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TUNING THE TRAITS OF NICKEL OXIDE NANOPARTICLES EMPLOYING GREEN SYNTHESIS USING BRASSICA OLERACEA L. VAR. CAPITATA FOR EXPLORING THE ANTIMICROBIAL, ANTICANCER AND SUPERCAPACITOR APPLICATIONS

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Abstract:

The Nickel Oxide (NiO) nanoparticles are synthesized using green synthesis method utilizing *Brassica oleracea* L. *var. capitata* leaf extracts. The X-ray diffraction studies revealed that synthesized NiO-NPs exhibits face centered cubic structure. SEM images of NiO-NPs exhibited agglomerated structures. TEM images of NiO-NPs revealed spherical morphology with variable size ranging from 5-50 nm. FT-IR spectrum was used to confirm the various functional group. The absorbance spectral studies reveal existence of three important peaks at wavelength 545 nm, 589 nm and 636 nm. The antibacterial activity of NiO-NPs against Gram +ve *Staphylococcus aureus* (NCIM2079), *Bacillus subtilis* (NCIM2250) and Gram –ve *Pseudomonas aeruginosa* (MTCC3541), *Escherichia coli* (NCIM2065) was further studied. Anticancer activity of NiO-NPs against lungs cancer cell (NRU-A549) was examined. The IC_{50} value was found to be 326.4 µg/ml. The synthesized NiO-NPs were further studied for electrochemical and supercapacitor application.

Key Words	*Brassica oleracea* L.*var.capitata*, green synthesis of NiO-NPs, antimicrobial activity, anticancer activity, supercapacitor application
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exploring the antimicrobial, anticancer and supercapacitor applications. *Nature Environment and Pollution Technology*, 24(3), B4261. https://doi.org/10.46488/NEPT.2025.v24i03.B4261

1. Introduction

Metal oxide nanoparticles have garnered significant attention due to their distinct optical, electronic and physiochemical characteristics(Balgude & Mardikar, 2021; Kumar et al., 2020; Mardikar et al., 2021; Mardikar et al., 2020; Mone et al., 2020). Recently, there has been a growing interest in producing nanoparticles of Fe, Co and Ni owing to their exceptional properties and wide-ranging applications in fields such as sensors, memory storage devices, photocatalytic processes, drug delivery systems, catalysis, magnetic resonance imaging and even cancer cell treatment(Baek & Cho, 2017; Ewais et al., 2017; Farzin et al., 2020; Lokesh et al., 2016; Magdalane et al., 2016; Manikandan et al., 2015; Prabhakar, 2023; Sreekanth et al., 2020; Tahir et al., 2022; Zhang et al., 2017). Metal oxides find utility in diverse sectors, with biomedicine being a particularly promising area(Iqbal et al., 2021). Researchers are actively exploring eco-friendly and non-hazardous methods to synthesize materials for treating tumours and cancer cells, leveraging the enhanced adsorption capabilities of these nanoparticles to induce cytotoxic effects through medium starvation. Their unique properties like surface area, metal ion releasing and adsorbing ability. It is also utilized as the competent material in the elimination of pathogens from wastewater, owing to its primary characteristics such as stability, gradual release of metal ions from the nanoparticle, and effective biological properties(Ahmad & Rawat, 2023). Nanoparticles exhibit various properties that distinguish them from bulk materials, including surface area to volume ratio, electro-optical, magneto-optical, chemical, and mechanical properties. These properties serve as a powerful tool in combating bacteria(Binns, 2021; McNamara & Tofail, 2017). Numerous antimicrobial nanoparticles have demonstrated their effectiveness against infectious diseases in vitro and animal cells(Kalashgarani & Babapoor, 2022). The antibacterial activity is not only dependent on the metal oxide used but also on the nature of the bacterial species. Bacteria can be classified into Gram-positive (+) and Gram-negative (-) based on their cell wall structure. Gram-positive cells have thick layered walls (20-50 nm), while Gram-negative bacteria have thin layered walls. In this study, an attempt was made to synthesize NiO nanoparticles with a small particle size and large surface area, possessing good antibacterial and anti-inflammatory properties. NiO nanoparticles have garnered significant interest in recent research due to their high chemical stability, electrocatalytic properties, superconductance characteristics, and electron transfer capability. Nickel oxide is a p-type semiconductor metal oxide with a band gap ranging from 3.6 to 4.0 eV(Goel et al., 2020). It is an environmentally active material, finding applications in the adsorption of hazardous dyes and inorganic pollutants(Pandian et al., 2015). Additionally, due to their anti-inflammatory properties, they are employed in the field of biomedicine(Uddin et al., 2021). NiO nanoparticles have been found to exhibit toxic effects on bacteria and microalgae(Isaacoff & Brown, 2017; Liu et al., 2022), attributed to their potential to induce oxidative stress and release nickel ions (Ni^{2+}) inside the cell(Berhe & Gebreslassie,

2023; Waseem et al., 2022). NiO nanoparticles have been associated with cytotoxic effects due to their unique properties such as surface area, metal ion release, and adsorption ability.
Many techniques, like co-precipitation, electro deposition, sol-gel method and solvothermal method, have been created for producing nanoparticles with increase reaction times and high energy consumption(Shang et al., 2017; Sharma et al., 2018). The conventional method of combustion is a self-sustaining exothermic reaction which produces various metal oxides in large amounts(Ahmad et al., 2022; Samrot et al., 2021). There are several environmentally eco-friendly methods for synthesis of metal nanoparticles. The use of less hazardous chemicals or natural materials in place of toxic chemicals is one of the green approach. The nanomaterials are synthesized by using micro-organism, plant extract. The green synthesis of nanoparticles mainly plant extract is used and the extensive use of chemicals is avoided(Ahmed et al., 2016; Akarca et al., 2020; Gupta et al., 2020; Habtemariam & Oumer, 2020; Kar & Ray, 2014; Sheikholeslami, 2022). Plant leaves and petals have a hardness that acts as a bio template, controlling the size of the nanoparticles and prevent their aggregation. The plant consists of many fatty acids, vitamins, amino acids and nutrients, glucosinolates(Dai et al., 2019) and phenolics (flavonoids, anthocyanins, proanthocyanides and cinnamates) which are responsible for capturing the metal ion.

The bottom-up approach for the synthesis of nanoparticles mainly deals with the creation of NPs from the assembly of miniature matter viz. atoms and molecules into novel nuclei, which further develop into a particle having nanoscopic sizes using different chemical and biological techniques(Pandit et al., 2022). The manipulation of the biological synthesis route is considered an innovative supernumerary for the physicochemical synthesis routes. This technique utilizes natural biomass sources like micro and macro-organisms (fungi, algae, bacteria, and selected viruses), biomolecules (lipids, pigments, proteins, poly saccharides, etc.), and plants as biologic-reducing and stabilizing agents for the green production of NPs with minimum contamination(Hamida et al., 2021; Jeevanandam et al., 2022). The clear expansion of NPs synthesis options and green methods that make use of various biological sources is quite breathtaking. Various remarkable features of the green (bio-synthesis) technique are the nonexistence of noxious chemical compounds being employed as stabilizing or reducing agents resulting in biocompatibility, no noxious yield being created from this procedure using low energy consumption at a sensible cost and being highly scalable(Hamida et al., 2021). The applicability of bio-NPs facilitated by the green route is more analogous to metal oxide nanoparticles with unconventional properties such as quantum size, miniature size, quantum tunnelling, and surface effects(Ali et al., 2023; Berta et al., 2021). Additionally, features such as enhanced morphologies, high yield, and enduring stability in metal oxides are achieved by employing a greener method(Nadeem et al., 2020).

