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Synthesis and Characterization of ZNO Nanoparticles Using Neem Leaf Extract as a Green Mediator

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ABSTRACT

The need for environmentally sustainable methods has prompted researchers to explore green chemistry approaches in nanotechnology. Zinc oxide (ZnO) nanoparticles, known for their exceptional antimicrobial, optical, and catalytic properties, have diverse applications in fields ranging from medicine to food preservation. This journal outlines the synthesis and characterization of ZnO nanoparticles using neem (*Azadirachta indica*) leaf extract as a green mediator. The bioactive compounds in neem serve as reducing and stabilizing agents, facilitating an eco-friendly and cost-effective process. The neem leaf n-Hexane extract was characterised the concentration of these phytochemicals was 6.20 mg/100g in saponins and the trend continued through to the ZnO neem-leaf nanoparticles synthesised. The zinc oxide synthesized by neem leaf extracts and the neem leaf extracts itself had excellent microbial inhibitive potency. The nanoparticles were characterized using advanced techniques to confirm their properties, providing insights into their potential applications.

Key words: ZnO, nanoparticles, Neem leaves, Green mediator, antimicrobial

Introduction

The demand for sustainable practices in the synthesis of nanoparticles has prompted researchers to explore biosynthetic methods using natural materials (Mirzaei and Darroudi., 2017). This article focuses on the green synthesis of zinc oxide (ZnO) nanoparticles using neem leaf extract (*Azadirachta indica*) and the subsequent characterization of these nanoparticles (Ingale and Chaudhari., 2013). The process capitalizes on the phytochemical constituents of neem, which act as reducing and stabilizing agents, providing an eco-friendly alternative to conventional chemical synthesis methods (Agarwal et al., 2017).

Neem (*Azadirachta indica*) is a versatile plant with various bioactive compounds, including alkaloids, glycosides, and terpenoids (Ramesh et al., 2015). The neem leaf extract has been widely used in traditional Indian medicine for its antiseptic, antibacterial, and antifungal properties. Zinc oxide (ZnO) nanoparticles have been extensively used in various applications such as electronics, cosmetics, and biomedicine due to their unique optical, electrical, and chemical properties. However, traditional chemical synthesis methods for ZnO nanoparticles often involve the use of toxic and hazardous chemicals, which poses a significant environmental and health risk (Ujah et al., 2021). In contrast, green synthesis methods using natural extracts have gained attention in recent years due to their potential to produce nanoparticles with improved properties and minimal environmental impact. In this study, we explored the potential of neem leaf extract as a green mediator for the synthesis of ZnO nanoparticles (Jamdagni, and Rana, 2018).

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 antimicrobial examination, to ascertain its ability of preserving food to be a substitute for the synthetic preservatives.

Materials and Methods

Neem leaves were collected, washed, air dried and pounded into fine powder. An extract was prepared by soaking the powder in n-Hexane at (1:3 w/v), followed by filtration. The Figure 1 below outlines the extraction of neem leaves. The extracts were further characterized using GCMS and FTIR for phytochemical and functional biomolecules in the extracts.

