Anti-breast carcinoma effects of green synthesized nickel nanoparticles from *Alhagi sparsifolia* leaf aqueous extract: a pre-clinical trial study

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Abstract

Introduction: In the study, nickel nanoparticles were green-synthesized using the *Alhagi sparsifolia* leaf aqueous extract.
Material and methods: The synthesized Ni nanoparticles were characterized by analytical techniques including SEM, UV-Vis., and FT-IR. The nanoparticles were formed in a spherical shape with the average size of 16.19 nm.
Results: In the antioxidant test, the IC_{50} values of Ni nanoparticles and BHT against DPPH free radicals were 316 and 231 µg/ml, respectively. In the cellular and molecular part of the study, the cells treated with Ni nanoparticles were assessed by MTT assay for 48 h as regards the cytotoxicity and anti-human breast cancer properties on normal (HUVEC) and breast cancer cell lines, i.e. lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453). The viability of the malignant breast cell line decreased dose-dependently in the presence of Ni nanoparticles. The IC_{50} values of Ni nanoparticles were 477, 548, and 605 µg/ml against lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453) cell lines, respectively.
Conclusions: After clinical study, nickel nanoparticles containing *Alhagi sparsifolia* leaf aqueous extract may be used to formulate a new chemotherapeutic drug or supplement to treat the several types of human breast cancer.

Key words: anti-human breast cancer effects, antioxidant properties, chemotherapeutic drug, cytotoxicity activities, green formulation, nickel nanoparticles.

Introduction

For many years, herbal medicines have been used and are still used in developing countries as the primary source of medical treatment. Plants have been used in medicine for their natural antiseptic properties. Thus,

research has been performed investigating the potential properties and uses of terrestrial plant extracts for the preparation of potential nanomaterial based drugs for diseases including cancer [1–4]. Many plant species are already being used to treat or prevent development of cancer. Multiple researchers have identified species of plants that have demonstrated anticancer properties with a particular focus on those that have been used in herbal medicine in developing countries [5–9]. Recently, the anticancer effects of *Tinospora cordifolia*, *Sophora subprostrata*, *Euphoria hirta*, *Barleria prionitis*, *Lubinus perennis*, *Maytenus boaria*, *Cephaelis acuminate*, *Phyllanthus niruri*, *Solanum seaforthianum*, *Boswellia serrate*, *Lavandula officinalis*, and *Cephalotaxus harringtonia* drupacea have been proved [10].

In recent centuries, the application of nanotechnology has played an important role in the development of science. Nanoparticles are widely used because of their high surface-to-volume ratio, small size, and excellent reactivity. One of the most important advances in nanotechnology is the production and application of nanoparticles in the biological sciences [10–13]. Nanoscience has shown that if we reduce the size to nanometers, unique properties such as optical properties, electrical conductivity, hardness, and chemical reactions will be obtained [10–12]. There are three methods to synthesize nanoparticles: biological, chemical, and physical. Chemical and physical methods are time-consuming and costly. In addition, these methods use some toxic additive chemicals that cause adverse effects on medical applications by adsorption on the surface. Applying the principles of green chemistry has decreased the use of toxic compounds or hazardous solvents, provided optimal regeneration conditions and ameliorated materials for the chemical processes, and raised new sources for green synthesis [12–14]. Therefore, one of the primary goals of green nanotechnology is to produce nanomaterials without harm to human health or the environment, and to develop and design nanomaterials and products that are suitable solutions to environmental problems. The synthesis of nanoparticles by similar biological methods results in greater catalytic activity and limits the use of toxic and expensive chemicals [10–13]. In biological methods, plant extracts, enzymes or proteins carrying natural resources are used to produce or stabilize nanoparticles. The nature of the materials used to make nanoparticles influences the shape, structure and morphology of these nanoparticles [10–14]. Biological systems involved in the green synthesis of nanoparticles include plants and their derivatives, as well as microorganisms such as algae, fungi, and bacteria. Plant parts such as roots, leaves, stems, fruits, and small parts such as the kernel and skin of the fruit are suitable to synthesize nanoparticles because their extracts are rich in phytochemicals that act as stabilizing and reducing substances [10–13]. The use of natural plant extracts is a cheap and environmentally friendly process and does not require intermediate groups. Short time, no need for expensive equipment, precursors, high purity product and excellent quality without impurities are the features of this method. It is possible very quickly, at room temperature and pressure as well as easily on a large scale. Bio-reduction in the conversion of base metal ions is carried out by various plant metabolites such as alkaloids, phenolic compounds, terpenoids and coenzymes [14, 15]. Nanoparticles centered on inorganic materials such as magnetic metals, their oxides and alloys, and semiconductors have the most studies and potential in biomedicine from diagnosis to treatment of diseases [11–13]. Nanoparticles are generally effective in a wide variety of sectors, and if their production is based on green chemistry, they have great applications in the fields of food, medicine, cosmetics and health. The effects of nanoparticles should be predictable, controllable and achieve the desired results with minimal toxicity. Metallic nanoparticles used in treatment and diagnosis, in addition to being non-toxic, must be biocompatible and stable *in vivo*. Also, by making appropriate changes in the surface of metallic nanoparticles, they will have a wide range of applications by binding to biomolecules and various carriers to cross the cell membrane and target the desired part of the body. One of the important points in the production of nanoparticles is the use of cost-effective and efficient precursors [12–15].