Brassica oleracea L.*var.capitata* belongs to *Brassicacea* family, genus *Brassica* and species *oleracea* L. *Brassica oleracea* L.*var.capitata* contain alkaloids, amino acids, flavonoids, glucoside, phenol, tannin, steroids and carbohydrates. The phenolic compounds are most important groups of plant metabolites compound(Arun et al., 2021).

Supercapacitors has been considered one of the most promising options owing to simple design and capacity to supply the electricity demand(Uke, Mardikar, et al., 2021; Yu et al., 2015). Transition metal oxides such as NiO are the most promising electrode materials in energy storage devices. They have a large surface area. A variety of substances can be found easily and are eco-friendly to the environment(Lu et al., 2017; Uke, Chaudhari, et al., 2021). These metals are very significant because they provide a clear increase in capacitance through the tuning and control of different imperfections at interfaces and surfaces(Hashem et al., 2022; Uke et al., 2020).

Here in the present study green synthesis of NiO nanoparticles from *Brassica oleracea* L.*var. capitata* extract as a reducing agent has been reported and their antimicrobial and anticancer activity has been evaluated. Synthesized NiO nanoparticles also showed good supercapacitor application.

2. Experimental Methods

2.1.Chemicals and materials

Nickel nitrate hexahydrate ($Ni(NO_3)_26H_2O$), distilled water, *Brassica oleracea* L. *var.capitata* extract were used for synthesis. Nickel nitrate hexahydrate ($Ni(NO_3)_26H_2O$) was procured form Loba Chemie and were used as received.

2.2. Preparation of extract of *Brassica oleracea* L.*var.capitata*

10 g of dehydrated leaf powder of *Brassica oleracea* L.*var.capitata* were taken in soxhlet and it was attached with 250 ml round bottom flask. During extraction, 100 ml distilled water was used as a solvent. The round bottom flask was heated at a constant temperature of 60 ^oC during extraction. After completion of all cycles of soxhlet extractor, the extract was collected in 250 ml beaker & this aqueous extract was utilized further for green synthesis of NiO NPs.

2.3.Green synthesis of NiO- NPs

100 ml 0.1 M nickel nitrate solution was taken in 250 ml round bottom flask and kept on magnetic stirrer. 50 ml of aqueous leaf extract of *Brassica oleracea* L .*var. capitata* was added dropwise under constant magnetic stirring. After complete addition, the mixture was stirred for 90 mins. Further, the reaction mass was isolated by centrifugation at 1500 rpm for 15 minutes. Supernatant liquid was discarded and Ni-NPs were collected. The obtained precipitate was washed several times with ethanol and distilled water. After washing the precipitate was dried and calcinated at 700 ^oC for 2 hours. The calcined product was kept in sealed container bottle and were utilized for further characterization.

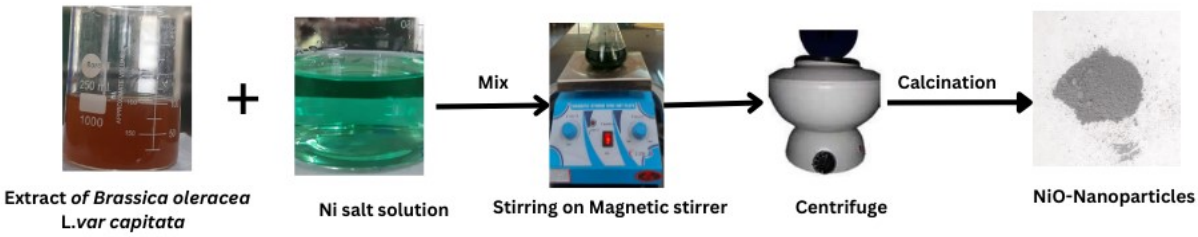

Fig.1-Schematic representation of preparation process of NiO-NPs

2.4. Fabrication of NiO electrode

The preparation of NiO electrodes involved the use of grade 304 stainless steel (SS) as the substrate. Prior to its application, the SS was subjected to a cleaning process with detergent and subsequently immersed in 4N HNO_3. This was followed by a series of washings with acetone and deionized distilled water (DDW). The cleaned substrate was then dried in an oven. A doctor blade was employed to apply a uniform layer of a mixture consisting of the active material (the prepared electrode material), poly (vinylidene fluoride) (PVDF), and carbon black (acetylene black) in weight ratios of 75%, 15%, and 10%, respectively, in dimethyl formamide (DMF) onto the stainless-steel substrate. The electrodes were then dried overnight at a temperature of 60 °C(Ezhilarasi et al., 2016; Karpagavinayagam et al., 2022). After this drying process, the weight of the electrode, measuring 1 x 1 cm^2, was found to be approximately 2 mg.

Electrochemical analysis was performed using the EmStat4S. LR electrochemical workstations and the Potentiostat-Galvanostat model PG12110 (Techno Science, Instrument). For the electrochemical characterization, an electrochemical cell was constructed with the fabricated electrode serving as the working electrode, a platinum electrode as the counter electrode, and an Ag/AgCl electrode as the reference electrode. A 1 mol/L Na_2SO_4 aqueous solution was utilized as the electrolyte. The electrochemical assessments of the prepared electrodes were conducted using various techniques, including cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS). The specific capacitance (Cs, Fg^{-1}) was determined using the CV and GCD methods, as
outlined in equations (1) and (2), respectively. Additionally, the energy density (ED, $Whkg^{-1}$), power density (PD, Wkg^{-1}), and Coulombic efficiency were calculated using equations (3), (4), and (5), respectively.

$$Cs = \frac{1}{mv(Vmax - Vmin)} \int_{Va}^{Vc} I(v)dv \quad (1)$$

$$Cs = \frac{Id \times td}{\Delta vm} \quad (2)$$

$$E = \frac{0.5 \times Cs(V^2max - V^2min)}{3.6} \quad (3)$$

$$P = \frac{E \times 3600}{td} \quad (4)$$

$$\eta = \frac{td}{tc} \times 100 \quad (5)$$

Where 'V' refers to the scan rate, and'(V_{max} - V_{min})' denotes the operational potential window in volts. 'V_{max}' represents the cathodic current, while 'V_{min}' represents the anodic current. The variable 'm'indicates the mass deposited in grams, and 'I(v)' represents the current in milliamperes (mA). The Id represents the current density ($mAcm^{-2}$) and td and tc represent the discharge and charge time of the electrode in GCD curve, respectively.

3. Characterization Techniques

The crystal structure of green synthesized NiO-NPs was confirmed by powder X-ray diffraction having Rigaku Mini Flex 600 X-Ray diffractometer. It operated at a voltage of 40 kV and a current of 50 mA with Cu-K radiation over a 2θ range of 20° to 90°. The UV-visible spectrum was recorded by JASCO V-750 of Serial No. D084261799 having accessory USE-753 ranging in 200-900 nm. The functional group of synthesizes NiO-NPs were identified by

SHIMADZU FT-IR having frequency range in 400-4000 cm^{-1}. The surface morphology of green synthesized NiO-NPs was identified by scanning electron microscopy (SEM, model-JSM-IT500LA, Jeol Japan), elemental detection spectroscopy (EDS), high resolution scanning electron microscopy (HR-TEM, JEOL ASIA PTE-LTD, model JEM 2100 PLUS).

