH AFRICA.

[2]Department of Biological and Environmental Sciences, Walter Sisulu University, Mthatha, 5117, SOUTH AFRICA.

[3]Department of Business Management and Economics, Faculty of Economics and Financial Sciences.

[4]Department of Chemistry, University of Fort Hare, Alice, 5700, SOUTH AFRICA.

[5]Vunikhaya (PTY)LTD, Burnshill Location, Keiskammahoek, 5670, SOUTH AFRICA.

Correspondence

Ayodeji O. Oriola

Department of Chemical and Physical Sciences, Walter Sisulu University, Mthatha, 5117, SOUTH AFRICA.

E-mail: aooriola@gmail.com

Adebola O. Oyedeji

Department of Chemical and Physical Sciences, Walter Sisulu University, Mthatha, 5117, SOUTH AFRICA.

E-mail: aoyedeji@wsu.ac.za

History

- Submission Date: 18-10-2024;
- Review completed: 10-12-2024;
- Accepted Date: 21-12-2024.

DOI : 10.5530/pj.2024.16s.236

Article Available online

http://www.phcogj.com/v16/i6s

INTRODUCTION

Metal oxide nanoparticles (MONPs) are a fascinating class and diverse form of nanomaterials, due to their wide applications in physical, chemical, material, agricultural, biological and biomedical sciences.[1] Currently, different physical and chemical processes are widely used to synthesize MONPs, which allow one to obtain particles with the desired characteristics. However, these production methods are usually expensive and are potentially hazardous to the environment and living organisms.[2] Thus, there is an urgent need to find an eco-friendly, simple, and cost-effective alternative method for the synthesis of nanoparticles (NPs).[3] Phytochemicals from plants are non-toxic, reproducible biological resources, which are considered safe for human and environmental use.[4] The use of different bioresources extracted from plants have been identified as an alternative approach for the synthesis of NPs instead of the physical and chemical methods which uses hazardous, expensive, and ecologically dangerous materials.[5] A commonly synthesized material that has received lots of attention in recent years is zinc oxide nanoparticles (ZnO NPs).

Zinc oxide nanomaterials are a multifunctional material, owing to their remarkable physical and chemical properties, which include high photostability and electrochemical coupling coefficient, broad range of radiation absorption, biocompatibility, biodegradability, and low toxicity.[5] They have also been reported to show remarkable biological properties, such as antidiabetic, anticancer, antibacterial, antifungal, antioxidant, and anti-inflammatory activities.[6–8] They have extensive applications in solar cells, gas sensors, piezoelectric devices, sunscreens anti-reflection coatings, photocatalysis and biotechnology.[9–14] The use of plant extracts in the green synthesis of ZnO NPs is gaining more research interest in recent times due to their bio-functionality, environmental sustainability, simplicity, and cost-effectiveness.[15] Several medicinal plants such as *Zingiber officinale* (ginger), *Azadirachta indica*, *Medicago sativa* and *Anisochilus carnosus*, are utilized nowadays as biogenic material in the synthesis of ZnO NPs.[16–19] They have also been used in drug delivery and as antibacterial, anticancer, antidiabetic, and agricultural agents.[15,20–22] At low concentrations, ZnO NPs synthesized using green approaches have shown outstanding antioxidant and anti-inflammatory properties compared to their chemically synthesized counterparts.[23,24]

In normal metabolism levels, free radicals and antioxidants are balanced.[24] However, the overproduction of free radicals results in oxidative damage, leading to a range of chronic diseases such as cancer, diabetes, and inflammation.[25–27] Therefore, antioxidants play an important role in inhibiting and scavenging free radicals which may be-come harmful

Cite this article: Diniso T, Oriola AO, Miya GM, Kuria SK, Hosu YS, Oyedeji OO, et al. Citrus Extract-Mediated Zinc Oxide Nanoparticles and Their Capacity to Attenuate Free Radicals and Inflammation. Pharmacogn J. 2024;16(6)Suppl: 1222-1233.

to human cells, thus providing protection against infections and inflammatory diseases.[28] Although inflammation, including acute and chronic inflammation, is the body's defense response to external stimuli such as pathogens, irritants, or infections,[29] persistent inflammatory responses may lead to chronic inflammation-related diseases such as cardiovascular, neurodegenerative, and inflammatory bowel diseases.[30] Currently, inflammation therapy is mainly based on chemical medicine including non-steroidal anti-inflammatory drugs such as Diclofenac, Ibuprofen, and Indomethacin.[31] However, long-term use of these drugs possesses various side effects, which include gastrointestinal damages (gastric ulcers, and bleeding), liver and kidney dysfunction, and skin diseases.[32,33] Therefore, the use of medicinal plants as biogenic materials in nanoparticle synthesis is attracting much interest in recent time because of their remarkable biological properties, vis a viz antioxidant and anti-inflammatory activities, while also presenting little or no side effect.[34,35]

Citrus plants (family Rutaceae) are among the widely distributed fruits worldwide, having about 160 genera and 1300 species.[36] It is widely grown in the tropical and sub-tropical areas of the world, with an annual production of approximately 102 million tons per year.[37] Citrus fruits are known to contain bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils, which are responsible for protective health benefits.[38] Citrus fruits and their by-products (peels and leaves) are of high economic and medicinal value because of their multiple uses, such as in the food industry, cosmetic and folk medicine.[39] They have been reported to have good antioxidant and anti-inflammatory activities.[40,41]

In this study, ZnO NPs were green synthesized using the peel and leaf aqueous extracts of Nova manadarin (*Citrus reticulata*), Satsuma mandarin (*C. unshiu*) and Eureka lemon (*C. limon*), while *in vitro* antioxidant and anti-inflammatory activities were determined and evaluated. To the best of our knowledge, this study is the first to report on the biogenic synthesis of ZnO NPs using the leaf and peel aqueous extracts of the three citrus plants as well as their antioxidant and anti-inflammatory properties.

MATERIALS AND METHODS

General experimental procedure

All reagents and solvents were purchased from Sigma-Aldrich (Pty) Ltd. (Johannesburg, South Africa) and Merck (Pty) Ltd. (Johannesburg, South Africa) through a licensed local supplier, Shalom Laboratories and Supplies, South Africa. Antioxidant absorbance was measured on a 680-BioRad Microplate Reader (Serial Number 14966, Irvine, CA, USA).

Plant materials and extraction

Fresh leaves and fruits of Nova mandarin (*C. reticulata*), Satsuma mandarin (*C. unshiu*) and Eureka lemon (*C. limon*), were collected from Greenwood Citrus Farm located in Fort Beaufort, Eastern Cape Province, South Africa, with GPS coordinate (Latitude, 32° 46' 26.61"S; Longitude, 26° 37' 58.44"E) on 23rd June 2023. The plants were authenticated at Walter Sisulu University Herbarium situated in the Department of Botany. The voucher numbers, TD/2023/001, TD/2023/002 and TD/2023/003, were assigned to the herbarium specimens of the three citrus plants, respectively. The collected leaves and peels were air-dried at room temperature (≈25°C) before they were milled into powder. A 50 g portion of the powdered leaves and peels were macerated in 300 mL of distilled water at 50°C for 1 h with constant stirring. They were filtered to obtain the aqueous extracts and stored at 4°C until needed for further use.

Green synthesis of ZnO NPs

The biosynthesis of ZnO NPs was performed following the method described by Dejen et al.[42] with some modifications. In a 250 mL conical flask, 50 mL suspension of each citrus extract was added carefully to 50 mL of a 0.122 M aqueous solution of [$(CH_3COO)_2$ Zn·$2H_2O$]. The pH of the solution was maintained at 12 by dropwise addition of 1M NaOH solution while stirring for approximately 2½ h. Thereafter, the solution was centrifuged three times at 12000 rpm for 15 min, each time using a mixture of distilled water and ethanol to remove impurities and minimize aggregation size. The collected precipitate was dried in an oven set at 100°C for 1 h. It was further heated in a furnace at constant temperature of 400°C for 2 h to ensure stability of the colloidal system. The resulting dried NPs were powdered and stored in an air-tight container until needed for further analysis.

Characterization of the biosynthesized ZnO NPs

To ascertain the successful green synthesis of ZnO NPs, different characterization techniques were employed. Vibrational spectra of the biosynthesized nanomaterials were recorded using Universal ATR sampling accessory spectrum 100 FT-IR spectrometer (Perkin Elmer Inc., Waltham, MA, USA). The UV analysis was performed on the Perkin Elmer UV-Vis absorption spectrometer (Perkin Elmer Inc., Waltham, MA, USA). The crystallinity of the biosynthesized ZnO nanoparticles was characterized by coating samples with gold-palladium for 2 min in the Denton air vacuum sputter coater (Denton Desk V, USA) at 30 mA. The sample was placed into the Apreo volume scope (Thermo-Fischer, Netherlands). The nano-images were acquired using the Xt Microscopy software (Thermo-Fischer Scientific, Waltham, MA, USA). The particle size and morphology of the synthesized products were analyzed on a TECNAI G2 (ACI) scanning electron microscope with an accelerating voltage of 200 kV. The elemental composition and the morphology of the prepared nanomaterials were studied using FEI Quanta FEG 250 field emission gun microscope operating at 15 kV with the Energy Dispersive X-ray (EDX) spectra obtained using Oxford Inca software (Oxford Instruments, Abingdon, UK).