In the current research, the properties of nickel nanoparticles formulated by *Alhagi sparsifolia* leaf aqueous extract against common breast cancer cell lines, i.e. lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453), were evaluated.

Material and methods

Green synthesis and chemical characterization of Ni nanoparticles

First, the dried leaves of *Alhagi sparsifolia* were ground. Then, 75 g of the sample was macerated in 800 ml of boiling water for 5 h. After that, the extract was filtered and evaporated to a concentrate. Finally, the extract was placed in a freeze drier for 72 h. The obtained extract, a brown powder, was kept in cold place.

The green synthesis of nickel nanoparticles (NiNPs) was carried out according to a previous

study [38]. 20 ml of *Alhagi sparsifolia* extract (1 g in 10 ml of deionized water) was added to 50 ml of 15 mM $NiSO_4 \times 6\ H_2O$ in a flask. Then, to adjust the pH, 2 ml of NaOH (1%) was added dropwise and shaken for 25 min. Next the flask was put in an ultrasonic bath (75 W) for 30 min. The NiNPs were formed as a green precipitate during the reaction time. The NiNPs were washed with water three times and then centrifuged at 10000 rpm for 15 min. Finally, the precipitate was dried in an oven at 45 °C.

Antioxidant activities of Ni nanoparticles

The free radical scavenging test was first performed by Blois in 1958, and after some modification by numerous studies in its current form. The DPPH method is one of the most widely used methods for estimating antioxidant content. DPPH is a stable radical that reacts with hydrogen atom compounds. This test is based on the inhibition of DPPH, which causes the decolorization of DPPH solution by adding radical species or antioxidants. DPPH changes color from purple to yellow by taking an electron from the antioxidant compound. The free radicals in DPPH are adsorbed at 517 nm, which follows Beer Lambert's law, and decreased absorption is linearly related to the amount of antioxidants; the higher the amount of antioxidants, the more DPPH is consumed and the more purple turns yellow [15].

The degree of inhibition of DPPH radicals was evaluated by Shaneza *et al.* [15]. For this purpose, solutions with different samples of the Ni nanoparticles of variable concentrations (0–1000 µg/ml) as well as synthetic antioxidant BHT in methanol solvent were prepared. The test method was that 1 ml of DPPH methanolic solution (at a concentration of 1 mM) was added to 4 ml of the extract and the resulting mixture was stirred vigorously. The test tubes were placed in a dark place for 60 min. After this period, the absorbance was read at 517 nm. Finally, the DPPH radicals' percentage inhibition of the Ni nanoparticles was calculated by the formula below [15]: Inhibition (%) = (Sample A/Control A) × 100.