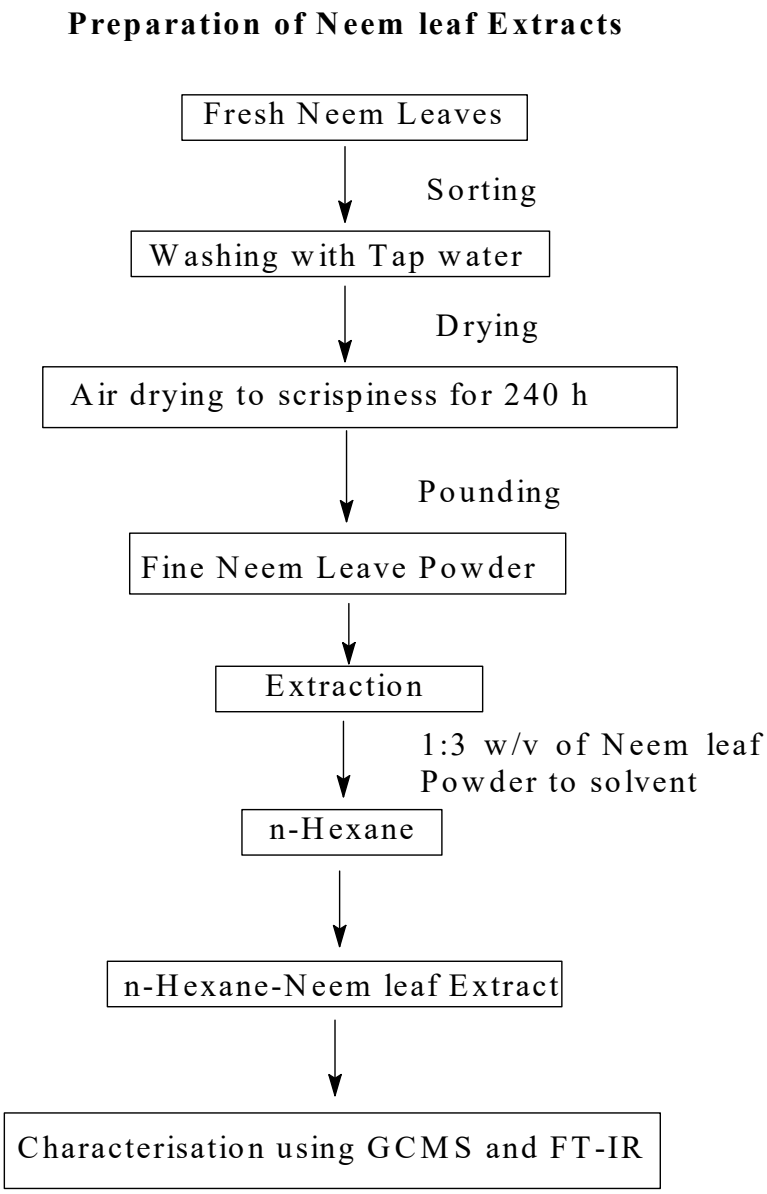


Figure 1: Preparation and extraction of neem leaves

Synthesis of ZnO NPs mediated with neem leaf extracts

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 which dissolved in 35.0 cm^3 of distilled water (Makarov et al., 2014). The reaction mixture was kept on a magnetic stirrer for 6 hours (Figure 2).

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.

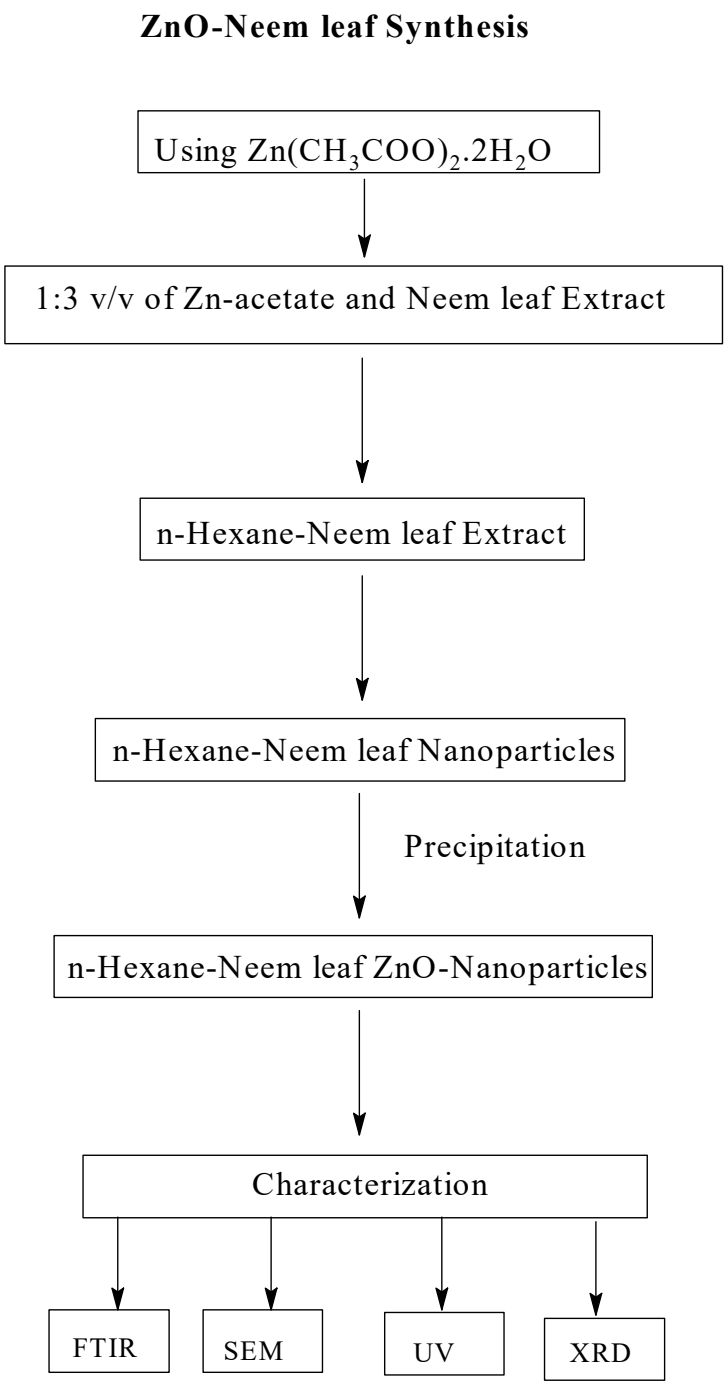


Figure 2: Zinc oxide nanoparticle mediated with neem leaf extract

Characterization techniques employed included UV-Vis spectroscopy to assess the optical properties, Fourier-transform infrared spectroscopy (FTIR) to identify functional groups, X-ray diffraction (XRD) to determine crystalline structure, and scanning electron microscopy (SEM) to analyze morphology (Sharma, et al., 2016).

