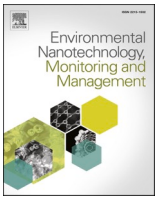
Environmental Nanotechnology, Monitoring & Management 18 (2022) 100674

Contents lists available at ScienceDirect

Environmental Nanotechnology, Monitoring & Management

journal homepage: www.elsevier.com/locate/enmm



A review on current trends in the green synthesis of nickel oxide nanoparticles, characterizations, and their applications

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ARTICLE INFO

Keywords:
Green synthesis
Nanoparticles
Phytochemicals
Plant extract

ABSTRACT

Over the current decade, nanotechnology has been most promising research field, showing encouraging applications in the photonics, electronics, medical, and catalyst industries. The nanoforms of metals/metal oxides and nanocomposites make them more effective and efficient tools than their bulky counterparts due to their specific physical, chemical, and optical properties. Recently, nickel oxide nanoparticles (NiO NPs) are gaining much more attention in the different research field due to their unique properties. NiO NPs were synthesized by physical and chemical methods, utilizing toxic chemicals as precursors. NPs so formed also have these toxic chemicals attached to their surfaces, thus reducing their biocompatibility, and making them biologically inactive. NPs synthesized using biological extract like, bacteria, fungi, plant extracts which have biomolecules act as chelating, stabilizing and capping agent, thus, forming bioactive and biocompatible nanomaterials. Therefore, the present review summarizes various green methodologies involved in the synthesis of NiO NPs, typical analytical techniques used for their characterizations, and their potential applications in different fields. Role of NiO NPs in shaping our day-to-day technologies has also been touched upon briefly.

1. Introduction

In the past few decades, nanotechnology has made tremendous progress. It deals with the utilization and production of materials at nanoscale dimensions. It can be defined as the ability to build devices, systems, and materials with atomic precision (Ahmad et al., 2020). The United States National Science foundation defines nanotechnology as studies of systems and materials having following key features:-

1. Dimensions: at least one dimension is between 1 and 100 nm.
2. Process: designing by processes which show fundamental control over physical and chemical properties of molecular structures.
3. Building block property: ability to combine and form larger structures (Alinezhad et al., 2020).

The significance of nanoscale science and technology is based on the fact that materials at nanoscale have properties (i.e., chemical, optical, mechanical, magnetic, and electrical) quite different from bulk material. In comparison to the bulk materials, nanomaterials show enhanced performance properties when they are used in similar applications. Nanotechnology has both present and futuristic applications in wide varying fields including biology, medicine, electronics, bottom-up technology (such as self-assembly), energy, environmental studies, etc. (Pakzad et al., 2019).

Nickel Oxide (NiO) is a transition metal oxide with cubic lattice structure (Hatamifard et al., 2016). It is a p-type semiconductor (Current is carried by positive holes) and is rarely stoichiometric (there are a small number of Ni^{+3} ions in the crystal lattice as well as Ni^{+2} ions) (Motahharifar et al., 2020). It has wide band gap, good thermal-chemical stability, and is anti-ferromagnetic in nature (Nasrollahzadeh et al., 2021).

Nickel oxide nanoparticles (NiO NPs) synthesis and their properties form an important area of research due to their superior ferromagnetic properties, high coercive forces, and chemical stability. NiO NPs form an

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https://doi.org/10.1016/j.enmm.2022.100674
Received 2 December 2021; Received in revised form 24 January 2022; Accepted 1 March 2022
Available online 8 March 2022

important part of present-day technology. They are used as cheap catalysts due to their natural abundance and ability to drive reactions through alternate pathways. They have innumerable biomedical applications including magnetic resonance imaging, cell separation, drug delivery, biomedical detection etc. (Jaji et al., 2020). Significant antibacterial properties have been reported against *Klebsiella pneumoniae*; *Pseudomonas aeruginosa* and *Bacillus subtilis*, *S. aureus* and many more (Yulizar et al., 2021; Iqbal et al., 2019). Similarly, Uddin et al. (Uddin et al., 2021) Reported antifungal activity of NiO NPs against *Aspergillus Niger*; *Alternaria altemata*, and *Fusarium oxysporum*. Many studies have successfully tested anti-cancerous and antiparasitic properties of NiO NPs. These include cytotoxicity study against A549 cell culture, impact of NiO NPs on Hela cancer cells, MCF-7 cancer line models and HepG2 cells (Lingaraju et al., 2020; Ariyanta et al., 2021; Angel Ezhilarasi et al., 2018; Ramalingam et al., 2019). Iqbal et al. showed NiO NPs can be antiparasitic against Leishman strains of Amastigotes, promastigotes, and Leishmania tropica. It also reported the compatibility of NiO NPs with macrophages and RBC's (IC_{50} value greater than 200 ug/ml). Studies have also revealed antioxidant nature of NiO NPs (Iqbal et al., 2019).

Apart from biomedical applications, NiO NPs show notable capacitance properties, thus acting as energy reservoir. Wastewater dye degradation is another field, where NiO NPs can be successfully employed. Photocatalytic degradation of 4-chlorophenol (endocrine disrupting chemical), Methylene blue, p-nitrophenol dye, Congo red etc. through NiO NPs has been noted (Haider et al., 2020; Yulizar et al., 2022). NiO NPs can also facilitate the seed germination; they are known to increase the seedling growth rate (Ariyanta et al., 2021). Thus, affirming their ability in modernizing agriculture. Fig. 1 displays the different applications of NiO NPs.

2. Methods involved in the synthesis of NiO NPs

In the field of nanotechnology, several different methods have been developed to synthesize NiO NPs, as shown in Fig. 2. Traditionally two techniques have been used for it. These are top-down approach and bottom-up approach. When breaking down bulk materials into nanoscale particles takes place, we say a top-down approach for NPs synthesis has been used. The most widely used methods in this approach include laser ablation, mechanical milling, nanolithography, sputtering and thermal decomposition etc. The major disadvantages of this approach are cost and difficulty to control the size of NPs. Thus, scientists prefer a bottom-up approach to synthesize NPs. In this approach, NPs are formed starting from atom-by-atom, molecule-by-molecule, and cluster-by-cluster (Uddin et al., 2021). Examples of this approach include, gas evaporation, co precipitation technique, solvent-gel etc. Generally, a hybrid of these techniques is used in the synthesis of NPs (Nasrollahzadeh and Mohammad Sajadi, 2016).

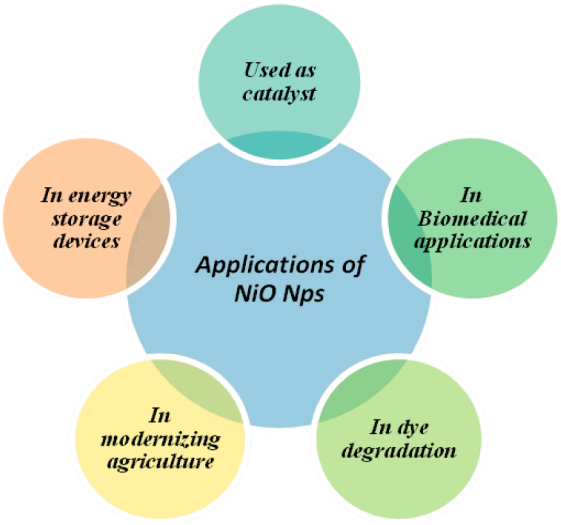

Fig. 1. Different applications of NiO NPs.

Syntheses of NiO NPs have been reported by various physical, chemical, and biological methods. Chemically for the synthesis of NiO NPs sol–gel technique is quite prominent. Zorkipli et al. synthesized NiO NPs using $Ni(NO_3)_2{\cdot}6H_2O$ as metal precursor, and isopropanol and ethylene glycol as chemical stabilizers. Surfactant TritonX-100 was also added to it to prevent agglomeration. Through continuous stirring (at pH 11) sol was formed, which was then heated to form gel. This gel was then dried at high temperature to produce NiO NPs (Zorkipli Mohd et al., 2016). Similar method has been used by Mahaleh et al. for the synthesis of NiO NPs by chemical precipitation method (Mahaleh et al., 2008). Kemary et al. through chemical reaction of Ni $(NO_3)_2{\cdot}6H_2O$ with hydrazine and alcohol (acting as complexing agents) formed Ni $(OH)_2$ as precursors which were then thermally decomposed to give NiO NPs (Kemary et al., 2013). Through the Co-precipitation method even without the use of external stabilizing, capping agents, or surfactants (Khandagale and Shinde, 2017), NiO NPs in basic solutions have been produced. Using polymerized complex technique Wongasprom et al. synthesized NiO NPs, in this method; citric acid was used as reagent and ethylene glycol as polymer stabilizer which also served as diffusion barrier (Wongsaprom and Maensiri, 2010). Interestingly, NiO NPs have also been synthesized using combustion route. In this study, Srikesh and co-workers fabricated NiO NPs through combustion route using glycine, glucose, and urea as fuels (Srikesh and Nesaraj, 2015).

An energy efficient method for the synthesis of NiO NPs was developed by Grabis et al. They utilized microwave assisted treatment of Ni $(NO_3)_2$ and urea solution for the synthesis of NiO NPs which were then calcined at 300–320 °C (Gabris et al., 2016).

In literature various physical methods have also been reported for the synthesis of NiO NPs. Laser ablation is one of the prominent methods for synthesis of NiO NPs. In this method the laser is used as an energy source, high energy produced by the laser is then concentrated at a specific point on a solid surface resulting in evaporation of light absorbing material. Laser ablation results in generation of high purity NPs (Nasrollahzadeh and Mohammad Sajadi, 2016). Quasi-spherical NiO NPs were fabricated by Mahdi et al. using laser ablation technique, dependence of their properties on laser energy (500, 600 and 700 mJ/pulse) were also determined (Mahdi et al., 2020). Among other approaches NiO NPs have also been synthesized by spray pyrolysis of water solutions of Ni precursors (e. g. nitrate, chloride, formate, or acetate) (Ukoba et al., 2018). Sputtering and chemical vapor deposition has also been used for the synthesis of NiO NPs (Moravec et al., 2011).