The IC_{50} factor was used to evaluate better the antioxidant activity, which indicates the concentration of the Ni nanoparticles that can reduce the concentration of the free radical DPPH. The initial is 50% of the initial value, and the lower the amount, the greater the antioxidant activity [15].

Anti-human breast cancer potential of Ni nanoparticles

In this research, we used the following cell lines to evaluate anti-human breast cancer and cytotoxic effects of Ni nanoparticles using an MTT method.

a) Human breast cancer cell lines: Lung well-differentiated bronchogenic adenocarcinoma (HLC-1), lung moderately differentiated adenocarcinoma (LC-2/ad), and lung poorly differentiated adenocarcinoma (PC-14).
b) Normal cell line: HUVEC.

Because nanoparticles are not directly soluble in 1640-RPMI medium and also the solvent of dimethyl sulfoxide nanoparticles (DMSO) itself has cytotoxic effects, to eliminate the effect of this substance on treated cells, its amount in the final solution is considered less than 1%. Dimethyl sulfoxide is not toxic at concentrations less than 1% and the concentration of this solvent is important in this regard. For this purpose, 1000 µg of nanoparticle was dissolved in 100 µl of dimethyl sulfoxide solvent after weighing. Then 1 ml of culture medium was added for better dissolution and finally the volume of solution was increased to 24 ml using culture medium: Then, successive dilutions of this stock were used in the proportions of 1–1000 µg/ml. Eleven concentrations were used for the cell lines [16, 17].

In this study, 100 µl of culture medium containing 10^4 cells per 96-well plate were placed. After 24 h, concentrations of 1–1000 µg/ml of nanoparticles were added to the cells, and incubated for 24, 48, and 72 h, respectively. After these times, 20 µl of MTT with a concentration of 5 mg/ml was added to each cell and incubated in the dark for another 4 h. After some time, the MTT medium was carefully removed, and 200 µl of acidified isopropanol was added to each plate to remove the purple formanes. After 15 min of incubation at room temperature, the light absorption of each well was read using an ELISA reader at 570 nm against a reference wavelength of 690 nm. The findings were reported as cell survival and IC_{50} (concentration that inhibits cell growth up to 50%) based on the concentration curve (µg/ml) [17].

It should be noted that the effect of each concentration of the nanoparticles on cell lines was investigated in five independent experiments. According to the values of light absorption obtained by the ELISA reader, the percentage of growth inhibition related to each concentration was calculated using the following formula: Cell viability (%) = (Sample A/Control A) × 100.

Finally, linear regression was done to gain IC_{50}, which indicates the nanoparticle concentration which causes 50% cancer cell growth inhibition. Using the curve, the line equation for cancer cells was obtained, respectively, then by replacing 50% inhibition in the equation, the IC_{50} value for cancer cells was obtained [17].

Ethics

This research was approved by Yan'an University Affiliated Hospital animal ethical committee, Approval No. YAUAH20201925.

Statistical analysis

SPSS statistical software version 22 was used for data analysis and the findings were determined as the mean ± standard deviation of 5 replications. Data were analyzed using one-way analysis of variance and the Duncan post hoc test and the significance level in the test was considered 0.05.

Results and Discussion

Chemical characterization of Ni nanoparticles

UV-Vis. analysis

The UV-Vis spectra of the green-synthetic Ni nanoparticles are presented in Figure 1. The surface plasmon resonance (SPR) of Ni nanoparticles was completed using UV-Vis. spectroscopy. The production of the biosynthetic Ni nanoparticles was observed. The advanced SPR bands at the wavelength of 321 nm proved the formation of the nickel nanoparticles. The bands are very close to those previously for the green synthesis of nickel nanoparticles [18, 19].

