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Research Article

Analysis of In-vitro antioxidant properties of Green synthesized Ni/reduced graphene oxide composite

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Abstract— Nickel nanoparticles (NiNPs) possess numerous health benefits that are useful in biomedical applications. Synthesis of NPs via green approaches has become prominent nowadays due to their cost effective, and eco-friendly manner. In the current study, Ni NPs were synthesized via a green approach. As a support to disperse and stabilize NPs, reduced graphene oxide (rGO) was used while preventing agglomeration. Then the in-vitro antioxidant activity of synthesized composites was examined. As the reducing and capping agent *Azadirachta indica* (Neem) extract was used in the synthesis process. All Ni/rGO composites synthesized possessed antioxidant activity in concentration dependent manner and activity was always greater than rGO. The highest activity was shown by Neem/NiNPs where inhibition was 78% for a 250 ug/ml sample. Energy Dispersive X-Ray (EDX) analysis, shows signals of Ni along with oxygen and carbon confirming the incorporation of NiNPs into composite. Among composites, the highest activity was shown by Ni/rGO (RT) where inhibition was 63% and EC_{50} was 35ug/ml. This contained only 17 weight% Ni. These results confirm that after the formation of the composite, even a lower amount of NiNPs show considerably high activity due to the formation of mono-dispersed layer of NPs and phytochemicals attached on rGO.
Antioxidant activity has greatly enhanced due to the synergetic effects between NiNPs and rGO sheets. These results suggest that Neem extract can be extensively used in green production of Ni/rGO composite which is an excellent candidate for safe biomedical, pharmaceutical as well as other industrial applications.

Keywords— Nanoparticles**,** *Azadirachta indica,* Antioxidant, Reduced Graphene oxide, Phytochemicals, Nickel

1. Introduction

Nanoparticles are special groups of materials that have one dimension that measures 100 nanometers or less and exhibit completely new or improved properties due to their extremely small size and large surface-to-volume ratio and morphology [1], [2]. Metal nanoparticles represent a major class of nanomaterials and are of great interest in electronics, catalysis, chemistry, energy, and especially in the biomedical field [3]. Metal nanoparticles are usually synthesized by the "Top-down" method and "Bottom-up" approaches. In "Top-down" methods, metal nanoparticles are synthesized from the bulk metallic materials by various chemical and physical methods such as mechanical grinding, laser ablation, and thermal decomposition to achieve size reduction. In the "Bottom-up" approach, metal nanoparticles are formed by stacking the metal atoms, which mainly includes chemical and biosynthesis [4].

Even though pure nanoparticles may be effectively produced by chemical and physical methods, most of these approaches require expensive or toxic reducing agents or stabilizers, toxic organic solvents or involve high temperature and pressure conditions. Consequently, there is a great demand for developing economical and environmentally friendly methods, and this aroused interest in using microorganisms and plant extracts in the synthesis of nanoparticles. This method is known as "green" since it does not produce harmful by-products, and among other advantages, it is eco-friendly, simple, and non-expensive [5], [6].

Many researchers have found the usage of plant leaf extracts, root, stem, bark, fruit, and flowers for the synthesis of metal nanoparticles. Nanoparticles produced by plant extracts are more stable and more varied in size and shape and also can be easily upgraded to large-scale production.

Azadirachta indica which is commonly known as Neem is a member of the Meliaceae family. Neem extract is used in the synthesis of various nanoparticles such as Silver (Ag), Gold (Au), Zinc oxide (ZnO), Nickel (Ni), etc. Terpenoids and flavanones are the main important phytochemicals that act as reducing and capping agents and also play an important role in stabilizing the nanoparticle. In this study, reduced graphene oxide supported Ni NPs were synthesized via a green approach using the leaf extract of *Azadirachta indica* and the antioxidant activity of the synthesized composites was evaluated.

Azadirachta indica shows a therapeutic role due to the presence of valuable active compounds such as azadirachtin, nimbolide, nimbin, nimbidin, nimbidol, ascorbic acid, salannin, and quercetin which play important roles in antimicrobial, anti-inflammatory, in the management of cancers and as free radical scavenging properties due to rich source of antioxidants. Therefore, it plays an important role in disease prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways [7].

There are many limitations in nanoparticle synthesis such as agglomeration of nanoparticles, control of crystal growth, morphology, and size [3], [8]. In the present study, graphene-based materials have been used which acts as a supportive material to overcome the main limitation of agglomeration of nanoparticles. The composite materials such as graphene/nanoparticles have unique thermal, mechanical, chemical, and electronic properties. Therefore, nanocomposites of graphene with metallic nanoparticles will have promising applications in bioelectronics, tissue engineering, drug delivery, antimicrobial materials, biosensing, and gene delivery [9].

GO is an important derivative of graphene and is applied as a substrate material to greatly improve the loading capacities of nanoparticles. Intercalation of metal nanoparticles between rGO layers can prevent the agglomeration of rGO nanosheets and act as a nano spacer between rGO sheets [10].

More attention has been gained by Ni NPs in the last decade due to their unique magnetic, chemical, and physical properties as well as their potential applications in various technological fields. Even though there are studies carried out for Ni NP Synthesis, there are only limited studies that have been done related to the green synthesis of Ni NPs using plant extracts. Green synthesized Ni NPs were found to possess reliable antioxidant and antibacterial activity as well as being non-toxic to animal cells, compared to chemically synthesized Ni NPs. In addition, it also can be used especially in biomedical applications including drug and gene delivery, magnetic resonance imaging, cell separation, biomedical detection, and diagnostics [11].