Results and Discussion

Results

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	**n-Hexane 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	**n-Hexane extract (mg/100g)**	**n-Hexane ZnO-neem NP (mg/100g)**
Saponins	0.01 ± 0.01^{e}	0.00 ± 0.00^{e}
Phenols	0.03 ± 0.00^{e}	0.00 ± 0.00^{e}
Tannins	0.07 ± 0.00^{e}	0.02 ± 0.00^{e}
Flavonoids	4.93 ± 0.12^{b}	3.63 ± 0.13^{a}
Alkaloids	8.65 ± 0.25^{a}	6.40 ± 0.62^{b}
Steroids	3.33 ± 0.02^{d}	2.04 ± 0.06^{c}
Terpenoids	5.08 ± 0.01^{c}	3.02 ± 0.01^{c}
Cardiac glycosides	0.02 ± 0.00^{e}	0.00 ± 0.00^{e}
P-Value	**0.06**	**0.00**

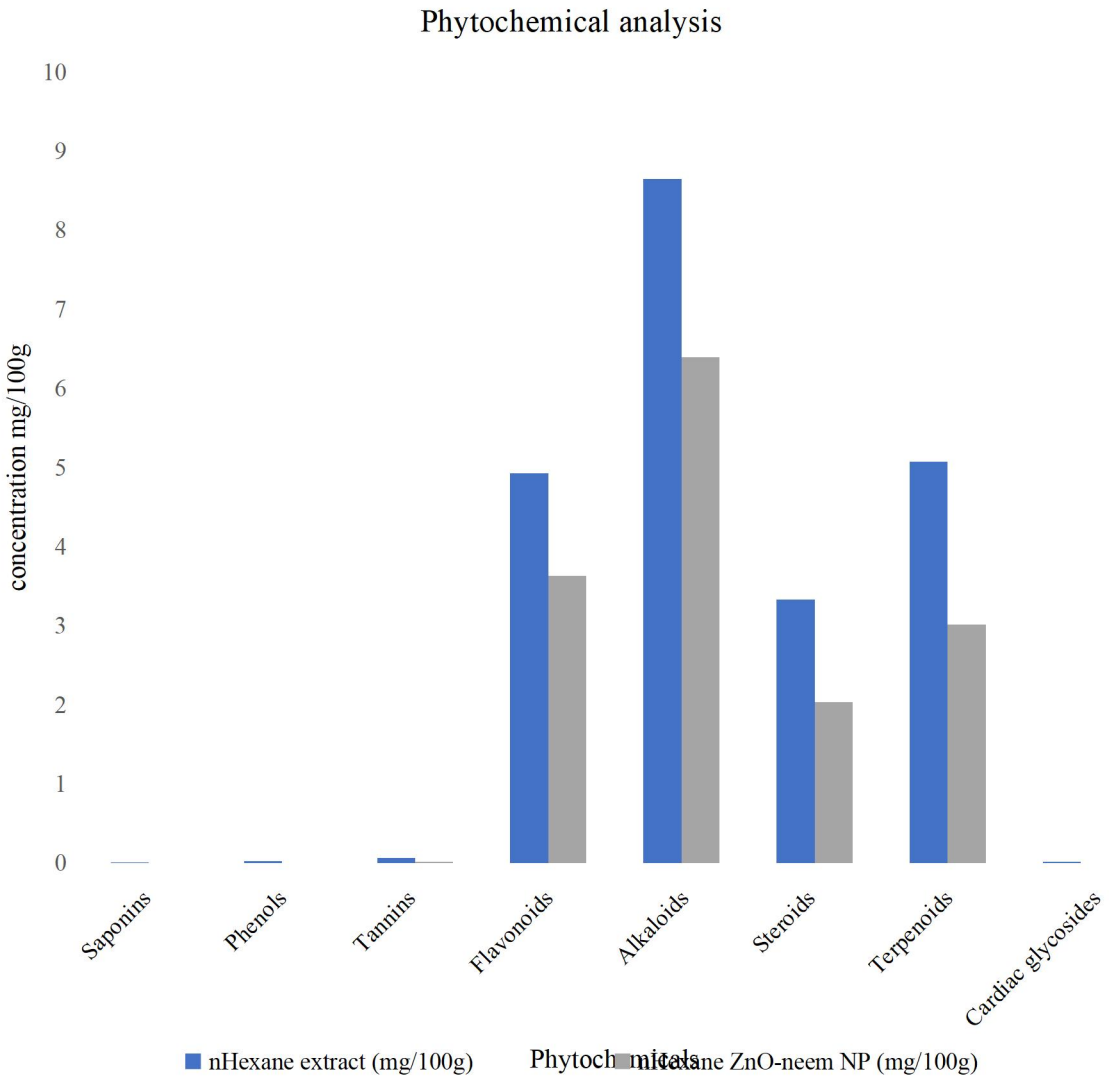


Figure 3: Quantitative analysis for phytochemical constituents present in the neem leaves using selected solvents and their respective nanoparticles

