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Green Synthesis of Nickel Oxide (Nio) Nanoparticles with *Brassica oleracea* Var. Capitata F. Rubra, Its Characterisation and Phytochemical Investigation

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Abstract

Most nanoparticles have diameters that lie within 1 to 100 nm. A variety of metal oxide nanoparticles can be synthesised chemically as well as biologically. NiO nanoparticles, which are synthesised chemically, tend to be highly toxic in their effects. In contrast, synthesis by the route of biogenesis or biomimetics is much more favourable than chemical synthesis. The pre-existing properties of purple cabbage are remarkably enhanced by NiO nanoparticles. Nickel oxide (NiO) nanoparticles were synthesised from the leaves of *Brassica oleracea* var. capitata. f. rubra (purple cabbage) by reducing Nickel nitrate hexahydrate solution for the formation of NiO nanoparticles. The analysis done using a Particle size analyser reveals the spherical morphology and the size of the NiO nanoparticles. The size and shape were studied under a Scanning Electron Microscope, which shows that the nanoparticle ranges from 34.5 nm to 89.6 nm. The formation of NiO nanoparticles was confirmed by the peaks obtained in Raman spectroscopy and UV-DRS analyser. The peaks that ensure the formation of NiO nanoparticles in Raman spectroscopy are 568.40 cm^{-1}, 1129.46 cm^{-1} and 1379.07 cm^{-1} and UV-DRS are 265.60 nm and 339.69 nm. Preliminary phytochemical analysis of the aqueous extract of purple cabbage leaves shows that there is an abundance of phytochemical content present in purple cabbage. From the preliminary phytochemical analysis of purple cabbage, it was observed that the aqueous purple cabbage leaf extract contains alkaloids, sterols, flavonoids, coumarin, tannins, cardiac glycosides, anthraquinones, saponins, quinones, and carbohydrates.

Keywords: Nanotechnology, Green Synthesis, Metal Oxide Nanoparticles, Nickel Oxide, Purple Cabbage, Phytochemical Analysis.

1. INTRODUCTION

The rapidly emerging field of nanoscience and nanotechnology focuses on the design, manufacture, characterisation, and distribution of nanoscale materials, which typically have diameters that range from 1 to 100 nm. These materials, ranging from 1 to 100 nm in diameter, are commonly called nanoparticles. Besides these biomedical applications of nanotechnology, it has highly relevant uses in a wider range of fields, including electronics, optics, biomedical science, drug delivery, mechanics, chemical industry, nonlinear optical devices, optoelectronic devices, catalysis, energy science, space industries, and photoelectrochemical applications [1–5].

These nanoparticles can be synthesised using various methods, including physical, chemical, and biological processes. Green synthesis is a clean, economical, environmentally benign, and sustainable method of producing nanomaterials [1–5]. High coercive forces, large specific capacitance, electrochemical stability, anti-ferromagnetic characteristics, good chemical stability, and magneto-crystalline anisotropy are just a few of the positive attributes that NiO nanoparticles possess [6]. Targeted drug delivery systems, antimicrobial and antifungal therapies, anti-inflammatory properties, and preventive and therapeutic activity against a range of acute and chronic illnesses are just a few of the uses for NiO nanoparticles.

Additionally, it is frequently employed in the decomposition of dyes, the calculation of chemical oxygen demand, and the elimination of contaminants from waste effluents from tanneries and the textile industry [6].

The two methods of nanoparticle synthesis are the top-down approach and the bottom-up approach. The top-down approach refers to the process of breaking bulk substances into nanosized particles. Many physical methods of synthesising nanoparticles are classified under a top-down approach. These physical methods are usually toxic in comparison with green synthesis [7]. The bottom-up approach refers to the production of nanoparticles from atoms. Many chemical and biological methods have been adopted for the synthesis of nanoparticles. The chemical method is way more toxic due to the high amount of chemicals used for its production. Biological methods are usually less toxic or non-toxic. This method is also called green synthesis [7]. The green synthesis method involves the use of plants, fungi, algae, etc. Every part of these has special properties that aid us in the production of nanoparticles by acting as a potential reducing or oxidising agent.

