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Research Article

PHYTOSYNTHESIS OF IRON NANOPARTICLES USING *GALIUM APARINE L.* EXTRACT: THEIR CHARACTERIZATION AND ANTIOXIDANT ACTIVITY

Merve BAT ÖZMATARA*[1]

[1]*Gebze Technical University, Dept. of Chemistry, KOCAELI;* ORCID: 0000-0002-6912-8825

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ABSTRACT

Nanoscience and technology are of great importance especially in terms of green synthesis of metallic nanoparticles, reducing the formation of by-products, chemical reagents and toxic solvents that have negative effects on the environment as well as on human health. In this study, iron oxide nanoparticles (FeNPs) were synthesized using *Galium aparine L.* extract. The nanoparticles were characterized by Ultraviolet-vis (UV-vis) spectroscopy and Fourier transform infrared spectroscopy (FT-IR). UV–Vis absorption spectrum of iron oxide nanoparticles display a peak in the region of 295–301 nm. FT-IR between 4000 and 400 cm^{-1} wavelengths exhibited exterior functional groups of FeNPs. The aim of this study was to evaluate antioxidant acitivities of FeNPs and *Galium aparine L.* extract. The antioxidant properties were evaluated using DPPH (1-1-diphenyl-2-picryl-hydrazyl), $ABTS^{+}$ (2,2'-azino-bis (3-ethyl benzo-thiazoline-6-sulphonic acid)) and DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) radical scavenging activity tests. According to the test results of DPPH (85.43%), $ABTS^{+}$ (75.28%) and DMPD (68.25%), FeNPs prepared with *Galium aparine L.* has higher antioxidant activity than *Galium aparine L.*
Keywords: *Galium aparine L.*, phytosynthesis, iron oxide nanoparticles, antioxidant activity.

1. INTRODUCTION

Oxidative stress is caused by a lack of balance between pro-oxidants and antioxidants. Excessive increase of reactive oxygenated species (ROS) with insufficient antioxidant defense increases oxidative stress and leads to various changes in biomolecules that define the disease state [1]. The use of herbs against diseases caused by the increase of oxidative stress is the research of today's drugs. The intake of antioxidants that reduce oxidative stress with natural foods such as fruits and vegetables is linked to the prevention of cancer and cardiovascular disease[2]. High use of plant foods is associated with a low risk of death from these diseases[3]. Polyphenols, an antioxidant compound, are the most important components of plants. The radical scavenging activity of polyphenols as oxygen quenchers, metal chelators, hydrogen donors and ferrous hemoglobin reducers is due to their redox properties [4, 5].

Galium aparine L. is a typical herb native to North America, Europe, and Asia. Anthraquinones, iridoids, alkanes, flavonoids, tannins, polyphenolic acids and vitamin C are

* Corresponding Author: e-mail: merve.bat@gtu.edu.tr, tel: (262) 605 30 60 / 3107

active ingredients of *Galium aparine L*. Consuming this plant contributes to the intake of natural antioxidants. Methanol extract of Galium aparine L has shown cytotoxic effects on human breast cancer cells [34.30±0.063, 55.67±0.131 and 71.14% for 100, 200 and 300 µg/ml] [6]. The hepatoprotective effects of *Galium aparine L.* was evaluated in carbon tetrachloride [CCl_4]-induced hepatic toxicity in rats. The results showed that *Galium aparine L.* possessed hepatoprotective effec ts [7]. The use of *Galium aparine L.* in the treatment of lymph swelling, tonsillitis, jaundice, wounds, cancer, fever, scurvy, hypertension and leukemia has been reported in different studies [8].

Nanoparticles are groups of many atoms or molecules, structures with diameters between 1 and 100 nm. Recently, iron, nickel, zinc, copper, silver and gold nanoparticles have attracted great attention due to their multitude of application areas [9]. Nanoparticles are synthesized by different methods and different precursors. Generally, nanoparticles are manufactured using chemicals that involve the use of toxic chemicals. With the increasing focus on the synthesis of nanoparticles with green synthesis, plant extracts have replaced toxic chemicals. Cost-effective and non-toxic green synthesis methods are preferred for the synthesis of iron nanoparticles because of the ability of plant extracts to act as stabilizing agents, reducing particle size and improving reactivity [10]. In this study, iron oxide nanoparticles were synthesized from *Galium aparine L.* for the first time, and this study revealed that iron oxide nanoparticles increase the antioxidant activity of the plant.