Antioxidant analysis of citrus-mediated ZnO NPs

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

The DPPH radical scavenging assay method was used according to Shen et al.,[43] with slight modifications. A solution of 0.135 mg/mL DPPH in methanol was prepared, and 1.0 mL of the DPPH solution was mixed with 1.0 mL of each of the test samples at varying concentrations (100.00, 50.00, 25.00, 12.50 and 6.25 µg/mL) in triplicates. The reaction mixture was thoroughly mixed and kept in a dark room for 30 min. The absorbance was measured at 515 nm on a 680-Bio-Rad Microplate Reader (Bio-Rad Inc., Hercules, CA, USA). *L*-Ascorbic acid was used as the positive control. Additionally, the leaf and peel aqueous extracts of the three citrus plants were also used as a control over similar concentration ranges. The DPPH radical scavenging activity of the test samples was determined based on the equation (1):

$$\%\ DPPH\ scavenging\ activity = \frac{(Ac - At)}{Ac}\ x\ 100\ (1)$$

where Ac = absorbance of negative control (methanol), and At = absorbance of test sample.

The inhibitory concentration (IC_{50}) of each test sample was determined from the concentration-% DPPH radical scavenging response plot.

Nitric oxide (NO) radical scavenging assay

The ability of the citrus extract based ZnO NPs to inhibit NO free radicals was determined using standard method.[44] ZnO NPs, prepared

using methanol, were evaluated at different concentrations (100.00, 50.00, 25.00, 12.50, and 6.25 µg/mL). They were added to sodium nitroprusside (2.0 mL, 0.2 mM) in triplicates. The reaction mixture was then incubated at 25°C for 3 h. Following the incubation, 0.5 mL of the mixture was combined with Griess reagent. Griess reagent consists of 0.33% sulphanilamide dissolved in 20% glacial acetic acid and mixed with 1 mL of naphthylethylenediamine chloride (0.1% w/v). The resulting complex formed by the mixture and Griess reagent was incubated at room temperature for 30 min. The absorbance of the complex was measured at 540 nm. *L*-Ascorbic acid was employed as the positive control, while the aqueous citrus extracts alone also served as a control over similar concentration ranges.

The percentage inhibition of NO radical was calculated based on equation (1), while the IC_{50} values were determined.

Ferric reducing antioxidant power (FRAP) assay

The FRAP activity of the green synthesized was evaluated using standard method.[45] Here, 1.0 mL of each of the test samples in methanol (100.00, 50.00, 25.00, 12.50 and 6.25 µg/mL) was added to the mixture containing 2.5 mL of phosphate buffer (0.2 M pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v) in triplicates. *L*-Ascorbic acid was used as the positive control. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (TCA) (10% w/v). It was then centrifuged at 300 rpm for 10 min. About 2.5 mL of the upper layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1% w/v). The experiment was conducted in triplicate, and absorbance was measured at 700 nm against a blank sample of only phosphate buffer. Thus, the ferric reducing power of the test samples at 100.00, 50.00, 25.00, 12.50 and 6.25 µg/mL concentration in methanol was determined as ascorbic acid equivalent (AAE) from the calibration curve of the *L*-ascorbic acid (positive control).

In vitro anti-inflammatory analysis of citrus extract-mediated ZnO NPs

The anti-inflammatory response of the six citrus-mediated ZnO NPs was carried out using the egg albumin denaturation assay method reported by Chatterjee et al.,[46] with modifications. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of the test samples including Diclofenac (positive control), so that the final concentrations become 100.00, 50.00, 25.00, 12.50 and 6.25 µg/mL in triplicates. Similar volume of double-distilled water served as negative control, while the various aqueous citrus extracts were tested to also serve as a control. The reaction mixtures were incubated at 37°C in a BOD incubator (Labline Technologies, Ahmedabad, Gujarat, India) for 15 min, and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. The percentage inhibition of protein (egg albumin) denaturation was calculated according to equation (2):

$$\%\ inhibition\ of\ EAD = \left[\left(\frac{Vt}{Vc}\right) - 1\right]\ x\ 100 \quad (2)$$

where Vt = Absorbance of test sample, and Vc = Absorbance of negative control, EAD = egg albumin denaturation.

The concentration of the test sample that exerted 50% inhibition of EAD represents the IC_{50} –a measure of the *in vitro* anti-inflammatory activity of the citrus mediated ZnO NPs.

Statistical analysis

Antioxidant and anti-inflammatory data were imputed on a Microsoft Excel version 365 (Microsoft® Corporation, Redmond, WA, USA). The data were expressed as the mean ± standard error of mean, SEM (n = 3) and analyzed using one-way analysis of variance (ANOVA), followed by Student Newman–Keul's post hoc test. The level of significance of the sampled groups was set at 95% confidence limit ($p < 0.05$).

Study workflow

The entire study from the green synthesis of ZnO NPs using the three citrus plants, through characterization, to free radical scavenging and anti-inflammatory analyses, is presented in a diagrammatic sketch as Figure 1, using the Microsoft Publisher version 365 (Microsoft® Corporation, Redmond, WA, USA Africa).

RESULTS AND DISCUSSION

Characterization of the biogenic nanoparticles

The characterization of the biosynthesized nanomaterials was carried out by using a number of techniques including X-ray diffraction analysis (XRD), Fourier infra-red spectroscopy (FTIR), ultraviolet-visible spectroscopy (UV-vis), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX).

UV-Visible analysis

The UV-Vis spectra of the green synthesized ZnO NPs using the peel and leaf extracts of C. reticulata (Nova mandarin), *C. unshiu* (Satsuma mandarin), and *C. limon* (Eureka lemon), are presented in Figures 2A–C. The zinc acetate dihydrate starting material (zinc precursor) showed absorption peaks at 208–238 nm, which are typical of the UV-Vis absorption peaks of zinc acetate.[47,48] The individual plant extracts used for the green nano-synthesis gave absorption peaks within the range 230–450 nm, which agree with the absorption peaks of conjugated compounds.[49,50] Furthermore, the absorption peaks at 387 and 415 nm in the citrus extract-mediated ZnO NPs (Figures 2A-C) correspond to the characteristic peaks for ZnO NPs ,[50–52] indicating the successful formation of ZnO NPs using citrus peel and leaf extracts.

FTIR analysis

The observed FTIR spectra obtained for the nanoparticles are presented in Figure 3. The vibrational bands at 600 and 460 cm^{-1} correspond to metal oxide (M–O) stretching and deformation vibration, respectively, as previously reported in extract-mediated ZnO NPs.[51,53] The hump at 3400 cm^{-1} corresponds to the presence of a hydroxyl (OH) functional group, while the peaks at 1600 cm^{-1} (C=O) and 1400 cm^{-1} (O-H bending vibration) could be due to phytochemicals in the citrus extracts.[50,53–55]

X-ray diffraction (XRD) analysis

The X-ray diffraction pattern obtained and presented in Figure 4 provides valuable information on the crystallinity of the biosynthesized ZnO NPs. The diffraction peak values (2θ) found at 31.77°, 34.42°, 36.25°, 47.53°, 56.60°, 62.86°, 66.38°, 67.96°, 69.10°, and 76.95° have been indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202) respectively. The observed intensity peaks in the diffraction pattern agree with the hexagonal wurtzite structure reported for ZnO NPs[56] and are consistent with the standard JCPD No 00-036-1451. In addition, the presence of these diffraction peaks shows that the biosynthesized ZnO NPs are crystalline and not amorphous in nature. In addition, the sharpness of the diffracted peaks observed in the obtained diffractogram suggests that the biosynthesized ZnO NPs exhibit a crystalline structure. Likewise, the absence of unwanted peaks indicates that the sample is free from impurities and is not amorphous. This report agrees with those in literature in which different plant extracts were used as mediating agents for ZnO NPs.[57,58]

SEM and TEM analyses

Microscopic evaluation of the citrus extract-based ZnO NPs confirmed

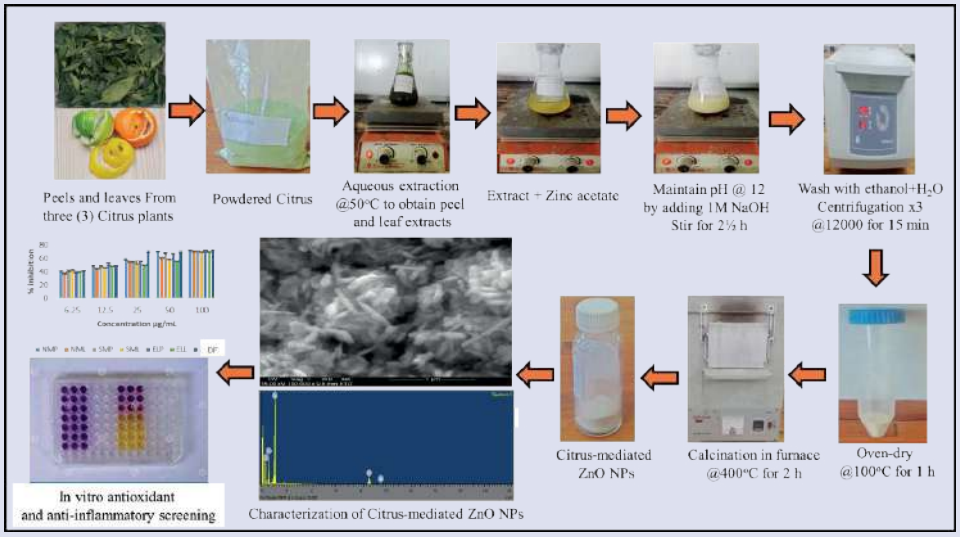

Figure 1. Flow chart for green synthesis of ZnO NPs using three citrus plants and their biological screening