In the current technological scenario chemical and physical methods dominate the synthesis practices of NPs. But there are many disadvantages associated with them. Generally physical methods require costly vacuum systems or equipment to prepare NPs. Another disadvantage of physical and chemical methods of synthesis are arduous reaction conditions such as specific range of pressure and temperature, high energy demands, use of inflammable solvents etc. also, chemical, and physical processes are not eco-friendly, mainly due to hazardous chemicals used for their synthesis, low yield of reaction, toxic by-products, and high energy requirements (Nasrollahzadeh and Sajadib, 2015). Moreover, NPs so produced also have these toxic chemicals attached to their surfaces, thus reducing their bio compatibility, and making them biologically inactive. Therefore, in recent years there has been a growing shift towards greener nano-synthesis, or bio-based/bio-inspired approaches (Khan et al., 2021).

In biological synthesis methods, NPs are generally produced from plant extracts, bacteria, fungi together with the precursors rather than using the traditional chemicals for reduction, stabilization, and capping purposes. The NPs produced from this technique are biodegradable, non-toxic and possess enhanced properties for biomedical application (Nasrollahzadeh et al., 2015).

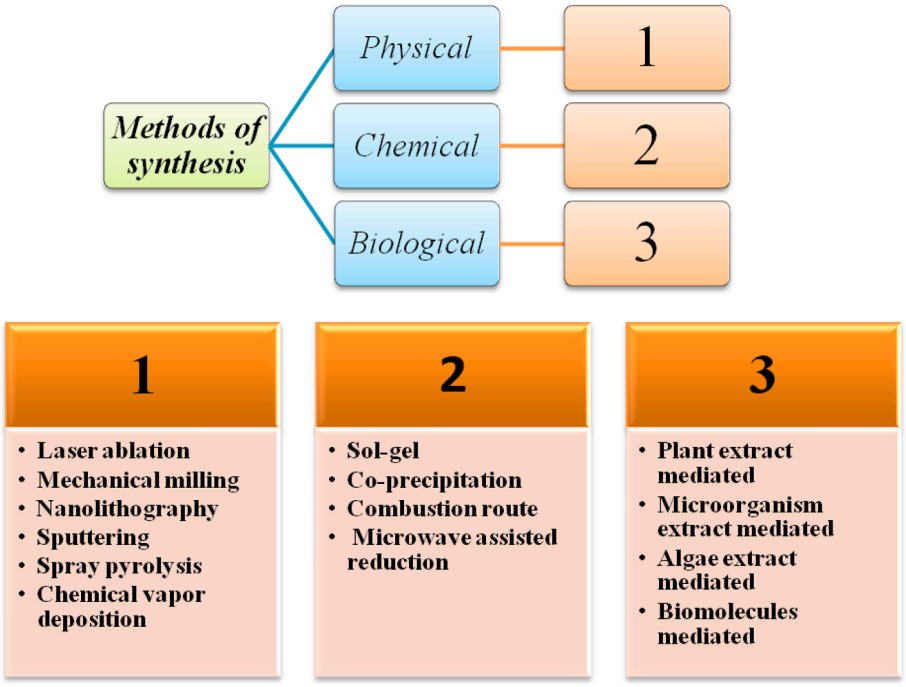

Fig. 2. Various available methods for the synthesis of NiO NPs.

In the biological method of synthesis, the reduction of metal ions takes place by the various biomolecules found in these extracts such as proteins/enzymes, polysaccharide, vitamins, amino acids. NPs produced are environmentally benign and the methods employed is cost-effective, safe, sustainable, and eco-friendly (Nasrollahzadeh et al., 2015).

3. Plant mediated synthesis of NiO NPs

Use of plant extracts for production of NPs has gained much attraction in the last 30 years. Various plant species have been reported in the biosynthesis of NPs. Plant mediated approach is gaining popularity as it is simple, ecofriendly, stable, rapid, and cost-effective. Generally, the plant mediated synthesis involves the addition of aqueous solution of metallic salts to the aqueous extract of plants, followed by continuous stirring for a few hours at room temperature (Nasrollahzadeh et al., 2015). Figs. 3 & 4 show the different mechanical steps for the NiO NPs synthesis from plant extract.

Different plants contain different composition and concentration of organic reducing agents. Therefore, in plant-mediated biogenic reduction of metal ions, a combination of water-soluble metabolites (like alkaloids, phenolic compounds, flavonoids and terpenoids) and coenzymes are used in the synthesis of NPs (Tajbakhsh et al., 2016). The Secondary metabolites like terpenoids, alkaloids, flavonoids and polyphenols act as a chelating agent. The hydroxyl group of flavonoids and

Fig. 3. Mechanism of the plant mediated synthesis of NiO NPs (Barzinjy et al., 2020; Imran Din and Rani, 2016).

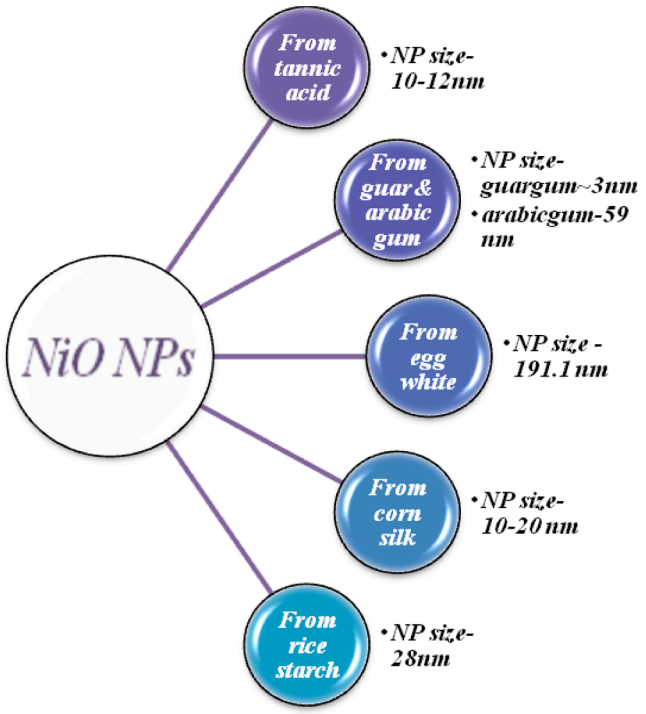

Fig. 4. Different steps involved in the synthesis of NiO NPs.

polyphenols act as capping agent and stabilize the synthesized NPs. The hydroxyl and carbonyl groups present in amino acid residue or in protein act as capping agent reduce agglomeration of NPs and stabilize them.

For the synthesis of NiO NPs a solution of metal precursor salt (generally $NiCl_2{\cdot}6H_2O$ or Ni $(NO_3)_2{\cdot}6H_2O$) is stirred vigorously with plant extract. This process generally takes place at higher temperature, certainly for a long period of time. After the reaction is complete, the mixture is then subjected to centrifugation. After centrifugation, the clear supernatant is discarded, and remaining pellets are collected. These pellets are then washed, oven dried and muffled to obtain nano-powder of NiO. For characterization of these NPs, various analytical techniques are used such as X-ray diffractometer (XRD), Fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray analysis (EDS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), particle size analyzer (PSA), and UV–visible spectroscopy (UV–Vis).

Habtemariam et al. synthesized NiO NPs using *V. amygdalina* leaf extract and $NiCl_2{\cdot}6H_2O$ salt solution as precursors (Habtemariam and Oumer, 2020). For this 50 ml of 0.1 M salt solution was prepared, to which 50 ml of leaf extract was added (1:1 ratio), at this point significant color change was observed. The resulting mixture was stirred using a magnetic stirrer for an hour under ambient conditions. After cooling down, the final mixture was centrifuged (at 3500 rpm) and the precipitate obtained was separated. The precipitate was washed with distilled water and ethanol, and oven dried. Finally, the powder is calcined at 500 °C for 2 h to collect NiO nano-powder. For characterization, XRD study, FTIR spectroscopy, and SEM were carried out.

NiO NPs have also been fabricated using root-based extract of *Raphanus sativus*. For obtaining root extract, dried roots were cut into small pieces and extract was then prepared in deionized water through a sterile grinder. The obtained crude extract was then filtered to remove impurities. To synthesize NiO NPs, 80 ml of 40 mM aqueous solution of $Ni(CH_3COOH)_2{\cdot}4H_2O$ was mixed with 20 ml of root extract. Resulting mixture was stirred for 10 min. pH of the solution was maintained at 10, and heating (at high temperature) and stirring was continued for another 30 min. After cooling for 12 h, the resulting mixture was washed with deionized water and ethanol. The solid product was oven dried and calcined in muffle furnace at 300 °C, 600 °C and 900 °C. The obtained nano-powder, for characterization is then subjected to XRD, study TEM, DRS, EDX and FTIR spectroscopy. NiO NPs were also analyzed for their antioxidant and antibacterial properties (Haq, 2021).

Using the stem of *Berberis Balochistanica* plant Uddin and co-workers were able to furnish NiO NPs. For the preparation of stem extract, the stem parts of the plant were dried in the oven (for 10 h at 40 °C). Later, the crushed fine powder (20.66 g) was thoroughly mixed with distilled water (200 ml). It was stirred for 12 h and then incubated in a water bath (40 °C) for 2 h. The extract was then filtered and centrifuged to remove the remaining aggregates. The prepared extract was then taken in a beaker. For the green synthesis of NiO NPs, 50 ml of extract was added with 0.3 M $Ni(NO_3)_2$ solution. The mixture was then magnetically stirred at 500 rpm for 3 hrs (60 °C). The resulting mixture was then centrifuged at 3000 rpm for 25 min (Fig. 3). The obtained pellets were then washed and incubated at 100 °C. Finally, the synthesized NPs were characterized and subjected for their antibacterial, antifungal, cytotoxicity, and stimulatory activities. Apart from leaf, root, and stem extract mediated synthesis of NiO NPs, fruit juice-based synthesis has also been reported. For instance, Ganganagappa et al. used *Limonia acidissima* natural fruit extract and $Ni(NO_3)_2{\cdot}6H_2O$ salt solution for the synthesis of NiO NPs (Kumar et al., 2021). Another interesting approach for green synthesis of NPs was developed by Mohseni et al. They reported the biosynthesis of Ni/montmorillonite nanocomposite using extract of *Allium jesdianum*. These nanocomposites were then used as nano-catalysts for the electrocatalytic oxidation of methanol (Hossein et al., 2020). Table 1 indicates the summary of used different plant extract in the NiO NPs synthesis with their obtained properties.