3.1.Antimicrobial Activity

The green synthesized NiO-NPs were examined for their antibacterial activity against Gram +ve *Staphylococcus aureus* (NCIM2079), *Bacillus subtilis* (NCIM2250) and Gram –ve *Pseudomonas aeruginosa* (MTCC3541), *Escherichia coli* (NCIM2065) by using agar diffusion method (Disc diffusion method). Overnight grown culture was prepared in the nutrient agar, composition of nutrient agar is peptone, yeast extract and NaCl. Loop full of culture was spread on nutrient agar plate. The zone of inhibition in mm was then calculated by Vernier Calliper.

3.2.Anticancer Activity

Cytotoxicity of the NiO-NPs on A-549 cell line was determined by NRU Assay. The cells (5000-8000 cells/well) were cultured in 96 well plates for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. Next day, medium was removed and fresh culture medium was added to each well of the plate. 5 µl of treatment dilutions of different concentrations (0-1000 µg/ml final concentration) were added to the defined wells and treated plates were incubated for 24 h. 10 µl of NRU (40 µg/ml in PBS) per 100 µl medium was added and incubated for 1 h. After that medium was removed then NRU was dissolved in 100 µl of NRU Destain solution. Finally, plates were read at 490/660 nm.

4. Result and Discussion

4.1.XRD Study of NiO-NPs

The powdered XRD pattern of green synthesized NiO-NPs from *Brassica oleracea L. var. capitata* are as shown in Fig.2. All the peaks appearing at 37.24, 43.29, 62.85, 75.38, 79.37 can be indexed to (111), (200), (220), (311) and (222) planes respectively which are very well matching with JCPDS Card no.04–0835. FCC (Face centered cubic) crystalline Bunsenite structure of NiO-NPs was observed. The crystallite size (*D*) was found to be 16.68 nm according to Scherer equation.

$$D=\frac{0.89\lambda}{\beta cos\theta}$$

Where λ is the wavelength of the x-ray, θ is the Bragg diffraction angle and β is the FWHM (Kaviyarasu et al., 2016). Lattice strain and interplanar distance (d) were found to be 0.00586 and 1.094 respectively. Besides these no impurities were observed which suggested the high purity of monophasic NiO nanoparticles. The reported literature studies indicate that the crystal size of green synthesized NiO nanoparticles was in between 8-43.9 nm (Kuchekar et al., 2018; Nouneh et al., 2011).

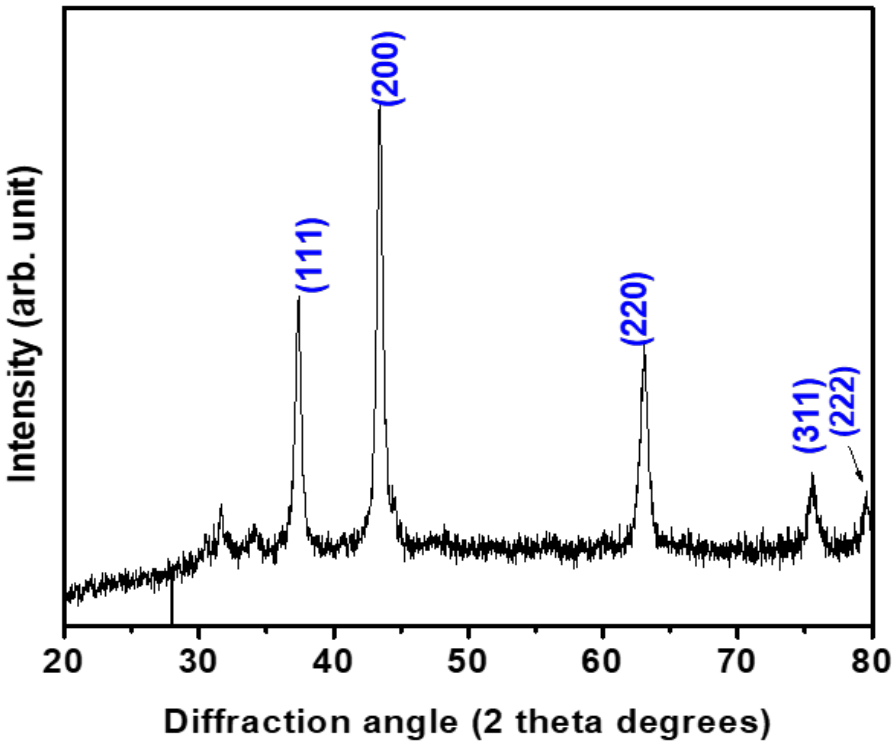

Fig.2- XRD pattern of green synthesized NiO-NPs

4.2. UV-Visible Analysis of NiO-NPs

NiO-NPs synthesized using *Brassica oleracea* L. *var. capitata* were analyzed using UV spectroscopic technique and the results obtained are shown in Fig.3. The synthesis of NiO-NPs using the green route was confirmed by observing the color change from green to dark brown which was further confirmed by UV-visible analysis. The results reveal emergence of three important peaks obtained at wavelength 545 nm, 589 nm and 636 nm. *(Kuchekar et al., 2018)* reported the absorption band at 656 nm owing to surface plasmon resonance. It was noticeable that broadening of the peak was evident at the 374 nm to 422 nm range corresponding to the SPR of nickel (Khalil et al., 2017).

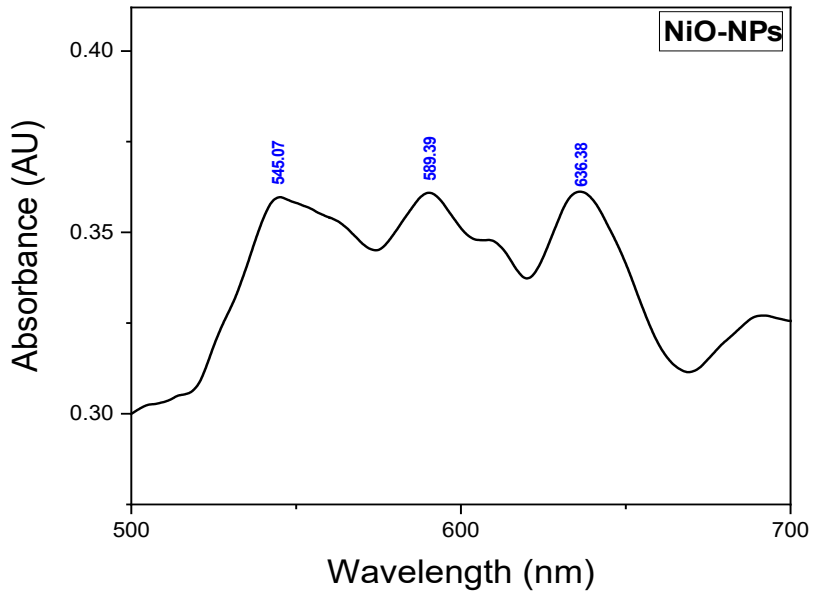

Fig.3- UV-visible Absorption Spectra of NiO-NPs

4.3. FT-IR Study of NiO-NPs

Fourier transform infra-red (FT-IR) analysis was carried out to identify the different functional groups present in NiO-NPs synthesized by *Brassica oleracea L. var. capitata* extract. The FTIR spectrum of synthesized NiO-NPs is shown in the Fig.4. The bands at 3026 cm^{-1} were corresponding to the –OH stretching. The band at 2950 cm^{-1} is due to stretching of C-H or N-H. The band at 1321 cm^{-1} corresponds to C-O stretching. The band at 1207 cm^{-1} and 1100 cm^{-1} corresponds to C-O and C=C stretching of aromatic ring and polyphenol. The band at 880 cm^{-1} can be ascribed to M-O bond stretching. The presence of -OH group indicates the presence of water molecule on NiO-NPs. (Huang et al., 2021; Iqbal et al., 2020; Uddin et al., 2021).