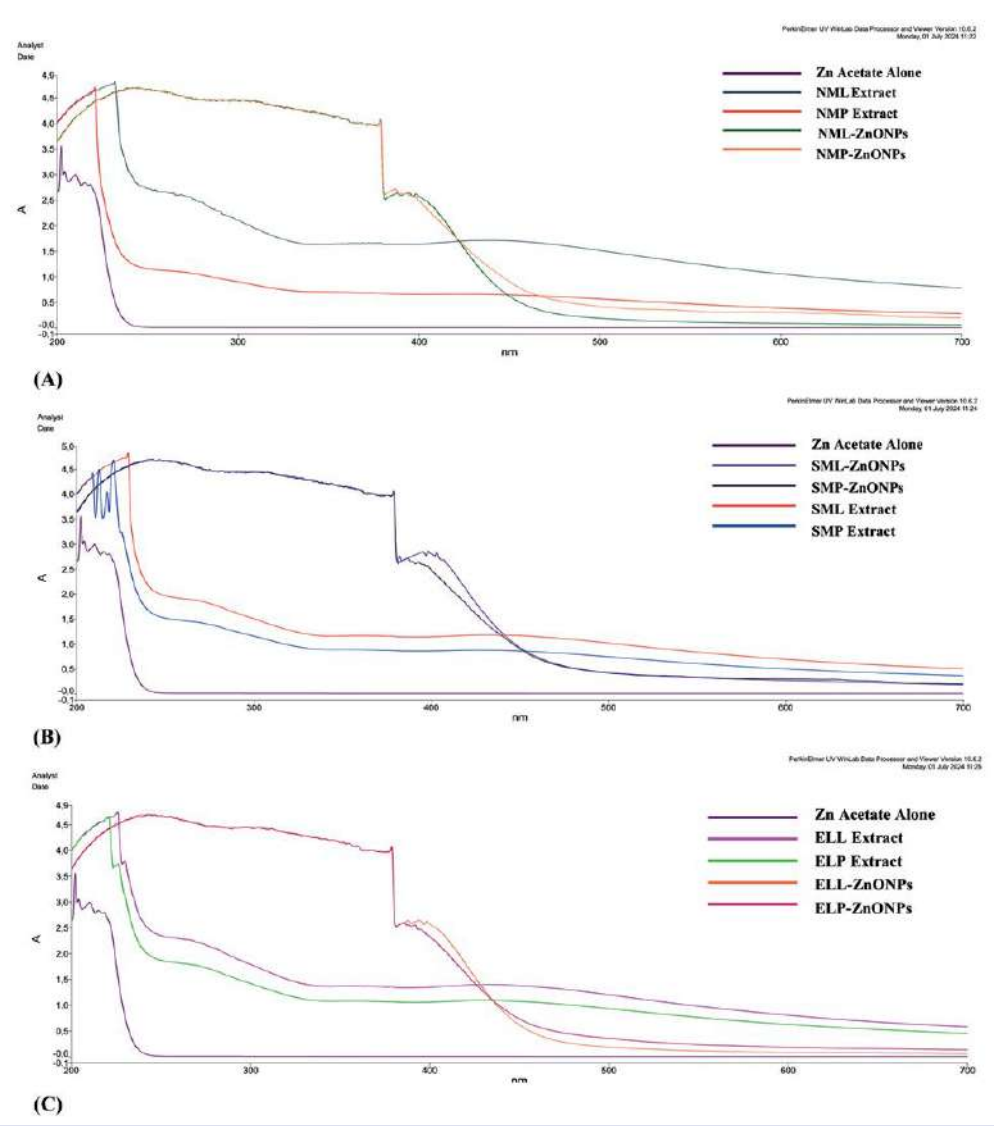

Figure 2. UV-Visible spectra of the green synthesized ZnONPs using three citrus species. (**A**) Nova mandarin leaf (NML) and Nova mandarin peel (NMP), (**B**) Satsuma mandarin leaf (SML) and Satsuma mandarin peel (SMP), (**C**) Eureka lemon leaf (ELL) and Eureka lemon peel (ELP).

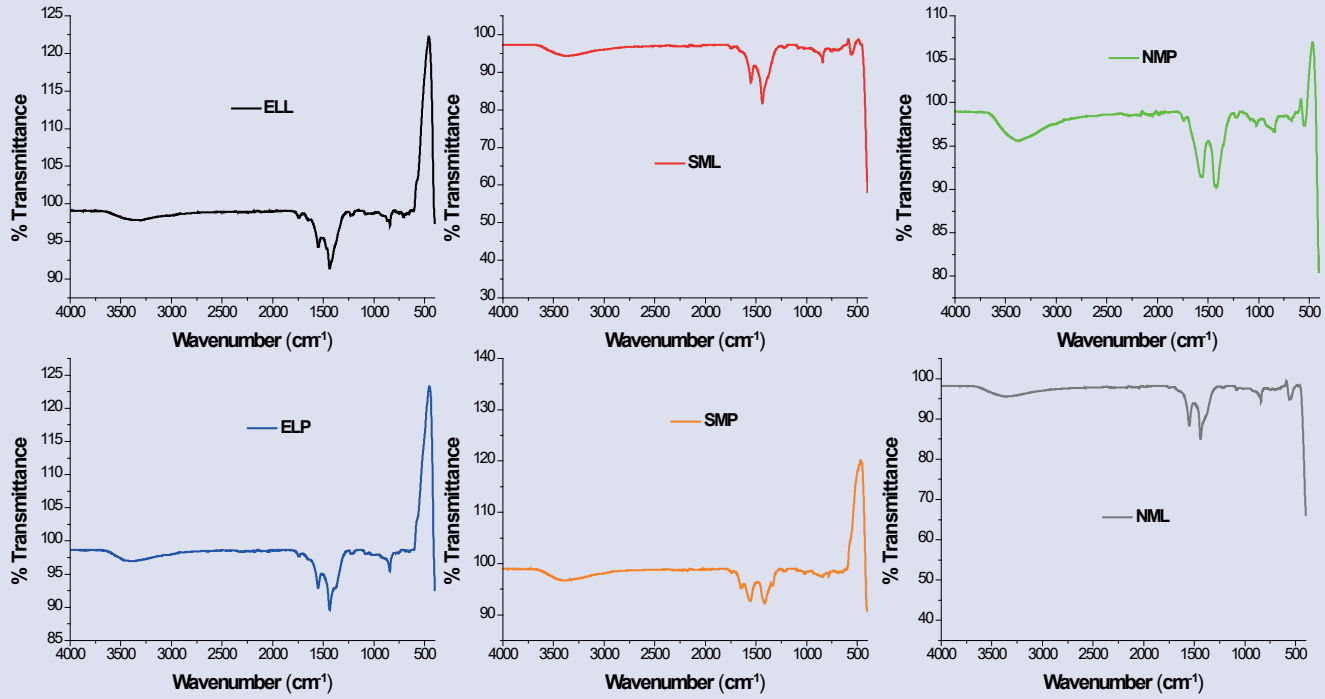

Figure 3. FTIR spectra of biosynthesized ZnO NPs using three different citrus species. Eureka lemon leaf (ELL), Eureka lemon peel (ELP), Satsuma mandarin leaf (SML), Satsuma mandarin peel (SMP), Nova mandarin leaf (NML), Nova mandarin peel (NMP).