4. Microorganism mediated synthesis of NiO NPs

An alternate method for the synthesis of NPs is utilizing microbes as reducing agents for the metal ion, either through extracellular approach or through intracellular one. Microorganism based route is comparatively new but has shown great potential, as this approach is eco-friendly, cost-effective, less energy consuming and non-toxic. In general, intracellular synthesis involves the transportation of metal ions into the cells of microorganisms which are then reduced to metal NPs. This is made possible by the action of various enzymes such as nitrate reductase. In case of extracellular synthesis of metal NPs, metal ions located at the cell wall or present in the growth medium are reduced, again through reductase enzymes secreted by microorganisms. Metal ions in this case need not to be transported into the cells. Not all microorganisms can produce the metal NPs as they may be lacking the ability or enzymes to reduce metal ions. So, screening of microorganisms becomes a difficult task in microbes-based NPs synthesis. Generally, microorganisms found in metal rich habitats are chosen, as these metals are highly resistant due to their uptake and stabilization by extracellular or intracellular enzymes (Bahrulolum et al., 2021). In literature various microorganisms including bacteria, fungi, microalgae, and yeasts have been reported for the synthesis of NiO NPs.

NiO NPs have been produced via extracellular synthesis of NiO NPs from *microbacterium sp.* MRS-1 (Wardani et al., 2019). This bacterium was isolated from nickel electroplating industrial effluent and was then utilized for the conversion of soluble $NiSO_4$ to NiO NPs of size 100–500 nm. The cells of MRS-1 were then utilized for the treatment of Nickel containing industrial effluent. Nickel removal efficiency was reported to be 95%. This shows the potential application of MRS-1 cells in bioremediation of Nickel containing wastewater and its ability to synthesize NiO NPs. Similar bio-reduction of Ni (II) ions to elemental Ni through a strain of Pseudomonas sp. MBR has also been reported (Zhang and Zhang, 2012).

Various fungi species have also been employed in the synthesis of NiO NPs, such as the use of dead biomass of the fungus Hypocrea lixii as a biological system for the fabrication of NiO NPs (Salvadori, 2015). NPs

Table 1
Plant extracts used for the synthesis of NiO NPs with their shape, size, and other features.

Plant (Part)	Precursors	Crystallite structure and size	Shape of NPs	Size of NPs	UV Absorption (λmax)	Applications	Ref.
Vernonia amygdalina (leaf)	$NiCl_2{\cdot}6H_2O$	Cubic, 17.86 nm	Octahedral	–	–	-	(Habtemariam and Oumer, 2020)
Arabic gum (gum)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 43 nm	Spherical and agglomerated	59 nm	312 nm	Antibacterial, Photocatalytic degradation, cytotoxic properties	(Sabouri et al., 2021)
Berberis balochistani (stem)	$Ni(NO_3)_2{\cdot}6H_2O$	Rhombohedral, 31.44 nm	Rhombohedral agglomerated	–	305 nm	Antibacterial, antifungal, cytotoxic potential, Stimulatory effect	(Uddin et al., 2021)
Opuntia ficus indica (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	Cubic, 16.01 nm	Spherical	20–35 nm	–	-	(Gebreinsae et al., 2021)
Lantana Camara linn (leaf)	$NiCl_2{\cdot}6H_2O$	Hexagonal phase, 21 nm	Hexagonal	–	245 nm	Antibacterial, Photocatalytic Degradation	(Feiona et al., 2021)
Rhamnus triquetra (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 25 nm	Spherical	65 nm	333 nm	Antibacterial, Antifungal, Antileshmanial, Anticancer, Antioxidant, Enzymes inhibition	(Iqbal et al., 2020)
Zingiber officinale and *allium sativum* (roots)	$Ni(NO_3)_2$	Hexagonal and FCC, Ginger- 32.9 nm Garlic- 29.92 nm	Pleomorphism with cubic and more spherical nanoparticles	16–52 nm for ginger 11–59 nm for garlic	350 nm	Antibacterial, Methylene blue degradation	(Haider et al., 2020)
Abutilon indicum (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	Simple cubic structure	Cubic and agglomerated	–	330 nm	Anticancer, Antibacterial, Antioxidant, Biocompatibility	(Khan et al., 2021)
Areca catechu (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 5.63 nm	Hexagonal and Rhombohedral	5–15 nm	–	Antidiabetic, Cytotoxic potential	(Shwetha et al., 2021)
Eichhornia crassipes (leaf)	$NiCl_2$	FCC	Spherical	9.1 nm	–	Role of NiO NPs in supplementing the ferementative hydrogen production by *Klebsilla* sp.	(Zhang et al., 2021)
Rosemarinus officinalis (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 11.6–15.5 nm	Quasi-spherical shaped	8.09 nm	365 nm	-	(Noukelag et al., 2021)
Raphanus sativus (root)	$Ni(CH_3COOH)_2.4H_2O$	Cubic, 22.37 nm	–	34.89 nm	346 nm	Antibacterial, Antioxidant	(Haq, 2021)
Calendula officinalis (leaf)	$NiSO_4{\cdot}6H_2O$	Crystalline, 16.9–43.9 nm	Spherical	60.39 nm	357 nm	Antioxidant, Cytotoxicity	(Zhang et al., 2021)
Leucas aspera (leaf)	$NiCl_2{\cdot}6H_2O$	Cubic, 40.438 nm	Spherical	–	–	Antibacterial	(Priya et al., 2020)
Limonia acidissima (fruit)	$Ni(NO_3)_2{\cdot}6H_2O$	Cubic, 10–21 nm	Spherical	23 nm	–	Antioxidant, Photocatalytic Degradation, Anti-angiogenic, Electrochemical sensing	(Kumar et al., 2021)
Stevia (leaf)	$Ni(CH_3COOH)_2.4H_2O$	FCC, 13.7 nm	Spherical	10–40 nm	–	Antibacterial, Antifungal, Antioxidant	(Srihasam et al., 2020)
Plectranthus Amboinicus (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 41.8 nm	Agglomerated porous shape	200 nm	350 nm	Photocatalytic, Sensor, Supercapacitor	(Radhakrishnan et al., 2020)
Nigella sativa (seed)	$Ni(NO_3)_2{\cdot}6H_2O$	Cubic, 20 nm	Spherical and agglomerated	10–50 nm	–	Catalytic degradation of 4 nitrophenol	(Boudiaf et al., 2021)
Capparis decidua (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$	FCC, 16.75 nm	Flower shaped structure	900 nm	410 nm	L-CHT removal	(Iqbal et al., 2021)
Allium jesdianum (leaf)	$Ni(NO_3)_2{\cdot}6H_2O$, Montmorillonite	FCC, 20.80 nm	Spherical	–	390 nm	Nano-catalyst for electrocatalytic oxidation of methanol	(Hossein et al., 2020)

were found deposited both intracellularly as well as extracellularly on the cell wall. The NPs were spherical in shape and were of average size 3.8 nm (extracellular ones) and 1.25 nm (intracellular ones). Bio-synthesised NPs show the potential of being used as sorbents for the removal of toxic metals from polluted sources. Another fungal species successfully used for the synthesis of NiO nanostructures is *Cladosporium cladosporioides* (Atalay et al., 2016). For this synthesis, fungus was grown in a 250 ml conical flask in 50 ml of potato dextrose broth, using a rotatory shaker (at 150 rpm for 72 h). This fermentation broth was then subjected to centrifugation (9000 rpm for 15 min), this led to the separation of mycelia biomass, which was then washed with deionised water. The crude cell extract was then collected in a beaker. For the synthesis of NiO NPs, at the rate of 10 ml/min 200 ml of 0.2 M $NiCl_2$ solution was added to the cell extract. The mixture was then stirred for 30 min. After this at the rate of 10 ml/ min 100 ml of 0.025 M of $NH_4.OH$ was added and the resulting mixture was stirred at 150 rpm for 72 hrs. The final mixture obtained was then centrifuged and precipitate was collected. The $Ni(OH)_2$/fungus precipitate was washed with deionized water and ethanol. The obtained pellets were then oven dried and calcined at 360 °C for 12 h. Subsequently, the obtained nanostructured NiO microtubes were subjected to various characterization techniques. The NiO NPs were also analyzed for its potential application as super-capacitor electrode material. In a similar fashion Ullah et al synthesized NiO NPs using fungus *Rhizopus nigricans* (Ullah et al., 2014).

Another innovative protocol for the synthesis of NiO NPs has been developed by Sabdouri et al. (Salvadori et al., 2016). They synthesized Ni/NiO NPs through a dead organic matrix of yeast *Rhodotorula mucilaginosa*, isolated from the Amazon region. Yeast cells were then grown in a 250 ml conical flask, containing 100 ml of YEPD broth, using a rotary shaker (150 rpm for 20 h). The fermentation broth was then subjected to centrifugation and the biomass was separated. Biomass obtained was washed with deionized water (living biomass) and collected in a beaker. To produce dead biomass, an appropriate amount of living biomass was autoclaved at 120 °C. Through various experiments optimized conditions (such as pH, temperature, initial Ni concentration, contact time, agitation rate, biosorbent dose etc.) were determined for the uptake of Ni, using 45 ml of 100 mg/ml Ni(II) ions test solution. After the desired contact time, through vacuum filtration Ni(II) solution was separated. The concentration of residual metal ion concentration was determined by optical emission spectroscopy. Efficiency of separation was calculated using initial and residual metal ion concentration. Structural characteristics of film forming NPs were determined using HRTEM, AFM, XPS, FTIR. Magnetic properties of NPs were analyzed through the Superconducting quantum interference device (SQUID).