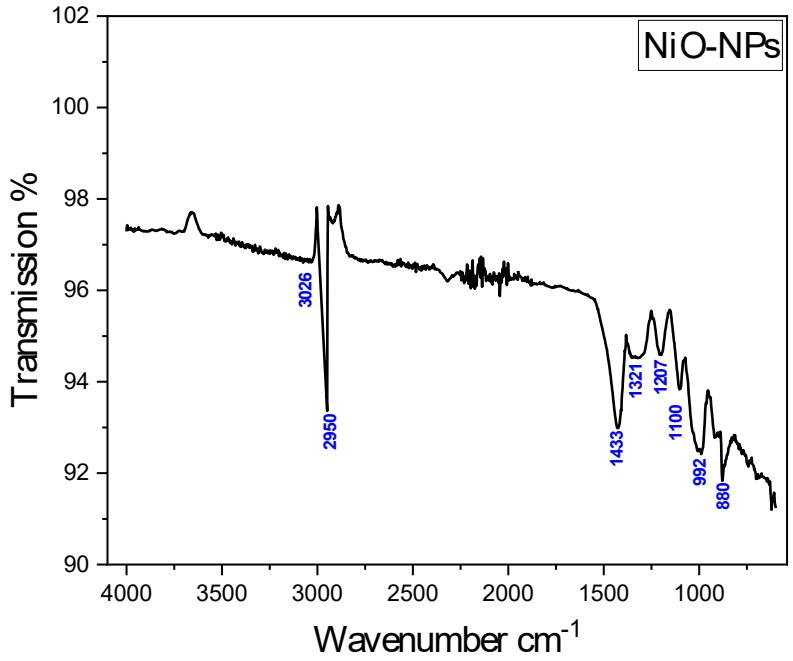

Fig.4-FT-IR Spectrum of Ni-NPs from *Brassica oleracea L. var. capitata*

4.4.Elemental Analysis of Green synthesized NiO-NPs

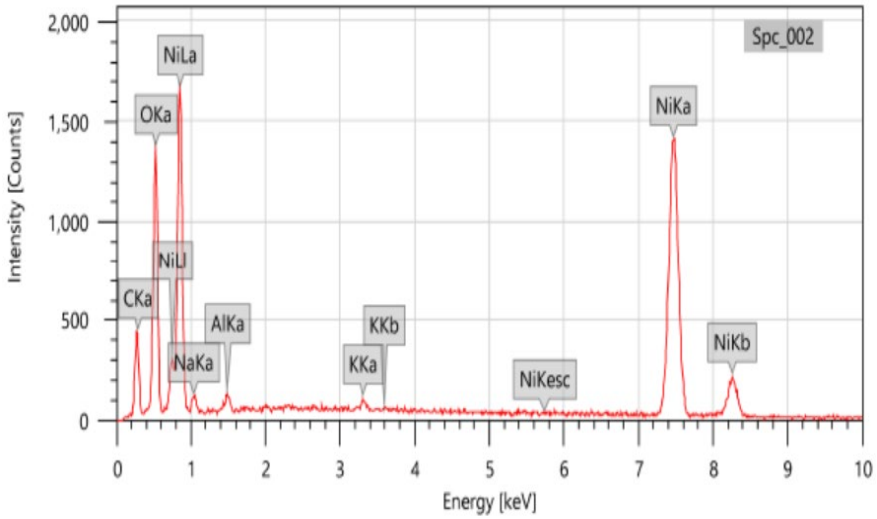

Fig.5- EDS of NiO-NPs

The quantitative and qualitative analysis of elemental composition was determined by energy dispersive spectroscopy (EDS) technique. Fig.5 showed the EDS elemental mapping of of NiO-NPs. The NiO-NPs exhibit stronger absorption peaks because of the surface plasmon resonance**.** In the EDS pattern, peaks pertaining to nickel (Ni) and oxygen (O) elements were predominantly observed suggesting the successful formation of NiO-NPs. The presence of carbon and oxygen can be ascribed to the organic compounds present in the plant extract (Alamgeer et al., 2018). The elemental composition of green synthesized NiO-NPs by using *Brassica oleracea L. var. capitata* are shown in table-1 which are in close agreement with the elemental composition of NiO reported by (Pandian et al., 2015).

Table-1- Elemental Composition of Synthesised NiO-NPs

Element	Line	% Mass	Atom%
C	K	15.87±0.13	34.20±0.29
O	K	22.88±0.20	37.02±0.32
Na	K	1.90±0.09	2.14±0.10
Al	K	0.74±0.04	0.71±0.04
K	K	0.42±0.03	0.28±0.02
Ni	K	58.19±0.41	25.66±0.18
Total		100.00	100.00

4.5.Particle Size Study of NiO-NPs

The dynamic light scattering was used to determine particle size of green synthesized NiO-NPs using *Brassica oleracea* L. *var. capitata*. The average particle size was found be 429.2

nm as shown in Fig.6. Sudhasree S. *et al(Sudhasree et al., 2014; Uddin et al., 2022)* reported that average particle size of Ni-NPs by green and chemical methods were found to be 2695 nm with PDI of 1.31 and 2453 nm with PDI of 0.401 respectively.

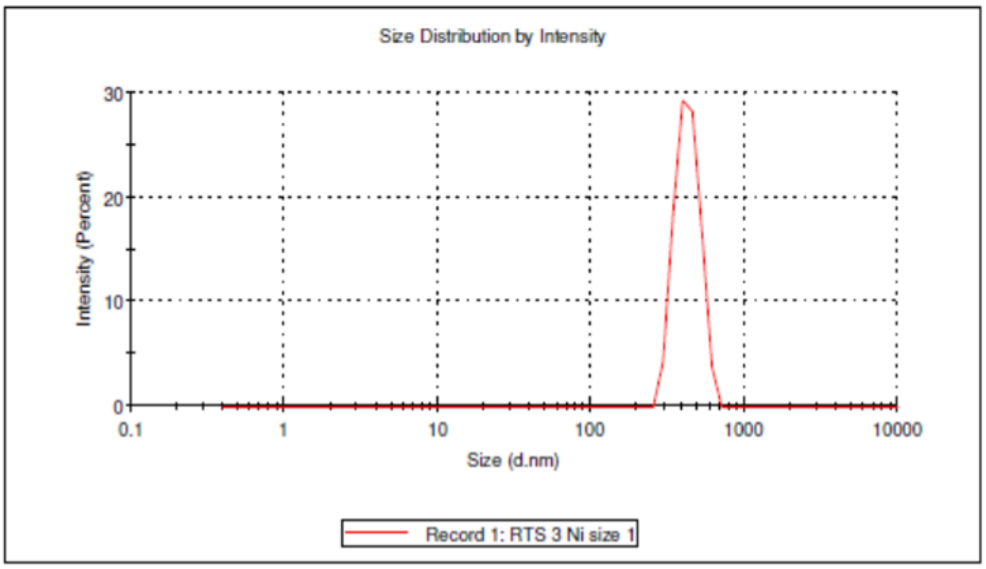

Fig. 6- Particle size distribution of green synthesized NiO-NPs

4.6.SEM Study of NiO-NPs

Low- and high-resolution SEM images of as-synthesized NiO-NPs are depicted in Fig.7 (a-d). Low resolution images reveal that the NiO nanoparticles exhibit agglomerated structures. More insights into these agglomerated structures can be found by close observation of high-resolution SEM images as shown in Fig.7 (c-d). It can be seen that the agglomerated structures are formed due to clustering of small nanoparticles. In the literature it was reported that agglomerated structure may be due to the magnetic interaction and polymeric adherence of NPs(Uddin et al., 2022).