Green synthesis is a bottom-up approach that aids in the synthesis of desired nanoparticles that have various applications in the fields of nanotechnology, nanomedicine, nanoscience and toxicology. It has great advantages over synthetically produced nanoparticles. Biologically synthesised nanoparticles are less toxic than chemically synthesised nanoparticles [8]. Green synthesis is an environmentally sustainable method of nanoparticle production. It is also called 'Biomimetic synthesis', or 'Biogenic synthesis'.

Purple cabbage (*Brassica oleracea* var. capitata f. rubra) is red cabbage. Purple colour is obtained from the phytochemical anthocyanins. It is widely used as a natural colourant. It is rich in antioxidants, anti-inflammatory, anti-cancer, and antidiabetic. Purple cabbage leaf extract is rich in alkaloids, sterols, flavonoids, coumarin, tannin, cardiac glycosides, anthraquinones, saponin, quinone, and carbohydrates [9]. Purple cabbage is highly favourable as it is low-calorie, nutrition-rich, and high-fibre. It is one of the best natural acid-base indicators. NiO nanoparticles synthesised using a biological bottom-up approach make it an environmentally sustainable product and show fewer toxic effects. This enhances the preexisting properties of purple cabbage.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Extract

Red cabbage was purchased from local markets in Chennai, Tamil Nadu.

The red cabbage was washed with distilled water and cut into fine pieces. The finely cut pieces are air-dried. About 20 g of finely chopped red cabbage was weighed accurately and added to 200 ml of distilled water. The mixture was heated at 80°C for about half an hour and mixed continuously with a magnetic stirrer.

The mixture thus obtained was filtered using Whatman No. 1 filter paper. Purple cabbage leaf extract was obtained as filtrate. The purple cabbage leaf extract obtained was stored in the refrigerator at 4°C for further use.

2.2. Green Synthesis of NiO Nanoparticles

50 ml of 0.1 mol of Nickel nitrate hexahydrate was prepared.

50 ml of purple cabbage extract was added to 50 ml of 0.1 mol Nickel nitrate hexahydrate and heated at 80°C for 4 hours. The mixture was continuously mixed using a magnetic stirrer. The clear green colour solution turned olive green.

The solution obtained was allowed to settle for an ageing period of 48 hours and filtered with Whatman No.1 filter paper. The residue thus obtained was dried at 60°C for 25 minutes. A dry fine powder of NiO nanoparticles was obtained and stored in an Eppendorf tube. A pictorial representation of the green synthesis of Nickel Oxide nanoparticles using B*rassica oleracea* var. capitata f. Rubra is given below in Figure 1.

2.3. Phytochemical Analysis of *Brassica oleracea* var. capitata f. Rubra

The phytochemical analysis of *Brassica oleracea* var. capitata f. rubra, also known as purple cabbage, reveals the presence of various bioactive compounds. This analysis is crucial in understanding this plant's medicinal and therapeutic potential. The phytochemical screening was conducted using standard protocols and reagents.

Test for Alkaloids

Hager's Test:

A few drops of Hager's reagent (saturated picric acid solution) were added to 2 ml of the respective plant extract. A bright orange-yellow colour is observed confirming the presence of alkaloids [10].

Test for Tannins

Alkaline Reagent Test:

Two millilitres of 1N NaOH were added to two millilitres of extract samples. The presence of tannins was verified by the appearance of yellow to red colour was observed [10].

Test for Anthraquinone

Anthraquinone Test:

A few drops of 2% Hydrochloric acid were added to the tested plant and a red colour was observed confirming the presence of Anthraquinone [10].

