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Evaluation of Antimicrobial and Antioxidant Properties of Green-Synthesized ZNO Nanoparticles For 'Ibadan' Sweet Orange (*Citrus Sinensis*) Preservation

Abah S. E.[1,2*], Okibe F. G.[2,3], and Kyenge B. A.[2]

[1]Department of Chemistry, Faculty of Physical Sciences, Joseph Sarwuan Tarka University, P. M. B. 2373, Makurdi, 970101, Nigeria
[2]Department of Chemistry, Centre for Food Technology and Research (CEFTER), Benue State University, Makurdi, 970101, Nigeria
[3]Department of Chemistry, Faculty of Physical Sciences, College of Health Sciences, P. M. B., 145, Otukpo, 972261, Nigeria
** Email address: stiko88@gmail.com*
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ABSTRACT

The demand for sustainable and effective methods for food preservation has led to the exploration of nanotechnology, particularly in the synthesis of zinc oxide (ZnO) nanoparticles. The analysis of the phytochemical constituents of neem leaf extracts shows that saponins has high yield of 17.15 mg/100g, followed by flavonoids which yielded 7.72 mg/100g, while steroids were least extracted with a record of 2.81 mg/100g. Saponins retained 16.20 mg/100g, flavonoids retained 7.41 mg/100g while steroids retained 2.04 mg/100g after synthesis to ZnO nanoparticles. This study investigates the antimicrobial and antioxidant properties of ZnO nanoparticles synthesized using neem (*Azadirachta indica*) leaf extract, emphasizing their application in extending the shelf life of perishable foods. The green synthesis method ensures eco-friendliness, while the bioactive properties of the nanoparticles provide enhanced preservation efficacy. The results demonstrate that the ZnO nanoparticles effectively inhibit microbial growth and mitigate oxidative spoilage, making them a promising candidate for food preservation applications.

Key words: **Antimicrobial, Food preservation, Nanoparticles, ZnO, Neem leaf extracts**

Introduction

Food spoilage due to microbial contamination and oxidation is a significant global challenge, leading to substantial economic losses and food insecurity. Traditional preservation techniques often rely on chemical preservatives, which may pose health risks and environmental concerns (Cai, et al., 2007; Elmer, and White, 2016).

Zinc oxide (ZnO) nanoparticles have gained attention for their strong antimicrobial and antioxidant properties. The use of green synthesis methods, particularly employing plant extracts like neem leaf extract, offers an eco-friendly alternative to conventional nanoparticle production. Neem leaves are rich in bioactive compounds such as flavonoids, terpenoids, and phenolic acids, which not only aid in nanoparticle synthesis but also enhance their functional properties (Ajitha, et al., 2016)

Plants also include bioactive secondary metabolites such as aldehydes, ketones, terpenoids, polyphenols, tannins, polysaccharides, flavonoids, proteins, amines, and alkaloids, which serve as stabilizing and capping agents, and can reduce metal ions into metal nanoparticles, synthesizing required NPs with previously reported beneficial properties (Akhtar, et al 2011; Geetha, et al., 2016; Zhou, et al., 2023)

Ibadan sweet orange, a widely grown and consumed fruit, is highly perishable due to its high moisture content and susceptibility to spoilage (Kalpana, and Rajeswari, 2017). The preservation of fruits and vegetables is of utmost importance to ensure their availability and quality throughout the year. Traditional methods of preservation, such as refrigeration and chemical treatments, have limitations and may pose potential health risks (Zhang, et al., 2013; Ramesh, et al., 2015)

The short shelf life of Ibadan sweet orange poses a significant challenge to its storage and transportation, leading to considerable post-harvest losses. The existing preservation methods are either inefficient or present harmful effects on human health (Rajiv, et al., 2013). Nanotechnology-based approaches have shown great potential in enhancing the shelf life of perishable fruits by inhibiting microbial growth and delaying physiological processes associated with senescence (Zhou, et al., 2023). Therefore, there is a need to explore novel and effective approaches for extending the shelf life of Ibadan sweet orange

This study evaluates the antimicrobial and antioxidant efficacy of green-synthesized ZnO nanoparticles for potential applications in food preservation.

The scope of the study focused on the extraction, screening/quantification, characterization, and application of zinc oxide (ZnO) nanoparticles that have been synthesised using neem leaf extracts for shelf-life extension of Ibadan sweet oranges.

The neem leaf extracts were done using methanol as solvent, the extract was purified and concentrated to the required concentration. Half of the extract was used independently for the treatment of sweet orange (Ibadan sweet) fruits and for the synthesis of the zinc oxide nanoparticles. A total of sixty kilogrammes (60.0 kg) of orange fruits (20.0 kg each for control, methanol extracts, and methanol ZnO NPs) were treated to enhance the shelf-life of the Ibadan sweet oranges for 25 days.

Materials and Methods

Water bath, beakers, distilled water, methanol, filter paper, mortar and pestle, oven (incubator), airtight containers, fridge, weighing balance, Zinc oxide acetate, pipette, spatula, hot plate, volumetric flask, crucible, test tubes, burette.