Apart from bacteria, fungi, and yeast-based NPs synthesis, algae mediated NPs production has also been reported. Moavi et al. (Moavi et al., 2021) synthesized NiO NPs using red marine algae extract (gathered from areas of Bushehr province, Iran) and $NiCl_2{\cdot}6H_2O$ solution as precursors. Table 2 listed the various microorganism species used in the NiO NPs synthesis and obtained different properties.

5. Biomolecules and other green source mediated synthesis of NiO NPs

Apart from plant and microorganism mediated synthesis of NiO NPs, researchers have also developed other benign, eco-friendly, and cost-effective methods utilizing green sources like, amino acids, polysaccharides, chitosan, gums, tannic acids etc. Fig. 5 shows the NiO NPs synthesis using different biomolecules.

Suvith et al. produced nanostructured NiO using tannic acid, which was then used as nanocatalysts for degradation of methylene blue dye (Suvith et al., 2019). Tannic acid being a biodegradable, environmentally benign substance has been frequently used for green synthesis of NPs. Similar synthesis has been reported for nanocomposites of Nickel Oxide and Silver (NiO-Ag) (Devi et al., 2017). Tannic acid is a polymer of glucose and gallic acid molecules. Since, tannic acid has a lot of –COOH groups, it has the ability to release a number of highly reactive Hydrogen atoms, which then are used in the reduction process. The $–COO^-$ ions formed in this process along with the remaining polymer get attached on the surface of Metal NPs, acting as surfactants, and stabilizing the formed NPs (Nasrollahzadeh et al., 2014).

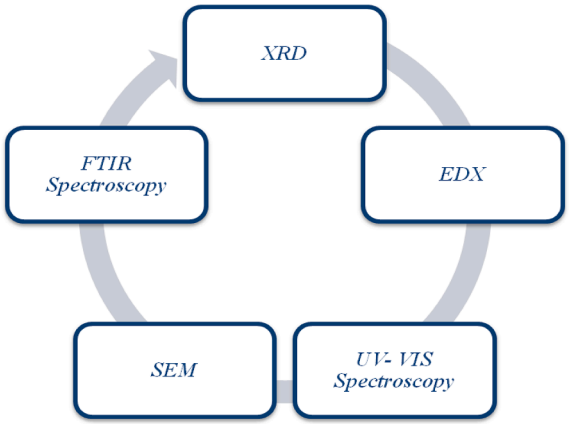

Fig. 5. Synthesis of NiO NPs from different biomolecules.

In the literature gums have also been reported being used as reducing, capping, and stabilizing agents, for the synthesis of NiO NPs. Baranwal et al. (Baranwal et al., 2018) successfully formed NiO NPs using Guar gum as a modifier through direct precipitation method. Arabic gum mediated synthesis of NiO NPs has also been studied by Sabouri and Co-workers (Sabouri et al., 2021). Chemically, gums are heterogeneous polysaccharides having complicated structure, which on hydrolysis gives sugars and uronic acids. These gums contain carbohydrate polymers with hydroxyl, carbonyl, and carboxyl functional groups, which are generally involved in the reduction of metal ions to metal NPs. These polysaccharides are further involved in the stabilization of NPs (Nasrollahzadeh et al., 2020).

Another important biodegradable natural polymer used in the synthesis of NiO NPs is starch. Reducing properties of starch is associated with the production of glucose units at higher temperature and in basic conditions. Glucose molecules so produced reduces the metal ion to metal NPs and itself gets oxidized to carboxylic acids (Marroquin et al., 2019). Williams et al. fabricated NiO NPs via sol–gel method using rice starch as bio-template (Williams et al., 2019).

Chitosan, a derivative of chitin (derived from crustacean shells, such as those from prawns or crabs) has also been employed in the synthesis of NiO NPs (Shahcheraghi et al., 2016; Sabouri et al., 2019). Chitosan plays an important role in polymerization and as an end point agent in the growing phase of particles. It also prevents agglutination, increases stability and reduces toxicity. These NPs were then analyzed for photocatalytic degradation property and cytotoxic potential (Sabouri et al.,

Table 2
Various microorganism species used for the synthesis of NiO NPs with their shape, size and other features.

Microorganisms	Precur-sors	Crystal structure and size	Shape of NPs	Size of NPs	UV Absorption (λ_{MAX})	Applications	Ref.
I) Bacteria							
Microbacterium sp. MRS-1	$NiSO_4$	Rhombohedral	Flakes	100–500 nm	400 nm	Bioremediation of toxic Ni ions.	(Wardani et al., 2019)
II) Fungi							
Hypocrea lixii	$NiCl_2{\cdot}6H_2O$	–	Spherical	1.25 nm 3.8 nm	–	Biosorbent for the removal of toxic Ni ions	(Salvadori, 2015)
Cladosporium cladosporioides	$NiCl_2$	FCC, 9.9 nm	Microtubes	–	~ 210 nm	potential application as supercapacitor electrode material	(Atalay et al., 2016)
Rhizopus nigricans	Ni $(NO_3)_2{\cdot}6H_2O$	FCC,	Spherical	~40 nm	–	-	(Ullah, 2014)
III) Yeast							
Rhodotorula mucilaginosa	$NiCl_2{\cdot}6H_2O$	–	Spherical	5.5 nm	–	Nano bioremediation of metals from wastewater	(Salvadori, 2016)
IV) Algae							
Red marine algae	$NiCl_2{\cdot}6H_2O$	FCC, 18 nm	Uniform, smooth and non-spherical	32.64 nm	330 nm	Nanocatalysts in the synthesis of pyridopyrimidine derivatives	(Moavi et al., 2021)

2019).

Amino acids and protein rich egg white biological fluid has also been utilized in the fabrication of NiO NPs (Sabouri et al., 2021). It was then investigated for their cytotoxicity, removal of methylene blue dye from aqueous solution and their antimicrobial activity. Structural properties of amino acids (like lysosome, albumin) play an important role in stabilizing NPs.

Agro wastes like Zea mays L. silk or corn silk (rich in phenolic compounds) has also been used as an effective reducing agent in the production of NiO NPs. The supercabattery properties of NiO NPs were then studied using cyclic voltammetry, electrochemical impedance spectroscopy and galvanostatic charge discharge cycles. (73).

5.1. Limitations of the green synthesis of Metal Oxide NPs

Although the use of plant extract in the synthesis of metal oxide NPs provide a very good cost effective, clean approach for the synthesis of metal oxide NPs but it has certain limitations. In some cases the plant extract contains a very small amount of Phytochemicals which play crucial roles as capping, chelating and stabilizing agent. In such conditions the experimental conditions like pH, concentration of extract and time are modified to achieve the satisfactory result.

6. Characterization of NiO NPs

Different characterizations used generally in the analysis of NiO NPs have shown in Fig. 6.

6.1. X-ray diffraction studies

One of the most widely used techniques for characterization of NPs is X-ray diffraction (XRD). It generally provides information regarding the crystalline structure, lattice parameters, nature of phase and crystalline grain size. For measuring the size of crystallite generally Scherrer equation is used. It relates the sub micrometer size of crystallites in a solid to the broadening of a peak in a diffraction pattern. One of the major advantages of using XRD technique is that it provides volume averaged values. Also sample composition can be easily determined by comparing the peaks with reference patterns available from the International Centre for Diffraction Data (ICDD) database. However, amorphous substances cannot be analyzed through this technique and very broad peaks are observed for particles of size less than 3 nm (Mourdikoudis et al., 2018). Table 3 listed the different XRD data obtained by various plants part NiO NPs including different angles, precursors, diameters etc.

6.2. UV–Visible spectrum

UV–Visible is an important tool used for identifying, characterizing, and studying nanomaterials. It is a technique which is used to quantify the amount of light (which lies in the UV–Visible range) absorbed or scattered (called extinction) when passed through the sample. Optical properties of NPs depend upon the shape, concentration, size, agglomeration state and refractive state near the surface of NPs (Gupta and Asha, 2020). NPs of noble metals (like silver, gold etc.) show intense scattering and absorption of light. It is because of LSPR (Localized Surface Plasmon Resonance), in noble metals it arises when photons of a certain frequency induce collective oscillations in the conduction electrons present on the surface of NPs (Raja and Barron, 2021). Strong bands appear in NiO NPs UV–Visible spectrum because of electron transitions taking place from the valence band to conduction band (Barakat et al., 2013).

In the literature, UV absorption peaks for NiO NPs are known to be observed at different wavelengths. For NiO NPs synthesized from the bark of Spirostachys africana UV–Visible spectroscopy peak was observed at 240 nm (Lefojane et al., 2020). In another research study