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

S/ N	Experimental frequencies (cm^{-1})	Samples	Bonds	Functional groups
		nHex-ZnO NP		
1	3826.35 3774.68 3772.89 3772.89	3826.35 3772.89	O - H Stretch vibrations	Alcohols
2	3572.29	3572.29	O - H Stretch vibrations	Phenols
3	3387.11 3340.80 3309.96	3387.11 3363.97 3340.80	O - H Stretch, H-bonded	Alcohols and Phenols, flavonoids

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

Keynotes; *nHex-ZnO NP; n-Hexane-neem leaf extract-ZnO nanoparticles.*

Table 4: Composition of major bio-active components present in ZnO nanoparticles of n-Hexane 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	6.394	2-Piperidonone, N-[4-bromo-n-butyl]	234.13	$C_9H_{16}BrNO$	Alkaloid	2.03
2	6.841	Tridecanoic acid	214.34	$C_{13}H_{26}O_2$	Ester	1.22
3	7.354	Hexadecanoic acid	256.40	$C_{16}H_{32}O_2$	Fatty acid	2.67
4	7.765	Oleic acid	282.47	$C_{18}H_{34}O_2$	Fatty acid	0.23
5	7.914	Phytol	296.53	$C_{20}H_{40}O$	Diterpene alcohol	2.00
6	8.232	Undec-10-ynoic acid	182.26	$C_{11}H_{18}O_2$	Ester	2.11
7	8.754	Trans-13-Octadecenoic acid	282.46	$C_{18}H_{34}O_2$	Fatty acid	1.15
8	8.922	9,17- Octadecenoic Acid	282.46	$C_{18}H_{34}O_2$	Fatty acid	2.32

9	9.215	Trans-13-Octadecenoic acid	282.46	$C_{18}H_{34}O_2$	Fatty acid	2.11
10	9.434	z-(13,14- Epoxy) tetradec-11-en-1-ol acetate	268.39	$C_{16}H_{28}O_3$	Alcohol Esters	0.48

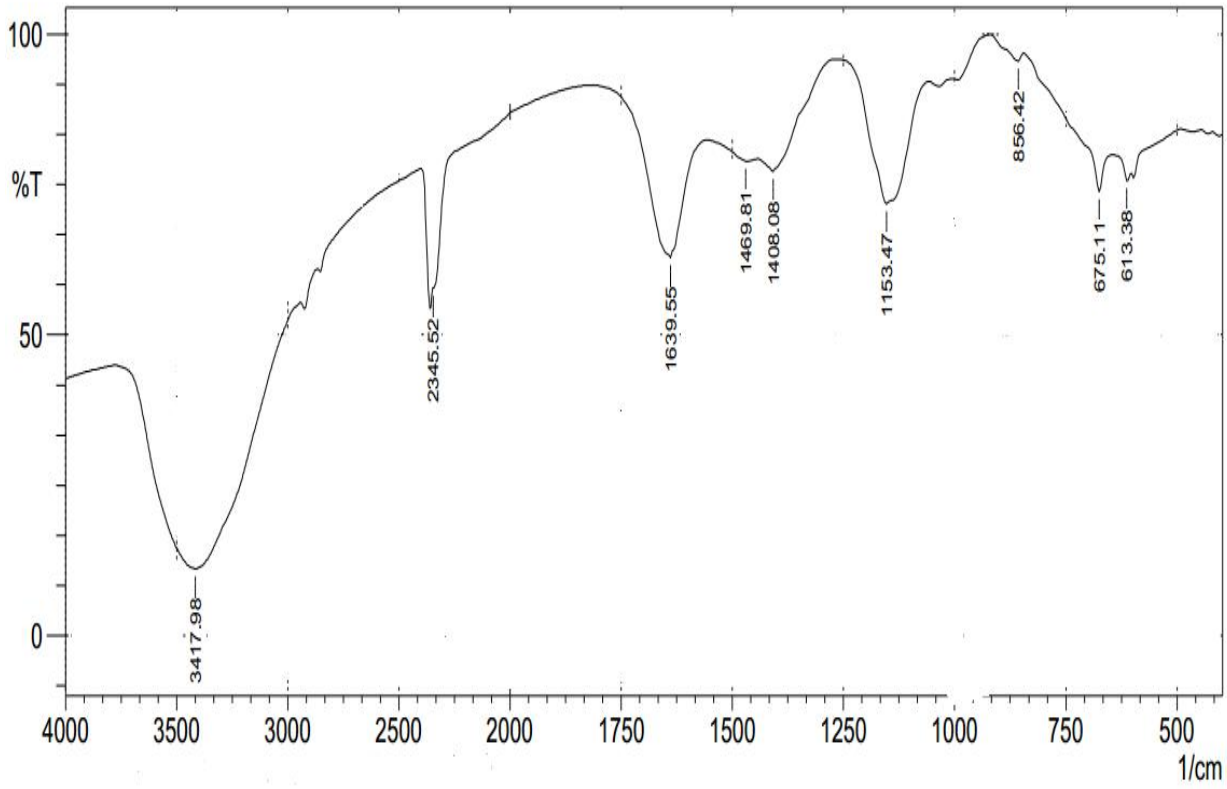
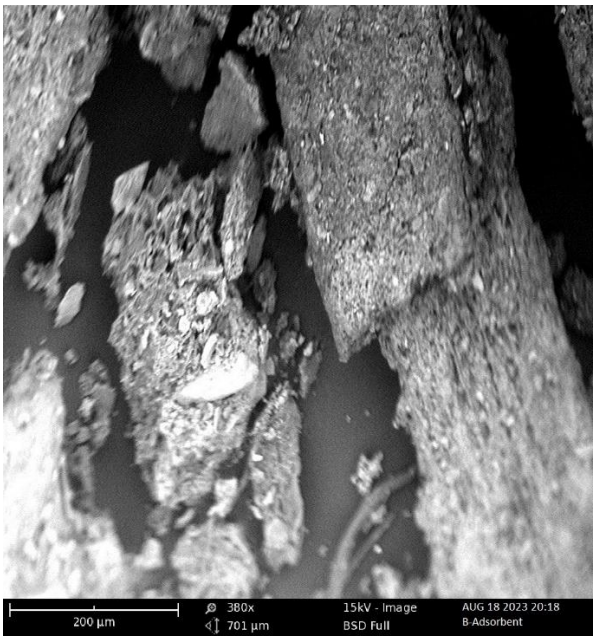