Methods

Ibadan sweet orange fruits for the study were sampled, using the random sampling method, based on high demand by sellers and consumers of these oranges, to address the challenges of postharvest loses. The samples were further prepared and analysed for shelf-live studies using instrumental (FTIR, UV/Vis, SEM, XRD and GCMs), proximates, physiochemical, microbial and statistical analysis were done using the latest version of SPSS.

Synthesis of ZnO Nanoparticles

The neem leaves collected were washed thoroughly with tap water and later with distilled water. It was then air-dried at room temperature for a period of 10 days. The dried leaves were then powdered using a mortar and a pestle and then stored in an airtight container at room temperature until further use. One kilogramme of neem leaf powder was soaked for 48 hours in 3.0 litres of methanol for neem leaf methanol extract.

Then, the soaked samples were filtered using the Whatman no. 42 filter paper. The concentration of the filtrates was done by placing in a water bath at a temperature of 50 $^{\circ}$C and for a period of 3 days where evaporation of the extract was achieved.

In the synthesis of neem leaf extract mediated ZnO nanoparticles (ZnO NPs), 15.0 cm^3 of leaf extract was added to 2.159 g of Zn acetate dihydrate dissolved in 35.0 cm^3 of distilled water.

The reaction mixture was kept on a magnetic stirrer for 6 hours. After 6 hours, 2.0 M NaOH (4.0 g of NaOH pallet in 50.0 cm^3 of Milli-Q water) was added to the solution and it was placed in incubator at 60 $^{\circ}$C with magnetic stirring for overnight while mixture was centrifuged at 14, 000 rpm for 15 minutes.

Precipitate was subjected to washing with alcohol and distilled water three times each. Precipitate was dried in an incubator at 40 – 50 $^{\circ}$C and fine powdered was prepared with the help of ceramic pestle and mortar. The fine powder was then used for characterization.

Treatment of sweet orange fruits with neem leaf extract mediated ZnO NPs

The fresh sweet orange fruits of uniform size and colour were selected and washed under running tap water. The sweet orange fruits were distributed in 2 lots with the 3rd as control and studied at ambient conditions. Each of the lots were 20 kg, and the selected fresh fruits were dipped in 1000.0 cm^3 solution prepared for coating with neem leaf extracts as well as ZnO nanoparticles for 2 minutes; 90 ppm for methanol neem leaf extracts and 75 ppm for methanol neem leaf extract mediated zinc oxide nanoparticles (Ujah.et al., 2021).

These were then placed in their respective lots, and studied at 5 days intervals for a period of 25 days. The above concentrations for sweet orange preservation were arrived at through a comprehensive pre-laboratory analysis.

UV-Visible Analysis

Optical properties of ZnO NPs were determined by preparing an aqueous solution using UV-visible spectrophotometer (Perkin Elmer, model λ-35, Waltham, Massachusetts, USA). The optical property of zinc oxide nanoparticles was analysed by ultraviolet and visible absorption spectroscopy in the range of 200 – 800 nm as a function of amount of neem leaf extract and reaction time (Salam et al., 2014).

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The samples were scanned for the presence of functional groups using Agilent Technologies, Happ-Genzel FT-IR spectrophotometer. The molecular functional vibration of chemical groups present in the sample was recorded with Happ-Genzel FT-IR spectrophotometer, ranging from 4000-650 cm^{-1} (Ammar, et al., 2017).

Scanning electron microscopy (SEM) Analysis

The structural morphology of zinc oxide nanoparticles was examined and measured by Scanning Electron Microscopic (SEM) using TM-1000, Hitachi, Japan. An aliquot of each sample was fixed on a carbon-coated copper grid, and the film on the SEM grid was then dried by fixing it under a mercury lamp for 5 minutes. The instrument was equipped with an energy

dispersive spectrum (EDS) to ensure the presence of nanoparticles (Jamdagni, et al., 2018).

X-ray diffraction (XRD) analysis

Crystal structure of synthesized particles was determined using X-ray diffractometer (XRD-6000, Shimadzu, Japan) operated at 40 kV and 30 mA with Cu Kα radiation (wavelength - 0.15406 nm) (Elumalai, and Velmurugan, 2015). The crystalline domain size was estimated from the width of the XRD peaks, using the Debye-Scherrer formula, D ¼ K λ/β cos θ; where, D is the average crystallite domain size perpendicular to the reflecting planes, K is the crystallite shape factor (approximated as 0.94), λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle (Vanathi et al., 2014).

Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was carried out using GC-MSQP2010 PLUS SHIMADZU, Japan machine. Helium was used as a carrier gas at a flow rate of 1.5 mL/min and at 250 °C injector temperature. Ion source temperature was maintained at 230 °C with purge flow of 3.0 mL/min. The oven temperature was programmed initially at 80 °C for 1 minute, then programmed to increase to 200 °C at a rate of 10 °C/minute, ending with a 5 minutes isothermal of 280 °C. The samples were analyzed and data evaluated using Total Ion Count (TIC) for compound identification (Chukwuebuka, and Chinenye, 2015).