In this study, we focused on the green synthesis of Ni/rGO nanocomposite material which has broad antioxidative activity due to the combined effect of Neem leaf extract, Ni NPs, and GO material. Therefore, this nanocomposite can be used in biomedical, pharmaceutical as well as other industrial applications.

2. Related Work

A recent study was done by Jagpreet Singh *et al*., (2018) in South Korea, regarding the synthesis of metals and their oxide nanoparticles. They have discussed the fundamental processes and metal oxide nanoparticles using natural extracts. They have mentioned the role of biological components, and essential phytochemicals as reducing agents and solvent systems. The stability or toxicity of nanoparticles and the associated surface engineering techniques for achieving biocompatibility have also been discussed. They have emphasized their applications in environmental remediation about antimicrobial activity, catalytic activity, removal of pollutants dyes, and heavy metal ion sensing [12].

A study conducted by Hesham R. El-Seedi *et al.,* (2019) on metal nanoparticles fabricated by green chemistry using natural extracts describes eco-friendly methods for the preparation of biogenic NPs and the known mechanisms for their biosynthesis. They have mentioned the use of bacterial and fungal extracts in the synthesis of NPs and the parameters that influence the rate of particle production, size and morphology have been discussed. The importance and uniqueness of NP-based products as well as their commercial applications in various fields are briefly featured [13].

S. Sudharsree *et al*., (2014) have conducted research on the synthesis of Ni nanoparticles by the chemical and green route and their comparison with respect to biological effect and toxicity. In this study, they have synthesized Ni NPs using both green and chemical approaches. *Desmodium gangeticum* extract has been used for the synthesis of Ni NPs in the green approach. They have noted that both processes have resulted in NPs of the same nature without significant differences. Green synthesis has given better NPs with reduced size and better mono-dispersity than the chemical method. Also, they exhibit better antioxidant and anti-microbial properties which makes them reliable [14].

Bharat Pokhrel *et al.,* (2015) in India, have done another study on investigations of the antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. The presence of numerous bioactive compounds in *Azadirachta indica* causes its wide spectrum biological activity. Various phytochemicals have been detected using a qualitative screening and the total phenols, flavonoids and proanthocyanins have been quantified. The antioxidant activity of the methanolic extract of the plant had also been determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and it was found that crude extract of *Azadirachta indica* can be used as a good source of antioxidants [15].

A study was done by V. Helan *et al.,* (2016) in India on Neem leaves mediated preparation of NiO nanoparticles and its magnetization, coercivity and antibacterial analysis. They used the phytoconstituents present in the neem leaf for the biosynthesis of Nickel Oxide nanoparticles (NiO-NPs). The X-ray Diffraction (XRD) patterns have revealed the presence of polycrystalline nature of samples while Transmission Electron Microscopy (TEM) has revealed their shape of oblong with 12 nm in size. They have quantified the percentage of Nickel and Oxygen using elemental analysis and have studied the antibacterial activity using agar diffusion assay against *Escherichia coli* and *Staphylococcus aureus* [16].

B. A. Abbasi *et al.,* (2019) in Pakistan, have done a study concerning plant-mediated synthesis of NiO nanoparticles via *Geranium wallichianum* as both reducing and capping agent. Biological applications such as strong antifungal, antibacterial and cytological activities have been shown by NiO NPs for in vitro biological assays. They had used different characterization techniques to study the formation, crystalline structure, elemental composition, surface charge and stability [17].

Recent research has been done by Yan Huang *et al.,* (2021) on the green synthesis of Ni nanoparticles using *Fumaria officinalis.* They have used various techniques such as Field emission Scanning Electron Microscopy (FE-SEM), EDX, XRD, UV-Visible spectroscopy, and Fourier Transform Infrared (FT-IR) spectroscopy to assess the properties of synthesized Ni nanoparticles. The study shows that the Ni nanoparticles exhibit significant antioxidant and anticancer activity against ovarian cancer. As per the study, Ni NPs of *Fumaria officinalis* extracts can be utilized for the production of novel chemotherapeutic drugs in the treatment of ovarian cancer [18].

A recent study was done by A.A. Olorunkosebi *et al*., (2021) on the optimization of GO and comparative reduction using the green approach. In this study, GO were synthesized using three techniques and had been reduced using two reducers' extracts from neem and pumpkin leaves. The obtained GO and rGO had been characterized using FTIR spectroscopy, Raman spectroscopy, UV–visible spectrophotometry, EDX and SEM. They have found that all three methods used are capable of producing GO with various levels of oxidation. FTIR spectra of rGO showed that the C—O signature of GOs was eliminated after reduction. Other characterization results of the rGO revealed that both the extracts of Neem and Pumpkin are good reducing agents, which can replace the use of hazardous chemicals [19].

A study has been performed by Gusbin Lou *et al*., (2021) to assess the anti-bacterial and anti-corrosion properties of Ni-GO/ Ni-rGO composite coating on Manganese (Mn) steel. It shows that Ni-GO/ Ni-rGO composite can enhance the antibacterial property and have better anti-corrosion ability compared to the uncoated Mn steel and pure Ni coating [20].

