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Characterization and Microbial Activity of Nickel Oxide Nanoparticles Synthesized from Aquatic Hydrilla Plant Leaves

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ABSTRACT

Nickel chloride as a precursor and leaf extract from the aquatic plant Hydrilla verticillata have been used to synthesize nickel oxide nanoparticles. The color shift of the resultant solution most likely served as an indication of the reaction's progress. Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), and Transmission Electron Microscope (TEM) were used, and using powder X-ray diffraction methods (XRD), the nanoparticle's size was ascertained to be 19.15 nm. The produced NiO-NPs microbial activity is investigated. NiO-NPs had an inhibitory impact on *S. aureus* and *A. niger* that was similar to that of streptomycin.

Keywords: Hydrilla aquatic plant, Green synthesis of nickel nanoparticles, Microbial effect.

INTRODUCTION

In recent years, there has been a huge increase in the interest in nickel oxide nanoparticles across many scientific areas owing to their different chemical, physical, and biological properties. Nickel oxide nanoparticles have been synthesized using a variety of physicochemical techniques because of their wide range of uses in diverse sectors. Nanotechnology represents one of the most groundbreaking scientific advancements in the 21st century that spans multiple disciplines. It focuses on manipulating matter at the nanoscale, typically measuring less than 100 nanometers (nm). NPs of certain metals or metal oxides are frequently utilized as treatments to cure various diseases and advance human health because of their antibacterial properties. These nanoparticles exhibit strong antimicrobial properties, making them effective against bacteria, viruses, and fungi.[2] The unique properties and wide-ranging applications of nanomaterials have attracted the attention of numerous scientific and technological fields.[3] Bacteria can multiply and spread in various environments, posing a significant health threat to living organisms through infections. High mortality

and morbidity rates associated with bacterial infectious diseases have created a strong need for potent antimicrobial medications.[4,5] In this context, nanoparticles (NPs) produced using eco-friendly methods have demonstrated promising effectiveness.[6] Nickel is one of the earliest known transition metals with the ability to store hydrogen, exhibit magnetism and catalyze chemical reactions.[7] Using the aquatic plant Hydrilla verticillata, Sable *et al.,* reported the extracellular biosynthesis of silver nanoparticles (Ag-NPs) for the first time.[8] In their most recent work, Gidey Berhe and Tadesse Gebreslassie (2023) examined the production of nickel oxide nanoparticles (NiO) utilizing a variety of plant extracts, such as those from Moringa oleifera and okra. Their study demonstrated the photocatalytic, magnetic, and antibacterial properties of the synthesized nanoparticles, highlighting their potential for a range of biological uses. The authors further highlighted the value of plant-mediated synthesis in tackling current health issues by offering a thorough analysis of the cytotoxic effects on cancer cells.[9] Numerous physiologically harmful diseases have been treated using aquatic Hydrilla verticillata, which has been shown to have good therapeutic properties and various nutrients.[10-11]

EXPERIMENTAL

Materials

Freshly aquatic Hydrilla plants (Fig. 1(a)) were collected from the Department of Botany, Milind College of Science, Aurangabad (MH) India.

Sodium hydroxide (pellet 99%) and nickel(II) chloride hexahydrate (99% purity) were purchased from Sigma-Aldrich, Merck and Molychem companies of high purity and used directly in the reaction protocol without further purification.

Preparation of Hydrilla Leaf Extract

Freshly collected hydrilla plants were washed with double-distilled water to remove surface salts, cut into leaves, and dried in the shade. Double-distilled water (100 mL) was used to homogenize 10 g of *H. verticillata* leaves, which were boiled for two hours at 90°C in a water bath. The resulting aqueous leaf extract was cooled to room temperature. Finally, Whatman No. 1 paper was used to filter the extract (Figure 1(b)).