Figure 4: FT-IR spectral for n-Hexane neem leaf extracts



igure 5: SEM image of n-Hexane neem leaf extract mediated ZnO NPs at 380x magnification

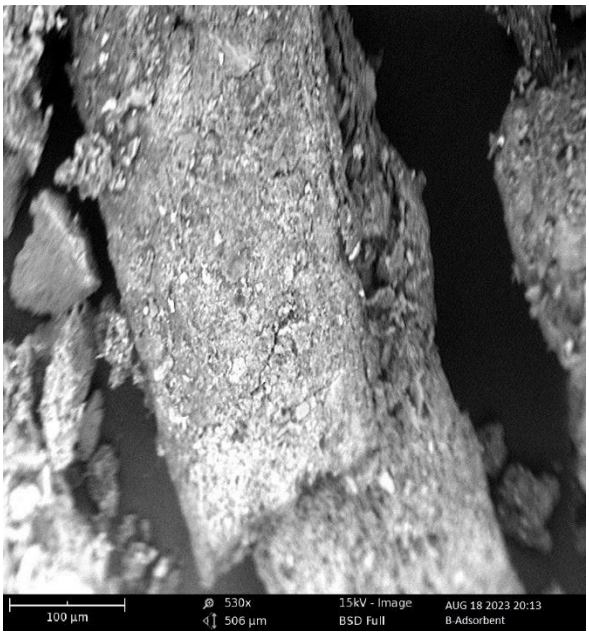


Figure 6: SEM image of n-Hexane neem leaf extract mediated ZnO NPs at 530x magnification

Microbial Analysis

The results for the microbial analysis of both the control and the inoculated substrates in the study are presented in Tables 5 to 7.

Table 5: Result for microbial count

Key: NG = No growth

Days	**Control ×10³cfu/g**	**n-Hexane–neem extract ×10³cfu/g**	**n-Hexane–ZnO NP ×10³cfu/g**
0	NG	NG	NG
5	NG	NG	NG
10	15	NG	NG
15	188	78	56
20	242	108	98
25	388	338	295