A.W. Anwar *et al.,* (2015) in Pakistan, have done a study on the simple and inexpensive synthesis of rGO - Ag, Ni nanocomposites via green methods. In this study, rGO- nanoparticle (Ag, Ni) composites have been successfully synthesized by cost-effective and environmentally friendly methods. The green method in which neem leaf extract was used as a reducing agent for the in-situ reduction of metal salts and GO as well as Ultra Violet laser irradiation mediated reduction also had been done. The deposition of Ag and Ni NPs on rGO has been examined by Raman spectroscopy, XRD, and SEM analysis for characterization, and both techniques were found efficient. Usage of neem leaf extract is a cheap, efficient, and non-toxic method to deposit metal nanoparticles on the rGO surface [10].

3. Theory

3.1 Preparation of Neem extract

Fresh Neem (*Azadirachta indica)* leaves were harvested and washed 3 times with distilled water to remove dirt and unwanted materials. A quantity of 50 g air-dried fresh Neem leaves was measured using the electronic analytical balance and they were crushed using the mortar and pestle. Then crushed Neem leaves were added into a beaker and mixed with 70% Ethanol to obtain a total volume of 300 ml. It was heated at 60^{o}C for one hour & 30 minutes while stirring using a magnetic stirrer. The resultant ethanol extract was allowed to cool, and was filtered through Whatman filter paper and stored in refrigerator for synthesis reactions.

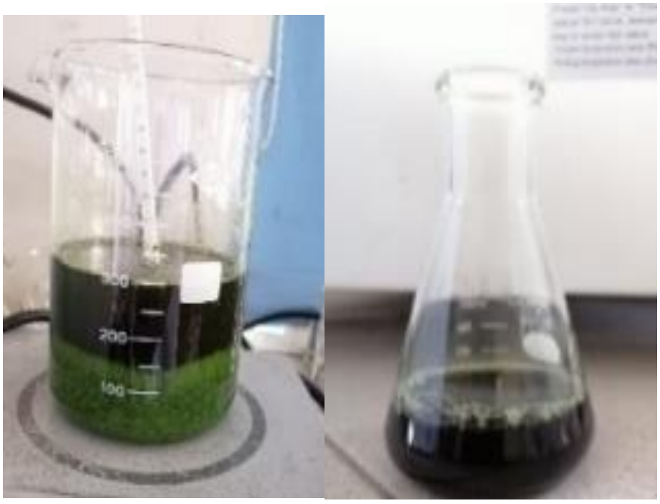

Figure 1: Preparation of the Neem (Azadirachta indica) leaf extract

3.2 Preparation of 0.25M $NiCl_2$ solution

5.925 g of $NiCl_2.6H_2O$ was measured to a watch glass using an electronic analytical balance. It was transferred into a volumetric flask and dissolved in distilled water and 100 ml of 0.25 M $NiCl_2$ solution was prepared. The solution was mixed well and labeled properly.

3.3 Synthesis of Nanoparticles

3.3.1 Synthesis of Ni nanoparticles using Neem leaf extract

100 ml of Ethanol Neem extract was mixed with 100 ml of above prepared 0.25M $NiCl_2$ solution in a beaker. It was heated at 60 ^{o}C for 3 hours and stirred for 24 hours using a magnetic stirrer. Absorbance values of the solution were taken at specific time intervals using UV-Visible spectrophotometer. This solution was kept at room temperature (RT) for a week and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and the precipitate was dried in hot air oven at about 60 ^{o}C until it results solid precipitate.

3.3.2 Synthesis of Ni/rGO nanocomposite: at room temperature - Ni/rGO (RT)
Graphene oxide (GO) solution was prepared by dissolving 0.1 g of Graphene Oxide in 100 ml distilled water. This solution was sonicated for 20 minutes and mixed with above prepared $NiCl_2$ solution and sonicated for 20 minutes. 100 ml of Neem extract was added in dropwise manner into the GO/$NiCl_2$ solution while stirring and continued stirring for 24 hours without heating by using the magnetic stirrer. This solution was kept at RT for a week and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and the precipitate was dried in a hot air oven at about 60 ^oC until it deposited as a solid precipitate.

3.3.3 Synthesis of Ni/rGO nanocomposite: heating at 60 ^0C-Ni/rGO (60 ^0C)
Graphene oxide solution was prepared using 0.5 g of Graphene Oxide in 100 ml of 70% Ethanol. This solution was sonicated for 20 minutes and it was mixed with above prepared 0.25M $NiCl_2$ solution and sonicated for 20 minutes. Then 100 ml of Neem extract was added in to that $NiCl_2$/ Graphene oxide mixture drop wisely while stirring and heated at 60 °C for 4 hours and stirred for 3 days without heating. This solution was kept at RT for a week and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and the precipitate was dried in a hot air oven at about 60 ^oC until it deposited as a solid precipitate.

3.3.4 Synthesis of Ni/rGO nanocomposite: sonication at room temperature -Ni/rGO (RT-sonicated)
Graphene oxide solution was prepared by dissolving 0.5 g of Graphene Oxide in 100 ml of 70% Ethanol. This solution was sonicated for 20 min and it was mixed with above prepared $NiCl_2$ solution and sonicated for 20 minutes. Then 100 ml of Neem extract was mixed with that solution and again sonicated for 4 hours. The sonicated solution was stirred till the next day morning. This solution was kept at RT for a week and centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and the precipitate was dried in a hot air oven at about 60 ^oC until it deposited as a solid precipitate.

*All the prepared solutions and precipitates were kept in dark in order to preserve their properties.