2. MATERIALS AND METHODS

2.1. Instrumentation

The antioxidant activity of *Galium aparine L.* extracts was measured using a UV-Vis spectrophotometer (SpectraMax Plus 384 Microplate Reader, California, USA). A vortex mixer, model IKA MS3 which is from Germany and FT-IR (Perkin-Elmer Spectrum 100, Wellesley, MA, USA) were used.

2.2. Materials and Chemicals

Galium aparine L. plant was bought from herbalist in Istanbul, Turkey. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), [2,2'- azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS), iron (III) chloride ($FeCl_3$) were purchased from Sigma. N,N-dimethyl-p-phenylenediamine dihydrochloride (DMPD) was purchased from Merck.

2.3. Extraction

In this study, *Galium aparine L*. leaves were extracted by maceration. 5 grams of *Galium aparine L.* was weighed and extracted by soaking in 20 mL distilled water for 1 day. At the end of the extraction, the samples were filtered and stored at 4°C.

2.4. Synthesis of iron oxide nanoparticles

20 mL of *Galium aparine L.* water extract was mixed with 20 mL of 0.001 M aqueous $FeCl_3$ solution in a 1 : 1 ratio. The mixture was incubated at 50°C-60°C for 20 min with shaking. The resulting black color showed the formation of iron oxide nanoparticles [11].

2.5. Characteriz ation of nanoparticles

2.5.1. UV-vis spectroscopy

Uv-vis spectra was recorded using UV-vis spectrophotometer in the range of 200-850 nm at room temperature. The purpose of UV-vis spectra was to observe the formation of iron nanoparticles [12].

2.5.2. FT-IR spectroscopy

FT-IR was used for the analysis in the range of 4000-400 cm^{-1}.According to this analysis of FeNPs, the bonds responsible from formation of iron oxide nanoparticles are detected.

2.6. Determination of antioxidant activity

2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

DPPH free radical scavenging activity is frequently used to evaluate the antioxidant capacity of compounds. The Brand-Williams method was used to test whether sample will bleach the stable DPPH radical [13].0.75 mL of plant extract was added over 1.50 mL of DPPH solution prepared in ethanol (0.05 mM). The mixture was kept at room temperature for 30 minutes. The absorbance values at 514 nm wavelength were measured in the spectrophotometer. The scavenging activity of DPPH radical was calculated using the following formula:

$$DPPH \text{ scavenging } (\%) = [\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}] \times 100 \quad \text{(Eq.1) [14]}$$

$A_{control}$ shows the absorbance of the control (DPPH solution without sample) and A_{sample} shows the absorbance of the test sample.

2.6.2. ABTS (2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity

This method, developed by Arnao et al, is based on the reduction of radical and color loss by adding antioxidants on the $ABTS^{\bullet+}$ radical cation formed by $K_2S_2O_8$ oxidation of ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]. Blue/green colored $ABTS^{\bullet+}$ radical, gives strong absorption in 600-750 nm [15].

An equal volume of 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution were mixed, then it was kept in the dark for 12 hours at room temperature. 1 mL of ABTS radical solution was diluted by adding approximately 60 mL of methanol to an absorbance of 1.1 ± 0.02 at a wavelength of 734 nm. 150 µL of sample solutions, 2850 µL of $ABTS^{\bullet+}$ radical solution was left in the incubation for 2 hours in the dark. The control solution was prepared using distilled water instead of the sample. The absorbance values at 734 nm wavelength were measured in the spectrophotometer. In the calculations, ABTS% radical scavenging effect was found with the following formula.

$$ABTS^{\bullet+} \text{ scavenging } (\%) = [\frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}}] \times 100 \quad \text{(Eq.2) [14]}$$

$A_{control}$ shows the absorbance of the control (ABTS solution without sample) and A_{sample} shows the absorbance of the test sample.