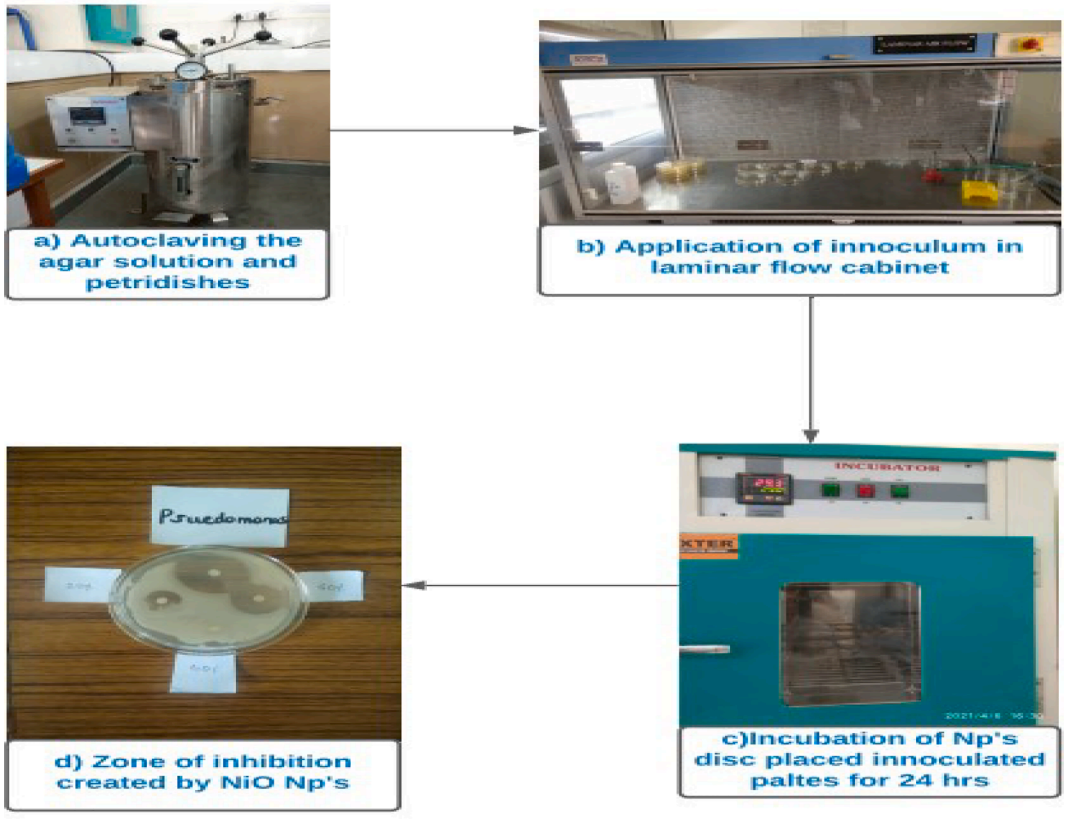

Fig. 6. Typical characterization tools used for NiO NPs analysis.

Table 3
NiO NPs crystallite structure, angle of diffraction and brief experimental conditions.

Plant (Part)	Precursors	Annealing temperature (°C)	Diffraction angles (2|)	Diameter and Identification	Ref.
Veronia amygdalina (leaf)	$NiCl_2{\cdot}6H_2O$	500	35.66°, 37.22°, 43.24°	17.86 nm, FCC	(Habtemariam and Oumer, 2020)
Arabic gum (gum)	Ni $(NO_3)_2{\cdot}6H_2O$	500	36.9°, 42.8°, 62.6°, 75.3°, 79.2°	43 nm, FCC	(Sabouri et al., 2021)
Opuntia Ficus indica (leaf)	Ni $(NO_3)_2{\cdot}6H_2O$	500	37.14°, 43.35°, 62.89°, 75.49°, 79.26°	16.01 nm, Cubic	(Gebreinsae et al., 2021)
Lantana Camara Linn (leaf)	$NiCl_2{\cdot}6H_2O$	400	37.21°, 43.29°, 62.88°	21 nm, hexagonal phase	(Feiona et al., 2021)
Rhamnus triquetra (leaf)	Ni $(NO_3)_2{\cdot}6H_2O$	–	36.52°, 43.44°, 63.11° 76.28°, 79.2°	FCC	(Iqbal et al., 2020)
Areca catechu (leaf)	Ni $(NO_3)_2{\cdot}6H_2O$	500	37.23°, 43.29°, 62.88°, 75.45°	5.63 nm, FCC	(Shwetha et al., 2021)
Rosmarinus officinalis (leaf)	Ni $(NO_3)_2{\cdot}6H_2O$	500	37.249°, 43.276°, 62.876°, 75.416°, 79.409°	11.598 nm–15.527 nm, bunsenite phase	(Noukelag et al., 2021)
Red marine algae	$NiCl_2{\cdot}6H_2O$	450	37.45°, 43.15°, 62.77°, 75.34°, 79.64°	18 nm, FCC	(Moavi et al., 2021)

synthesizing NiO NPs from Berberis balochistani absorption maximum was noted to be at 305 nm (Uddin et al., 2021). Similarly, in the synthesis of NiO NPs from the extract of Zingiber officinale and Allium sativum, the UV–Vis peak was at 350 nm (Haider et al., 2020). In case of NiO NPs synthesis from Rhamnus Virgata and Ageratum conyzoides the peaks were reported at 332 nm (Iqbal et al., 2019) and 324 nm respectively (Wardani, et al., 2019). When NiO NPs were prepared with the extract of Geranium wallichianum UV–Visible peak was observed at 259 nm (Abbasi et al., 2019). Other NiO NPs synthesized from different plant extracts, show absorption maximum in UV–Vis spectrum at 319 nm (Sabouri et al., 2019); 362 nm (Yulizar et al., 2022); 285 nm (Mamuru et al., 2015) and 330 nm (Khan et al., 2021). So, from the above discussion it can be assumed that NiO NPs generally show UV absorption peaks in the range from 240 to 370 nm.

6.3. FTIR spectroscopy

FTIR is a spectroscopic technique which involves measuring the functional groups of the sample molecules through IR radiations. An IR spectrum is represented in a graph with absorbance and transmittance on the vertical axis and frequency or wavelength on the horizontal axis. Position of bands in the IR spectrum reveals important information regarding functional groups, nature and strength of bonds, molecular structures, and their interactions. For NPs FTIR spectroscopy is an important characterization tool as, it helps in identifying various biomolecules present on the surface of NPs and thus assess the reducing and capping environment on molecular level (Mourdikoudis et al., 2018).

Generally, for Metal-Oxygen bond IR bands are observed in the range of 400–4000 cm^{-1} (Feiona et al., 2021). As it is quite evident from the Table 4 below, absorption bands for NiO are in the range 400–1100 cm^{-1}. Typically, these bands arise due to stretching, bending, wagging and other vibrational modes of Ni–O bond. Apart from Metal-Oxygen bands observed in the IR spectrum, frequencies corresponding to various other functional groups (like –OH stretching modes, –N–H and –C–H absorption bands, –C=C– vibrational bands) are also detected.

Table 4
FTIR absorption bands for red marine algae mediated synthesis of NiO NPs.

Entry	Functional group	Absorption band (cm^{-1})
1.	O–H, N–H stretch	3411
2.	C–H stretch	2945
3.	C=O stretch	1640
4.	C=C stretch	1545
5.	S=O stretch	1322
6.	C–O stretch	1193, 1030
7.	Ni–O stretch	525, 685

Moavi et al. (Moavi et al., 2021) synthesized NiO NPs using red marine algae extract, and $NiCl_2{\cdot}6H_2O$ as precursors. The results of fingerprint regions showing main functional groups present on the surface of freshly prepared NiO NPs has been tabulated below (Table 4 & 5).

6.4. Scanning electron microscopy (SEM)

For characterization of NPs SEM is one of the most extensively used techniques. Principle of electron microscopy is similar to optical microscopy, with a difference being in optical microscopy visible light is used as an incident source compared to accelerated electrons in electron microscopy (Amidon et al., 2009). The small wavelength of the electron beam makes even very small features of the sample visible. Signals obtained through electron-sample interaction reveal information regarding surface morphology (texture), and chemical composition of the sample (Sagadevan and Koteeswari, 2015).

In the literature, a lot of variation in surface morphology and microstructure of NiO NPs is observed. Feiona et al. (Feiona et al., 2021) synthesized NiO NPs using *Lantana Camara linn.* Leaf extract. The surface morphological features were analyzed by SEM. The images recorded at 500–10000 magnification, showed hexagonal structure with high agglomeration. Habtemariam et al. (Habtemariam and Oumer, 2020) reported octahedral structure with FCC geometry (bunsenite phase) when SEM was performed on the synthesized NPs. Uddin and coworkers (Uddin et al., 2021) utilized Berberis balochistani for the fabrication of NiO NPs. SEM analysis of the fabricated NPs showed the formation of highly agglomerated NPs.

Arabic gum mediated synthesis of NiO NPs resulted in formation of spherical and agglomerated NPs (Sabouri et al., 2021). Again, spherical

Table 5
Absorption bands corresponding to Ni−O vibrational modes for various green synthesized NiO NPs.

Plants (part)	Absorption bands (cm^{-1})
V. amygdalina (leaf) (Habtemariam and Oumer, 2020)	1094.8
Berberis balochistani (stem) (Uddin et al., 2021)	616.36
Lantana Camara Linn (leaf) (Feiona et al., 2021)	473
Rhamnus triquetra (leaf) (Iqbal et al., 2020)	545.17 and 657.43
Zingiber officinale and allium sativum(roots) (Haider et al., 2020)	978
Abutilon indicum (leaf) (Khan et al., 2021)	430
Eichhornia crassipes (leaf) (Zhang et al., 2021)	426 and 926
Rosmarinus officinalis (leaf) (Noukelag et al., 2021)	569, 924 and 1031
Raphanus sativus (root) (Haq, 2021)	545.28, 468.12 and 618.43
Red marine algae (Moavi et al., 2021)	525 and 685
Arabic gum (gum) (Sabouri et al., 2021)	421

NPs with some degree of agglomeration (due to high surface energy and high surface tension) were formed using cactus extract as a reducing agent. Surface morphology of phytochemically reduced (using *Zingiber officinale* and *Allium sativum* extract) NiO NPs were analyzed using FE-SEM (Haider et al., 2020). The synthesized NPs pleomorphism with cubical and more spherical shape (less than 50 nm) having slight agglomeration. Khan et al. (Khan et al., 2021) produced NiO NPs with *Abutilon indicum* leaf extract and Ni $(NO_3)_2{\cdot}6H_2O$ as precursors. SEM results showed that NiO NPs are appropriately dispersed, are highly crystalline and have cubic structure.

SEM micrograph of NiO NPs produced using Raphanus sativus (Haq, 2021) extract demonstrated that NiO NPs were uniformly distributed, had spherical shapes and were slightly agglomerated. Haq et al. also concluded that most likely the agglomeration is a result of heat produced during the combustion process, consequent gas evolution, interparticle forces and magnetic interaction between particles.

6.5. EDX analysis

The energy dispersive X-ray technique (EDX) is a microanalysis technique used for identification and elemental compositional analysis of a specimen. Elemental identification is done by measuring the number and energy of X-rays emitted by specimens, caused due to being excited by an electron beam (Scimeca et al., 2018). EDX systems are generally used in combination with electron microscopy techniques like SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy) (Roger and Welker, 2012).