Antimicrobial Activity

Exactly 1.0 g of the sample was weighed and incorporated into the first test tube containing 1.0 cm^3 of normal saline and labeled stock, with the use of syringe 1.0 cm^3 was drawn from stock and transferred to a second test tube and labeled A (10^{-1}), this serial fold dilution was performed to give B (10^{-2} cm^3), C (10^{-3} cm^3), D (10^{-4} cm^3) and E (10^{-5} cm^3). From the test tubes 10^{-2}, 10^{-3}, 0.1 cm^3 of the diluents were drawn and inoculated into labeled petri dishes respectively (Anbuvannan et al., 2015a).

The growth media (agar and broth) was prepared and sterilized in an auto clave (121 ºC for 15 minutes). The growth media was cooled to about 50 ˚C in a water bath and poured into the petri dishes for bacterial growth. It was allowed to solidify and incubated inverted at 35 °C for 24 to 48 hours. Chloramphenicol (0.1 m^3) was added to the SDA at 50 °C to suppress bacterial growth and allow growth of fungi only.

The SDA was poured into petri dishes for fungi growth. It was incubated at 27 °C for 5-7 days. The colonies of both bacterial and fungal growth was counted and recorded. The number of colonies was multiplied by the number of times the original cm^3 of bacteria was diluted divided by the volume of the culture plate. This is formulated as; Colony forming unit cfu/mL = (no. of colonies × dilution factor)/volume of culture plate (Anbuvannan et al., 2015a).

Microbial analysis of the extracts (Agar Well Diffusion Method)

Broth media were measured and dissolved in appropriate volume of distilled water, following the manufacturer's guideline and were sterilized by autoclaving. Pour plate technique was used; about 1.0 m^3 of the standardized inoculum was mixed with the medium in a sterile container to ensure that the test organisms were evenly distributed and poured into sterile petri dishes and allowed to gel. Each plate contained equal volume of the media. The antibacterial activity of the plant extract were determined in accordance with standard agar-well diffusion method, (Dhandapani, et al., 2012; Ammar, et al., 2017).

Determination of zones of inhibition

A cork borer (0.6 cm) was used to bore wells on the agar medium after which 0.1 m^3 of the extract solution were dispensed into the wells. The plates were incubated at 37 °C for bacterial activity, the plates were observed for zones of inhibition after 24 hours. This implied that any clear zone of inhibition observed was due to the activity of the extract. There was a control to test the activity of the solvent used to dissolve the extract to ensure that the activity was not due to action of solvent on the test organisms (Zakpaa, et al., 2010).

Plates were read by measuring observed clear zones (area without growth) of inhibition around the wells containing the extract. Measuring rule in millimetre were used to take the measurement from the edge of the well to the end of the clear zone of inhibition. The estimation of MIC (Minimum Inhibitory Concentration**)** and MBC (Maximum Bactericidal Concentration) of the crude extracts were carried out by standard method, (Arruda, et al., 2015), were the tubes without bacterial growth were cultured in their appropriate agar and incubated appropriately to check for those that will revive and develop colonies. Those that did not revive and grow were recorded as bactericidal (Chukwuebuka, and Chinenye, 2015).

Antioxidant Activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

Application in Food Preservation

Freshly harvested Ibadan sweet orange fruits were coated with a ZnO nanoparticle solution and stored under ambient conditions. The microbial load and oxidative changes were monitored over a period of 25 days.

Results and Discussion

Neem leaf extract

The phytochemical constituents present in the neem leaves was carried out and results are presented in tables 1 – 2.

Table 1: The qualitative analysis for phytochemical constituents present in the neem leaves

Phytochemicals	Methanol extract
Saponins	+++
Phenols	++
Tannins	+
Flavonoids	+++
Alkaloids	++
Steroids	++
Terpenoids	++
Cardiac glycosides	+

+++: Most present; ++: Moderately present; +: Least present; –: Absent

Table 2: The quantitative analysis for phytochemical constituents present in the neem leaves and in the ZnO-neem nanoparticles

Phytochemicals	Methanolic extract (mg/100g)	Methanol ZnO-neem NP (mg/100g)
Saponins	17.15 ± 0.37^{a}	16.20 ± 0.81^{a}
Phenols	3.28 ± 0.11^{f}	3.15 ± 0.08^{e}
Tannins	5.37 ± 0.13^{d}	3.22 ± 0.10^{e}
Flavonoids	7.72 ± 0.18^{b}	7.41 ± 0.04^{b}
Alkaloids	6.93 ± 0.40^{c}	6.22 ± 0.66^{c}
Steroids	2.81 ± 0.15^{gh}	2.78 ± 0.22^{f}
Terpenoids	4.75 ± 6.63^{e}	4.05 ± 6.63^{d}
Cardiac glycosides	2.92 ± 0.00^{hg}	2.92 ± 0.00^{f}
P-Value	**0.02**	**0.00**

Characterization of ZnO Nanoparticles

The synthesized ZnO nanoparticles were monitored by UV-Vis spectroscopy with a characteristic peak at ~370 nm. XRD analysis revealed a hexagonal wurtzite structure, and SEM images showed nanoparticles with an average size of 20-50 nm. FTIR spectra indicated the presence of bioactive compounds from neem, enhancing nanoparticle stability.