3.4 Characterization of synthesized samples
Physicochemical properties of the synthesized samples were evaluated via various material analysis techniques. All the samples were dried well at 60^oC in hot air oven before the characterization.

(a)UV-Visible spectroscopy
UV-Visible spectroscopy was initially used to confirm the formation of nanoparticles and absorbances were taken during the experiments.

(b)Fourier Transform Infrared (FTIR) Spectroscopy
Infra-red spectra of samples were analyzed by Fourier Transform Infra-Red spectrometer (Bruker alpha (10031705) with specifications of scan range 4000-650 cm-1; 32 scans and 8 cm^{-1} resolution).

(c)Thermogravimetric Analysis (TGA)
TGA measurements were taken under a temperature range of RT-800^0C, with a heating rate of 10^0C per minute under N_2.

(d)Scanning Electron Microscopy (SEM) & Energy Dispersive Xray Analysis (EDX)
Surface morphological study was carried out using Carl Zeiss Evo 18 research scanning electron microscopy attached with energy dispersive X-ray analyzer.

3.5 DPPH radical scavenging assay
1,1-diphenyl-2-picrylhydrazyl (DPPH) is a radical, present in its monomer form in a solid state, as well as in solution. The radical is soluble in different organic solvents, but not in water. It usually dissolves in methanol, ethanol, or their aqueous mixtures.

The neutralization DPPH test is based on donating electrons from the antioxidants in order to neutralize the DPPH radical. The reaction is accompanied by changing the DPPH color measured at 517 nm, and discoloration acts as an indicator of antioxidant activity by the DPPH neutralization method is often reported as EC_{50}, which is defined as the efficient concentration of the antioxidant necessary to reduce the initial DPPH concentration by 50% [21].

4. Experimental Method

The Antioxidant activity of the synthesized nanoparticles was assessed using the DPPH radical scavenging assay. A DPPH solution with 0.004% concentration was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol. A concentration series of, 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml were prepared by dissolving each of the synthesized nanoparticles in 70% ethanol. Ascorbic acid was used as the standard solution. One millilitre of the sample was mixed with 4 mL of DPPH solution. The mixture was shaken vigorously and incubated at 25 °C for 30 minutes in the dark. The absorbances were taken after 30 minutes at 517 nm using 70% ethanol as the blank solution in a UV-Visible Spectrophotometer. A control was prepared without a sample. The DPPH scavenging activity of each of the samples was calculated using the following equation;

$$\text{Free radical scavenging activity (RSA)\%} = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where A_0 is the absorbance of the control and A1 is the absorbance of the sample mixture.

5. Results and Discussion

5.1 Characterization of synthesized composites
a) UV-Visible spectroscopy

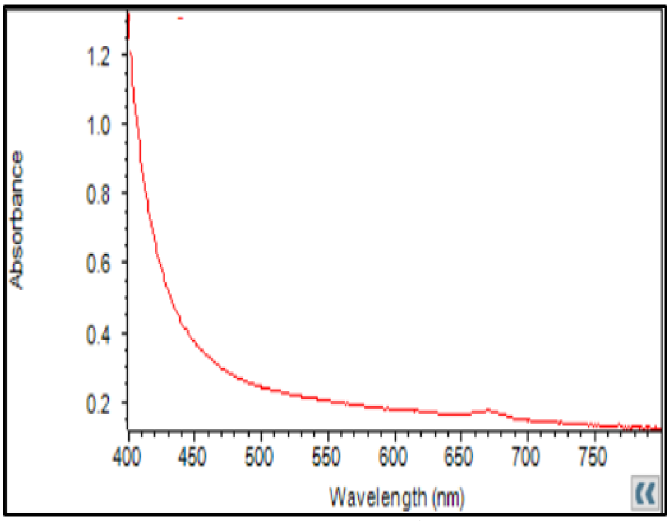

Figure 2: UV-visible spectrum of Neem extract

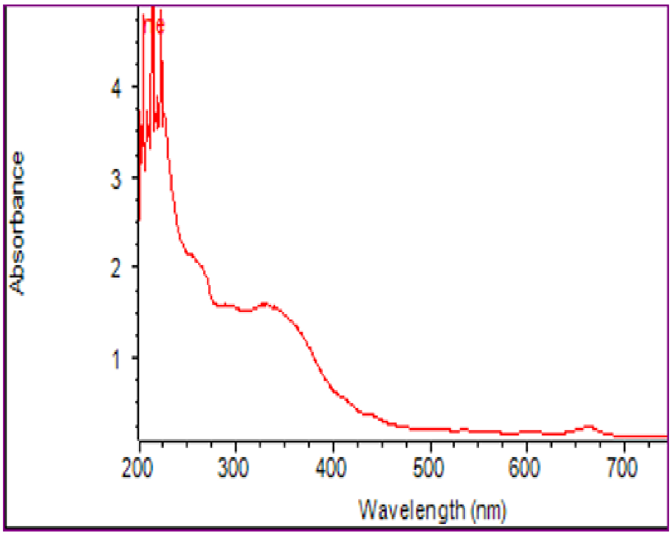

Figure 3: UV-visible spectrum of NiNPs reduced by Neem extract

Figures 2 and 3 show, UV-Visible spectra of Neem extract and NiNPs reduced by Neem extract, respectively. The formation of the nanoparticles was confirmed by the colour change of the solution from dark green to black. This is supported by UV absorption spectra. The absorption spectrum of neem extract shows chlorophyll pigments. Two broad peaks observed at 460 nm and 660 nm confirm the presence of both chlorophyll a and chlorophyll b.