For the characterization of NPs, EDX is an important tool. It helps in detecting elements present in the specimen. For NiO NPs, EDX analyses giving weight percentage of the elements is presented in Table 6.

In the EDX analysis of green synthesized NiO NPs using *Berberis balochistani* stem extract, a strong peak was observed for Ni (79.19%) and O (15.56%) by weight. The presence of C (4.5%) and K (0.74%) was also affirmed from the graph 10. Gibretinsae el al. through EDX spectroscopy confirmed the presence of Ni and O. Peaks corresponding to Cu, Cl, Ca and K were also observed, these are attributed to cactus plant extract (Gebretinsaea et al., 2020). For the characterization of NiO NPs synthesized from *Punica grantanum L.* (pomegranate) juice extract EDX technique was used. Elemental mapping of Biosynthesized NPs showed peaks for Ni (47.32%), O (56.28%) and Au by weight (Barzinjy et al., 2020). In the case of NiO NPs fabricated from Rhamnus triquetra leaf extract EDS analysis showed strong signals at 0.94 keV, 7.08 keV, and 8.12 keV, confirming the presence of Ni and O (Iqbal et al., 2020). Table 6 summarizes the EDX results for NiO NPs synthesized from different biological routes.

Table 6
EDX analysis of various biological extract mediated NiO NPs.

Biological Extract	Annealing temperature	Element	Weight %	Ref.
Berberis balochistani (leaf)	100 °C	Ni O C K	79.19% 15.56% 4.5% 0.74%	(Uddin et al., 2021)
Punica granatum L. (Fruit)	600 °C	Ni O Au	47.32% 56.28%	(Barzinjy et al., 2020)
Arabic Gum	500 °C	Ni O	73.23% 26.77%	(Sabouri et al., 2021)
Zingiber officinale and *Allium sativum* (roots)	90 °C	Ni O C Zn	54.69% 27.81% 18.06% 0.55%	(Haider et al., 2020)
Abutilon indicum (leaf)	500 °C	Ni O C N	-	(Khan et al., 2021)
Areca catechu (leaf)	500 °C	Ni O C, Mg, Al, S, Cl, K, Ca	46.99% 19.26%	(Shwetha et al., 2021)
Leucas aspera (leaf)	400 °C	Ni O	45.49% 54.51%	(Priya et al., 2020)
Egg white	400 °C	Ni O	51.64% 48.36%	(Sabouri et al., 2020)
Allium jesdianum (Leaf)	–	Ni O Si Al Na, Ca	3.29% 56.22% 29.62% 9.18% 0.96%, 0.73%	(Hossein et al., 2020)
Rosmarinus officinalis (leaf)	500 °C	Ni K, Na Cl C, O, N	-	(Noukelag et al., 2021)

7. Applications of NiO NPs

There are different applications of NiO NPs. Some of them were discussed here.

7.1. Biomedical applications of green synthesized NiO NPs

7.1.1. Antibacterial activity

For the evaluation of antibacterial activity of green synthesized NPs, disc diffusion method also known as agar diffusion method (ADM) is the most widely used one. In this method, a sample to be tested for its antimicrobial potential, diffuses from its reservoir through the agar medium nucleated with the test microorganism. Generally, a filter paper disk is used as a reservoir and is placed above agar medium. If the sample has potency for antimicrobial activity, a zone of inhibition is formed around the filter paper disc after incubation. The more is the microbiological activity of a sample; more is the diameter of the inhibition zone (Horváth et al., 2016).

Generally, for the antibacterial study of synthesized NPs following procedure is followed:

- Preparation of agar medium

To begin with MHA (Mueller-Hinton Agar) is prepared using dehydrated medium and distilled water, complete dissolution of medium is ensured through continuous heating and agitation. For sterilization, the medium is autoclaved for 2 h at 121 °C. The medium is then taken out and a small amount of it is dissolved in distilled water to determine pH (generally neutral medium is preferred). The agar medium is then cooled from earlier temperature to around 40 °C-50 °C. It is then poured into sterile glass petri dishes up to a depth of 4 mm. In order to get rid of excess moisture the agar medium is then dried at around 30–35 °C in an incubator for about 30 min. The prepared agar plates are then used for further studies.

- Application of Inoculum

Inoculum of different bacteria is then evenly applied with the help of a cotton swab to agar medium petri dishes, in the laminar flow cabinet (to avoid contamination by other undesired bacterial strains). The petri dishes are then allowed to dry up for 3–5 min.

- Study of antibacterial activity of NiO NPs

Various solutions of NiO NPs of different concentrations are prepared. Then with the help of sterilized forceps, filter paper discs dipped in NPs solutions are placed on the inoculated plates, along with a disc dipped in antibiotic. These plates are then placed in an incubator for 24 h at 37 °C. After full incubation, the samples are assessed by measuring their particular zones of inhibition for different bacteria. Fig. 7 involves the different steps used in the antibacterial analysis of NiO NPs.

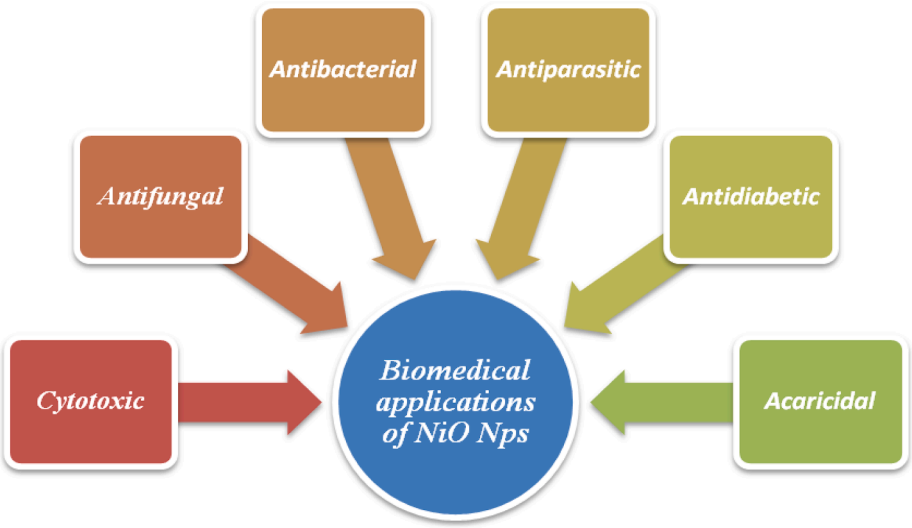

Fig. 7. Steps involved in the antibacterial analysis of NiO NPs.

Researchers have reported significant antimicrobial properties of NiO NPs. For instance, superior antibacterial activity of NiO NPs synthesized using *Abutilon indicum* leaf extract was observed against gram negative bacterial strains of *E. coli* and *B. bronchiseptica* and gram positive strain of *B. subtilis* and *S. aureus* (Khan et al., 2021), in comparison to plant extract and chemically synthesized NiO NPs at the same

Table 7
Antibacterial analysis of various biological extract mediated NiO NPs.

Biological extract	Agar medium	Bacterial strains	Maximum zone of inhibition	NiO NPs conc.	Reference
Berberis balochistani (leaf)	Muller-Hinton agar	Gram negative- P.vulgaris Gram positive- S.aureus	~15 mm ~15 mm	1000ug/ml 1000ug/ml	(Uddin et al., 2021)
Rhamnus triquetra (leaf)	–	Gram negative- E.coli K. pneumonia P.aeruginosa Gram positive- S.aureus Bacillus subtilis	~21 mm ~18 mm ~14 mm ~19 mm ~20 mm	1100ug/ml 1100ug/ml 1100ug/ml 1100ug/ml 1100ug/ml	(Iqbal et al., 2020)
Zingiber officinale and *allium sativum* (roots)	Muller-Hinton agar	Gram positive- S.aureus	~4.95 mm Zingiber officinale ~5.75 mm Allium sativum	1 mg/50ul 1 mg/50ul	(Haider et al., 2020)
Abutilon indicum (leaf)	Muller-Hinton agar	Gram negative- E.coli B.bronchiseptica gram positive- B.subtilis S.aureus	18 ± 0.58 mm 21 ± 0.45 mm 22 ± 0.32 mm 23 ± 0.77 mm	40 mg/ml 40 mg/ml 40 mg/ml 40 mg/ml	(Khan et al., 2021)
Raphanus sativus (roots)	–	Gram negative- E.coli P.aeruginosa Gram positive- S.aureus Bacillus subtilis	 4.175 mm 2.576 mm 2.189 mm 2.176 mm	 10 mg/ml in ethane diol 10 mg/ml 10 mg/ml 10 mg/ml	(Haq, 2021)
Leucas aspera (leaf)	Muller-Hinton agar	Gram negative- E.coli Gram positive- S.aureus	 26 mm 18 mm	 1000ug/ml 1000ug/ml	(Priya et al., 2020)
Stevia rebaudiana (leaf)	Lysogeny broth agar	Gram negative- E.coli gram positive- B. subtilis S. pneumonia	 16 mm 12 mm 14 mm	 0.1 mg/ml 0.1 mg/ml 0.1 mg/ml	(Srihasam et al., 2020)
Euphorbia heterophylla (leaf)	Nutrient agar medium	Gram positive- S. aureus Gram negative- E. coli Pseudomonas desmolyticumKlebsiella aerogenes	 8.23 ± 0.15 mm 9.23 ± 0.15 mm 7.33 ± 0.09 mm 7.10 ± 0.21 mm	 600ug/ml 600ug/ml 600ug/ml 600ug/ml	(Lingaraju et al., 2020)

concentration level. This might be due to the participation of phytomolecules adsorbed on the surface along with NPs participating in the inhibition of bacterial growth. Table 7 shows the different antibacterial analysis of various extract mediated NiO NPs.