Table 3: Functional groups present in ZnO NPs blends of neem leaf extracts

S/ N	Experimental frequencies (cm^{-1})	Samples		Bonds	Functional groups
		MeOH neem leaf extract	MeOH-ZnO NP		
1	3826.35	3826.35	3774.68	O - H Stretch vibrations	Alcohols
	3774.68	3772.89	3772.89		
	3772.89				
	3772.89				
2	3572.29	3572.29	3572.29	O - H Stretch vibrations	Phenols

3	3387.11 3340.80 3309.96 3363.97 3371.68	3387.11 3363.97 3340.80	3387.11 3309.96 3371.68	O - H Stretch, H-bonded	Alcohols and Phenols, flavonoids
4	2885.60	2885.60	2885.60	O-H Stretch	Carboxylic Acids
5	2978.18 2970.48	2978.18 2970.48	2978.18 2970.48	C-H Stretch	Alkanes
6	1782.29 1658.84 1685.84 1643.41	1782.29 1658.84 1685.84	1658.84 1685.84 1643.41	C=O Stretch (Carbonyls)	Aldehydes, Saturated Aliphatics, Carboxylic Acid, Esters
7	1419.66	1419.66	1419.66	C - C Stretch (in ring)	Aromatics
8	1381.08	1381.08	1381.08	C-H Rock	Alkanes
9	1273.05	1273.05	1273.05	C-N Stretch	Aromatic amines
10	1087.89	1087.89	1087.89	C-O Stretch	Alcohols, Carboxylic acid, Esters, Ethers
11	879.57	879.57	879.57	=C– H Bend	Alkenes

Keynotes; *nHex-ZnO NP; nHexane-neem leaf extract-ZnO nanoparticles, MeOH-ZnO NP methanol-neem leaf extract-ZnO nanoparticles.*

Table 4: Composition of major bio-active components present in methanol extracts of neem leaf using GC-MS

Peak No	Retention Time(s)	Name of the compound	Molecular Weight	Molecular Formular	Class of compound	Peak area (%)
1	9.454	Hexadecanoic acid, methyl ester	270.45	$C_{17}H_{34}O_2$	Fatty acids methyl esters	0.58
2	11.932	Methyl 10-Trans,12-cis Octadecadienoate acid	310.47	$C_{19}H_{34}O_3$	Ester	1.87
3	14.276	11-Octadecenoic acid, methyl ester	296.49	$C_{19}H_{36}O$	Ester	1.02
4	16.467	9,17-Octadecadienal	264.45	$C_{18}H_{32}O$	Fatty acid	1.24
5	19.451	n-Propyl-11 octadecenoate	282.47	$C_{21}H_{40}O_2$	Alkanal	2.13
6	23.636	Oleic Acid	282.47	$C_{18}H_{34}O_2$	Fatty acid	1.74
7	26.783	1,19-Eicosadiene	278.50	$C_{20}H_{38}$	Alkene	2.30

8	29.172	9-Octadecenoic acid (Z)-2,3 dihydroxypropyl ester	356.54	$C_{21}H_{40}O$	Fatty acid	1.77
9	33.436	2-Methyl-Z,Z-3,13-octadecadienol	266.46	$C_{19}H_{36}O$	Fatty acids methyl esters	2.11
10	36.492	Erucic acid	338.57	$C_{22}H_{42}O_2$		2.32
11	39.332	E-11-Hexadecenal	238.41	$C_{16}H_{30}O$	Alkanal	2.56

Table 5: Composition of major active bio-components present in ZnO nanoparticles of methanol neem leaf extracts using GC-MS

Peak No	Retention time (s)	Name of the compound	Molecular Weight	Molecular Formular	Class of compound	Peak area %
1	6.254	7-Octen-2-one	270.45	$C_{17}H_{34}O_2$	Ketone	0.22
2	6.652	1,15-Pentadecanediol	244.41	$C_{15}H_{32}O$	Ester	1.26
3	6.835	9-Octadecenal	266.46	$C_{18}H_{34}O$	Alkanal	1.64
4	7.213	cis-11-Hexadecenal	238.41	$C_{16}H_{30}O$	Alkanal	2.01
5	7.425	E-2-Octadecadecen-1-ol	268.48	$C_{18}H_{36}O$	Alcohol	1.22
6	7.681	1-Cyclohexylnonene	208.38	$C_{15}H_{38}$	Ketone	0.43
7	7.922	13-Octadecenal	266.46	$C_{18}H_{34}O$	Alkanal	2.11
8	8.326	9-Octadecenoic(Z)-, 2,3-dihydroxypropyl ester Acid	356.54	$C_{21}H_{40}O$	Fatty acid	1.67
9	8.543	2-Methyl-Z,Z-3,13-octadecadienol	266.46	$C_{19}H_{36}O$	Fatty acids methyl esters	0.26
10	8.701	Oleic Acid	282.47	$C_{21}H_{40}O_2$	Fatty acid	2.11
11	8.923	cis-Vaccenic acid	282.46	$C_{18}H_{34}O_2$	Fatty acid	1.45

Scanning Electron Microscopy (SEM) analysis

The SEM image of the zinc oxide nanoparticles (ZnO NPs) mediated with neem leaf extracts of selected solvents so as to analyze the morphology and size. The results are presented in Figures 1 and 2.