Figure 3 shows the absorbance spectrum of Ni NPs. It can be clearly seen that the surface plasmon resonance (SPR) of Nickel NPs centered in figure 3 at 330 nm and at 335 nm. The position of the SPR band in UV Visible spectrum depends on the particle size, shape and its interaction with medium and the extent of charge transfers between medium & particles.

b) Fourier Transform Infrared Spectroscopy

Figure 4 shows FTIR spectrum of NiNPs reduced by Neem extract. The FTIR spectral results displayed different peaks at 460, 1058, 1160, 1400, 1632, 2926 and 3443 cm^{-1}.

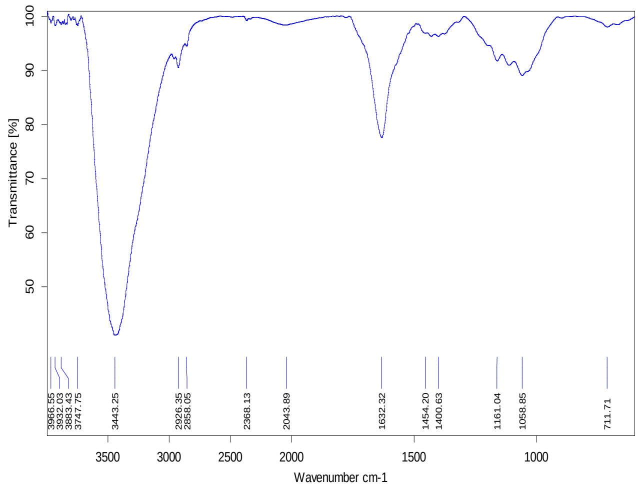

Figure 4: FTIR spectra of Ni/rGO (RT) nanocomposite

c) Thermogravimetric Analysis (TGA)

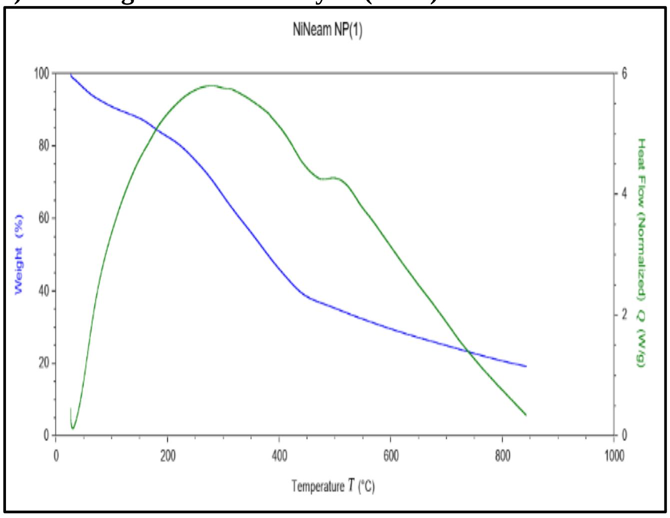

Figure 5: Thermogravimetric Analysis curve of Ni NP

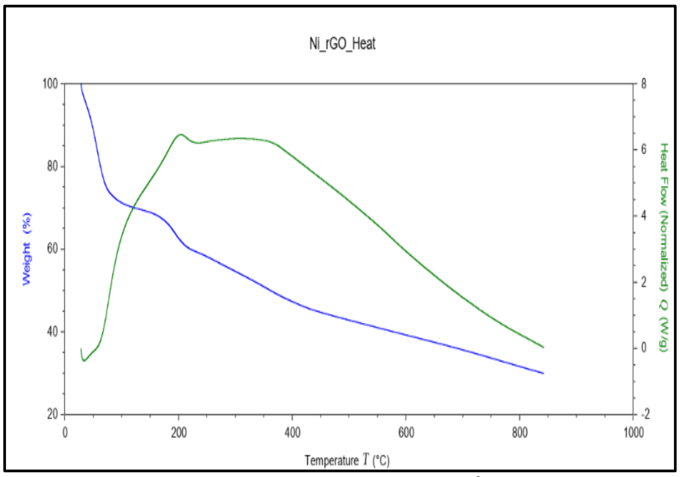

Figure 6: Thermogravimetric Analysis Ni/rGO (60 ^{0}C) nanocomposite

d) Scanning Electron Microscopy (SEM)

The surface morphology, size and elemental composition of Ni/rGO nanocomposites were studied using Scanning Electron Microscopy and Energy Dispersive Xray analysis technique. It can be seen that the surface of rGO is covered with Ni NPs (Figure 7).

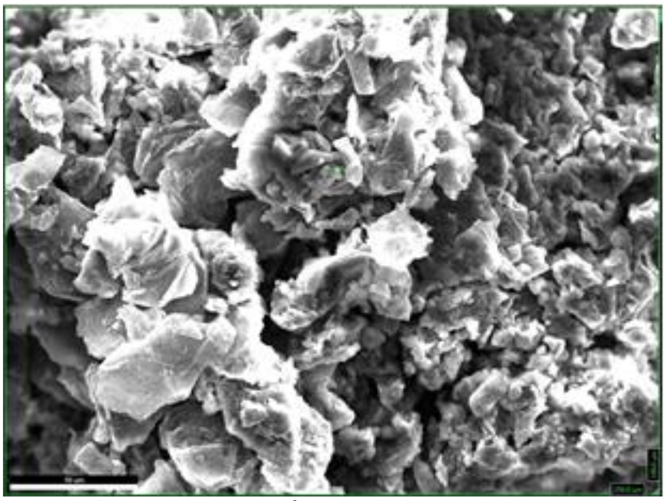

Figure 7: SEM image of Ni/rGO (RT) nanocomposite

e) Energy Dispersive Xray analysis (EDX)

The EDX analysis was used to detect the chemical composition of Ni/rGO Nanocomposite. The EDX profile shows the signals of Ni along with oxygen and carbon. Chlorine is also present.