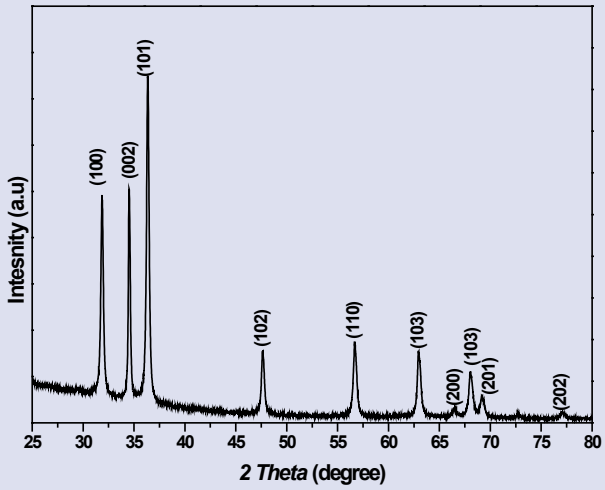

Figure 4. XRD diffraction pattern of Nova mandarin peel extract mediated ZnO NPs

the formation of a rod-like morphology arranged in bundles but tend to agglomerate into spherical particles, with sizes between 9.4 and 35.1 nm (Figure 5). The rod-like appearance is characteristic of ZnO NPs, as previously reported.[59–61]

TEM is the most used technique for determining the particle size of nanorods. It is also used to determine the shape and dispersibility of nanoparticles. Studies have shown that nanorods activities are influenced by the particle size, as small particle size enhances the activities of nanorods and vice versa.[62] Thus, the ZnO NPs synthesized using the peel and leaf extracts of three citrus plants showed nanomaterials of particle size range 35–55 nm with rod-like shape, which tends towards spherical shape upon agglomeration. Although agglomeration was also observed for all the images, this observation corroborates the result obtained for the SEM analysis of the nanomaterials (Figure 5) and are similar to previous studies on biosynthesized zinc oxide nanorods.[59, 63]

EDX analysis

In this study, EDX analysis was used to determine the elemental composition of the citrus mediated ZnO NPs. The spectrum (Figure 6) showed the occurrence of C, O, and Zn peaks at 0.25, 0.51, and 1.01 keV. The Zn and O components of the NPs showed weight percentage values of 78.05 and 11.00%, which agree with previous reports on green synthesized ZnO NPs.[64,65.] Furthermore, the characteristic Zn peaks in the acquired EDX spectrum at 1.01, 8.71 and 9.57 keV, are due to

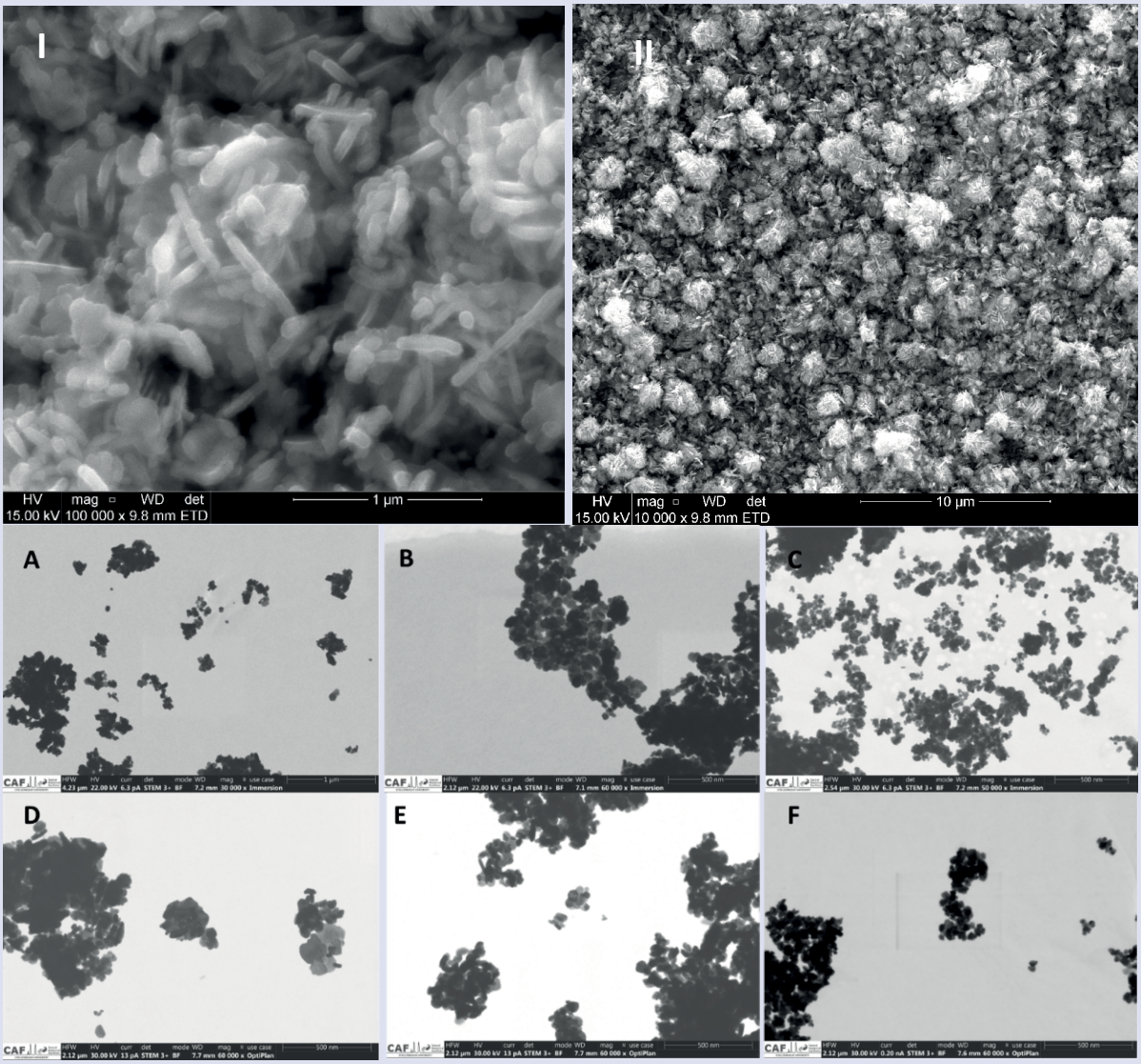

Figure 5. SEM and TEM micrograph images of citrus extract mediated ZnO NPs at different magnifications. SEM images: (**I**) rod-like morphology of Nova mandarin peel (NMP) extract synthesized ZnO NPs arranged in bundles, (**II**) agglomeration of the NMP extract based ZnO nanorods into spherical particles. TEM images: (A) SMP-Satsuma mandarin peel, (B) SML-Satsuma mandarin leaf, (C) NMP-Nova mandarin peel, (D) NML-Nova mandarin leaf, (E) ELP-Eureka lemon peel, and (F) ELL-Eureka lemon leaf.

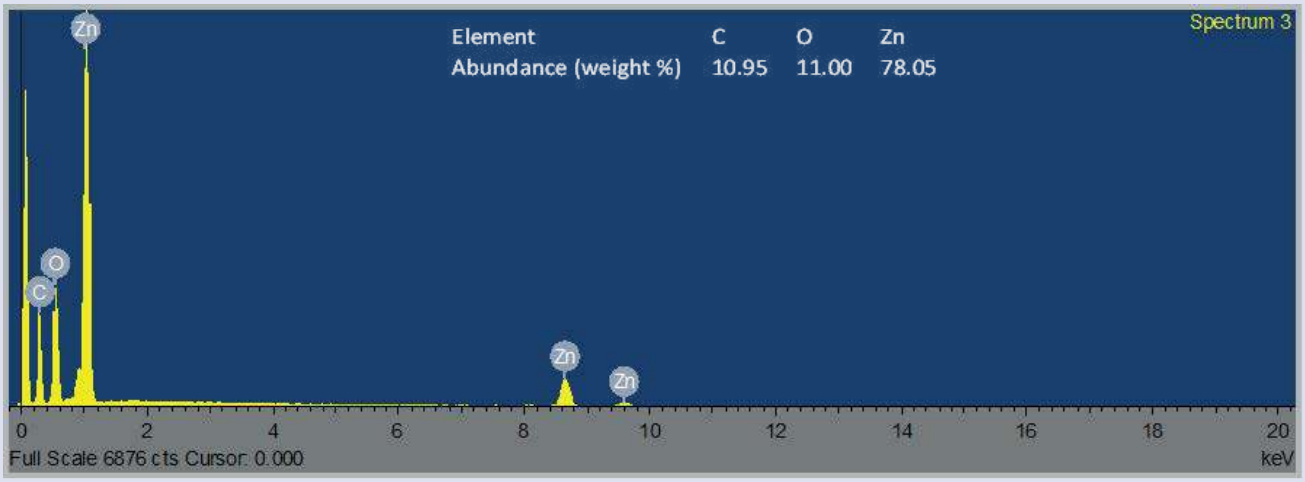

Figure 6. EDX spectrum of Nova mandarin peel extract-mediated ZnO NPs showing the weight % of elements in the nanoparticles.

surface plasmon resonance effect, which is typical for the absorption of ZnO nanostructures.[66]

Free radical scavenging capacity

DPPH radical scavenging activity

The method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging is a widely employed technique to assess the antioxidant potential of natural products and other substances. The assay is based on the ability of a substance to donate hydrogen atoms, thereby reducing the DPPH radical, which is initially deep violet in color, to a light-yellow color. This reduction can be quantitatively measured using spectrophotometry.[43] The test samples showed a steady concentration-dependent increase in the percentage inhibition of the DPPH radical from 43.64% to 73.9% with increase in concentration from 6.25–100 µg/mL (Figure 7). At a concentration of 100 µg/mL, NMP extract-mediated ZnO NPs displayed the highest DPPH percentage inhibition (73.9±0.82%,) among the green synthesized ZnO NPs. However, it was lower in percentage inhibition compared to that of *L*-ascorbic acid, which demonstrated 78.03±0.50%.