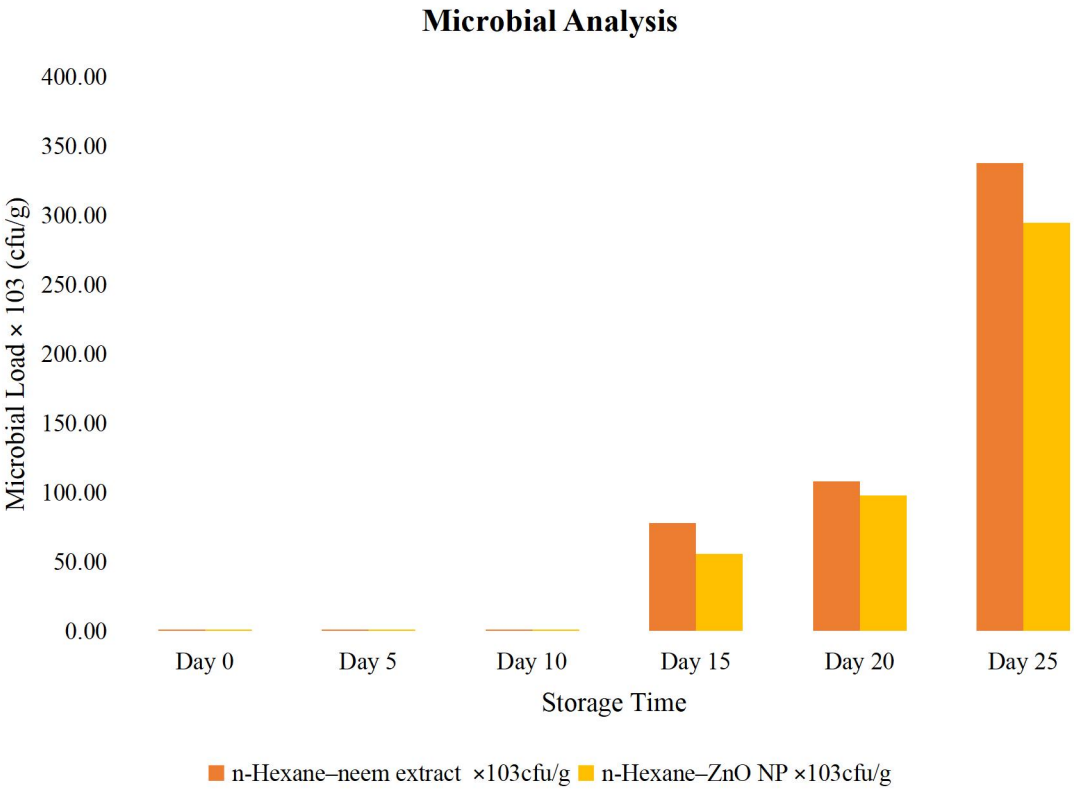


Figure 7: Microbial analysis of the selected samples

Table 6: Antimicrobial susceptibility test – Zones of inhibition (mm)

Microbial specie	**n-Hexane-neem leaf extract**	**n-Hexane-neem leaf extract-ZnO NPs**
Staphylococcus	13.00 ± 1.4[a]	15.50±2.12[a]
Bacillus	8.00 ± 00[d]	12.00 ± 1.41[d]
Klebsiella	12.00 ± 1.4[b]	15.50± 2.12[a]
Pseudomonas	7.50. ± 0.71[f]	13.00±1.41[c]
Proteus	9.00 ± 1.41[e]	12.00 ± 0.00[d]

Saccharomyces	10.00 ± 0.00^{d}	12.00 ± 0.00^{d}
Mucor	11.00 ± 1.41^{c}	14.00 ± 5.66^{b}
Aspergillus	2.50 ± 0.71^{g}	09.00 ± 0.00^{f}
Fusarium	7.50 ± 0.71^{f}	10.00 ± 0.00^{e}
P-value	**0.03**	**0.01**

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

Table 7: Microbial load of the substrates

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}
n-Hexane extract	68.10 ± 0.15^{a}	6.23 ± 0.03^{b}	3.41 ± 0.05^{b}
n-Hexane ZnO NP	18.62 ± 0.05^{c}	3.05 ± 0.04^{d}	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, $\geq 10^6$ 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 ($p>0.05$)

Discussion

The FT-IR spectral for n-Hexane neem leaf extracts

UV-Vis spectroscopy indicated a strong absorption peak at approximately 365 nm, confirming the formation of ZnO nanoparticles. FTIR analysis revealed characteristic absorption bands attributed to O-H and C=O functional groups, indicating the presence of biomolecules in neem extract that assist in the reduction process. XRD patterns showed a hexagonal wurtzite structure typical of ZnO, with an average crystallite size calculated using the Scherrer equation. SEM images revealed uniform spherical nanoparticles with a diameter of around 30 nm (Chukwuebuka, and Chinenye, 2015; Kalpana, and Rajeswari, 2017).

While, the ZnO NPs mediated with n-Hexane neem leaf extract where amorphous, Figures 5 and 6 show their SEM same images with lumpy particles with irregular shapes and sizes at magnification levels of 380 and 530 µm respectively.