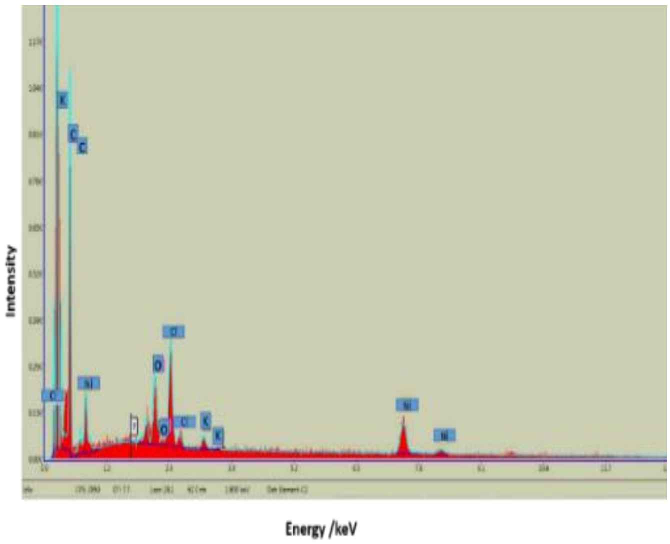

Figure 8: EDX spectrum of Ni/rGO (RT) nanocomposite

Table 1. EDX results of Ni/rGO (RT) nanocomposite

Element	Weight%	Atomic %
C	54.22	80.00
Cl	12.57	8.37
O	13.83	3.40
Ni	17.24	6.93
K	2.14	1.29

5.2 Antioxidant activity: DPPH radical scavenging activity

The UV absorption spectrum of DPPH revealed maximum absorption at 517 nm, when scanned between 240 nm and 700 nm.

Table 2: Results of DPPH free radical scavenging assay

Sample	Concentration (ug/ml)	Absorbance	Inhibition %
Ni/Neem NP	100	0.274	56.08
	150	0.219	64.90
	200	0.167	77.41
	250	0.166	77.56
Ni/rGO (60 ^0C)	100	0.329	47.27
	150	0.280	55.12
	200	0.321	56.68
	250	0.299	59.59
Ni/rGO (RT – Sonicated)	100	0.321	48.55
	150	0.321	56.15
	200	0.322	56.48
	250	0.294	60.27
Ni/rGO (RT)	100	0.303	51.44
	150	0.279	55.28
	200	0.299	59.59
	250	0.275	62.83
Ascorbic Acid	10	0.076	85.82
	25	0.080	85.07
	50	0.020	96.26
	75	0.01 6	97.01
	100	0.01 7	96.82
Control	-	0.536	-

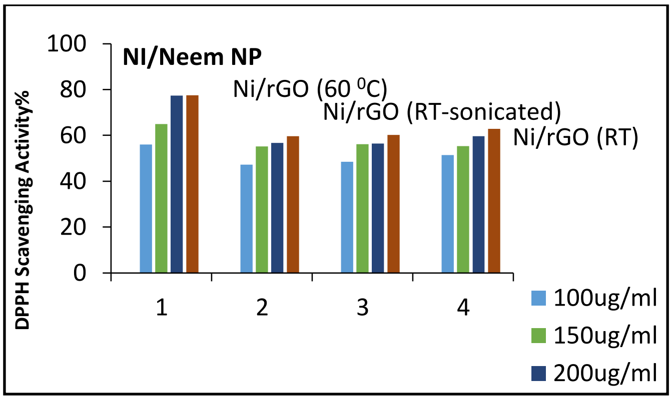

Figure 9: Antioxidant activity results of synthesized nanocomposites in terms of % DPPH scavenging at different concentrations

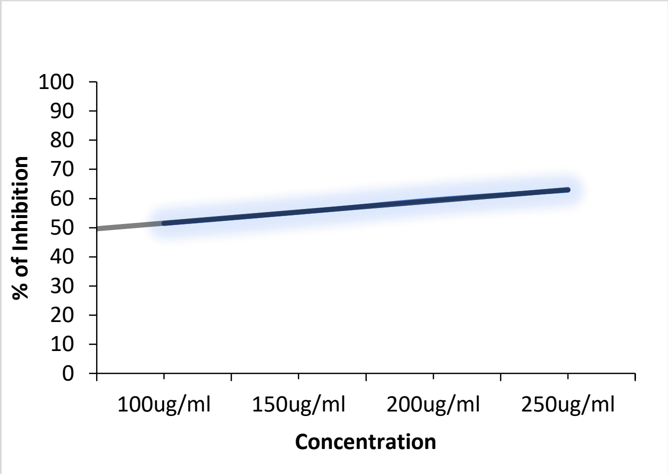

Figure 10: Antioxidant activity results of Ni/rGO (RT) nanocomposite in terms of % DPPH scavenging (% of inhibition) at different concentrations

*EC_{50} is 35ug/ml.