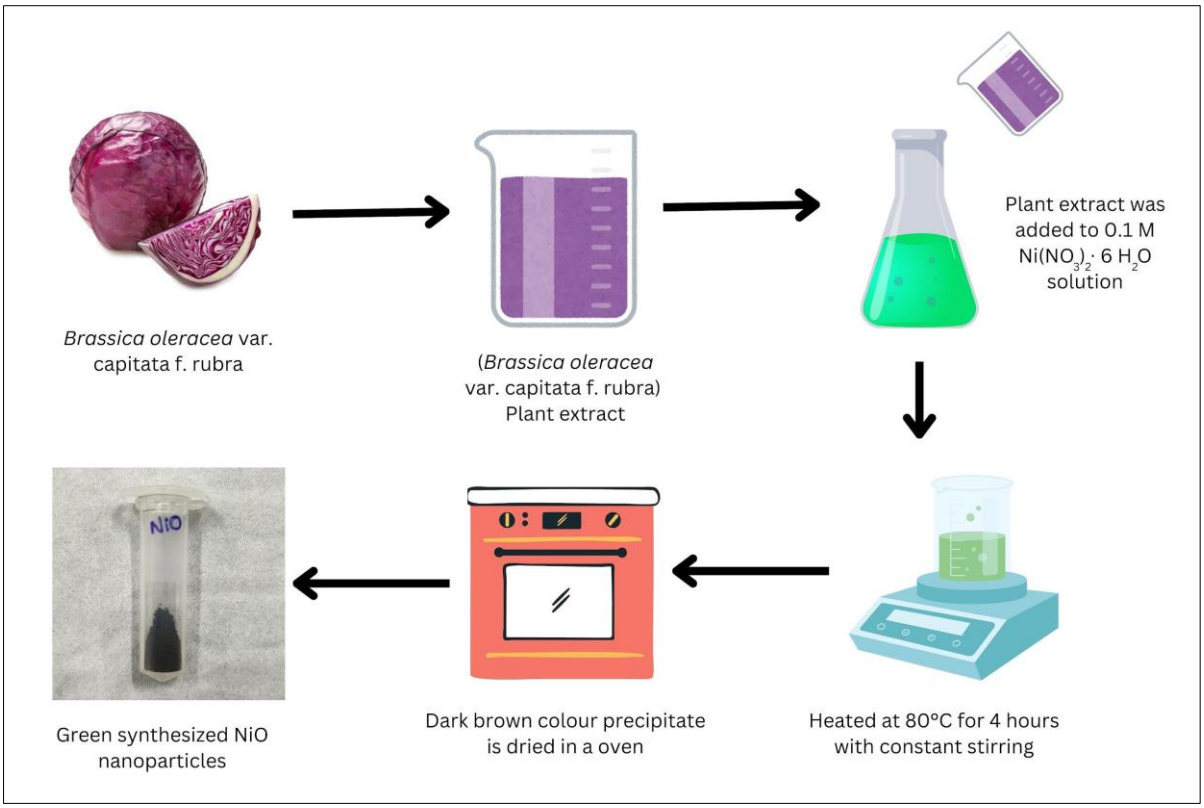

Figure 1: Green synthesis of Nickel Oxide nanoparticles using *Brassica oleracea* var. capitata f. Rubra

Test for Anthocyanin
Anthocyanin Test:

To the plant extract, a few ml of concentrated sulfuric acid was added and a yellowish orange was observed confirming the presence of anthocyanin [10].

Test for Coumarins
Coumarin Test:

1ml of 10% sodium hydroxide was added to 1 ml of plant extract and yellow colour was observed authenticating the presence of coumarins [10].

Test for Quinone
Quinone Detection Test:

To 1 millilitre of plant extract, 1 millilitre of concentrated sulfuric acid was added and a red colour was observed confirming the presence of quinones [10].

Test for Saponin
Saponin Test:

2mg of sodium bicarbonate was added to the aqueous extract and shaken vigorously. Froth formation confirms the presence of Saponin [10].

Test for Cardiac Glycosides
Baljet's Test:

A few drops of Baljet's reagent (1% picric acid, ethanol and NaOH) were added to 2-3 mg of the sample and an orange colour was observed affirming the presence of cardiac glycosides [11].

Test for Flavonoids
Flavonoid Test:

After boiling the 1g powdered sample in 10 ml of distilled water for 5 minutes, it was filtered while still hot. One millilitre of the cooled filtrate was mixed with a few drops of a 20% Sodium Hydroxide Solution and a yellow colour was observed [11]. This confirms the presence of flavonoids.

Test for Sterol:
Salkowski's Test:

To form a lower layer, 2 to 3 drops of strong sulfuric acid were applied and a reddish-brown colour was observed at the interphase confirming the presence of a steroidal ring [12].

Test for Carbohydrates
Molisch Test:

After reacting a few millilitres of plant extract with the Molisch reagent, concentrated sulfuric acid was applied to the test tube walls. The formation of a violet colour ring was observed at the junction confirming the presence of carbohydrates [12].

Test for Protein
Ninhydrin Test:

The ninhydrin reagent was added and heated in a few millilitres of plant extract. No characteristic colour change was observed indicating the absence of protein [13].

Xanthoproetic Test:

Concentrated nitric acid was added to 2 ml of plant extract and heated. No colour change was observed which indicates the absence of protein [13].