FT-IR analysis

The FT-IR spectrum of nickel nanoparticles is shown in Figure 2. The formation of Ni nanoparticles is proved by the presence of the peaks at wavenumbers of 452 and 561 cm^{-1}. These peaks are attributed to bending vibration of Ni-O. Similar peaks with some differences in the wavenumber have been reported for green-synthetic Ni nanoparticles by other research groups [20–22]. The other peaks in the spectrum are attributed to the functional groups of different organic compounds in *Alhagi sparsifolia* extract, which are linked to the surface of Ni nanoparticles. The presence of secondary metabolites such as phenolic, flavonoid, and triterpenes in *Alhagi sparsifolia* extract has been reported previously [7–9]. The peaks at 3425 and 2931 cm^{-1} are related to O-H and aliphatic C-H stretching; the peaks from 1411 to 1680 cm^{-1} correspond to C=C and C=O stretching, and the peaks at 1193 and 1107 cm^{-1} could be ascribed to -C-O and C-O-C stretching.

SEM analysis

The morphology of Ni nanoparticles was assessed by the SEM technique. Figure 3 presents the SEM of Ni nanoparticles. The images show the spherical shape for the nanoparticles with average particle size of 16.19 nm. Furthermore, the nanoparticles are aggregated. In our literature review, 10 to 100 nm was reported for biosynthesis of nickel using plant extracts as the capping agent [23–29].

Cytotoxicity, anti-human breast cancer, and antioxidant activities of Ni nanoparticles

Cancer is recognized as one of the leading causes of death in today's society and several drugs have been introduced to treat this disease, but most common cancers are not yet controllable and this disease imposes huge costs on the patient and society [30–32]. The main factor in the