Green synthesized Ni NPs have shown reduced size and better mono-dispersity compared to chemically synthesized counterparts. It has been found that green synthesized Ni NPs have antioxidant and antibacterial activity [14]. Plant extracts are used as reductants and stabilizers in synthesizing metal nanoparticles due to the advantages such as low cost, environmental friendliness, sustainability, ease of operation, high stability, and more uniformity in size than chemical and physical methods. Importantly, biosynthesized metal nanoparticles have a superior significance in biomedical applications where non-toxicity is strictly required [4].

Due to its rich source of antioxidants, Neem is considered to have a health-promoting effect [7]. The aqueous extract of neem has prevented the NPs from agglomeration by their action as capping agents [22]. As a reducing agent and capping agent Neem can be used in the synthesis of a vast range of NPs [23]. The usage of neem leaf extract is a cheap, efficient, and non-toxic method to deposit metal nanoparticles on the GO surface [10]. Ni NPs have more significance due to their excellent magnetic properties, cytotoxic behavior, antibacterial properties, antioxidant and anticancer activity. There are many biomedical applications of GO-based nanocomposites including antibacterial materials, biofuel, and bio sensing materials, particularly in the diagnosis of diabetes, contact lenses, etc. And also widely used in other fields such as material science and electronics[9]. It has been found that due to the synergetic effects between supported Ni NPs and rGO sheets, Ni-rGO hybrids exhibit better catalytic activity than pure Ni NPs [24].

In the current study, we have focused on the synthesis of a Ni/rGO nanocomposite using a green approach which can be considered as a cost-effective and eco-friendly method. Neem leaf extract was used as the reducing agent in the synthesis of Ni nanoparticles. We decided to use GO with to obtain a mono-dispersed single layer of Ni NPs which aids in enhancing the properties and the efficiency of synthesized material.

As the first step, Ni NPs were synthesized by heating and stirring Neem ethanol extract with $NiCl_2$ solution at 60^0C. Color changes and UV-Visible spectroscopy results confirmed the formation of Ni NPs. Synthesis of Ni/rGO nanocomposites was carried out under different conditions to compare their properties and thereby to obtain the nanocomposite with the best properties. GO and $NiCl_2$ solutions were mixed with Neem extract in 3 beakers under different synthesis conditions to obtain nanocomposites with different properties.

Sonicated Ni/rGO composite, heated Ni/rGO composite and the Ni/rGO composite at room temperature (RT) were synthesized by sonicating, keeping the solution at 60^0C and by keeping the solution at room temperature respectively. Color changes and the UV-Vis spectroscopy results confirmed the formation of the nanocomposites. All the samples were kept undisturbed at room temperature for a week and allowed them to get precipitated. Finally, the precipitates were separated after centrifugation and dried in a hot air oven to obtain the expected Ni/rGO nanocomposite. At the end of the process, Ni/rGO nanocomposites were synthesized successfully.

Piu Das *et al.*, (2019) in India, have done a study on rGO-Ni nanocomposite. The existence of different oxygen-containing functional groups on GO and the successful reduction of GO to rGO in the rGO–Ni nanocomposites were confirmed by FTIR spectroscopy. The FTIR spectrum of GO has exhibited the stretching frequency of hydroxyl, epoxy, alkoxy, and O–H bending at 3300, 1147, 1100 and 1618 cm^{-1} respectively [25].

Amer Al-Nafiey *et al.*, (2019) in Iraq, have done a study on one step synthesis of Ni/rGO nanocomposite. They have found that there are differences in FTIR spectra of GO and the reduction product. For GO, the 3418 cm^{-1} observed belongs to O–H stretching vibration with strong and broad absorption. Also, 1737 cm^{-1} observed belongs to C=O stretching of -COOH groups situated at the edges of GO sheets. The absorption at 1420 cm^{-1} may be due to tertiary C–OH groups. The absorption band at 1080 cm^{-1} is recognized to stretching vibrations of -CO groups, while the band at 1623 cm^{-1} is recognized as C=C stretching vibrations as part of the ring breathing mode in the GO skeleton and the band at 1228 cm^{-1} is recognized as stretching vibration of C-C. In comparison, for the rGO/NiO NPs, the band O-H was decreased, while a vibration band C=C at 1623 cm^{-1} which shifted to 1587 cm^{-1}, and the band C-C at 1228 cm^{-1} shifted to 1233 cm^{-1}. Furthermore, the band C=O at 1737 cm^{-1} decreased and shifted to 1717 cm^{-1}, and the C–OH, and C–O band of GO disappeared. It was clearly noted that the chemical reduction with nickel chloride with GO was reduced to rGO and that appears in the decreases in the peaks of rGO [26].

FTIR spectra of Ni NPs reduced by Neem extract. (Figure 4) have been recorded in the range of 400-4000 cm^{-1}. The FTIR spectral results displayed different absorption peaks at 460, 1058, 1160, 1400, 1632, 2926 and 3443 cm^{-1}. When compared to the FT-IR spectra of GO, peak intensities due to alkoxy and hydroxyl groups have been decreased in the FT-IR spectrum of rGO according to the previous studies. Peaks given by C-O stretching, C=O stretching, and O-H stretching in GO disappeared and decreased dramatically in the FT-IR spectrum of rGO due to the removal of oxygen-containing functional groups. The disappearance of these oxygenated groups in the nanocomposite confirms the successful formation of the product. Thus, the FTIR results collaborated with the successful synthesis of the Ni/rGO composite.