3. RESULTS AND DISCUSSION- CHARACTERIZATION STUDIES AND PHYTOCHEMICAL ANALYSIS

The produced NiO nanoparticles were characterised using Particle Size Analyzer (PSA), Scanning Electron Microscopy (SEM) examinations, UV Spectroscopy, and Raman Spectroscopy.

3.1. Particle Size Analyser

Using a Malvern particle size analyser, the size of NiO nanoparticles is in the range of 0.1-10000 nm. When the green synthesised NiO nanoparticles were analyzed using Particle Size Analyzer, one peak was observed at 295.3 nm with an intensity of 100% (Figure 2). Large particle size is due to the agglomeration of the nanoparticles. Size can be reduced by sonication [14].

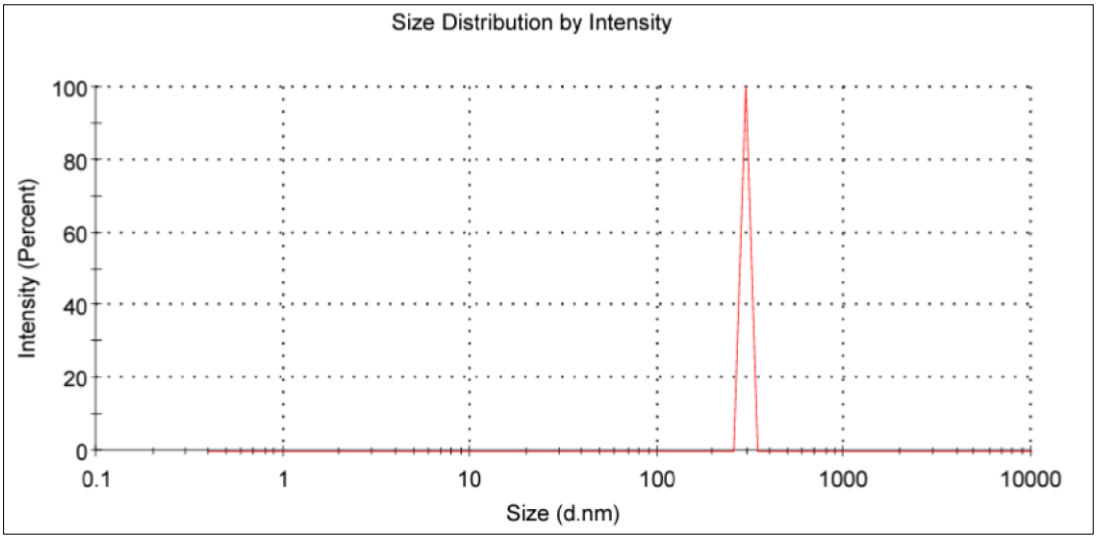

Figure 2: Graphical representation of intensity (per cent) against the size of NiO nanoparticles obtained from the PSA analysis

3.2. UV DRS Analysis

By analysing with UV Spectroscopy, the absorption spectrum of the nanoparticles was analysed and obtained peaks at 265.60 nm and 339.69 nm [15], (Figure 3). This verifies that NiO nanoparticles are forming. Previous research on Nickel Oxide nanoparticle [15], suggests that an absorption peak for NiO nanoparticles should fall between 265 and 346 nm. The peaks of the absorption boundary at 265.60 nm and 339.69 nm, which are read using Origin 8.5 software, verify the fabrication of NiO nanoparticles [15]. The quantum confinement of NiO nanoparticles accounts for the absorption peak at 339.69 nm [15].