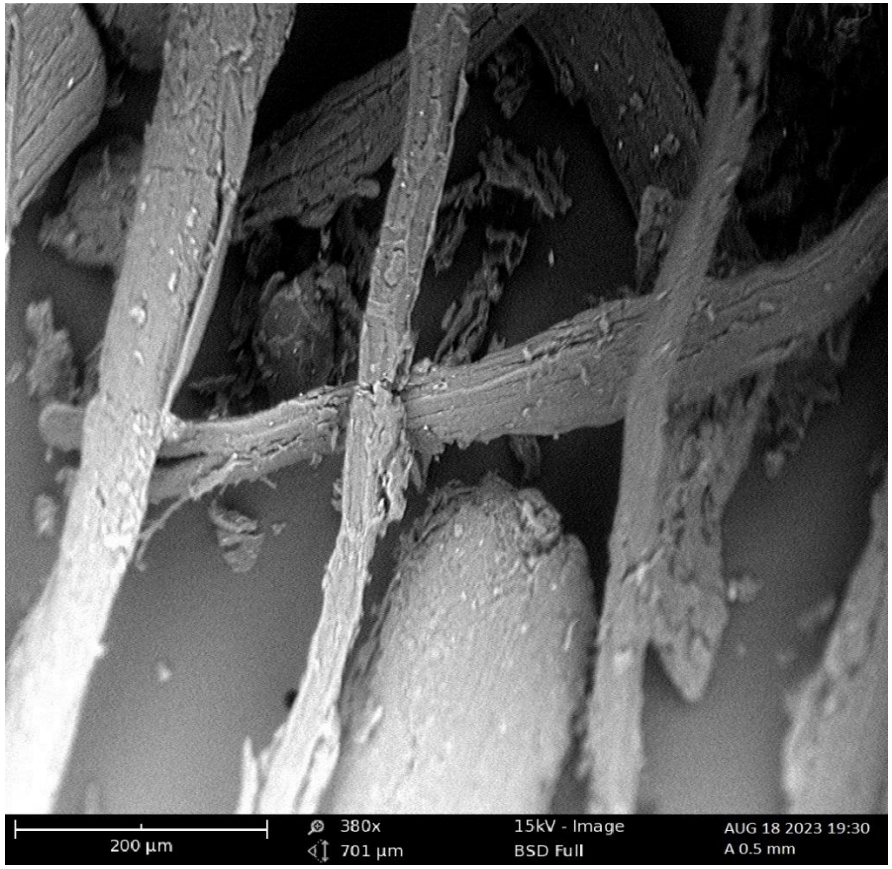

Figure 1: SEM pictures of ZnO-methanol neem nanoparticles at 380× magnification

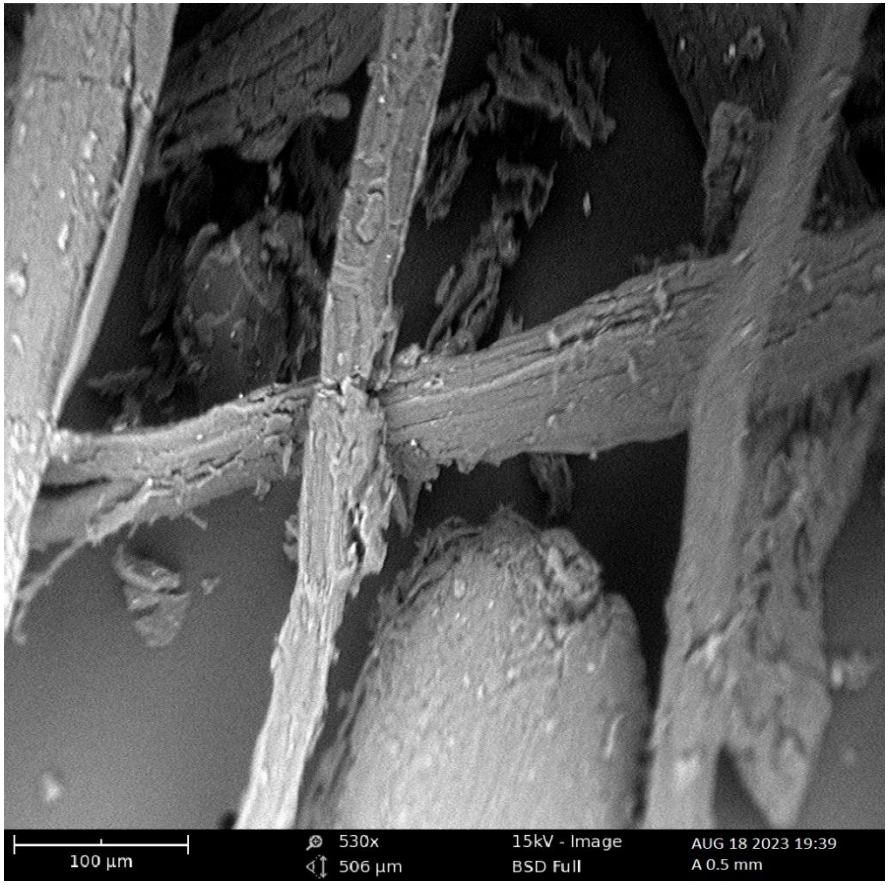

Figure 2: SEM pictures of ZnO-methanol neem nanoparticles at 530× magnification

Antimicrobial Activity

The nanoparticles exhibited strong antimicrobial activity, with significant zones of inhibition against *E. coli* (16 mm), *S. aureus* (18 mm), *and P. aeruginosa* (15 mm). The activity is attributed to the nanoparticles' ability to generate reactive oxygen species (ROS), disrupting microbial cell walls and membranes Chukwuebuka, and Chinenye, 2015).