Fig. 1(a). *H. verticillata* and 1(b) *H. verticillata* leaf extract

Procedure Green Synthesis of NiO Nanoparticles

For the green synthesis of NiO-NPs, 100 mL distilled water (0.1 M) nickel(II) chloride solution $Ni(Cl)_2.6H_2O$ was stirred for 15 min, and then 10 mL of the leaf extract was added dropwise. The mixture was progressively stirred for 1 h at 80°C temperature using a magnetic stirrer at 2000 rpm in an oil bath. The pH of the solution was adjusted by adding a 0.5 M NaOH solution dropwise until the pH of the solution reached 10. The mixture was stirred for 2 h, the resulting solution changed from greenish to yellowish green, and a precipitate was obtained. Subsequently, the solution was cooled to room temperature. After cooling down, the solution was centrifuged at 4000 rpm for 10 minute. The precipitate was then calcined for 2 h at 400^{0}C in a muffle furnace. A fine black powder was obtained meticulously and preserved for future research (Figure 2).

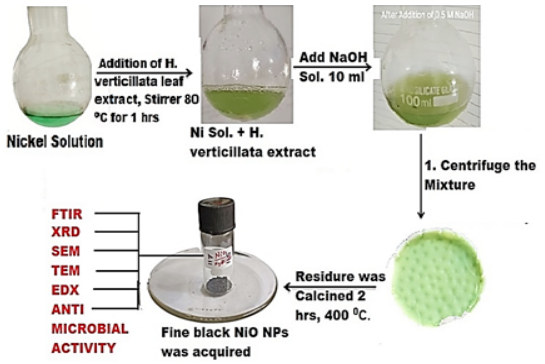

Fig. 2. A schematic diagram illustrating the green synthesis steps of NiO nanoparticles

Characterization of NiO nanoparticles

The production of NiO-NPs from *H. verticillata* leaf was validated by physical characterization instruments. NiO-NPs were crystallo graphically elucidated using X-ray diffraction (Brukar D8-Advanced Diffractometer). The functional groups of Synthesized NiO-NPs and *H. verticillata* leaf extract were evaluated using (Bruker Alpha FT-IR). NiO-NPs morphological features, including size

and topology, were analyzed using FE-SEM (Nova Nano-SEM NPEP303) and TEM (PHILIPS CM200). Energy dispersive X-ray spectroscopy (EDX, Bruker, XFlash 6I30) indicated the elemental composition and chemical purity.

RESULTS AND DISCUSSION

Visible Observation

NiO-NPs were formed from *H. verticillata* leaf extract added to Ni-Solution when the color of the solution was slightly hazy and green in color changed from greenish to yellowish green (Fig. 2). After centrifugation for 10 min, the residue was calcined for 2 h at 400^{0}C in a muffle furnace. A fine black substance was obtained and appropriately collected.

X-ray diffraction (XRD) spectrum

The X-ray diffraction pattern of NiO-NPs is displayed in (Fig. 3) as it was scanned between 30° and 70° at an angle 2θ (2 theta) degrees. The diffraction peaks observed at the theta angles of 36.40°, 47.74°, 56.76°, and 63.60° were assigned to the (111), (200), (220), and (311) planes, respectively. This pattern confirms the crystallinity of the green-synthesized NiO nanostructures. The cubic phase of NiO (JCPDS card no. 47-1049/4-835).[12-14]

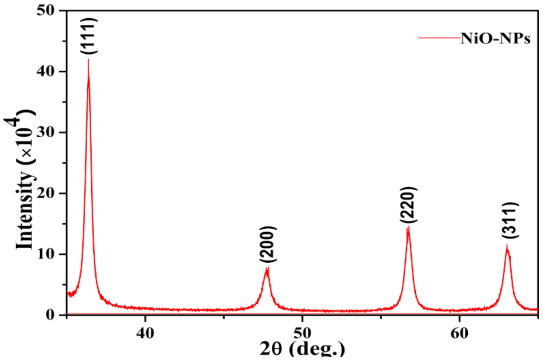

Fig. 3. XRD spectrum of NiO-NPs

The average crystallite size of the NiO-NPs was found to be 19.15 nm using Scherrer's equation. The size of the nanocrystals was determined using the Debye-Scherrer equation, which showed that the surface-area-to-volume ratio was high. The equation is as follows:

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

In this equation, where D represents nanoparticle crystalline size, K is the crystallite shape factor approximation of 0.9. The wavelength of the X-rays (λ), β is the whole breadth at half maxima (FWHM) of peaks, and θ is the Braggs' angle (deg.).