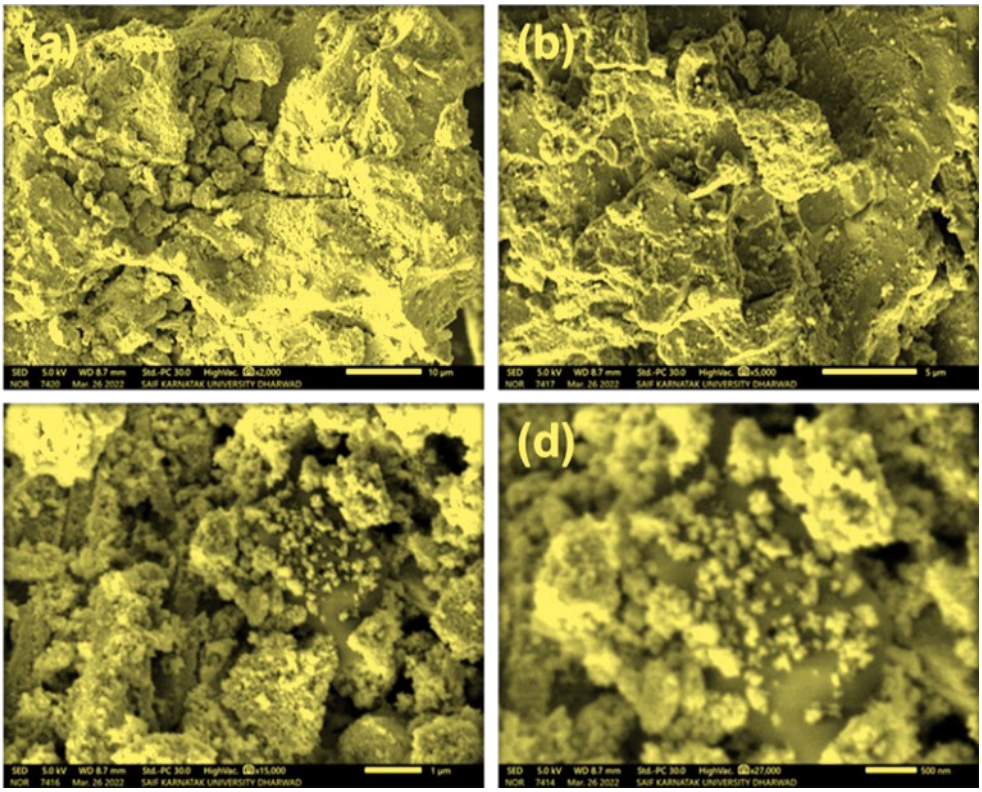

Fig.7 (a-d). Low- and high-resolution SEM images of synthesized NiO-NPs

4.7.TEM Study of NiO-NPs

The morphological investigations were further confirmed by studying the synthesized NiO-NPs by TEM. Fig.8-(a-b) shows low- and high-resolution TEM images, in which it is clearly observed that the synthesized NiO nanoparticles exhibits spherical morphology with variable sizes ranging from 5-50 nm. Here in present case the particle sizes obtained using PSD and TEM varies significantly from 5-50 nm for TEM to 490 nm for PSD. These results can be attributed to the green synthetic architecture employed for the synthesis of NiO nanoparticles. It has been reported that the extract of medicinal plants are rich source of phytochemicals compounds (Iqbal et al., 2020) These phytochemicals act as a fuel to synthesize smaller dimensions nanoparticles (Al-Zaqri et al., 2022; Moradnia et al., 2024).

Although several techniques have been used to measure the size of the particles, each technique has its own advantage and limitation(Domingos et al., 2009). TEM expresses the primary particle size of particles under study. However, DLS measurements are often done in order to determine the true state of particles in media especially if they will be applied/studied in media. Herein present, since the NiO particles have been synthesized using green method it might be tendency of these particles to agglomerate in an aqueous solution and preserving their monodispersed owing to the surface charge and functional groups present on them. Another reason for significant difference between the particle sizes observed by DLS and TEM can be attributed to DLS being an intensity-based technique, and this puts higher emphasis on the larger particle sizes whereas TEM is a number-based technique, and will thus show stronger emphasis of the smallest components in the size distribution.