Further evaluation of antioxidant activity to gain more insight on the possible contributory role of the various plant extracts on their respective ZnO NPs is presented in Table 1. The study revealed that the various citrus extracts contribute to the free radical scavenging activities of the ZnO NPs. Apart from ELL extract (IC_{50} = 16.71±1.28 µg/mL), which exhibited a significantly ($p<0.05$) better activity than its green synthesized ZnO NPs (22.52±0.67 µg/mL), the ZnO NPs synthesized with the peel and leaf extracts of Nova mandarin and Satsuma mandarin as well as those from Eureka lemon peel extract exhibited better activity compared to their respective crude extracts. The best activity was demonstrated by NMP-based ZnO NPs, with an IC_{50} of 7.61±0.69 µg/mL. Previous studies have shown that ZnO NPs prepared with natural citrus extract, such as the Eureka lemon juice, exhibit more antioxidant activity than those capped with citric acid, with the latter showing 50% inhibition of DPPH at 500 µg/mL.[67] Also, Nova mandarin

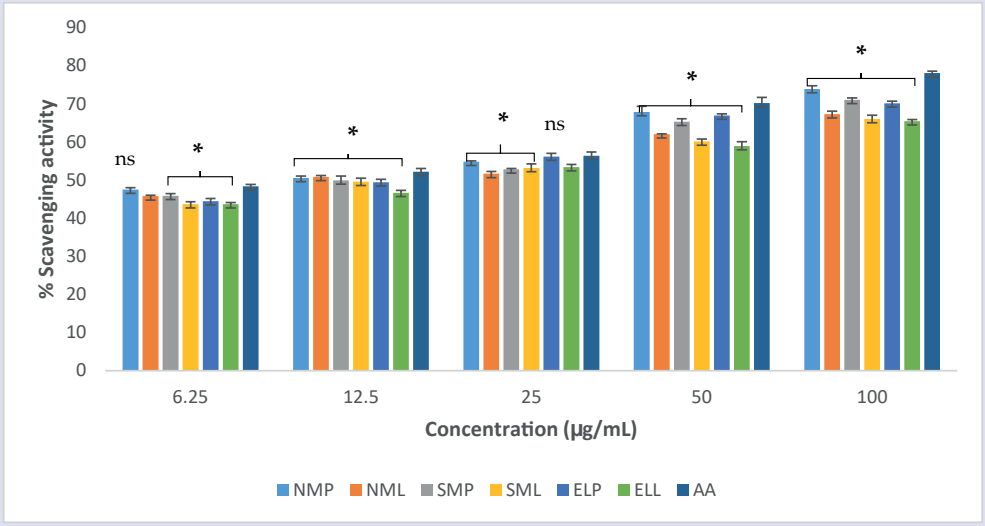

Figure 7. DPPH radical scavenging activity of Synthesized ZnO NPs from various Citrus leaf and peel extracts. Data are expressed as mean ± SEM (n = 3), NMP – Nova mandarin peel, NML – Nova mandarin leaf, SMP – Satsuma mandarin peel, SML – Satsuma mandarin leaf, ELP – Eureka lemon peel, ELL – Eureka lemon leaf, AA – *L*-ascorbic acid, * $p<0.0001$ – data on bars are significantly different from positive control (AA), ns – no significant difference to the positive control (AA) at $p>0.0001$.

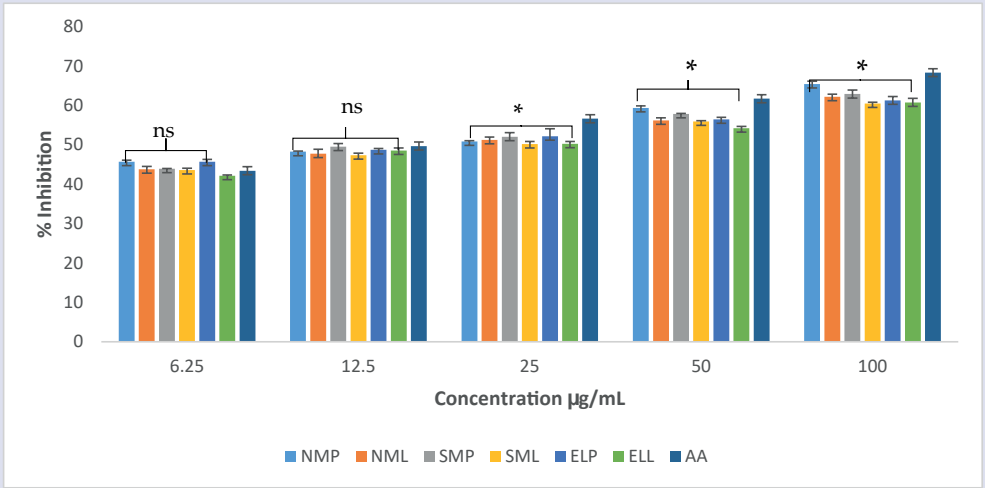

Figure 8. Nitric oxide inhibitory effect of citrus extract-mediated ZnO NPs. Data are expressed as mean ± SEM (*n* = *3*), NMP – Nova mandarin peel, NML – Nova mandarin leaf, SMP – Satsuma mandarin peel, SML – Satsuma mandarin leaf, ELP – Eureka lemon peel, ELL – Eureka lemon leaf, AA – Ascorbic acid, * $p<0.0001$ – data on bars are significantly different from positive control (AA), ns – no significant difference to the positive control (AA) at $p>0.0001$.

leaf extract-based ZnO NPs have been reported to show notable DPPH, radical scavenging activity with an IC_{50} of 33.5±0.99 mg/mL.[68] Thus, our study findings underscore the considerable antioxidant potential of citrus extract based ZnO NPs, especially those prepared with Nova mandarin peel extract, positioning it as a promising source of natural antioxidants.

The color changes from deep violet to light yellow serves as a visual and spectrophotometric indicator of the antioxidant activity. The ability of the extract to mimic the behavior of the standard antioxidant further supports its efficacy in scavenging free radicals. Overall, these results contribute valuable insights into the antioxidant properties of Nova mandarin peel as well as other biosynthesized nanorods in this study and highlight their potential applications in various fields, including functional foods and pharmaceuticals. Further research may delve into the specific compounds responsible for the observed antioxidant activity and explore potential synergistic effects within the plant extracts.

Nitric oxide inhibitory activity

The capacity of the synthesized ZnO NPs to inhibit the activity of inducible nitric ox-ide synthase (iNOS) by free radical scavenging activity was demonstrated in the NO radical inhibition test, a key player in inflammatory processes and oxidative stress. The Nova mandarin peel (NMP) ZnO NPs gave the best activity, having demonstrated 65.52 ± 0.69% inhibition of NO radical. (Figure 8). Comparatively, ZnO NPs from Satsuma mandarin, another citrus variety, also exhibited notable NO inhibition, albeit slightly lower at 62.92 ± 0.99%. To better understand the antioxidant capacity of each citrus based ZnO nanorods and the contributory role of their crude extracts, the IC_{50} values of all the test samples were presented (Table 1). Unlike the result obtained for the DPPH radical scavenging activity, the leaf extracts based ZnO NPs from the three Citrus plants exhibited significantly ($p<0.05$) higher IC_{50} values than their control extracts. This translates to a reduced antioxidant activity when the extracts were biosynthesized with ZnO NPs, suggesting that some of the chemical constituents present in NML, SML and ELL extracts might not have been fully functionalized in the ZnO NPs to be able to act as reducing agents against the NO free radical. Conversely, the peel extract mediated ZnO NPs showed better activity against NO, based on their significantly ($p<0.05$) lower IC_{50} values compared to their control extracts. Overall, the NMP extract based ZnO NPs gave the best activity against NO radical in all the Citrus plants tested, with an IC_{50} value of 19.93±0.40 µg/mL. Our findings highlight the variability in the antioxidant activity of ZnO NPs de-rived from different citrus sources. The differences may be attributed to the variation in the phytochemical composition of the three citrus plants, emphasizing the importance of selecting specific Citrus varieties for nanoparticle synthesis to harness optimal antioxidant properties. The superiority of NMP extract based ZnO NPs in the NO radical inhibition test could be linked to the unique phytochemical profile of Nova mandarin peel. Citrus peels are known to contain a myriad of bioactive compounds, such as flavonoids and phenolic acids, which are renowned for their antioxidant and anti-inflammatory properties.[38–40] The NPs derived from NMP likely retained and concentrated these bioactive compounds, contributing to their enhanced NO scavenging ability.

This study not only highlights the potential of NMP synthesized ZnO NPs as a source of antioxidant activity but also underscores the importance of considering the specific citrus variety when exploring NP applications in medicine and therapeutics. Further investigations into the underlying mechanisms of the observed NO inhibition and

Table 1. Antioxidant activities of Citrus synthesized ZnO NPs with their control extracts.