FTIR Spectrum (Functional group studies)

Figure 4 FT-IR spectra of NiO-NPs indicate an absorption peak at 449 cm^{-1}, which corresponds to the Ni-O stretching vibration mode. The Peak at 1619 cm^{-1} might be due to the C = C bond.

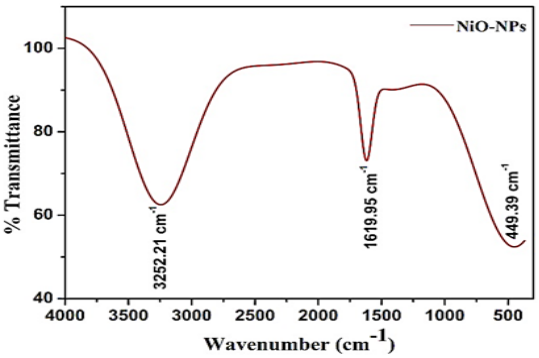

Fig. 4. FTIR specturm of NiO-NPs

In addition, the band at 3252 cm^{-1} represents the O-H stretching of alcohols and phenols.[15-18] The FTIR spectra and XRD data of the biologically produced NiO-NPs were consistent.

SEM and EDX spectrum

Utilizing a scanning electron microscope (SEM), the surface morphological properties of the generated NiO-NPs were investigated. The aggregation of irregularly shaped nanoparticles is shown in (Fig. 5(a)) SEM micrographs. Additionally, the particles exhibited a hexagonal form with some degree of agglomeration, which might be explained by the high surface tension and energy of the NiO nanoparticles. Using the energy dispersive X-ray (EDX) technique, the fundamental composition of the produced NiO NPs is displayed in (Fig. 5(b)). This chart indicates that the sample contains Ni and O. Furthermore, the weak unassigned peaks corresponding to O, N, and S might be caused by the plant phytochemicals present in the sample. EDX examination confirmed the presence of Ni and O signals in the Nickel Oxide Nanoparticles derived from Hydrilla verticillata leaves (Table 1).

According to this table, the main constituents of the sample were found to be sulfur (1.65%), oxygen (24.32%), and nickel (74.03%).

Table 1: EDX Analysis of the Compositions Major Elements

Element	%Weight	%Atomic
Ni	74.03	42.18
O	24.32	56.63
S	1.65	1.19

EDX, which can precisely measure Ni and O and display the Ni and O peaks without any contaminants, was used to confirm the purity of NiO-NPs.[19,20] This finding revealed an assistance proposal for NiO production that may be taken into account in addition to FTIR and XRD analyses.