Generally, it is observed that gram positive bacteria are more susceptible to the antibacterial effect of NiO NPs than gram negative bacteria. A probable reason for this is the variation in the chemical structure of cell walls in both types of bacteria, also there is a difference in their susceptibility towards different concentrations of metal oxide NPs (Khan et al., 2021). Exact mechanism behind the antibacterial activity of NiO NPs is still quite unclear. Many studies have suggested that the NiO NPs release highly reactive Ni^{+2} cations which get attached to the negatively charged cell wall of bacteria. The strong bonding between cations and cell membrane leads to the demise of bacterial cells (Khan et al., 2021).

7.1.2. Antifungal activity

In the literature antifungal properties of NiO NPs is well known. Uddin et al (Uddin et al., 2021) used poisoned food technique for the analysis of antifungal properties of NiO NPs, synthesized using *Berberis balochistani* leaf extract. For this fungal pathogen (*Aspergillus niger*, *Alternaria alternate* and *Fusarium oxysporum*) were cultured using autoclaved sabouraud dextrose agar media (SDA). For the preparation of poisoned plates different concentrations (100, 500, 1000 ug/ml) of NiO NPs solution was added to the SDA media. It was then shaken properly and poured into petri dishes for solidification. In each of the solidified media plates a disc with 7-day old fungal growth was placed, non-treated SDA media and fluconazole treated media are used as negative and positive control correspondingly. After incubation of plates for 3 days at 27 °C, mycelial growth was measured in each plate (in mm). For the calculation of % inhibition formula used was.

$$\%\text{inhibition} = (FC - FN/FC) \times 100$$

Where FC is increase in average fungal growth in the control and, FN is increase in average fungal growth in poisoned plates.

Dose dependent response was observed in all three fungi. *A. alternata* was found to be most susceptible against NiO NPs (1000 ug/ml) with a % inhibition of 71.25%, followed by *A. niger* (%inhibition of 39.51%) and *F. oxysporum* was not so vulnerable even at high concentration of NiO NPs (1000 ug/ml). Rhamnus triquetra mediated NiO NPs (Iqbal et al., 2020) also showed dominant antifungal properties. Disc diffusion method was used to analyze the antifungal potential against the fungal strains of (*A. flavus*; *M. racemosus*, *C. albicans*, *A. niger*, *F. solani*). Maximum zone of inhibition was reported at a concentration of 1100 ug/ml in all media plate and was in the order *C. albicans*, followed by *A. niger*, *F. solani*, *A. flavus* and least is for *M. racemosus*. Srihasam et al. (Srihasam et al., 2020) studied antifungal potency of NiO NPs again by using disc diffusion method, with potato dextrose agar as medium (Fig. 8). Fungal strains used were *A. niger* and *A. fumigatus*. Antifungal activity against Candida, a pathogenic yeast found in the gastrointestinal tract of humans has also been reported (Radhakrishnan et al., 2020).

7.1.3. Cytotoxic activity

Cytotoxicity refers to the toxicity developed due to the action of various medical devices (like chemotherapeutic agents) on living cells. This may be caused due to leaching of toxic substances or from direct contact. Cytotoxic studies play an important role in determining various biomedical applications of NPs (Ramakrishna et al., 2015).

NiO NPs synthesized using Arabic gum (Sabouri et al., 2021)were

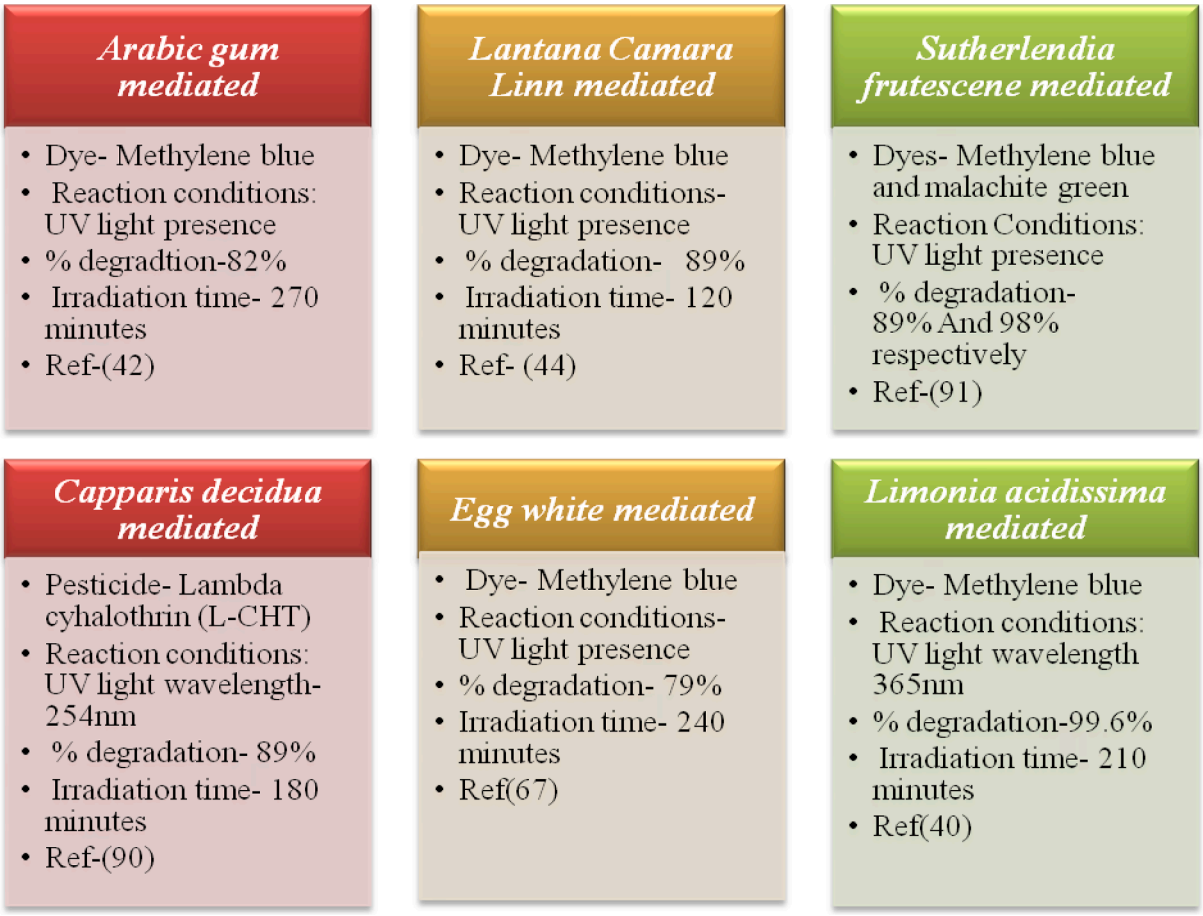

Fig. 8. Various aspects of biomedical applications of NiO NPs.

analyzed for their cytotoxic properties on normal nervous neurospheres (NC's) cell and cancer human glioblastoma cancer (U87MG) cell lines. MTT assay was applied for the investigation of cytotoxic activity of NiO NPs, absorbance for color produced was then measured using spectrophotometer. Using MTT method cell viability and IC50 value were calculated. The obtained results showed that NiO NPs eliminated 50% of US7MG cells at the concentration of 16ug/ml. Maximum inhibition for Normal nervous neurospheres was observed at NPs concentration of 125ug/ml, which was approximately 40%. For US7MG cell line maximum inhibition came out to be around 61% at a concentration of 62 ug/ml.

Another unique approach to study cytotoxic effects of *Berberis balochistani* mediated NiO NPs was developed by Uddin et al. (Uddin et al., 2021). They used Brine shrimp cytotoxicity assay, in which 20 mature brine shrimp larvae were shifted to different glass vials having varying concentrations of NiO NPs. After 24 h, alive brine shrimps were counted. After this using Graph Pad software lethality concentration (LC50) and % mortality was calculated. Considerable dose dependent cytotoxic response was observed for NiO NPs with IC50 value 10.40ug/ml.

By using the MTT colorimetric method, cytotoxic potential of Abutilon indicum mediated NiO NPs (Khan et al., 2021) was determined; it was done against HeLa cancer cells. Cytotoxic effect was comparable to anticancer drugs and presented more than 50% cytotoxicity. Maximum cytotoxicity was observed at 120ug/ml. Shwetha et al. (Shwetha et al., 2021) synthesized NiO NPs using Areca catechu leaf extract. Cytotoxic activity of NiO NPs was analyzed for human lung cancer line (A549). Significant cytotoxicity against the human lung cancer line was observed with IC50 value 93.349 ug/ml in a dose dependent manner (from 0 to 100 ug/ml).

Cytotoxic activity was also determined for *Calendula officinalis* mediated NiO NPs (Zhang et al., 2021). It was determined by using MTT assay against distal esophageal adenocarcinoma (FLO-1), gastroesophageal junction adenocarcinoma (ESO26), human caucasian esophageal carcinoma (OE33), and human esophageal squamous cell carcinoma (KYSE-270) cell lines. Excellent anti-cancer and cytotoxic properties were reported against esophageal carcinoma cell lines. IC50 values observed were 380, 263, 229, and 251 μg/mL, respectively.

7.1.4. Photocatalytic degradation

Wastewater treatment is a huge environmental problem nowadays. Conventional techniques used for the treatment of dye-contaminated water such as flocculation, reverse osmosis, ion exchange, activated carbon adsorption, are very expensive and generate a lot of amine residues. On the other hand, NPs such as NiO NPs show excellent photocatalytic properties and lead to the degradation of dyes. Photocatalysis is different from normal catalysis as in this Oxygen accepts electrons in the presence of photons, generated by the photocatalytic degradation of dyes (Sarkar et al., 2020; Iqbal et al., 2021). In literature various studies have been reported, utilizing the fabricated NiO NPs for the photocatalytic degradation of dyes. Generally, the method followed for this analysis is; initially a solution of dye is prepared. It is divided into two or more samples; a fixed amount of NiO NPs is added to each of them and is stirred thoroughly so that proper dispersion can take place. One of these samples is kept in dark and another one is exposed to UV light. At a fixed interval of time, a small amount of liquid from the sample is taken, centrifuged, and then UV absorbance for supernatant is measured. The percentage degradation for the dye is then calculated by using following equation:-.

$$\%Degradation = (Ao - At)/Ao \times 100$$

Where Ao is initial absorbance and at is absorbance at time t.