Citrus plant	Extract-mediated ZnO NPs with their control extracts	IC_{50}±SEM (µg/mL)	
		DPPH	NO
Nova mandarin	Peel extract-ZnO NPs	7.61 ± 0.69 ^{b}	19.93 ± 0.40 ^{b}
	Control (peel extract)	9.91 ± 0.70 ^{c}	25.17 ± 1.58 ^{d}
	Leaf extract-ZnO NPs	13.91 ± 0.42 ^{d}	26.23 ± 0.79 ^{d}
	Control (leaf extract)	22.81 ± 2.12	23.61 ± 1.02 ^{c}
Satsuma mandarin	Peel extract-ZnO NPs	12.71 ± 0.65 ^{d}	20.64 ± 0.61 ^{b}
	Control (peel extract)	14.99 ± 0.87 ^{e}	33.79 ± 2.63 ^{fg}
	Leaf extract-ZnO NPs	18.05 ± 0.83 ^{f}	29.53 ± 0.43 ^{d}
	Control (leaf extract)	23.17 ± 2.26 ^{g}	35.18 ± 1.97 ^{g}
Eureka lemon	Peel extract-ZnO NPs	15.08 ± 0.66 ^{e}	20.11 ± 0.86 ^{b}
	Control (peel extract)	17.11 ± 1.08 ^{f}	28.62 ± 1.17 ^{e}
	Leaf extract-ZnO NPs	22.52 ± 0.67 ^{g}	31.53 ± 0.59 ^{f}
	Control (leaf extract)	16.71 ± 1.28 ^{ef}	22.87 ± 1.44 ^{bc}
Positive control	L(+)-Ascorbic acid	4.17 ± 0.88 ^{a}	13.39 ± 0.92 ^{a}

Data are expressed as Mean ± SEM (n=3), values with different alphabets in superscript are considered significant at $p<0.05$.

Table 2. Ferric reducing activity of nanoparticles synthesized from three Citrus species.

Conc. (µg/mL)	µgAAE/mg					
	NMP	NML	SMP	SML	ELP	ELL
6.25	122.16 ± 2.69 ^{d}	107.03 ± 2.49 ^{b}	112.97 ± 3.55 ^{c}	101.08 ± 3.26 ^{a}	115.41 ± 4.54 ^{c}	105.14 ± 3.41 ^{ab}
12.5	137.84 ± 3.48 ^{c}	117.57 ± 2.43 ^{b}	118.92 ± 2.72 ^{b}	109.73 ± 2.80 ^{a}	121.08 ± 2.72 ^{b}	116.22 ± 3.28 ^{b}
25	143.24 ± 4.96 ^{d}	134.05 ± 4.31 ^{c}	137.57 ± 4.27 ^{a}	120.27 ± 2.67 ^{a}	135.14 ± 3.42 ^{c}	127.84 ± 2.83 ^{b}
50	150.81 ± 3.08 ^{c}	141.35 ± 3.57 ^{a}	140.54 ± 5.23 ^{ab}	137.84 ± 2.71 ^{a}	147.57 ± 5.56 ^{bc}	138.92 ± 2.60 ^{a}
100	169.78 ± 4.65 ^{c}	152.97 ± 3.04 ^{ab}	158.65 ± 4.76 ^{b}	146.67 ± 3.40 ^{a}	164.32 ± 6.58 ^{bc}	149.46 ± 1.94 ^{a}

Data are expressed as mean ± SEM ($n = 3$). Synthesized ZnO nanoparticles: NMP – Nova mandarin peel, NML – Nova mandarin leaf, SMP – Satsuma mandarin peel, NML – Nova mandarin leaf, ELP – Eureka lemon peel, ELL – Eureka lemon leaf, AAE – Ascorbic acid equivalent, values having different alphabets in superscript are considered significant at $p<0.05$, concentration (conc.).

Table 3. ***In vitro*** **anti-inflammatory activity of Citrus extract-mediated ZnO NPs.**

Citrus plant	Extract-based ZnO NPs with extract alone (control)	IC_{50}±SEM (µg/mL)
Nova mandarin	Peel extract-ZnO NPs	14.80 ± 1.29 [ab]
	Control (peel extract)	21.17 ± 2.11 [d]
	Leaf extract-ZnO NPs	28.44 ± 1.04 [e]
	Control (leaf extract)	37.51 ± 2.33 [f]
Satsuma mandarin	Peel extract-ZnO NPs	17.92 ± 0.40 [c]
	Control (peel extract)	29.36 ± 2.35 [e]
	Leaf extract-ZnO NPs	25.58 ± 2.91 [e]
	Control (leaf extract)	18.01 ± 1.13 [c]
Eureka lemon	Peel extract-ZnO NPs	16.77 ± 0.79 [bc]
	Control (peel extract)	22.81 ± 1.72 [d]
	Leaf extract-ZnO NPs	29.90 ± 0.94 [e]
	Control (leaf extract)	17.44 ± 0.95 [c]
Positive control	Diclofenac	12.37 ± 1.67 [a]

Data are expressed as mean ± SEM (n=3), values with different alphabets in superscript are significantly different at p<0.05.

the identification of key bioactive compounds in citrus plants ZnO NPs could provide valuable insights for the development of novel antioxidant and anti-inflammatory agents with potential biomedical applications.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) of an extract is a measure of its ability to donate electrons; thus, it may serve as an important indicator of the antioxidant activity of natural products.[44] In the ferric reducing antioxidant assay, the ZnO NPs from NMP, ELP and SMP exhibited good reducing activity (Tables 2). At the highest concentration of 100 µg/mL, they significantly (p<0.05) reduced Fe^{3+} radicals at 169.78±4.65, 164.32±6.58 and 158.65±4.76 µgAAE/mg, respectively. The observed reducing activity is indicative of the ability of these citrus mediated ZnO NPs to donate electrons and neutralize Fe^{3+} radicals. This property is crucial in the context of antioxidant defenses as it suggests the potential of these nanoparticles to counteract oxidative stress by mitigating the effects of reactive oxygen species. The slight variation in reducing activity among the different citrus peel NPs may be attributed to the unique phytochemical composition of each Citrus species. Citrus peels are known reservoirs of bioactive compounds such as flavonoids and phenolic acids, which contribute to their antioxidant potential. Thus, the NPs derived from the peel extract of these citrus species likely encapsulate and concentrate these bioactive compounds, influencing their overall ferric reducing antioxidant activity.

The findings of this ferric reducing antioxidant assay emphasize the promising antioxidant properties of citrus peel nanoparticles, particularly Nova mandarin, Eureka lemon, and Satsuma mandarin. The ability to effectively reduce Fe^{3+} radicals suggest the potential applicability of these NPs in various fields, including functional foods, pharmaceuticals, and nanomedicine. Further research could delve into identifying the specific bioactive components responsible for the observed reducing activity and explore potential synergistic effects within these citrus peel nanoparticles for enhanced antioxidant efficacy.

In vitro anti-inflammatory activity

The egg albumin denaturation assay is based on the idea that substances with anti-inflammatory properties may be able to stabilize protein structures by inhibiting its denaturation, thus preventing inflammation and tissue damage in biological systems.[69]

At 100 µg/mL, ZnO NPs green synthesized with NMP demonstrated the highest activity of 71.73±0.76% inhibition of lipid peroxidation (Figure 9). The observed high inhibition of lipid peroxidation aligns with the general antioxidant trends seen in other assays in previously published studies. Thus, our findings suggest that NMP extract based ZnO NPs can neutralize and break the chain reactions associated with lipid peroxidation, indicating a robust antioxidant defense mechanism. This result is consistent with a previously published report on biosynthesized ZnO NPs.[56]

A summary of the anti-inflammatory activities of the three Citrus extracts based ZnO NPs alongside their control extracts is presented in Table 3. The NMP extract based ZnO NPs showed the best anti-inflammatory activity, with an IC_{50} value of 14.80±1.29 µg/mL. This activity was comparable (p>0.05) to those demonstrated by Diclofenac (IC_{50} = 12.37±1.67 µg/mL) and ELP extract mediated ZnO NPs (IC_{50} = 16.77±0.79 µg/mL). The result further explains the significant role that the NMP extract played in the overall anti-inflammatory activity displayed by its ZnO nanorods. It is worthy of mention that the leaf extract of Satsuma mandarin and Eureka lemon showed better anti-inflammatory activities than their ZnO NPs, which may suggest that some of their constituents are not fully utilized as anti-inflammatory agents in the green synthesized ZnO NPs. The unique phytochemical composition of NMP and the other citrus plant parts, possibly concentrated and preserved in the nanoparticle form, likely contributes to this elevated activity. The nanoparticles may act as effective scavengers of reactive oxygen species, preventing oxidative damage to lipids and thereby preserving the integrity of cellular membranes.[70]

The results further emphasize the potential applications of citrus plant parts biosynthesized ZnO NPs in combating oxidative stress-related conditions including inflammatory diseases. As lipid peroxidation is implicated in various diseases, including neuro-degenerative disorders and cardiovascular diseases, the demonstrated inhibition by these nanoparticles opens avenues for their use in pharmaceutical and nutraceutical formulations. Future investigations could delve into elucidating the specific molecular mecha-nisms underlying the inhibitory effects of these ZnO NPs on lipid peroxidation, providing deeper insights into their therapeutic potential. Lastly, a thorough toxicology studies on these Citrus based ZnO NPs may be necessary, since metal oxide NPs including ZnO NPs, have been reported to show a high potential to accumulate in marine food chains, causing serious toxicity to marine ecosystems.[71]

CONCLUSION

We describe a straightforward, inexpensive, and eco-friendly procedure for the green synthesis of ZnO NPs using the peel and leaf aqueous extracts of Nova mandarin, Satsuma mandarin and Eureka lemon. In this study, the citrus extract-mediated ZnO NPs were subsequently characterized and evaluated for free radical scavenging and anti-

inflammatory properties. The characterization by FTIR, UV-Vis, SEM, TEM, EDX and XRD confirmed the successful green synthesis of ZnO NPs, with distinctive features, such as a metal-oxygen vibrational band and rod-like morphology with particle sizes ranging from 35 to 50 nm. Biologically, they showed considerable free radical scavenging and anti-inflammatory capacity, with Nova mandarin peel extract ZnO NPs exhibiting the highest activity. Thus, the study findings highlight the biological contribution of natural extracts such as Nova mandarin peel extract in the green synthesis of ZnO NPs. The demonstrated biological activities of the NPs further underscore their antioxidant and anti-inflammatory potentials.