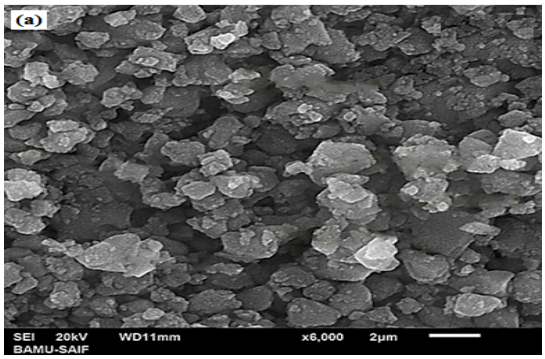

Fig. 5(a). SEM pictures of NiO-NPs

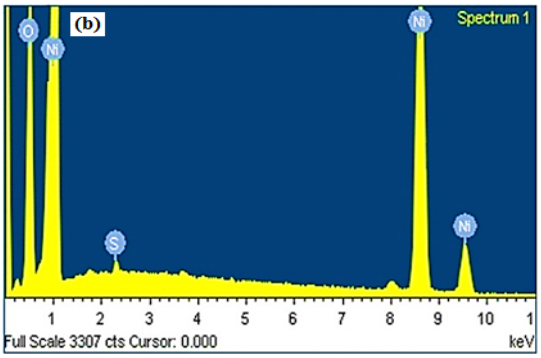

Fig. 5(b). EDX pictures of NiO-NPs

TEM and SAED spectrum

The microstructural properties of the produced NiO-NPs were examined using the transmission electron microscopy (TEM) technique. The TEM image of NiO-NPs is displayed in Fig. 6(a), while the selected area electron diffraction (SAED) pattern is displayed in Fig. 6(b). The majority of particles, according to TEM images, have pseudo-spherical structures that correspond to their shapes Fig. 6(a). Bright spots in the shape of concentric circles are visible in the SAED pattern for NiO-NPs shown in Fig. 6(b). Thus, this pattern demonstrated that the NiO-NPs as produced were polycrystalline[21].

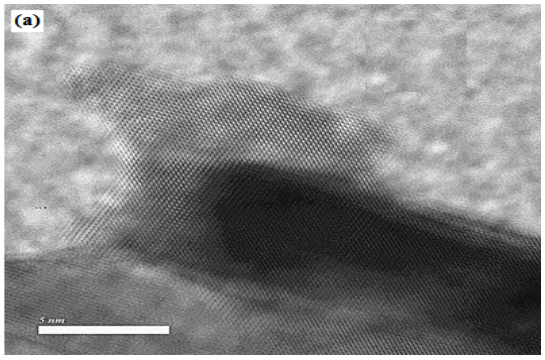

Fig. 6(a). TEM images of NiO-NPs

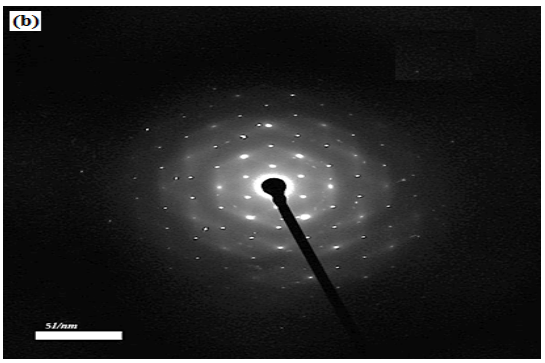

Fig. 6(b). SAED images of NiO-NPs

Greenly produced NiO-NPs have a large specific surface area, a small particle size, and a high dispersity, which makes them a viable material for biological applications.

Antimicrobial Activity

Using a disk diffusion experiment, the antibacterial activity of the biosynthesized NiO-NPs was evaluated against two *Gram-positive* bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and two *Gram-negative* bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* bacteria.[22] The findings were compared using Streptomycin (10 micrograms per disk) as the reference standard. The antibacterial activity was tested using nutritional agar from Hi-media. Composition (gL^{-1}). Sodium chloride 5.0: Beef extract 10.0: Penton 10.0 (pH 7.2).

The biosynthesized NiO-NPs were further tested for anti-fungal action against Aspergillus niger using an agar diffusion assay.[23,24] Streptomycin (100 units per disk) is used as the reference standard. The antifungal activity is assessed using Sabouraud Agar Media and DMSO as a control solvent. The diameter of the zone is measured using Vernier Calliper. Zone of inhibition (mm) data are displayed in (Table 2).

Table 1: Anti-bacterial and Anti-fungal Screening of NiO-NPs

Compound	Microorganism	Strain Name & Strain Reference	Zone of Inhibition (ZOI) (mm)	Streptomycin	Amphotericin B
NiO-NPs	*Gram-positive* bacteria	*S. aureus* (NCIM 2079)	12	25	NA
		B. subtilis (NCIM 2250)	NIL	24	NA
	Gram-negative bacteria	*E. coli* (ATCC 25922)	NIL	26	NA
		P. aeruginosa (NCIM 2719)	NIL	28	NA
	Fungi	*A. niger* (ATCC 16404)	13	NA	NA

Streptomycin was used as a reference molecule to evaluate the produced NiO-NPs' antifungal and antibacterial effectiveness. NiO-NPs showed greater antifungal efficacy, according to the data. The antibacterial and antifungal qualities of the NiO-NPs were evaluated (Figures 7, 8 & 9).