Mechanism involved in the photocatalytic degradation of NiO NPs has been well reported by Ganganagappa et al. (Kumar et al., 2021). They suggested enhanced photocatalytic activity is a result of decrease in energy gap between conduction and valence band. This facilitates the transfer of electrons from photocatalyst to oxygen, which then converts into radicals. Oxygen radical is a strong oxidizing agent which oxidizes dyes into oxides of Carbon and mineral acids. Furthermore, in presence of photons dye converts to its cationic form and undergoes degradation (Fig. 9 & Table 8).

NiO NPs show excellent reusability and barzinjy et al reported that the NiO NPs were reused in the photocatalytic degradation of methyl orange dyes up to seven cycles. In order to reuse the NiO NPs first it was isolated from the reaction mixture by centrifugation method then it was dried at 80 0C for 1 h and reused (Barzinjy et al., 2020).

7.1.5. Miscellaneous applications

Due to their unique properties NiO NPs have garnered a lot of attention owing to their potential applications in a number of fields such as: catalysis, ceramic materials, battery cathodes, active optical fibers, gas sensors, and electrochromic films etc. (Hatamifard et al., 2016). NiO NPs have now become an integral part of present-day technology. Various research studies done regarding these wide varying applications are discussed in this section.

Gunasekaran et al. (Gunasekaran et al., 2021) used Opuntia-Ficus-indica leaf extract to prepare NiO NPs. High specific capacitance (644 Fg^{-1} (~89.4 $mAhg^{-1}$) at 0.5 Ag^{-1}) and specific energy (25Wh kg^{-1}) was noted for asymmetric NiO–NPs|| activated carbon (AC) supercapacitor cell. Also, for the device high coulombic efficiency (124%) even after 10,000 cycles was observed. With KOH as electrolyte NiO—NPs| The AC device was able to work up to 1.4 V. Thus, NiO nanocomposites were formed using a cost effective, environmentally friendly technique, and were successfully utilized in the formation of an energy storing device. Similar study has been reported by Nwanya et al. (Nawnya et al., 2020). They used Zea mays lea silk mediated NiO NPs to form an asymmetric supercabattery device, using activated charcoal as the negative and NiO NPs as positive electrode. Specific capacity was reported to be 29Cg^{-1} at a specific current of 0.25 Ag^{-1}. This study again confirmed the potential application of NiO NPs in electrochemical storage devices.

NiO NPs have also been used for various electrical applications. To enhance the electrical properties of NiO NPs Williams et al. (Williams et al., 2019) doped NiO NPs with polyaniline (PANI), which were derived using rice starch. DC conductivity of NiO- PANI Composites showed remarkable improvement, from $1.015x10^{-4}$ S/cm^{-1} for pure NiO NPs to $2.1129x10^{-2}$ S/cm^{-1} at 90 °C.

NiO NPs are very active catalysts (Motene et al., 2021), in comparison to other transition metals and noble metals, using Ni as a catalyst is cheaper. It is due to abundance in which Ni is present, and the ability to precede reaction via alternate pathway and thus creating new chemical transitions (Dander and Garg, 2017). Barzinjy el al. (Barzinjy et al., 2020) used NiO NPs as a catalyst for the degradation of environmentally hazardous methyl orange dye, with sodium borohydride as reducing agent. Similar catalytic degradation was observed by Haider et al. (Haider et al., 2020) for methylene blue dye, with NiO NPs as catalyst and sodium borohydride as reducing agent. Also, Ni/montmorillonite nanocomposites formed by Allium jesdianum leaf extract have been successfully used as a catalyst in electrocatalytic oxidation of methanol (Hossein et al., 2020). Apart from decomposition and degradation reactions NiO NPs have also been used as a catalyst for the synthesis of pyridopyrimidine derivatives (Moavi et al., 2021).

NiO NPs are well known for their biomedical applications. Abdel-Ghany et al. (Abdel-Ghany et al., 2021) synthesized NiO NPs using Melia azedarach fruit extract. These NPs were then evaluated for their in vitro acaricidal activity against different developmental stages (egg, nymph, larva and adult) of camel tick (Hyalomma dromedarii). This species of camel tick is responsible for spreading the deadly virus, causing Crimean-Congo hemorrhagic fever. Significant acaricidal activity was reported on eggs, larvae, engorged nymphs and fully fed females of H. dromedarii. Similarly significant Antileishmanial potential of Rhamnus triquetra mediated NiO NPs (Iqbal et al., 2020) against leishmania parasite L. tropica. Aluminum doped NiO NPs showed

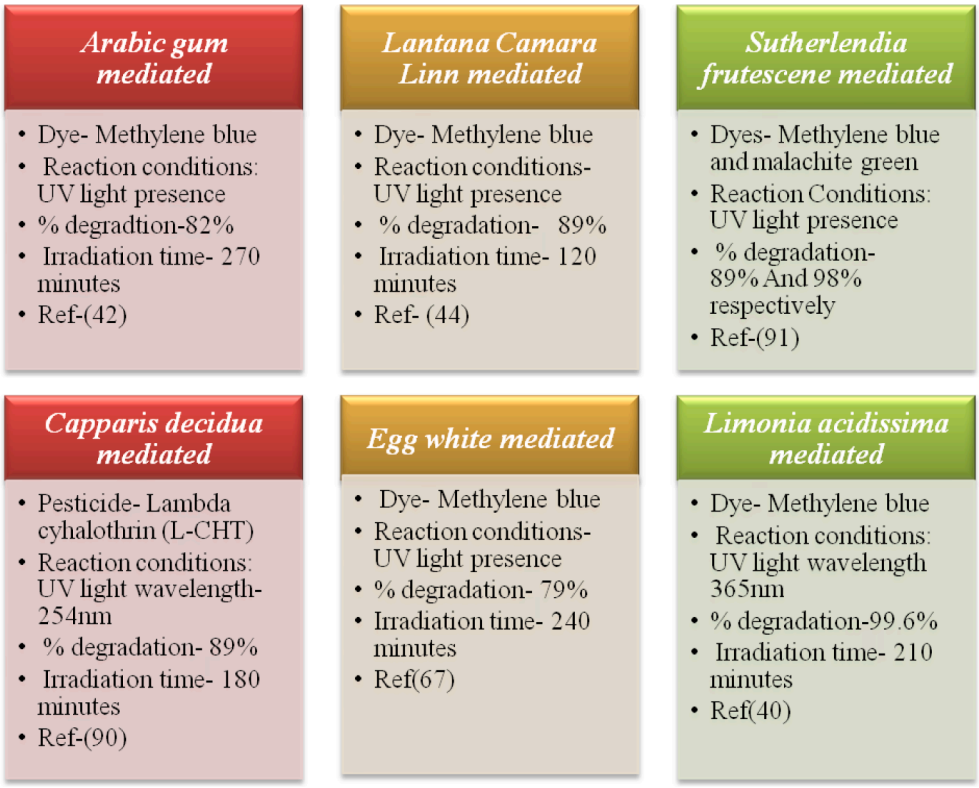

Fig. 9. Photocatalytic applications of NiO NPs.

Table 8
Photo catalytic Application of NIO NPs.

S.NO	Name of Plant /Microorganism Used in the Synthesis	Name of Dye	Irradiation Time (minutes)	% Degradation	Reference
1.	*Arbic Gum*	Methylene Blue	270	82%	(Sabouri et al., 2021)
2	*Lantana camara Linn*	Methylene Blue	120	89%	(Feiona et al., 2021)
3	*Sutherlendia frutescene*	Methylene Blue	–	89%	(Sarkar, 2020)
4.	*Sutherlendia frutescene*	Malachite Green	–	98%	(Sarkar, 2020)
5.	*Capparis Decidua*	Lambda Cyhalothrin	180	89%	(Ramakrishna et al., 2015)
6	*Egg White*	Methylene Blue	240	79%	(Nasrollahzadeh et al., 2020)
7.	Limonia acidissima	Methylene Blue	210	99.6%	(Kumar et al., 2021)

significant antipseudomonal activity when analyzed against Pseudomonas aeruginosa (Irum et al., 2021). Shwetha UR and coworkers (Shwetha et al., 2021) reported significant α amylase inhibitory effectiveness of NiO NPs. Thus, affirming the remarkable antidiabetic potential of Areca catechu mediated NiO NPs.

8. Conclusions

Green mediated synthesis has become the most popular approach for the formation of NiO NPs. Even among the plethora of biological extracts available for the chelating, stabilizing and capping of NiO NPs, plant extracts are mostly used as they are ecofriendly, nontoxic and result in a cost-effective method of synthesis. Irrespective of the species involved, most of the plant derived extracts are capable of fabricating NPs. But the mechanism and compounds mainly responsible for these abilities of plant extracts are not well known. Thus, further research should be promoted to figure out the mechanistic details of the process involved in the synthesis of NPs. NiO NPs have shown potential applications in biomedicines and chemotherapeutics. Therefore, cytotoxic and biocompatibility studies of NiO NPs become much more important. With increasing applications of NiO NPs in a number of fields, proper environmental assessment of these NPs should also be prioritized. Hence, the methodologies, applications and analytical techniques discussed in this review paper will help in advancing this already galloping field of nanotechnology.

9. Future prospects

Green synthesis of NiO NPs by using plant extract or other biological material is one of the most suitable and appropriate methods. There are so many plants that extract is used as a precursor in the synthesis of the NiO NPs.

However, some points are still needed to investigate in future research work.

(1) The Exact mechanism which established the role of plant extract in the synthesis of NiO NPs is not fully understood yet.
(2) The effect of different parts of the plant in the synthesis of NiO NPs like the color of the flowers is also needed to further investigate.
(3) The effect of Temperature and the reaction time on the morphology of NiO NPs is also needed to investigate.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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