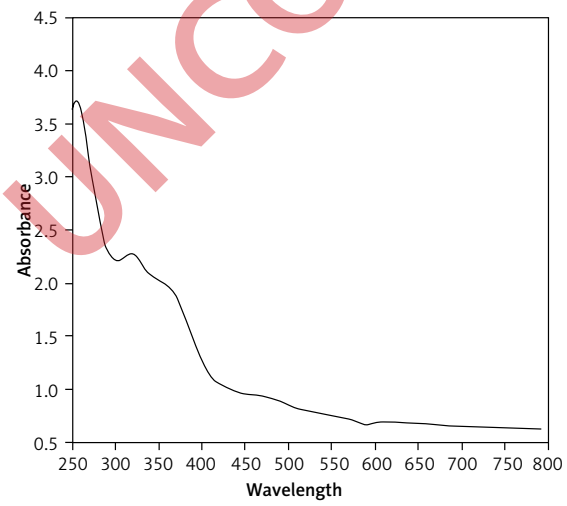

Figure 1. UV-Vis. Spectrum of biosynthesized Ni nanoparticles

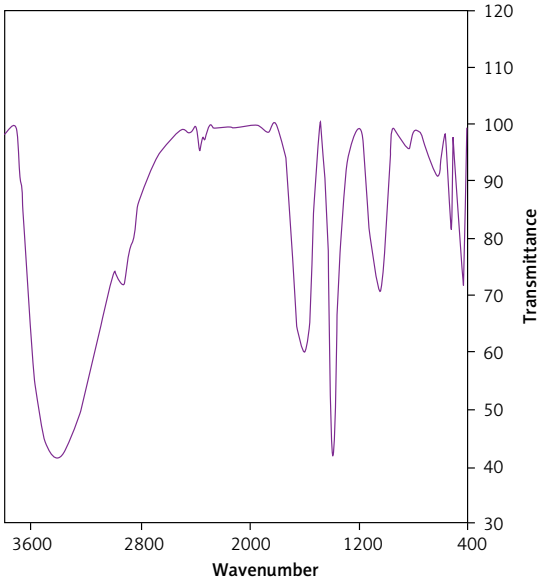

Figure 2. FT-IR spectra of biosynthesized Ni nanoparticles

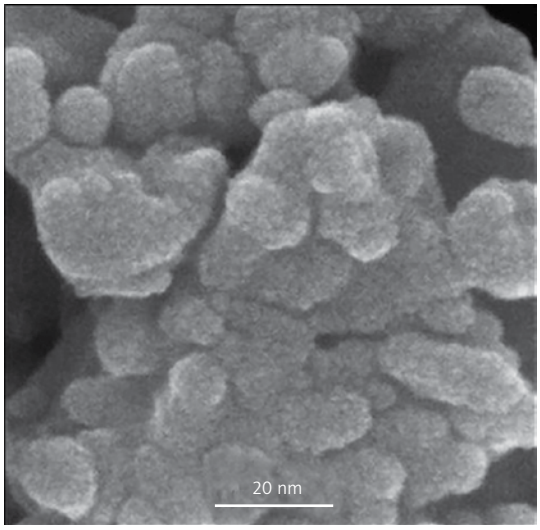

Figure 3. SEM image

development and progression of cancer has not yet been precisely identified; however, the available data suggest that metabolic disorders in the tissue and immune disorders may be involved in the development and exacerbation of this disease. In addition, metabolic disorders in the production and excretion of oxygen free radicals are important factors affecting cancer cells [33–35]. Free radicals are destructive compounds that are produced as a by-product by the body's chemical reactions and are destroyed by the body's defense system and enzyme system and antioxidants. However, in cases where the body's metabolic disorders and the production of free radicals are high and they are not destroyed by the neutralizing system, due to their instability, these compounds have a strong tendency to react with a variety of molecules in the body [31–34]. It is estimated that each cell in the human body is exposed to free radicals 10,000 times a day and DNA strands 5,000 times a day. Damage to cell components includes proteins (genetic disorder), fats (lipid oxidation), and cell membranes (permeability disorder); if the damage is not repaired, it leads to disruption of the chemical reaction and normal proteinization of the cell and the formation of harmful compounds and sometimes cancer cells in the body [35–38]. It is reported that thousands of cancer cells are produced daily in the human body that are killed by the body's defense system. In some cases, due to dysfunction of the above systems, cancer cells proliferate and conditions for cancer develop in different tissues [31–35]. According to the above, antioxidants play a vital role in preventing disorders caused by the effects of free radicals and thus the prevention and treatment of cancer. Antioxidants are a wide range of molecular compounds with complex properties that combine with and neutralize free radicals. More than 60,000 types of molecular antioxidants have been identified so far. Antioxidants can be effective in three known ways to prevent and treat cancer; 1. Destruction of free radicals; 2. Strengthening the immune system to destroy cancer cells; 3. Preventing adhesion of cancer cells to other cells and preventing their proliferation [32–36].

Table I. IC_{50} of Ni nanoparticles in anti-human breast cancer tests

Cells	Ni nanoparticles [µg/ml]
Human breast cancer cells:	
UACC-3133	477
UACC-732	548
MDA-MB-453	605

In this investigation, the cells treated with different concentrations of the nickel salt, *Alhagi sparsifolia* leaf aqueous extract, and Ni nanoparticles were assessed by MTT assay for 48 h as regards the cytotoxic properties on normal (HUVEC) and breast malignancy cell lines, i.e. lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453). The absorbance rate was evaluated at 570 nm, which represented viability on a normal cell line (HUVEC) even up to 1000 µg/ml for nickel salt, *Alhagi sparsifolia* leaf aqueous extract, and Ni nanoparticles (Table I and Figure 4). The viability of the malignant breast cell line decreased dose-dependently in the presence of nickel salt, *Alhagi sparsifolia* leaf aqueous extract, and Ni nanoparticles. The IC50 values of Ni nanoparticles were 477, 548, and 605 µg/ml against lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453) cell lines, respectively (Table 1 and Figure 4).

Many nanoparticles have pharmacological and biochemical properties, including antioxidant and anti-inflammatory properties, which appear to be involved in anticarcinogenic and antimutagenic activities [37–39]. It seems that the anti-human breast cancer effect of recent nanoparticles is due to their antioxidant effects. Because tumor progression is so closely linked to inflammation and oxidative stress, a compound with anti-inflammatory or antioxidant properties can be an anticarcinogenic agent [37, 38].