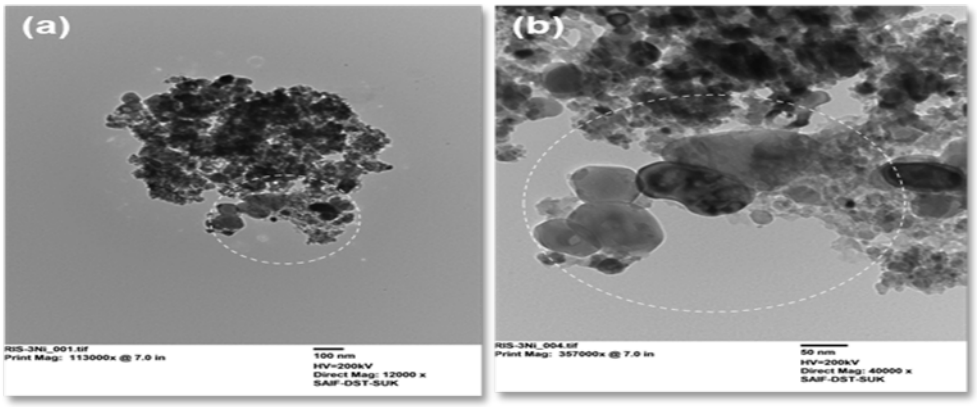

Fig.8- (a-b). Low- and high-resolution TEM images of synthesized Ni-NPs

4.8.Antimicrobial Assay

4.8.1. Antibacterial Activity

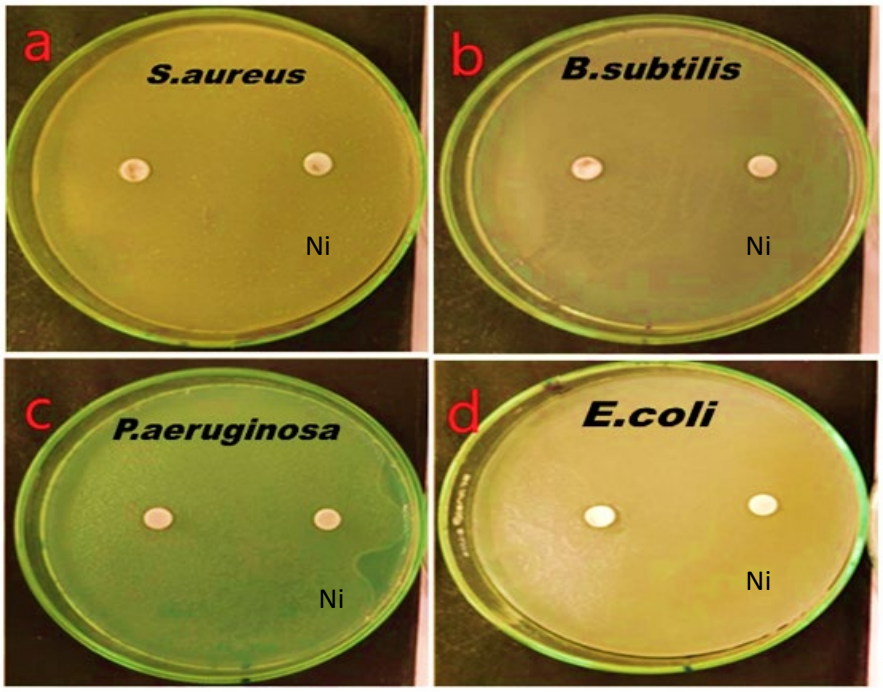

Fig.9 (a-d)-Antibacterial activity of green synthesized NiO-NPs against *S. aureus, B. subtilis, P. aeruginosa, E.coli*

Green synthesized NiO-NPs from extract of *Brassica oleracea* L. *var. capitata* were evaluated for their potential killing efficiency of both Gram positive (*S. aureus, B. subtilis*) and Gram negative (*P. aeruginosa, E.coli*) bacteria by performing disc diffusion method. The chloraimphenicol is used standard antibiotic as a positive control. The zone of inhibition resulting from green synthetic nanoparticles was used to prove the bactericidal efficiency. The synthesized NiO-NPs from extract of *Brassica oleracea* L. *var. capitata* showed that good bacterial activity of Gram positive *B. subtilis* whereas Gram negative bacteria i.e. *P. aeruginosa, E.coli* was showed that good bacterial activity as compared to Gram positive bacteria. NiO-NPs shows good antibacterial activity against Gram-ve bacteria *P. aeruginosa*.

The precise mechanism underlying the bactericidal activity of nanoparticles (NPs) has not yet been elucidated. However, it is hypothesized that these nanoparticles interact with bacterial cells, leading to alterations in the morphology of the cell membrane and an increase in permeability. This disruption interferes with the normal transport processes across the plasma membrane, ultimately resulting in cell death. Evidence suggests that biosynthesized nickel oxide nanoparticles (NiO-NPs), characterized by their small size and high stability, possess a large surface area to volume ratio. This property enhances their interaction with bacterial cells, thereby increasing their bactericidal efficacy through the release of Ni^{2+} ions. These ions can easily penetrate the cell wall, subsequently disrupting the electron transport system and affecting cellular components such as proteins and DNA, while also inducing oxidative stress, which ultimately leads to cell damage. Consequently, the bactericidal activity and stability of NiO nanoparticles are highly effective, indicating their potential application in antimicrobial coatings for various environmental and biomedical uses. (Nagajyothi et al., 2014). The overall mechanism is schematically represented in **Fig.10**

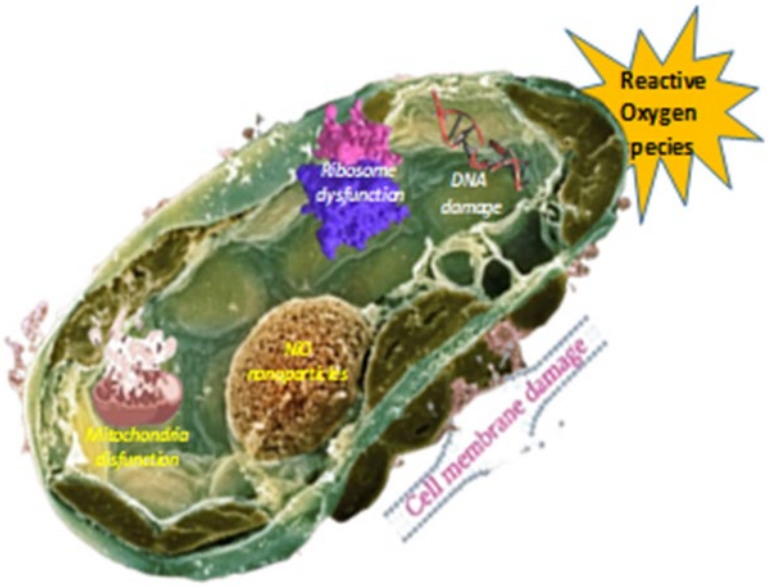

Fig. 10. The schematic diagramme representing proposed antibacterial mechanism of NiO-NPs

The anti-bacterial zone of inhibition in millimetre of NiO-NPs are shown in table-2. The data shows that NiO NPs contribute to the antimicrobial activity for the studied microorganisms. Efficacy of the NiO NPS is compared with previous studies in **Table 3.** It can be seen from the data that the green synthesized pristine NiO NPs exhibit comparable biological activity.
The anti-bacterial zone of inhibition in millimetre of NiO-NPs are shown in table-2.

Table-2- Antibacterial activity zone diameter of NiO-NPs

Sr. No	**Name of NPs**	**Zone of inhibition in millimetre (mm)**			
		S.aureus	***B.subtilis***	***P.aeruginosa***	***E.coli***
1	NiO-NPs	-	9.44	9.53	9.31

2	Chloramphenicol Standard sample	19.45	24.55	21.30	27.1

Table 3. Antimicrobial activity of NiO NPs against gram-positive (*S. aureus*) and gram-negative (*S. typhimurium*) compared with previous studies.

Sr. No.	Nanomaterial employed	Synthesis method used	Bacteria used for study	Observed Amoxicillin Zone of inhibition (mm)	References
01	Chitosan-based nanocomposite films incorporated with NiO nanoparticles	Solution combustion synthesis method	gram-positive (S. aureus)	17.61 ± 0.39	(Marand et al., 2021)
			gram-negative (S. typhimurium)	11.47 ± 0.04	
02	Cobalt doped NiO nanoparticles	*Murraya koenigii* mediated green synthesis	gram negative (Klebsiella pneumonia, Shigella dysenteriae)	15.5 & 17	(Elamin et al., 2022)
			gram positive (Staphylococcus aureus, and Bacillus)	7 & 14	
03	Nanostructured Ag/NiO composite	Using *W. somnifera* leaf extract (WSLE)	human pathogen Klebsiella pneumoniae	15.4	(Chinnaiah et al., 2024)
04	Nanostructured CdO-NiO composite	microwave-assisted precipitation method	Gram-negative bacterium Bacillus subtilis	27	(Karthik et al., 2018)
05	NiO nanoparticles	Using *Brassica oleracea L. var. capitata* extract	Gram positive *B. subtilis*	9.44	Present study
			Gram negative *P. aeruginosa E. coli*	9.53 & 9.31	

4.9. Anticancer Activity

The cytotoxic potential of NiO nanoparticles was evaluated using the Neutral Red Uptake (NRU) assay on A549 cancer cells. Exposure to NiO nanoparticles at concentrations ranging from 1 to 1000 µg/ml for 24 hours resulted in a concentration-dependent decrease in cell viability. The results of NiO showed that the cell viability is slightly decreased at a concentration of 10 mg/ml. A significant reduction of approximately 60% in cell viability was observed at the highest concentration (1000 µg/ml). These findings indicate that NiO nanoparticles exhibit cytotoxic effects by compromising lysosomal integrity, as assessed by the NRU assay. The observed cytotoxicity suggests that biosynthesized NiO nanoparticles endorse further investigation as potential anticancer agents.