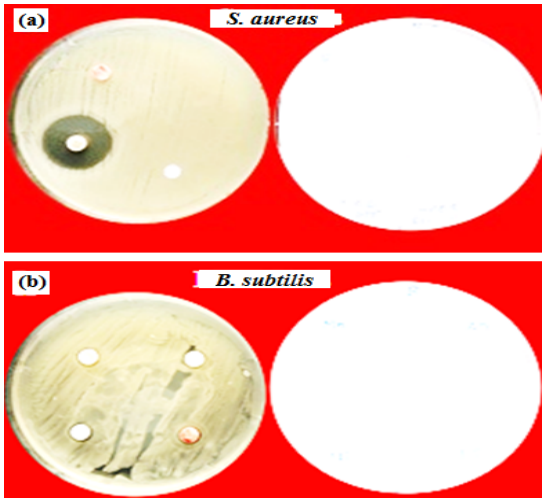

Fig. 7. Antibacterial effect of biologically synthesized NiO NPs against *Gram-positive* bacteria, (a) *S. aureus* and (b) *B. subtilis*

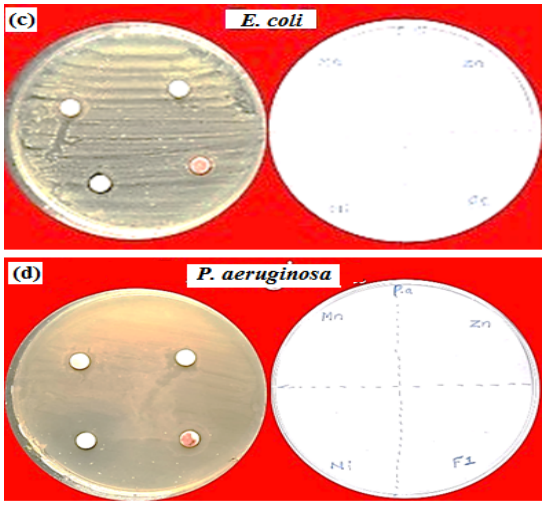

Fig. 8. Antibacterial effect of biologically synthesized NiO NPs against *Gram-negative* bacteria, (a) *E. coli* and (b) *P. aeuroginosa*

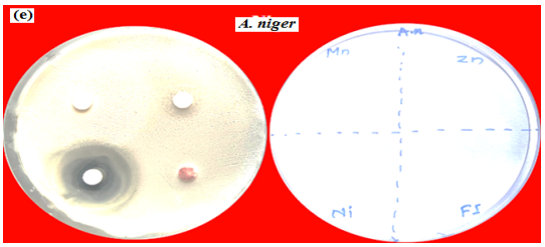

Fig. 9. Antifungal effect of biologically synthesized NiO NPs against *A. Niger*

The analysis of antibacterial activity *Gram-positive S. aureus* shows a 12 mm ZOI, and *B. subtilis* does not exhibit a zone of inhibition, and also *Gram-negative E. coli* and *P. aeruginosa* do not exhibit a zone of inhibition. The analysis of antifungal activity shows *A. niger* exhibits a 13 mm ZOI compared with the standard streptomycin.

For sustained performance, the activity is also reliant on the NPs' physicochemical characteristics. Generally, the effectiveness of a potential antibiotic depends on the particle dose, treatment duration, and administration technique.[25,26]

CONCLUSION

The green synthesis process used H. verticillata leaf extract to produce the Nickel oxide nanoparticles successfully. The XRD analysis revealed the Nickel Oxide nanoparticles formed a well-crystallite monoclinic structure, and the average crystal size of NiO-NP is approximately 19.15 nm. SAED and TEM image is verified the pseudo-spherical shape. The FT-IR spectrum shows the Ni-O absorbance band at wavelength is 449 cm^{-1}. Further, EDX analysis the data shows the N, S and O atom present. One of the most important Anti-bacterial and Anti-fungal results of the present study was that the synthesized NiO-NPs were effective against the *S. aureus* bacteria and *A. niger* fungi. This research article is significant in the field of synthesizing of NiO-NPs.

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Conflicts of interest

The authors state that there are no financial conflicts of interest.