Further *in vitro* assessment using human or animal cell lines, to elucidate the underlying molecular mechanisms of action, followed by *in vivo* efficacy and toxicity studies, will contribute to a comprehensive understanding of the therapeutic and safety profile of these NPs.

FUNDING

The authors thank the CSIR, and the National Research Foundation (NRF), South Africa, for providing funding support through the Citrus NRF UID-135455 grant.

ACKNOWLEDGEMENTS

The authors acknowledge the Research Directorates at Walter Sisulu University (WSU) and University of Fort Hare, South Africa, for funding the APC. The African Medicinal Flora and Fauna Research Niche Area, WSU and the NRF South Africa through the NRF incentive grant, are appreciated for their support.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Alhalili Z. Metal oxides nanoparticles: General structural description, chemical, physical, and biological synthesis methods, role in pesticides and heavy metal removal through wastewater treatment. Molecules. 2023;28(7):3086.
2. Yedurkar S, Maurya C, Mahanwar P. Biosynthesis of zinc oxide nanoparticles using Ixora coccinea leaf extract—a green approach. Open J Syn Theory App. 2016;5(1):1–14.
3. Rajkumar PV, Prakasam A, Rajeshkumar S, Gomathi M, Anbarasan PM, Chandrasekaran R. Green synthesis of silver nanoparticles using *Gymnema sylvestre* leaf extract and evaluation of its antibacterial activity. South Afr J Chem Eng. 2020;32(1):1–4.
4. Ahmed S, Ahmad M, Swami BL, Ikram S. Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. J Rad Res Appl Sci. 2016;9(1):1–7.
5. Wojnarowicz J, Chudoba T, Lojkowski W. A review of microwave synthesis of zinc oxide nanomaterials: Reactants, Process parameters and morphologies. Nanomaterials. 2020;10:1086.
6. Rai RS, Girish JP, Bajpai V, Khan MI, Elboughdiri N, Shanableh A, Luque R. An eco-friendly approach on green synthesis, bio-engineering applications, and future outlook of ZnO nanomaterial: A critical review. Environ Res. 2023;221:114807.
7. Gudkov SV, Burmistrov DE, Serov DA, Rebezov MB, Semenova AA, Lisitsyn AB. A mini review of antibacterial properties of ZnO nanoparticles. Front Phys. 2021;9:641481.
8. Ranjith Santhosh Kumar DS, Elango N, Selvaraju GD, Matthew PA, Palanisamy S, Cho H, Al Khattaf FS, Hatamleh AA, Roy AD. Mycosynthesis of zinc oxide nanoparticles coated with silver using *Ganoderma lucidum* (Curtis) P. Karst and its evaluation of *in vitro* antidiabetic and anticancer potential. J Nanomaterials 2022;3:1–9.
9. Kumar R, Umar A, Kumar G, Nalwa HS, Kumar A, Akhtar MS. Zinc oxide nanostructure-based dye-sensitized solar cells. J Mat Sci. 2017;52:4743–4795.
10. Leonardi SG. Two-dimensional zinc oxide nanostructures for gas sensor applications. Chemosensors 2017;5(2):17.
11. Huang R, Zhang S, Zhang W, Yang X. Progress of zinc oxide-based nanocomposites in the textile industry. IET Collaborat Intel Manufacture. 2021;3(3):281–289.
12. Kim KB, Kim YW, Lim SK, Roh TH, Bang DY, Choi SM, Lim DS, Kim YJ, Baek SH Kim MK, Seo HS. Risk assessment of zinc oxide, a cosmetic ingredient used as a UV filter of sunscreens. J Toxicol Environ Health Part B. 2017;20(3):155–182.
13. Makableh YF, Vasan R, Sarker JC, Nusir AI, Seal S, Manasreh MO. Enhancement of gas solar cell performance by using a ZnO sol–gel anti-reflection coating. Solar Energy Mat Solar Cells. 2014;123:178–182.
14. Hatamie A, Khan A, Golabi M, Turner AP, Beni V, Mak WC, Sadollahkhani A, Alnoor H, Zargar B, Bano S, Nur O. Zinc oxide nanostructure-modified textile and its application to biosensing, photocatalysis, and as antibacterial material. Langmuir. 2015; 31(39):10913–10921.
15. Xu J, Huang Y, Zhu S, Abbes N, Jing X, Zhang L. A review of the green synthesis of ZnO nanoparticles using plant extracts and their prospects for application in antibacterial textiles. J Eng Fibers Fabr. 2021;16.
16. Raj LFA, Jayalakshmy E. Biosynthesis and characterization of zinc oxide nanoparticles using root extract of *Zingiber officinale*. Orient J Chem. 2015;31(1):51–56.
17. Bhuyan T, Mishra K, Khanuja M, Prasad R, Varma A. Biosynthesis of zinc oxide nanoparticles from *Azadirachta indica* for antibacterial and photocatalytic applications. Mat Sci Semiconduct Process. 2015;32:55–61.
18. Wang XX, Wu L, Zhou P, Li C, Zhao LB, An W, Chen Y. Effect of ZnO nanoparticles on *Medicago sativa* at the germination stage. In Appl Mech Mat. 2014;665:583–586.
19. Anbuvannan M, Ramesh M, Viruthagiri G, Shanmugam N, Kannadasan N. *Anisochilus carnosus* leaf extract mediated synthesis of zinc oxide nanoparticles for antibacterial and photocatalytic activities. Mat Sci Semiconduct Process. 2015;39:621–628.
20. Sharma H, Kumar K, Choudhary C, Mishra PK, Vaidya B. Development and characterization of metal oxide nanoparticles for the delivery of anticancer drug. Artif Cells Nanomed Biotechnol. 2016:44(2):672–679.
21. Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R, Nandy P. Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. RSC Adv. 2015;5(7):4993–5003.
22. Mishra PK, Mishra H, Ekielski A, Talegaonkar S, Vaidya B. 2017. Zinc oxide nanoparticles: A promising nanomaterial for biomedical applications. Drug Disc Today. 2017;22(12):1825–1834.
23. Dangana RS, George RC, Agboola FK. The biosynthesis of zinc oxide nanoparticles using aqueous leaf extracts of *Cnidoscolus aconitifolius* and their biological activities. Green Chem Lett Rev. 2023;16:1.
24. Nagajyothi PC, Sang JC, In Jun Yang, TVM, Sreekanth KJK, Heung MS. Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using *Polygala tenuifolia* root extract. J Photochem Photobiol B: Biology. 2015;146:10–17.
25. Prasad S, Srivastava SK. Oxidative stress and cancer: Chemopreventive and therapeutic role of triphala. Antioxidants. 2020;9(1):72.

26. Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, Kumar S, Bhatti GK, Reddy PH. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Rad Biol Med. 2022.

27. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative stress and inflammation: What polyphenols can do for us? Oxidative Med Cell Longev. 2016.

28. Muthukumaran P, Abirami C. Antioxidant and free radical scavenging activity of *Azima tetracantha* Lam. Res J Pharm Dosage Forms Technol. 2013;5(6):315–319.

29. Okeleye BI, Nongogo V, Mkwetshana NT, Ndip RN. Polyphenolic content and *in vitro* antioxidant evaluation of the stem bark extract of *Peltophorum africanum* Sond (Fabaceae). Afr J Tradit Complement Altern Med. 2015;2(1):1–8.

30. Ma Z, Du B, Li J, Yang Y, Zhu F. An insight into anti-inflammatory activities and inflammation related diseases of anthocyanins: A review of both *in vivo* and *in vitro* investigations. Int J Mol Sci. 2021;22(20):11076.

31. Hussain T, Murtaza G, Yang H, Kalhoro MS, Kalhoro DH. Exploiting anti-inflammation effects of flavonoids in chronic inflammatory diseases. Curr Pharm Design. 2020;26(22):2610–2619.

32. Wang YH, Zeng KW. Natural products as a crucial source of anti-inflammatory drugs: Recent trends and advancements. Tradit Med Res. 2019;4(5):257–268.

33. Borquaye LS, Darko G, Laryea MK, Roberts V, Boateng R, Gasu EN. Anti-inflammatory activities of extracts from *Oliva* sp., *Patella rustica*, and *Littorina littorea* collected from Ghana's coastal shorelines. Cogent Biol. 2017;3(1):1364063.

34. Bai R, Yao C, Zhong Z, Ge J, Bai Z, Ye X, Xie T, Xie Y. Discovery of natural anti-inflammatory alkaloids: Potential leads for the drug discovery for the treatment of inflammation. Eur J Med Chem. 2021;213:113165.