The results for the microbial analysis of both the control and the treated samples in the study period are presented in Tables 6, 7 and 8.

Table 6: Result for microbial count

Days	Control $\times 10^3$cfu/g	Methanol– neem extract $\times 10^3$cfu/g	Methanol–ZnO NP $\times 10^3$cfu/g
0	NG	NG	NG
5	NG	NG	NG
10	15	NG	NG
15	188	76	32
20	242	102	85
25	388	326	280

Key: NG = No growth

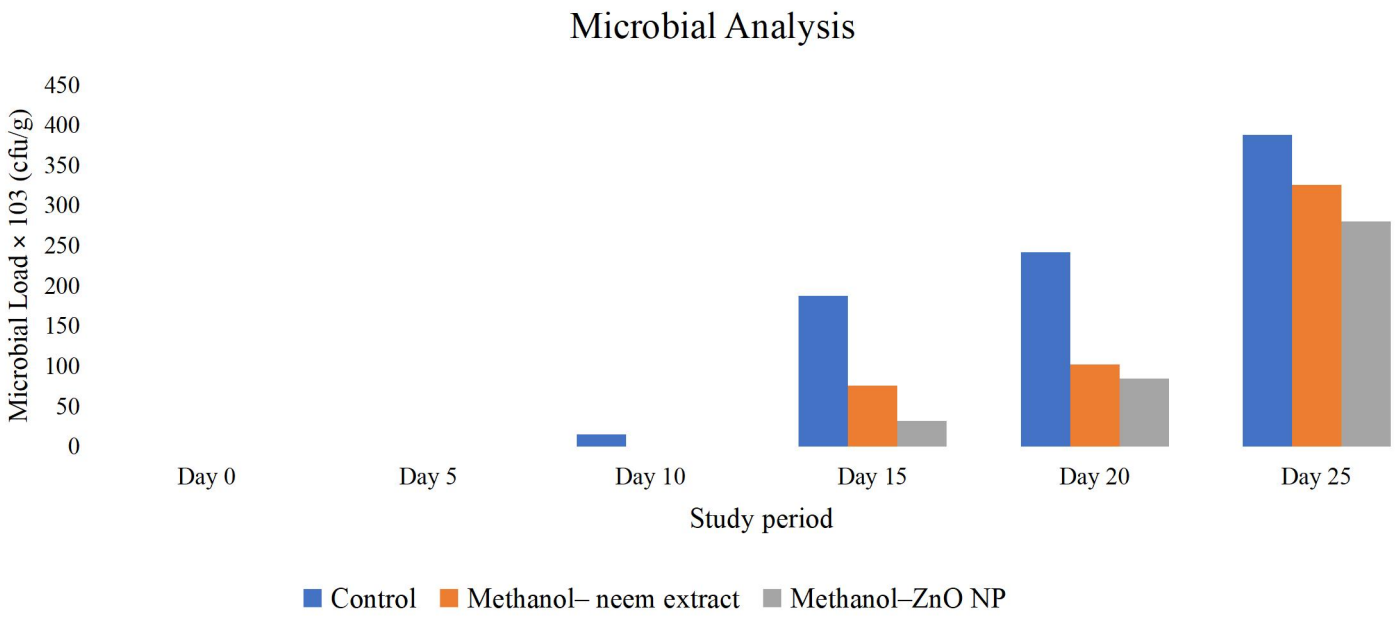

Figure 3: Microbial analysis of the selected samples

Table 7: Antimicrobial susceptibility test – Zones of inhibition (mm)

Microbial specie	Methanol-neem leaf extract	Methanol-neem leaf extract-ZnO NPs
Staphylococcus	14.50±2.29^{bc}	17.00 ± 1.4^{a}
Bacillus	11.50 ± 0.71^{ef}	14.00 ± 00^{d}
Klebsiella	15.00 ± 1.41^{a}	17.00 ± 1.4^{a}
Pseudomonas	10.00±0.00^{g}	15.50. ± 0.71^{cd}
Proteus	11.50±2.12^{ab}	14.00 ± 1.41^{cd}
Saccharomyces	13.00±1.41^{cd}	16.00 ± 0.00^{b}
Mucor	14.00 ± 1.41^{bc}	17.00 ± 1.41^{a}
Aspergillus	12.60 ± 0.00^{d}	12.50 ± 0.71^{def}
Fusarium	11.00 ± 1.41^{ef}	13.50 ± 0.71^{cde}
P-value	**0.09**	**0.08**

Values are mean ± standard deviation of triplicate determinations. Means within the sample column bearing different superscripts are significantly different ($p \leq 0.05$).