TGA was used to analyze the thermal characteristics of the synthesized composites. The Ni/rGO composites had a mass loss of approximately 30% in the temperature range of RT-100 ^{0}C. This can be due to the loss of moisture in the sample. The second loss can be seen with a 20% degradation between 150-250 ^{0}C due to the pyrolysis of oxygen-holding functional groups present in the plant extract giving CO_2, CO or H_2O vapors. A mass loss of 35% was visualized between 300-800 ^{0}C which can be mostly due to the combustion of carbonaceous materials [26].

As-prepared composite, rGO/NiO NPs and GO characterized by using FE-SEM and EDX. These techniques evaluate the surface morphology, size and the elemental composition of Ni/ rGO nanocomposites. The SEM images depict that the surface of the rGO is covered with Ni NPs. The surface morphology appears as a cluster of crystals due to the capping of Ni/rGO nanocomposites with phytochemicals present in *Azadirachta indica extract.* EDX mappings are done in order to further confirm the composition of the rGO/Ni NPs composite (Figure 8). EDX spectrum shows the presence of nickel, carbon, and oxygen. FE-SEM image for the same region that EDX spectrum illustrates that the graphene is coated by the Ni NPs [27].

The presence of carbon and oxygen originates from the biomolecules present in the aqueous leaf extract. The major element present in the nanocomposites is C. And also, it is much greater than the percentage of oxygen. The percentage of Nickel is small compared to carbon and oxygen. Chlorine is also present, which may be due to incomplete removal in the washing procedure. The amount of Ni incorporated into nanocomposite was found to be 17.24%.

Antioxidants protect cells against damaging effects caused by reactive oxygen species, decreasing the occurrences of some degenerative diseases like cancer, and heart diseases. The antioxidant activity of synthesized nanocomposites was examined by DPPH scavenging assay; while ascorbic acid was utilized as a positive control.

The ability to donate hydrogen is thought to be responsible for the antioxidant properties of synthesized nanocomposite. When DPPH accepts an electron from an antioxidant, the color of the solution changes from violet to yellow, which may be detected spectrophotometrically at 517 nm [28].

Ni NPs have shown good antioxidant properties and results of Ni/rGO nanocomposites have always shown higher antioxidant activities than GO. Concentration-dependent antioxidant activity can be observed with each sample including Ascorbic acid which was used as the standard. The highest activity has been observed with Ni/rGO nanocomposite which was synthesized at room temperature. EC_{50} of Ni/rGO (RT) sample was calculated as 35ug/ml. That is the antioxidant concentration required to obtain a 50% radical inhibition from that sample. The EDX analysis confirmed that the Ni/rGO composite shows a remarkably high antioxidant activity even with a lower amount of Ni in the sample.

Equation/Formula

Scavenging effect (%) or Percentage of Inhibition =[A_0-A_1/A_0] X 100. --- (1)

A_0 & A_1, are the absorbance of control and test sample solution respectively

6. Conclusion and Future Scope

Synthesis of nanoparticles via green approaches has become prominent in the modern scientific world due to their cost-effective, eco-friendly manner. Previous literature verifies this fact as they have conducted a vast range of research on this field. We were able to bring these efforts to the next level by successfully synthesizing eco-friendly, reduced GO supported Ni NPs via a green synthetic protocol using *Azadirachta indica* as the reducing and capping agent. The characterization of the synthesized material using UV-Visible spectroscopy, FT-IR, SEM, EDX, and TGA confirmed the formation of the expected composite. Furthermore, the excellent antioxidant activity of the Ni/rGO composite was proven by the DPPH assay. The highest antioxidant activity exhibited by the composite synthesized at RT which contained 17% Ni proved that the antioxidant activity is greatly enhanced due to the synergetic effects between supported NiNPs and rGO sheet Hence this study concludes that the Ni/rGO composite synthesized using leaf extract of *Azadirachta indica* can be an excellent candidate for biomedical, pharmaceutical as well as other industrial applications.

This study has focused on the synthesis of a novel Ni/rGO nanocomposite and it possesses good antioxidant properties. Therefore, we recommend further investigations for assessing their antioxidant activity and the ability to use them in therapeutic applications. The mechanism of the reduction process is determined using the chemical composition of the leaf extract. Further studies are needed to be carried out to compare the activity of the biologically synthesized nanocomposite and chemically synthesized counterparts. Also, it’s better to check how activity changes with temperature, concentration of the leaf extract and metal ion solution.

Also, studies should be extended to evaluate the stability of the synthesized nanocomposites and investigations are required to synthesize Ni/rGO nanocomposite with increased efficiency. Additional exploration is recommended to evaluate other properties except antioxidant activity such as antimicrobial activity and biocompatibility of the synthesized composite.

It is also important to extend the study to include an in-vivo antioxidant activity assessment to better understand the composite's effects in a biological system. Also exploring the potential of the Ni/rGO composite in drug delivery systems, wound healing, or as an anti-cancer agent is vital due to its antioxidant properties. In addition to that focusing on studying the feasibility of scaling up the production of Ni/rGO composites and evaluating their economic viability for commercial applications in various industries.

Data Availability
None

Conflict of Interest
We declare that we do not have any conflict of interest.

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Authors' Contributions
Author-1 and Author-2 reviewed literature and carried out the experiments. Author-1 prepared the first draft of the manuscript. Author-3 supervised the research work and reviewed and edited the manuscript and approved the final version of the manuscript.

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