In this study, we assessed the antioxidant properties of *Alhagi sparsifolia* leaf aqueous extract green-synthesized Ni nanoparticles by using DPPH as a common free radical. Antioxidants produced in the body fight free radicals with two systems: enzymatic defense and non-enzymatic defense. Superoxide dismutase, catalase, and

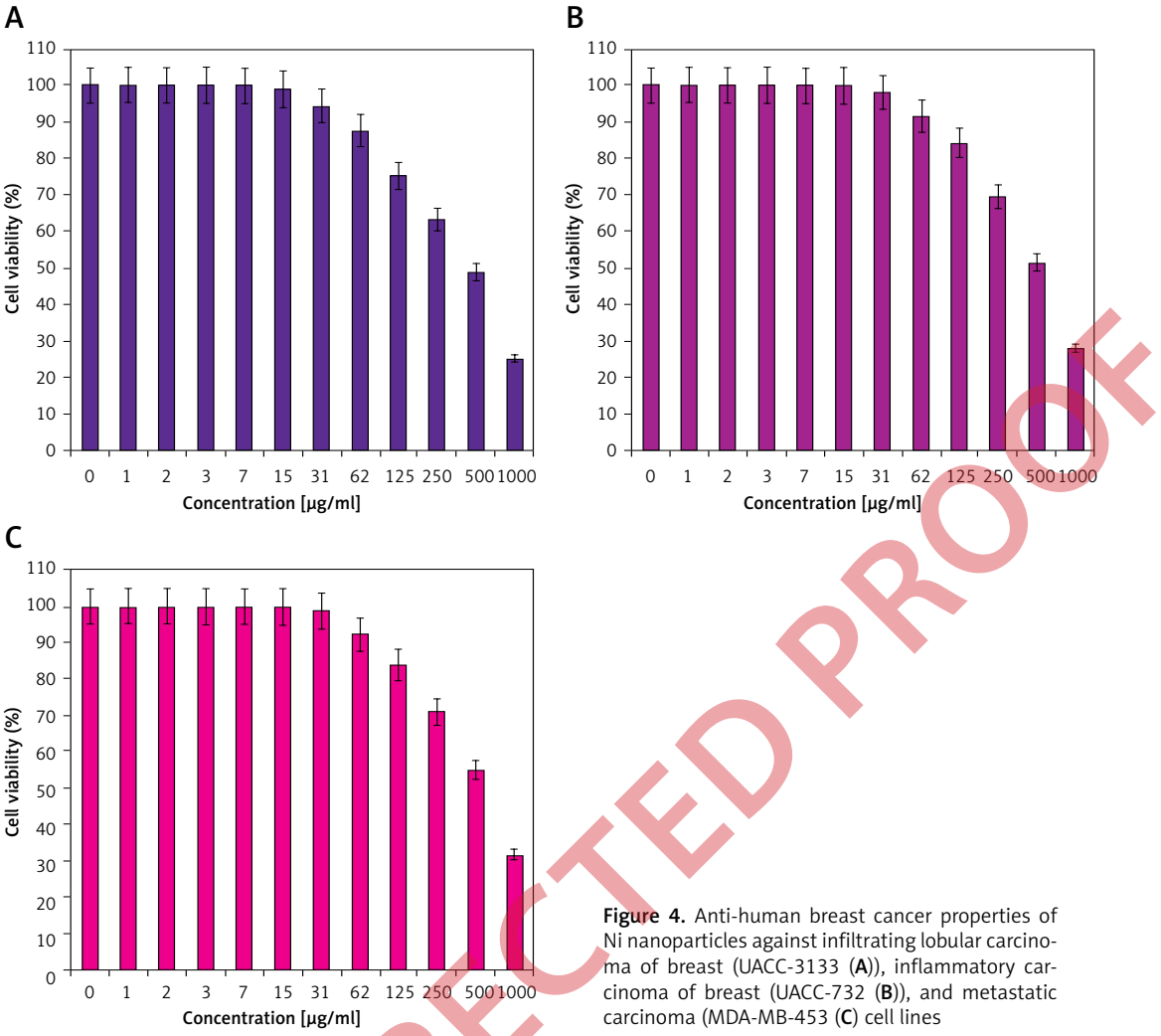

Figure 4. Anti-human breast cancer properties of Ni nanoparticles against infiltrating lobular carcinoma of breast (UACC-3133 (**A**)), inflammatory carcinoma of breast (UACC-732 (**B**)), and metastatic carcinoma (MDA-MB-453 (**C**) cell lines

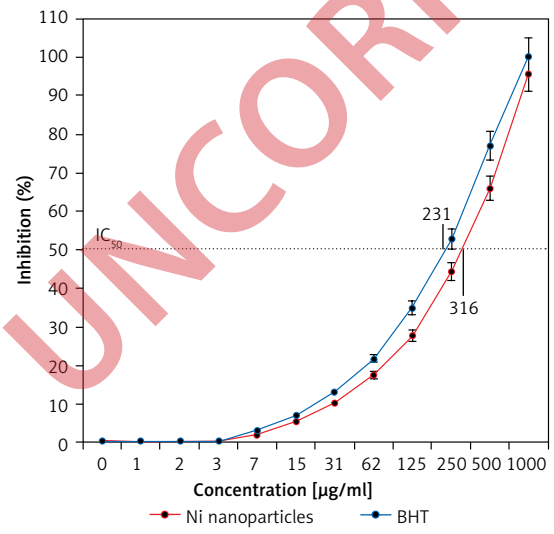

Figure 5. Antioxidant properties of Ni nanoparticles and BHT against DPPH

glutathione peroxidase metabolize lipid peroxide, hydrogen peroxide, and superoxide and prevent the production of toxic hydroxyl radicals [12, 15]. In non-enzymatic defense, there are two classes of antioxidants – fat-soluble (such as carotenoids and vitamin E) and water-soluble (glutathione and vitamin C) – that trap free radicals. These two systems help neutralize oxidants. However, oxidants can escape from antioxidants and damage tissues. In this case, the activated antioxidant repair system (comprising the enzymes lipase, protease, transferase and DNA repair enzymes), counteract the oxidant effects. However, due to deficiencies in the production of antioxidants in the body or due to physiopathological factors and situations (smoking, air pollution, UV radiation, diets containing high unsaturated fatty acids, inflammation, ischemia, bleeding, etc.) in which ROS are yielded in large quantities at the wrong place and time, oral antioxidants are needed to counteract the cumulative effects of oxidative damage [11, 12, 15].