Table 8: Microbial load of preserved orange fruits

Samples	Total Viable count (TVC) × 10^3cfu/g	Total Coliform Count (TCC)×10^2cfu/g	Total Fungi Count (TFC)×10^3cfu/g
Control	TNTC	9.08± 0.13^{a}	5.02± 0.03^{a}
Methanol extract	51.22± 0.34^{b}	4.01± 0.21^{c}	ND
Methanol ZnO NP	13.25± 0.33^{d}	1.07± 0.01^{e}	ND
P-Value.	**0.34**	**0.02**	**NS**

ICMSF guidelines: Stipulated values of < 10^5 for bacteria and 10^3- 10^4 for fungi

Values < 10^5 cfu/g = Satisfactory, 10^5 to <10^6 cfu/g = Borderline, ≥10^6 cfu/g = Unsatisfactory. cfu/g: Colony-forming units per gram, TNTC: too numerous to count, ND: not detected

Sample means in the same column with different superscript are significantly different at (p>0.05)

Antioxidant Activity

The DPPH assay showed a high radical scavenging activity (80 % at 100 µg/mL), indicating the potential of the nanoparticles to prevent oxidative spoilage. The antioxidant activity is enhanced by phytochemicals from neem extract, which act synergistically with ZnO nanoparticles.

Table 9: Mean scores of sensory evaluation of selected oranges using the 9-point hedonic scale

Attributes	**5**			**10**			**15**			**20**			**25**		
	Ctrl	**MetOH**	**MetOH NPs**	**Ctrl**	**MetOH**	**MetOH NPs**	**Ctrl**	**MetOH**	**MetOH NPs**	**Ctrl**	**MetOH**	**MetOH NPs**	**Ctrl**	**MetOH**	**MetOH NPs**
Overall liking	8.43±0.05^{a}	8.61±0.05^{c}	8.93±0.05^{a}	6.62±0.11^{a}	7.90±0.05^{b}	8.73±0.05^{b}	0.00	7.43±0.05^{b}	8.20±0.05^{a}	0.00	6.71±0.08^{a}	7.01±0.08^{c}	0.00	0.00	6.71±0.08^{a}
Taste	8.22±0.32^{c}	8.65±0.13^{b}	8.76±0.30^{d}	6.21±0.26^{b}	7.70±0.13^{c}	8.61±0.13^{c}	0.00	6.78±0.31^{dc}	8.15±0.31^{b}	0.00	6.25±0.01^{b}	7.25±0.01^{a}	0.00	0.00	5.21±0.01^{d}
Colour	8.18±0.25^{cd}	8.28±0.25^{d}	8.80±0.25^{c}	5.42±0.07^{d}	7.18±0.25^{e}	7.84±0.25^{d}	0.00	6.18±0.25^{f}	6.78±0.25^{f}	0.00	5.83±0.01^{e}	6.53±0.01^{e}	0.00	0.00	5.32±0.01^{c}
Aroma	8.25±0.13^{b}	8.75±0.13^{a}	8.87±0.13^{b}	5.46±0.02^{c}	8.35±0.13^{a}	8.78±0.13^{a}	0.00	7.65±0.13^{a}	7.88±0.13^{c}	0.00	6.10±0.05^{d}	7.10±0.05^{b}	0.00	0.00	6.10±0.05^{b}
Mouthfeel	7.47±0.02^{f}	7.67±0.02^{f}	7.98±0.02^{f}	4.40±0.42^{f}	7.27±0.02^{d}	7.64±0.02^{e}	0.00	6.79±0.02^{cd}	7.27±0.02^{d}	0.00	6.22±0.03^{c}	6.82±0.03^{d}	0.00	0.00	5.12±0.03^{e}
Aftertaste	7.90±0.34^{e}	7.94±0.34^{e}	8.46±0.34^{e}	4.81±0.08^{e}	7.14±0.34^{f}	7.84±0.34^{d}	0.00	6.34±0.34^{e}	7.14±0.34^{e}	0.00	5.14±0.01^{f}	6.14±0.01^{f}	0.00	0.00	4.10±0.03^{f}
P-Value	**NS**	**0.03**	**0.03**	**0.02**	**NS**	**0.03**	**0.00**	**0.02**	**NS**	**0.00**	**0.03**	**0.02**	**0.03**	**0.00**	**NS**

Values represent means of triplicate values ± s∂ (standard deviation)

Sample means with the same superscripts in a Column are not significantly different

Fresh orange fruit (Ibandan sweet orange) Preservation

Leaf extracts of *Azadiracta indica,* indicated that the plant is a rich sources of bioactive compounds such as tannins, saponins alkaloids, glycosides, flavonoid balsams, steroids, saponin glycocides and cardial glycocides (Ammar, et al., 2017). Similar bioactive compounds were also earlier observed in the seed, back and leaves of *Azadiracta indica* (Etebu, and Nwauzoma, 2014). The most important of these plants bioactive chemical constituents (That is, phytochemicals or infochemicals) are alkaloids; tannins, flavonoids, anthraquinonnes, and phenolic compounds (Patil, and Taranath, 2016).

The results of these studies provide empirical basis for the preservation of high quality sweet orange with ZnO nanoparticles blends with neem-leaf extracts and neem-leaf extracts. The moisture analysis conducted, clearly demonstrated that all the treated sweet oranges had the recommended moisture content, which will enhance the stability of the fruits (Patharka, et al., 2017).