Fig.11 showed that more than 50% of cell death at 1000 µg/ml of nanoparticles. The cytotoxic effects induced by nanoparticles at lower concentrations due to which the plant components

attached to the nanoparticles. The reduction in cell viability associated with elevated concentrations of NiO nanoparticles indicates a notable cytotoxic effect, resulting in the accumulation of internal cellular stress and ultimately culminating in apoptosis(Ahmad et al., 2015; Vani et al., 2018). The results obtained from this research work are also very well supported with various evidences for the cytotoxic effect of green synthesized metal nanoparticles by using *annona squamosa* leaf extract against the breast cancer mcf-7 cell line(Vivek et al., 2012). *Piper longum* leaf extracts against hep-2 cancer cell line (Jacob et al., 2012) and *morinda citrifolia* against hela cell lines in-vitro (Meli et al., 2019). The table 4 cytotoxicity effect of NiO-NPs on cancer cell by NRU assay A549.

Table 4-Cytotoxicity effect of NiO-NPs against cancer cell

Sr. No.	Name of Nanoparticles	IC50 value (µg/ml)
1	NiO-NPs	326.4

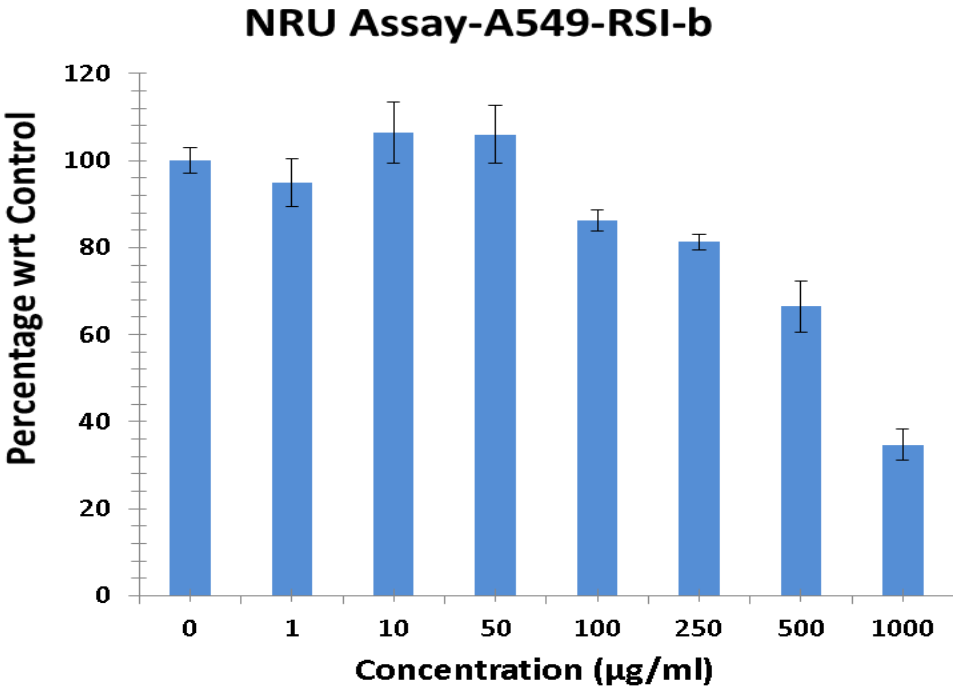

Fig.11- Anticancer Potential of NiO-NPs

4.10. Supercapacitor Study of as-synthesized NiO-NPs

Utilizing 1 M Na_2SO_4 as the electrolyte, cyclic voltammetry tests have been carried out. The cyclic voltammetry tests have been carried out on NiO electrodes in the potential range of 0 to 0.5 V. At the electrode/electrolyte interface (electric double layer) and electrode surface (via redox reactions), electrodes in an alkaline electrolyte store charge. The CV curve of NiO electrodes is shown in Fig.12 (a) for various scan rates. The Faradic nature of the NiO is confirmed by the clearly marked redox peaks on CV curves as opposed to the perfect rectangular shape of EDLC. Their corresponding anodic and cathodic scans do not have symmetric oxidation and reduction peaks. This is because the redox reaction is kinetically irreversible. The following redox process produces the charge storage function of NiO electrodes: electrodes in the potential range of 0 to 0.5 V. Galvanostatic charge-discharge curves at different current densities are shown in Fig.12 (b), and the specific capacitance obtained from CV at 5 mVs^{-1}, 10 mVs^{-1}, 25 mVs^{-1}, 50 mVs^{-1} and 100 mVs^{-1} 226 Fg^{-1}, 201.3 Fg^{-1}, 159.0 Fg^{-1}, 115.66 Fg^{-1} and 78.82 Fg^{-1} respectively.