The scavenging capacity of *Alhagi sparsifolia* leaf aqueous extract green-synthesized Ni nanoparticles and BHT at different concentrations expressed as percentage inhibition is indicated in Figure 5.

In the antioxidant test, the IC_{50} values of Ni nanoparticles and BHT against DPPH free radicals were 316 and 231 µg/ml, respectively (Figure 5).

In conclusion, the nanoparticles were characterized using common chemical techniques such as UV-Visible, FT-IR, and SEM. The SEM images indicated a spherical morphology for Ni nanoparticles with average size of 16.19 nm, which is well known as a sufficient size for synthetic nanoparticles.

The IC_{50} values of Ni nanoparticles and BHT against DPPH free radicals were 316 and 231 µg/ml, respectively. The viability of the malignant breast cell line decreased dose-dependently in the presence of Ni nanoparticles. The IC_{50} values of Ni nanoparticles were 477, 548, and 605 µg/ml against lobular carcinoma of the breast (UACC-3133), inflammatory carcinoma of the breast (UACC-732), and metastatic carcinoma (MDA-MB-453) cell lines, respectively. After clinical study, Ni nanoparticles containing *Alhagi sparsifolia* leaf aqueous extract can be utilized as an efficient drug in the treatment of breast cancer in humans.

Conflict of interest

The authors declare no conflict of interest.

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