REFERENCES

1. S. Jadoun.; R. Arif.; N.K. Jangid.; R.K., Meena., *Green synthesis of nanoparticles using plant extracts: A Review Environ. Chem. Lett.*, **2021**, *19*, 355-374.
2. Shnawa, B. H. Advances in the use of nanoparticles as anti-cystic echinococcosis agents: *A Review article. J. Pharm. Res. Int.*, **2018**, *24*, 1–14.
3. Harish, V.; Tewari, D. Gaur.; M. Yadav.; A. B.; Swaroop, S. Bechelany.; M. Barhoum.; A. Review on nanoparticles and nanostructured materials: Bioimaging, biosensing, drug delivery, tissue engineering, antimicrobial, and agro-food applications., *Nanomaterials.,* **2022**, *12*, 457.
4. Du, T.; Huang, B.; Cao, J.; Li, C.; Jiao, J.; Xiao, Z.; Wei, L.; Ma, J.; Du, X., & Wang, S., Ni Nanocrystals Supported on Graphene Oxide: Antibacterial Agents for Synergistic Treatment of Bacterial Infections., *ACS Omega.*, **2022**, *7*(22), 18339–18349.https://doi.org/10.1021/acsomega.2c00508
5. Rheima, A. M.; Al Marjani, M. F.; Aboksour, M. F., & Mohammed, S. H., *Evaluation of Anti-Biofilm* Formation Effect of Nickel Oxide.
6. Nanoparticles (NiO-NPs) Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)., *In Int. J. Nanosci. Nanotechnol.,* **2021**, *17*, 4.
7. Samiei, M.; Farjami, A.; Dizaj, S. M. Lotfipour, F. Nanoparticles for antimicrobial purposes in Endodontics: A systematic review of *in vitro* studies., *Mater. Sci. Eng.* C., **2016**, *58*, 1269–1278.
8. Zhao, J. Tian.; Y. Liu.; A. Song.; L. Zhao.; Z. The NiO electrode materials in electrochemical capacitor: A review. *Mater. Sci. Semicond. Process.*, **2019**, *96*, 78–90.
9. SABLE, N.; GAIKWAD, S.; BONDE, S.; GADE, A., & RAI, M. Phytofabrication of silver nanoparticles by using aquatic plant Hydrilla verticillata., *Nusantara Bioscience.*, **2012**, *4*(2).
10. Berhe, M. G., & Gebreslassie, Y. T. , Biomedical Applications of Biosynthesized Nickel Oxide Nanoparticles., *In International Journal of Nanomedicine.*, **2023**, *18*, 4229–4251). Dove Medical Press Ltd. https://doi.org/10.2147/IJN.S410668
11. Pal, D., & Nimse, S. B., Little known uses of common aquatic plant, *Hydrilla verticillata* (Linn. f.) Royle., **2006**, https://www.researchgate.net/publication/224934600
12. Bhoye, M.; Pansambal, S.; Basnet, P.; Lin, K. Y. A., Gutierrez-Mercado, K. Y., Pérez-Larios, A., Chauhan, A., Oza, R., & Ghotekar, S., Eco-Friendly Synthesis of Ni/NiO Nanoparticles Using Gymnema sylvestre Leaves Extract for Antifungal Activity., *Journal of Composites Science.*, **2023**, *7*(3). https://doi.org/10.3390/jcs7030105
13. Srivastava, N., & Srivastava, P. C. Realizing NiO nanocrystals from a simple chemical method., *Bulletin of Materials Science.*, **2010**, *33*(6), 653–656.https://doi.org/10.1007/s12034-011-0142-0
14. Shnawa, B. H.; Jalil, P. J.; Hamad, S. M., and Ahmed, M. H. Antioxidant, Protoscolicidal, Hemocompatibility, and Antibacterial Activity of Nickel Oxide Nanoparticles Synthesized by Ziziphus spina-christi., *BioNanoScience.*, **2022**, *12*(4), 1264–1278.https://doi.org/10.1007/s12668-022-01028-3