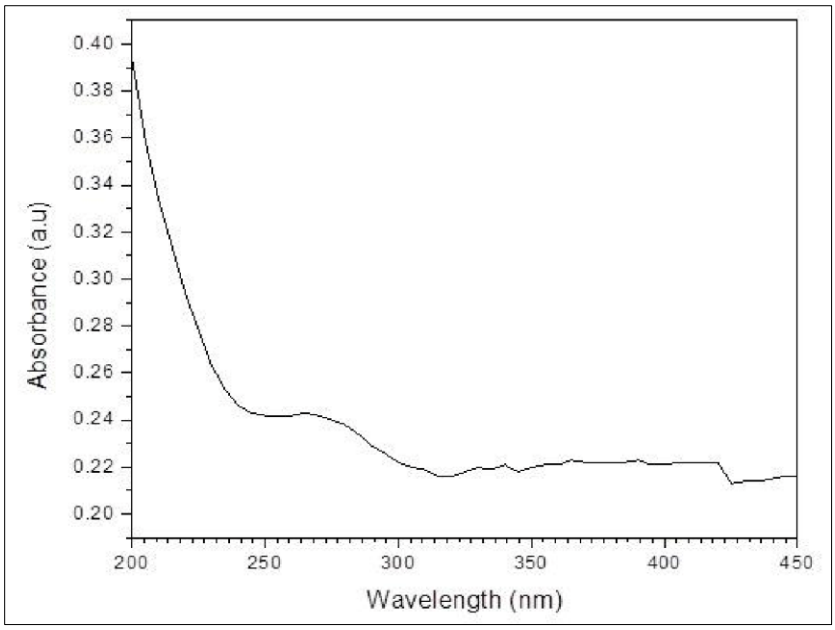

Figure 3: UV Spectroscope Analysis of NiO nanoparticles

3.3. Raman Spectroscopy

The obtained NiO NPs were subjected to Raman spectroscopy analysis to confirm the synthesis of NiO nanoparticles. The peaks were observed within the range of 0-4000cm^{-1}. The peaks were observed at 568.40 cm^{-1}, 1129.46 cm^{-1} and 1379.07 cm^{-1} and were read using Origin 8.5 software [15, 16] (Figure 4). These peaks were observed in previous literature and confirm the formation of NiO nanoparticles [15, 16].

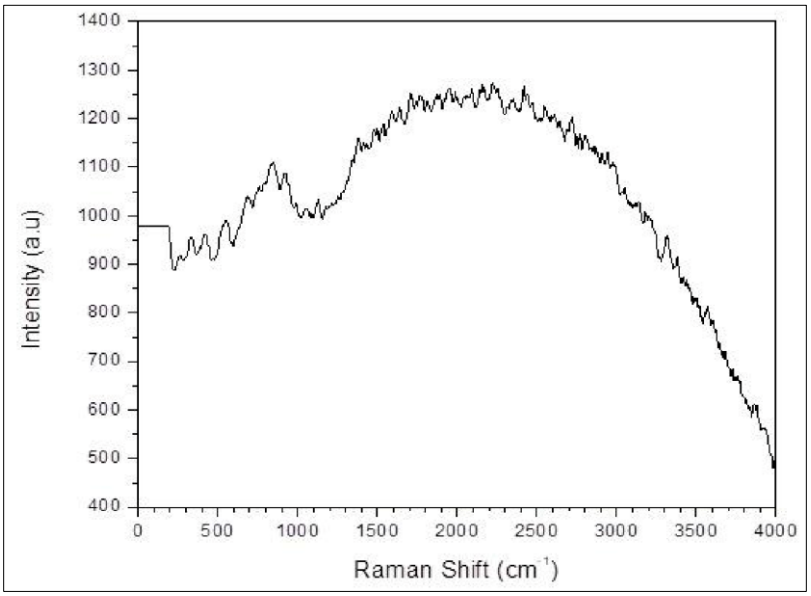

Figure 4: Raman Spectroscope Analysis of NiO nanoparticles

3.4. Morphological Analysis using SEM

The morphology of the nanoparticles is shown in (Figure 5). The SEM analysis aids us in concluding that the particles were highly agglomerated in nature. Due to the aggregation of minute particles, it has formed some larger particles. The SEM results after sonication and drying reveal that the nanoparticles are randomly distributed structures of around 34.5 nm to 89.6 nm and it is detected that the nanoparticles are homogeneous spherical-shaped particles [17]. In earlier studies, the shape and the average mean particle size observed from the SEM analysis were spherical. This is as per our SEM analysis results. So, we can confirm that these are NiO nanoparticles. The increased size of NiO nanoparticles occurs by aggregation and agglomeration of nanoparticles due to their larger surface area and higher number [17].