The sweet oranges had high vitamins C and A contents, all the sample categories were observed to be, relatively, good sources of vitamins A and ascorbic acid as they can provide the recommended dietary allowance for daily healthy living (Patharka, et al., 2017). The overall vitamin E contents for the sweet orange analyzed in this study was low which has further showed that no single plant food could provide all the required nutrients in recommended amounts and so there is the need to consume these fruits in combination with other dietary sources of nutrients to ensure an adequate nutritional status, thus reducing the problem of micronutrient deficiencies (Patel, et al., 2015).

The extracted neem leaves had significant amounts of phytochemicals present in them. The Phytochemicals present in these extracts and its synthesised products had great inhibitive potentials on the various rot processes in sweet orange during storage. The preserved sweet oranges generally had low microbial count, which could be explained as a result of the presence of the preservatives in line with the International Commission for Microbiological Specification for Foods (ICMSF) guidelines, the stored oranges were within satisfactory levels ($<10^5$ cfu/g) and are safe for consumption.

Results from sensory evaluation revealed that the judges had higher preference in aroma and mouth feel of the treated sweet oranges than they had for the control (untreated sweet oranges). This is because the judges were able to perceive the residual flavor and freshness of the treated sweet oranges unlike the control that does not have a strong flavour of freshness.

This finding suggests that fresh sweet oranges preserved with the extracts and blends of ZnO NPs might be preferred and acceptable over the naturally stored oranges.

FTIR spectra of ZnO nanoparticles

Infrared studies were carried out in order to ascertain the purity and nature of the metal nanoparticles (Tables 3 and 4). Metal oxides generally give absorption bands in fingerprint region of below 1000 cm^{-1} arising from inter-atomic vibrations. The peak observed at 3453.30 and 1118.15 cm^{-1} may be due to O-H stretching and deformation, respectively assigned to the water adsorption on the metal surface. The peaks at 1624.00, 620.93 cm^{-1} are corresponding to Zn-O stretching and deformation vibration, respectively (Rajakumar, et al., 2017). The metal-oxygen frequencies observed for the respective metal oxides are in accordance with literature values, confirming the formation of bio-mediated zinc oxide nanoparticles.

The fresh sweet samples before and after blending with the neem leaf extracts and its synthesised ZnO NP products were found to contain C-H stretch of alkanes at 2360.95 cm^{-1} and halogen compounds (Table 3), this informs that the neem leaf extracts and its ZnO products had no effects on the nutritional composition of the sweet oranges studied. No significant changes were recorded in the functional groups after blending with neem leaf extracts and ZnO NPs notably the presence of hydroxyl group at around 3855 - 3700 cm^{-1}, C-H stretch of alkanes which were located at 3000 – 2851 cm^{-1} and alkyl halides.

The occurrence of the active compounds revealed in the spectra obtained for all categories of samples after blending with neem leaf extracts and its mediated products. SEM images at 380 and 530 µm magnifications of the ZnO nanoparticles synthesised with methanol extract for the neem leaves revealed its crystalline morphology (Figures 1 and 2). The performance of the ZnO nanoparticles synthesised with methanolic neem extract was excellent.

GC-MS results (Tables 4 and 5) showed compounds that were common to all the extracts as oleic acid, and 2-Methyl-Z, Z-3, 13-octadecadienol and 9, 12-Octadecenoic acid (Z, Z) found to be common amongst the samples. We believe from that the biochemical changes that occurred in the FTIR spectra of the stored sweet orange fruits were as a result of the presence of these phytochemicals in the preservatives.

In this study, zinc oxide nanoparticles were successfully biosynthesized using plant extracts of neem leaves (*Azadirachta indica*), from a polar solvent. The resultant nanoparticles were characterized using UV – Visible spectroscopy, FTIR, XRD, SEM and GC-MS. The antibacterial activity of the ZnONPs were examined on these tough microbes (*Staphylococcus, Bacillus, Klebsiella, Pseudomonas, Proteus, Saccharomyces, Mucor, Aspergillus and Fusarium*) that causes detoriation on farm produce and it was discovered that zinc oxide synthesized by neem leaf extracts and the neem leaf extracts itself had excellent inhibitive potency (Tables 6-8) and Figure 3.

Orange fruits coated with ZnO nanoparticles showed a 65 % reduction in microbial load, while those immersed in methanolic neem leaf extract showed 50 % microbial reduction compared to untreated samples. The nanoparticles also delayed the oxidation of lipids and vitamins, maintaining the quality and shelf life of the food for up to 20 days under ambient conditions.

The statistical tests of the obtained research data also probed the findings to a common conclusion that, the sweet orange fruits were preserved by the extracts of neem leaves along side their ZnO NPs during the shelf-life extension studies, up to 20 days of storage.

Conclusion

Green-synthesized ZnO nanoparticles using methanolic neem leaf extract and methanolic neem leaf extract exhibit remarkable antimicrobial and antioxidant properties, making them a sustainable solution for food preservation. By inhibiting microbial growth and oxidative spoilage, these nanoparticles can effectively extend the shelf life of perishable foods. Future studies should focus on optimizing the application methods and scaling up the production for commercial use.

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