SEM provided further insight into the morphology and size details of the ZnO nanoparticle. The size of the particles was from nano to micron range and morphology of particles was nearly spherical in all ratio in n-Hexane extract mediated nanoparticles, and rod-like in shape, this is because the composition of the bioactive components were more (Sujita, et al., 2019). The size of the prepared nanoparticles were more than the size of nanoparticle (between 1-100 nm). This was because the proteins were bound to the surface of the nanoparticles (Ujah et al., 2021). As the ratio differs size also differs, this is because of the variation in the concentration of the phytochemical composites.

Qualitative and quantitative analysis of the neem-leaf extract

The neem leaf was extracted using an organinc solvent with good extractive abilities. The neem leaf n-Hexane extract were characterised as shown in Table 1, where the qualitative analysis of the neem-leaf extracts had the needed phytochemicals.

Similarly, the concentration of these phytochemicals was higher in the methanolic neem – leaf extracts with 6.20 mg/100g saponins and the trend continued through to ZnO neem-leaf nanoparticles synthesised as shown Table 1. Figure 3 show the distribution of the bioactive compounds of neem leaf that were extracted in the selected solvents as well as their respective ZnO nanoparticles synthesised.

Phytochemical analysis of selected samples

Phytochemicals also known as phytonutrients are naturally occurring substances found in plants (Ujah. et al., 2021). The results of the phytochemical screening of the neem leaf extracts from the selected solvents are shown in Tables 1 and 2. The results showed presence of flavonoids, alkaloids, tannins, phenols, saponins, steroids, terpenods and cardiac glycosides (oxalates).

The phytochemicals present in these samples can influence various body processes. They work together with nutrients and dietary fibre to protect the body against diseases, slow the aging process and reduce the risk of many diseases such as cancer, heart disease, stroke, high blood pressure etc.

(Kalpana et al*.,* 2017). These results bear similarities to ones obtained by Babatunde et al., (2019), in their aqueous neem leaf extracts saponins occurred the most, tannins and glycoside are moderate while alkaloid, flavonoids and reducing sugars are low.

The microbial load of the control became significant towards day 10 of storage with 15×10^3 cfu/g and 288×10^3 cfu/g at day 25, at this time; the fruits were completely unwholesome for consumption.

The neem leaf extracts (Table 5) all had growths at day 15 from 76×10^3 cfu/g to 230×10^3 cfu/g. The neem leaf extracts mediated ZnO NPs all had growths beyond day 15; 110×10^3 cfu/g at day 20 and 250×10^3 cfu/g for n-Hexane neem leaf extracts mediated ZnO NPs. The Figure 7, show the increase in microbial growth on the studied substrates.

The results for neem leaf extracts shows that, total coliform count which ranged from 1.07 to 6.01×10^2 cfu/mL with the highest total coliform count obtained from n-Hexane extract, 6.01×10^2 cfu/mL, while the least coliform count of 1.07×10^2 cfu/mL gotten from Methanol neem leaf extract mediated ZnO NPs.

The total viable count was too numerous to count (TNTC) in the control while, fungi were only found in n-Hexane neem extracts and the control, while it was not detected in the rest of the parameters under test. There was a significant difference in the microbial enumeration of the substrates under study at ($p < 0.05$).

Table 6, show the results of the antimicrobial susceptibility test – Zones of inhibition (mm). The antimicrobial activities of the extracts and mediated products (ZnO NPs) were measured using the agar diffusion method with film in a disc shape. The diameter of inhibitory zones surrounding the film discs was used to evaluate the inhibitory and antimicrobial activities of the films (Elumalai, and Velmurugan, 2015).

However, the incorporation of 5 % *w/w* neem leaves extract into the control demonstrated inhibitory activities against *Staphylococcus aureus* and *Aspergillus* with the diameter of inhibition zone of 17.00 ± 1.40 mm and 12.50 ± 0.00 mm, *Staphylococcus aureus* and *mucor* with the diameter of inhibition zone of 15.50 ± 2.12 mm and 9.00 ± 0.00 mm (n-Hexane-neem leaf extract-ZnO NPs), *Klebsiella* and *mucor* with the diameter of inhibition zone of 15.14 ± 1.14 mm, *mucor* and *Pseudomonas* with the diameter of inhibition zone of 17.00 ± 1.14 mm and 7.50 ± 0.71 mm, suggesting that the difference in the antimicrobial activities against bacteria was attributed to the lipid bilayer composition of bacterial strains and the degree of depolarization and permeability of the cell walls (Mohamad, et al., 2014; Chukwuebuka, and Chinenye, 2015).).

In addition, the antimicrobial activity of neem leaf extract-based ZnO NPs might be due to the presence of effective components such as carotenoids, phenolic compounds, flavonoids, triterpenoids, ketones, valavinoids, saponins, gilcosides, steroids, and tetra-triterpenoids azadirachtin in the neem leaves (Parra, and Haque, 2014). This could be attributed to the essential components of incorporated neem extracts in the ZnO NP matrix, which served as the principal antibiotics that could serve as a defensive mechanism against different pathogens (Geetha, et al., 2016; Babatunde, et al*.,* 2019).