35. Aswad M, Rayan M, Abu-Lafi S, Falah M, Raiyn J, Abdallah Z, Rayan A. Nature is the best source of anti-inflammatory drugs: Indexing natural products for their anti-inflammatory bioactivity. Inflamm Res. 2018;67:67–75.

36. Bora H, Kamle M, Mahato DK, Tiwari P, Kumar P. Citrus essential oils (CEOs) and their applications in food: An overview. Plants. 2020;9(3):357.

37. Mehl F, Marti G, Boccard J, Debrsu B, Merle P, Delort E. Differentiation of lemon essential oil based on volatile and non-volatile fractions with various analytical techniques: A metabolomics approach. Food Chem. 2014;143:325–335.

38. Oikeh EI, Omoregie ES, Oviasogie FE, Oriakhi K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Sci Nutr. 2016;4(1):103–109.

39. Mahato N, Sharma K, Sinha M, Cho MH. Citrus waste derived nutra-/pharmaceuticals for health benefits: Current trends and future perspectives. J Funct Foods. 2018:40:307–316.

40. Lv X, Zhao S, Ning Z, Zeng H, Shu Y, Tao O, Xiao C, Lu C, Liu Y. Citrus fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chem Century J. 2015;9(1):1–14.

41. Nasri M, Bedjou F, Porras D, Martínez-Flórez S. 2017. Antioxidant, anti-inflammatory, and analgesic activities of *Citrus reticulata* Blanco leaves extracts: An *in vivo* and *in vitro* study. Phytothérapie. 2017;2017:1–13.

42. Dejen KD, Zereffa EA, Murthy HA, Merga A. Synthesis of ZnO and ZnO/PVA nanocomposite using aqueous *Moringa oleifeira* leaf extract template: Antibacterial and electrochemical activities. Rev Adv Mat Sci. 2020;59(1):464–476.

43. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the Se-enriched *Bifidobacterium animalis*. Anaerobe. 2010;16:380–386.

44. Oriola AO, Miya GM, Singh M, Oyedeji AO. Flavonol glycosides from *Eugenia uniflora* leaves and their *in vitro* cytotoxicity, antioxidant and anti-inflammatory activities. Sci Pharm. 2023;91:42.

45. Kumar RS, Hemalatha S. *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. J Chem Pharm Res. 2011;3:259–267.

46. Chatterjee P, Chandra S, Dey P, Bhattacharya S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study. J Adv Pharm Technol Res. 2012;3:136–138.

47. Chinnasamy E, Indumathi N, Deepa K, Madhavan J, Senthil S. Growth and characterization of zinc acetate dihydrate formic acid single crystals. Res J Pharm Biol Chem. 2017;8(3):2030–2035.

48. Polash SH, Pyreddy S, Abraham AN, Mahasivam S, Bansal V, Varadi L, Bryant G, Shukla R. Impact of nucleic acid encapsulated MOF crystal phase on protein corona formation. Mater Adv. 2023;4:4761–4774.

49. Mohan S, Singh Y, Verma DK, Hasan SH. Synthesis of CuO nanoparticles through green route using Citrus limon juice and its application as nanosorbent for Cr(VI) remediation: Process optimization with RSM and ANN-GA based model. Proc Saf Environ Prot. 2015;96:156–166.

50. Naiel B, Fawzy M, Halmy MWA, Mahmoud AEID Green synthesis of zinc oxide nanoparticles using sea lavender (*Limonium pruinosum* L. Chaz.) extract: Characterization, evaluation of anti-skin cancer, antimicrobial and antioxidant potentials. Sci Rep. 2022;12:20370.

51. Fakhari S, Jamzada M, Fard HK. Green synthesis of zinc oxide nanoparticles: A comparison. Green Chem Lett Rev. 2019;12(1):19–24.

52. El-Belely EF, Farag MM, Said HA, Amin AS, Azab E, Gobouri AA, Fouda A. Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Arthrospira platensis* (Class: Cyanophyceae) and evaluation of their biomedical activities. Nanomaterials. 2021;11(1):95.

53. Kaningini GA, Azizi S, Nyoni H, Mudau FN, Mohale KC, Maaza M. Green synthesis and characterization of zinc oxide nanoparticles using bush tea (*Athrixia phylicoides* DC) natural extract: assessment of the synthesis process. F1000Res. 2021;10:1077.

54. Al-Naim AF, Sedky A, Afify N, Ibrahim SS. Structural, FTIR spectra and optical properties of pure and co-doped Zn1-x-yFexMyO ceramics with (M = Cu, Ni) for plastic deformation and optoelectronic applications. Applied Phys A. 2021;127:840.

55. Hamed R, Obeid R, Abu-Huwaij R. Plant mediated-green synthesis of zinc oxide nanoparticles: An insight into bio-medical applications. Nanotech Rev. 2023;12(1):20230112.

56. Adeyemi JO, Elemike EE, Onwudiwe DC. ZnO nanoparticles mediated by aqueous extracts of *Dovyalis caffra* fruits and the photocatalytic evaluations. Mater Res Express. 2019;6:125091.

57. Leta TB, Adeyemi JO, Fawole OA. Valorisation of pomegranate processing waste for the synthesis of ZnO nano-particles: antioxidant and antimicrobial properties against food pathogens. Mater Res Express. 2023;10:115401.

58. Adeyemi JO, Onwudiwe DC, Oyedeji AO. Biogenic synthesis of CuO, ZnO, and CuO–ZnO nanoparticles using leaf extracts of *Dovyalis caffra* and their biological properties. Molecules. 2022;27:3206.

59. Saini M, Mushtaq A, Yadav S, Rawat S, Rani N, Gupta K, Saini K. Green synthesis of rod shaped ZnO using extract of *Origanum majorana* leaf and investigation for antibacterial applications. IOP Conf Ser: Mater Sci Eng. 2022;1225:012048.

60. Khairnar N, Kwon H, Park S, Lee H, Park J. Tailoring the size and shape of ZnO nanoparticles for enhanced performance of OLED device. Nanomaterials. 2023;13:2816.

61. Iqbal Y, Mustafa MK, Wang J, Wang C. Orientation and actual growth mechanism of ZnO nanorods through hydrothermal method on gold seed layer. AIP Adv. 2021;11(12). https://doi.org/10.1063/5.0067231

62. Hembram KC, Kumar R, Kandha L, Parhi PK, Kundu CN, Bindhani BK. Therapeutic prospective of plant-induced silver nanoparticles: Application as antimicrobial and anticancer agent. Artif Cells Nanomed Biotechnol. 2018;46:S38–S51.

63. Diwan P, Sharma K, Puri NK. Green route synthesis and characterization of zinc oxide nanorods and its composite with PVA. Mat Today: Proc. 2022;62(6):3832–3836.

64. Albarakaty FM, Alzaban MI, Alharbi NK, Bagrwan FS, Abd El-Aziz ARM, Mahmoud MA. Zinc oxide nano-particles, biosynthesis, characterization and their potent photocatalytic degradation, and antioxidant activities. J King Saud Univ-Sci. 2023;35(1):102434.

65. Jamzad SFM, Fard HK. Green synthesis of zinc oxide nanoparticles: a comparison. Green Chem Lett Rev. 2019;12(1):19–24.

66. Khan AU, Malik N, Singh B, Ansari NH, Rehman M, Yadav A. Biosynthesis, and characterization of zinc oxide nanoparticles (ZnONPs) obtained from the extract of waste of strawberry. J Umm Al-Qura Univ Appl Sci. 2023;9:268–275.

67. Prashanth GK, Prashanth PA, Meghana R, Ananda S, Nagabhushana BM, Krishnaiah GM, Nagendra HG, Sathyananda HM, Mutthuraju M, Rajendra SC. Comparison of antimicrobial, antioxidant and anticancer activities of ZnO nanoparticles prepared by lemon juice and citric acid fueled solution combustion synthesis. BioNanoSci. 2019;9:799–812.

68. Shah NI, Jabeen N, Irum S, Ahmad KS, Tauseef I, Khan TF, Anwaar S, Shafique S, Haleem SK, Mehmood A, Hussain SZ. Environmentally benign and economical bio-fabrication of ZnO and Cr-doped ZnO nanoparticles using leaf extract of *Citrus reticulata* for biological activities. Materials Today Communicat. 2021;*27*:102383.

69. Madhuranga HDT, Samarakoon DNAW. *In vitro* anti-inflammatory egg albumin denaturation assay: An enhanced approach. J Nat Ayurv Med. 2023;7(3):1–6.

70. Yu Z, Li Q, Wang J, Yu Y, Wang Y, Zhou Q, Li P. Reactive oxygen species-related nanoparticle toxicity in the biomedical field. Nanoscale Res Lett. 2020;15(1):115.

71. Vasyukova IA, Zakharova OV, Chaika VV, Golokhvast KS, Gusev AA. Toxic effect of metal-based nanomaterials on representatives of marine ecosystems: A review. Nanotechnol Russia. 2021;16:138–154.

Cite this article: Diniso T, Oriola AO, Miya GM, Kuria SK, Hosu YS, Oyedeji OO, et al. Citrus Extract-Mediated Zinc Oxide Nanoparticles and Their Capacity to Attenuate Free Radicals and Inflammation. Pharmacogn J. 2024;16(6)Suppl: 1222-1233.