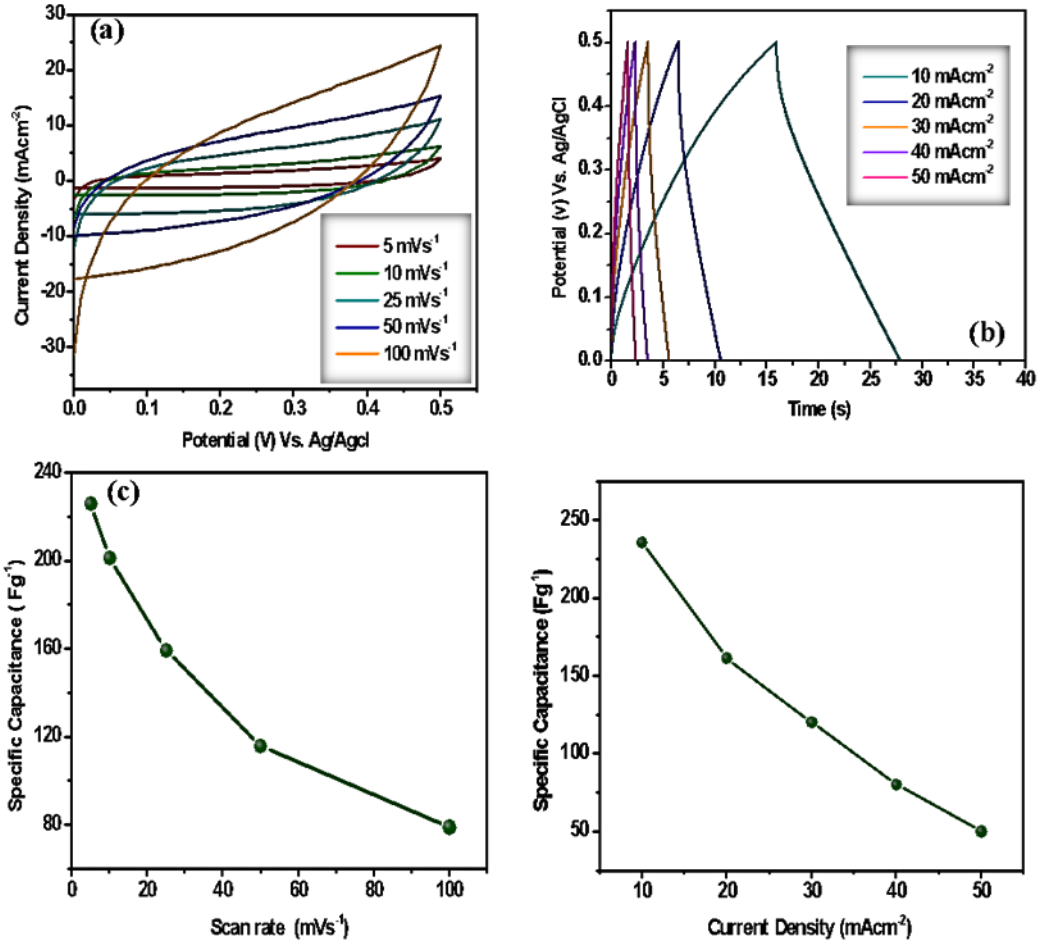

Fig.12- (a) Cyclic voltammetry curves at different scan rates, (b) Galvanostatic charge-discharge curves at different current density. (c) Specific capacitance Vs. Scan rate (d) Specific capacitance Vs. Current density

Moreover, the variation of specific capacitance with scan rate is demonstrated in Fig.12 (a-d). The specific capacitance, energy density, and power density of NiO Supercapacitor electrodes calculated from the Galvanostatic discharge curve is shown in Table-5. Fig.13 shows the Nyquist plot for the NiO electrode. From this graph, the electrolyte resistance and charge

transfer resistance for NiO electrodes in 1 Molar Na_2So_4 electrolyte is obtain 0.8 Ohm cm^{-2} and 3.2 Ohm cm^{-2}, respectively, the low electrolyte resistance and charge transfer resistance of NiO electrodes are highly desirable for the higher electrochemical performance of the electrode material. The obtained low values of electrolyte resistance, charge transfer resistance, high specific capacitance and energy density for NiO electrodes demonstrated the patentability of NiO in terms of its applicability as an electrode for supercapacitor applications. Table-5 the specific capacitance, energy density and power density of NiO- Supercapacitor electrode calculated from Galvanostatic discharge curve.

Table 5- Specific capacitance, energy density and power density of NiO-NPs

Current density ($mAcm^{-2}$)	**Specific capacitance (Fg^{-1})**	**Energy density (Wh Kg^{1})**	**Power Density (Wkg^{-1})**
10	235.4	5.23	1600
20	161.2	3.58	3200
30	120.2	2.66	4800
40	80.1	1.78	6400
50	50.6	1.1	8000

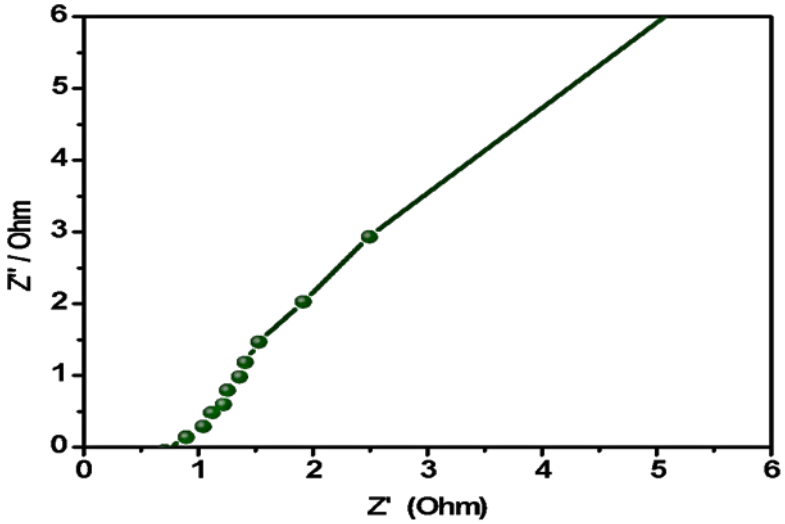

Fig.13- Nyquist Plot of NiO-NPs Electrode

A comparative summary of calculated specific capacitance with previous literature is provided in **Table 6** At the different current densities, the potential window for as-prepared NiO electrodes linearly increases during the charging process and linearly decreases during the discharge process, revealing good electro chemical properties. The as-prepared NiO NPs has spherical morphology, these special features provide more electroactive surface area for ion transportation.

Table ~~6~~: Comparative study of capacitive performance of as-synthesized NiO NPs electrode with literature reports

Sr No	Electrode Material	Method of synthesis	Electrolyte	Sp. capacitance (F g^{-1}), scan rate (mV s^{-1})/ current density (A g^{-1})	Ed (Wh kg^{-1})	Columbic efficiency (%), cycles,	Ref.
01	Cu_2O@MnO_2@NiO	Green synthesis using *Sarcophrynium Brachystachys* leaf extract	1.0 M Na2SO4	1.00 mV/s scan rate, 1092 F/g	12.74 W/kg at 0.5 A/g	-	(Obodo et al., 2024)
02	NiO@MnO_2 nanoparticles	Hydrothermal	0.5 M Na2SO4	343 (F g^{-1})	energy density of 7.62 Wh/kg with a power density of 249 W/kg	-	(Islam et al., 2024)
03	NiO bio-composite materials	Co-precipitation method	6.0 M KOH	348 F g^{-1}	-	-	(Avinash et al., 2021)
04	NiO/ZnO Nano-composite	Hydrothermal and subsequent calcination process	3 mol L^{-1} KOH	723 F g–1	-	75, 3000	(Zhu & Shao, 2018)
05	NiO NPs	Green synthesis using *Brassica oleracea* L. *var. capitata*	1 M Na_2SO_4	235 F g-1	5.23WhKg-1 with a power density of 1600 W/kg	-	Present study

5. Conclusion

In this research work, we have successfully synthesised NiO-NPs from an extract of *Brassica oleracea* L.*var.capitata*. Formation of NiO-NPs were confirmed by a change in the colour of the solution by UV-visible spectroscopy. The FT-IR spectrum shows different functional groups in synthesised NiO-NPs. The morphological study utilizing SEM and TEM revealed formation of spherical morphology. XRD results revealed successful formation of NiO nanoparticles utilizing green method. Particle sizes were determined using PSD which was found out to be 490 nm. The as-synthesized NiO NPs exhibited competing antibacterial and anticancer activities. Further, the as-synthesized NiO NPs were also employed for supercapacitor applications. The results demonstrated that, NiO electrodes exhibit superior electrochemical characteristics, including high specific capacitance, favourable energy, power densities, and low resistive losses. These findings highlight the potential of NiO as an efficient and robust electrode material for supercapacitor applications. The demonstrated high energy, power density performance, and low internal resistances position NiO as a promising candidate for future energy storage technologies. Further research and development could optimise these properties, making NiO-based supercapacitors viable for a wide range of practical applications in energy storage and management.

Author Contribution

Ruqquaiya Shaikh- Writing original draft, Investigation

Arif Pathan - Data Curation, Resources

Mohd. Anis – Interpretation of characterization data

Satish P. Mardikar – Investigation, resources, software
M. I. Baig - Data Curation
Mazahar Farooqui - Data Curation, Resources
R. D. Isankar – Conceptualization, methodology

Conflicts of Interest
There are no conflicts to declare.

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