Conclusion

In this study, zinc oxide nanoparticles were successfully biosynthesized using plant extracts of neem leaves (*Azadirachta indica*). 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.

Leaf extracts of *Azadiracta indica,* indicated that the plant is a rich sources of bioactive compounds.

The successful green synthesis of ZnO nanoparticles using neem leaf extract demonstrates a viable method to produce nanoparticles that possess potential applications in food preservation, given their antimicrobial properties (Jayaseelan, et al 2012; Stan, et al., 2015). The characterization results confirm the integrity and purity of the synthesized nanoparticles.

REFERENCE

Agarwal, H. Kumar, S. V. and Rajeshkumar, S. (2017). "A review on green synthesis of zinc oxide nanoparticles–an eco-friendly approach," *Resource-Efficient Technologies*, 3(4):406–413.

Babatunde, D. E., Otusemade, G. O., Ojewumi, M. E., Oluranti, A. and Oyeniyi, E. (2019). Antimicrobial activity and Phytochemical screening of neem Leaves and lemon grass essential oil Extracts. *International Journal of Mechanical Engineering and Technology (IJMET)*. 10(03): 882-889.

Chukwuebuka, E. and Chinenye, I. J. (2015). Biological functions and anti-nutritional effects of phytochemicals in living system. *J Pharm Biol Sci* 10(2): 10-19.

Elumalai, K. and Velmurugan, S. (2015). "Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.)," *Applied Surface Science*, 345:329–336.

Geetha, M. S., Nagabhushana, H. and Shivananjaiah, H. N. (2016). "Green mediated synthesis and characterization of ZnO nanoparticles using *Euphorbia Jatropha* latex as reducing agent," *Journal of Science: Advanced Materials and Devices*, 1(3):301–310.

Ingale A. G. and Chaudhari, A. N. (2013). "Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach," *Journal of Nanomedicine and Nanotechnology*, 4 (165)1–7.

Jamdagni, P. Khatri, P. and Rana, J. S. (2018). "Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes* arbor-tristis and their antifungal activity," *Journal of King Saud University–Science*, 30(2):168–175.

Jayaseelan, C., Abdul Rahuman, A. and Vishnu Kirthi, A. (2012). "Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 90:78–84.

Kalpana, V. N. and Rajeswari, V. D. (2017). "Biosynthesis of metal and metal oxide nanoparticles for food packaging and preservation: a green expertise," *Food Biosynthesis*, 293–316.

Makarov, V. V., Love, A. J. and Sinitsyna, O. V. (2014). "Green"nanotechnologies: synthesis of metal nanoparticles using plants," *Acta Naturae*, 6(1):20 - 27.

Mirzaei, H. and Darroudi, M. (2017). "Zinc oxide nanoparticles: biological synthesis and biomedical applications," *Ceramics International*. 43(1):907–914.

Mohamad, N. A., Arham, N. A., Jai, J. and Hadi, A. (2014). "Plant extract as reducing agent in synthesis of metallic nanoparticles: a review," *Advanced Materials Research*, 832:350–355.

Parra, M. R. and Haque, F. Z. (2014). "Aqueous chemical route synthesis and the effect of calcination temperature on the structural and optical properties of ZnO nanoparticles," *Journal of Materials Research and Technology*, vol. 3, no. 4, pp. 363–369.

Ramesh, M., Anbuvannan, M. and Viruthagiri, G. (2015). "Green synthesis of ZnO nanoparticles using Solanum nigrum leaf extract and their antibacterial activity," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 136:864–870.

Sharma, D., Sabela, M. I. and Kanchi, S. (2016). "Biosynthesis of ZnO nanoparticles using *Jacaranda mimosifolia* flower flowers extract: Synergistic antibacterial activity and molecular simulated facet specific adsorption studies," *Journal of Photochemistry and Photobiology B: Biology*, 162:199–207.

Stan, M., Popa, A. Toloman, D., Dehelean, A., Lung, I. and G. Katona, (2015). "Enhanced photocatalytic degradation properties of zinc oxide nanoparticles synthesized by using plant extracts," *Materials Science in Semiconductor Processing*, 39:23–29.

Sujita, M., Binita, M. and Ram, L. S. (2019). The antioxidant, antibacterial and GC-MS analysis of methanolic extract of *Azadirachta indica* Juss, The shaded dried powdered leaves of *Azadirachta indica* Juss. *The Pharma Innovation Journal,* 8(12):178-180

Ujah. I. I., Nsude, C. A., Ani, O. N., Alozieuwa, U. B., Okpako, I. O. and Okwor, A. E. (2021). Phytochemicals of neem plant (*Azadirachta indica*) explains its use in traditional medicine and pest control *GSC Biological and Pharmaceutical Sciences,* 14(02), 165–171.