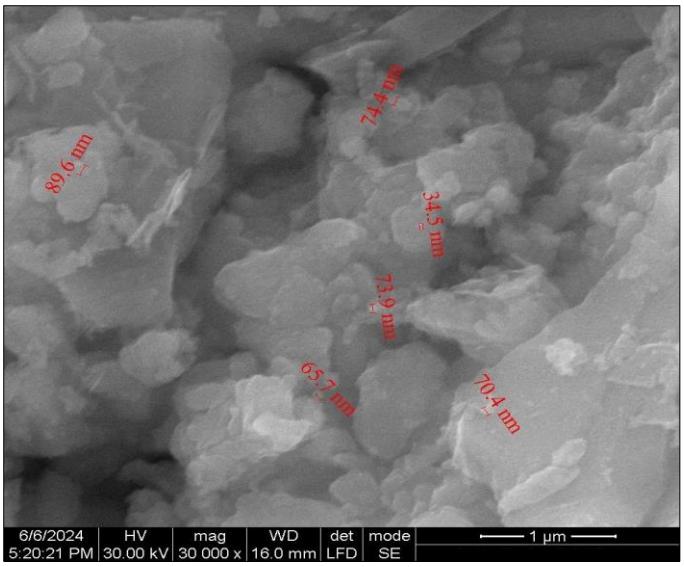

Figure 5: SEM Analysis of NiO nanoparticles

3.5. Phytochemical Analysis

The aqueous purple cabbage leaf extract of Brassica oleracea var. capitata f. rubra was shown to contain alkaloids, sterols, flavonoids, coumarin, tannins, cardiac glycosides, anthraquinones, saponins, quinones, and carbohydrates based on a preliminary phytochemical examination. These were confirmed by the colours observed in this study. Phytochemicals like flavonoids have various benefits and applications against a wide range of diseases [18]. It acts as a free radical scavenger and has anti-allergic, anti-microbial, anti-toxic, anti-cancer, and anti-cataract activity [18]. They have a wide range of biochemical actions and various pharmacological actions [19].

In this preliminary phytochemical analysis, phytochemicals present in purple cabbage (*Brassica oleracea* var. capitata f. rubra) were analysed. The phytochemical-rich nature of purple cabbage reveals its exclusive properties that make miracles by acting as a prophylactic and therapeutic agent for various diseases.

Numerous fundamental assays, including the Talcott test for sterols, the Anthraquinone test for anthraquinones, the Anthocyanin test for anthocyanin, the Coumarin test for coumarin, the Alkaline Reagent test for tannins, the Flavonoid test for flavonoids, the Anthraquinone test for anthraquinones, the Quinone detection test for quinone, the tests used were the Xanthoproteic test for proteins, the Molisch test for carbohydrates, the Ninhydrin test for acids, and the acid detection test [20]. These were noted in earlier research publications [10-13]. Table 1 provides an overview of the observed outcomes.

Table 1: **Summary of phytochemical investigation of *Brassica oleracea* var. capitata. f. rubra**

SUMMARY OF PHYTOCHEMICAL INVESTIGATION OF *Brassica oleracea* var. capitata. f. rubra. IN AQUEOUS EXTRACT	
CONSTITUENTS	**OBSERVATION**
ALKALOIDS	+
CARDIAC GLYCOSIDES	+
TANNINS	+
TEST FOR FLAVONOIDS	+
SAPONINS	+
STEROLS	+
ANTHRAQUINONES	+
ANTHOCYANIN	+
COUMARINS	+
QUINONE	+
CARBOHYDRATES	+
PROTEIN	-

4. CONCLUSION

The high toxicity of chemically synthesised nanoparticles can be overcome by green synthesis of metal oxide nanoparticles. In this study, NiO nanoparticles are synthesised biologically and characaterised. The applications of *Brassica oleracea* var. capitata. f. rubra extract proved to be an effective method for reducing the synthesis of NiO nanoparticles.

The NiO nanoparticles were examined using various techniques such as SEM, UV, Raman spectroscopy, and Particle size analyzer. SEM analysis revealed that the NiO nanoparticles synthesised range from 49 to 75 nm. This method of synthesis is an environmentally sustainable method of nanoparticle production.

The phytochemical analysis is crucial in understanding the medicinal and therapeutic potential of this plant. This analysis reveals the presence of Alkaloids, Cardiac glycosides, Tannins, Flavonoids, Saponins, Sterol, Anthraquinones, Anthocyanin, Coumarin, Quinone and Carbohydrates. Future application studies have to be done for the synthesised and characterised NiO nanoparticles to reveal the true prophylactic and therapeutic action of NiO nanoparticles synthesised using *Brassica oleracea* var. capitata. f. rubra.

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