15. Shwetha, U. R.; Rajith Kumar, C. R.; Kiran, M. S.; Betageri, V. S.; Latha, M. S.; Veerapur, R.; Lamraoui, G.; Al-Kheraif, A. A.; Elgorban, A. M.; Syed, A.; Shivamallu, C., & Kollur, S. P. , Biogenic synthesis of nio nanoparticles using areca catechu leaf extract and their antidiabetic and cytotoxic effects., *Molecules*., **2021**, *26*(9).https://doi.org/10.3390/molecules26092448
16. Patel KN.; Deshpande M.; Chauhan K.; Rajput P.; Gujarati VP.; Pandya S.; Sathe V.; Chaki SH. Effect of Mn doping concentra- tion on structural, vibrational and magnetic properties of NiO nanoparticles., *Adv Powder Technol*., **2018**, *29*(10), 2394.
17. Vishnukumar P.; Saravanakumar B.; Ravi G.; Ganesh V.; Guduru RK.; Yuvakkumar R. Synthesis and characterization of NiO/Ni3V2O8 nanocomposite for supercapacitor applications., *Mater Lett*., **2018**, *219*, 114.
18. Gondal M.; Saleh TA.; Drmosh Q. Synthesis of nickel oxide nanoparticles using pulsed laser ablation in liquids and their optical characterization., *Appl Surf Sci*., **2012**, *258*(18), 6982.
19. Shwetha, U. R.; Rajith Kumar, C. R.; Kiran, M. S.; Betageri, V. S.; Latha, M. S.; Veerapur, R.; Lamraoui, G.; Al-Kheraif, A. A.; Elgorban, A. M.; Syed, A., Shivamallu, C., & Kollur, S. P. Biogenic synthesis of NiO nanoparticles using areca catechu leaf extract and their antidiabetic and cytotoxic effects., *Molecules*., **2021**, *26*(9).https://doi.org/10.3390/molecules26092448
20. Bakhsh, H.; Buledi, J. A.; Khand, N. H.; Junejo, B.; Solangi, A. R.; Mallah, A. and Sherazi, S. T. H. NiO nanostructures based functional none-enzymatic electrochemical sensor for ultrasensitive determination of endosulfan in vegetables., *Journal of Food Measurement and Characterization*., **2021**, *15*(3), 2695–2704.https://doi.org/10.1007/s11694-021-00860-7
21. Karthik, K.; Shashank, M.; Revathi, V.; Tatarchuk, T. Facile microwave-assisted green synthesis of NiO nanoparticles from Andrographis paniculata leaf extract and evaluation of their photocatalytic and anticancer activities., *Molecular Crystals and Liquid Crystals.,* **2018**, *673*(1),70–80. doi:10.1080/15421406.2019.157849
22. Mayedwa, N.; Mongwaketsi, N.; Khamlich, S.; Kaviyarasu, K.; Matinise, N., & Maaza, M. Green synthesis of nickel oxide, palladium and palladium oxide synthesized via Aspalathus linearis natural extracts: physical properties & mechanism of formation., *Applied Surface Science*., **2018**, *446*, 266–272.doi:10.1016/j.apsusc.2017.12.116
23. Jorgensen J.H.; Turnidge J.D. Susceptibility Test Methods: Dilution and Disk Diffusion Methods, In Manual of Clinical Microbiology (11th Edition), Ed. Jorgensen J.H., Baron E.J., Pfaller M. A., Landry M. L., Murray P.R. ASM Press., **2015**, 1253-1272.
24. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disk Diffusion Methodology., **2024**, v12.0, EUCAST Development Laboratory.
25. Espinel-Ingroff A., Pfaller M.A. Susceptibility Test Methods: Yeasts and Filamentous Fungi, In Manual of Clinical Microbiology (12th Edition), Ed. Jorgensen J.H., Baron E.J., Pfaller M.A., Landry M.L., Murray P.R. ASM Press., **2017**, 2083-2098.
26. Huq, A.; Ashrafudoulla; Rahman, M.M. Balusamy, S.R.; Akter, S. Green synthesis and potential antibacterial applications of bioactive silver nanoparticles: A Review. *Polymers*., **2022**, *14*, 742