2.6.3. DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) radical scavenging activity

Fogliano method was applied for this antioxidant capacity test [16]. DMPD turns into cation radical form ($DMPD^{\bullet+}$) in acidic pH or in the presence of oxidant. After 100 mM DMPD solution was prepared, radical was formed by adding 100 mL of 0.1 M acetate buffer (pH 5.3) and 0.2 mL of 0.05 M $FeCl_3$ onto 1 mL of this solution. Next, the sample solution or the control solution which is prepared with water was then added. After 10 minutes, the absorbance at the 505 nm wavelength was measured in the spectrophotometer. $DMPD^{\bullet+}$ radical scavenging activity was calculated according to the formula below.

$$DMPD^{\bullet+} \text{ scavenging (\%)} = \left[\frac{Acontrol - Asample}{Acontrol}\right] \times 100 \quad \text{(Eq. 3) [14]}$$

3. RESULTS AND DISCUSSION

3.1. FT-IR and UV-vis spectra characterization

The FTIR spectrum of FeNPs and plant extract are shown in Fig. 1. The peaks at position of 3357.57 cm^{-1} represent the –OH bond stretching. The peaks at 1433.11 cm^{-1}, and 3691.57 cm^{-1} also represents the –OH bond stretching and bending from various phenolic and carboxylic group respectively present the plant extract. The shift in peak position in the range of 400–4000 cm^{-1} ensure that these functional groups containing compounds bound to the iron oxide surface. Metal oxygen bond formation is observed in the region of 400–850 cm^{-1}. Peaks at 618 cm^{-1} and 467 cm^{-1} correspond to the stretching vibration of Fe–O. The band at 618 cm^{-1} is associated with Fe-O-H stretching vibration of water molecules.1000-1400 cm^{-1}(1384 cm^{-1}) occurred because of C=C, OH, C–O, C=O or C–O stretching vibration. The band in 1630 cm^{-1} was formed due to the bending vibration of the water.

UV–Vis spectrum of FeNPs was presented in Fig. 2. The absorption peak at 298-301 nm indicates that iron oxide nanoparticles were formed.

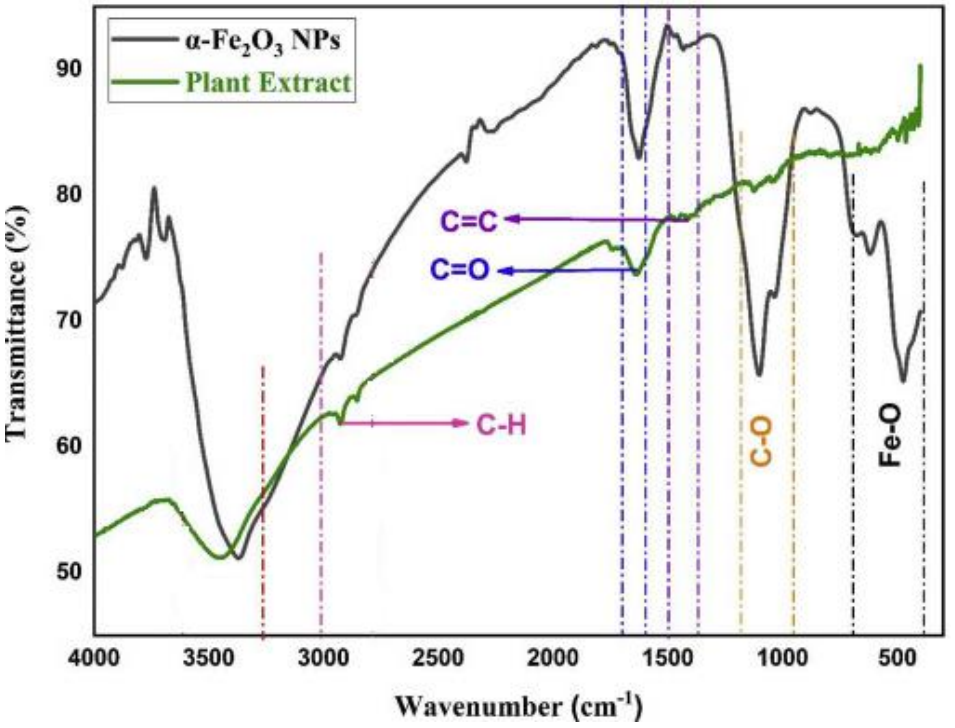

Figure 1. FT-IR spectrum of iron oxide nanoparticles and plant extract

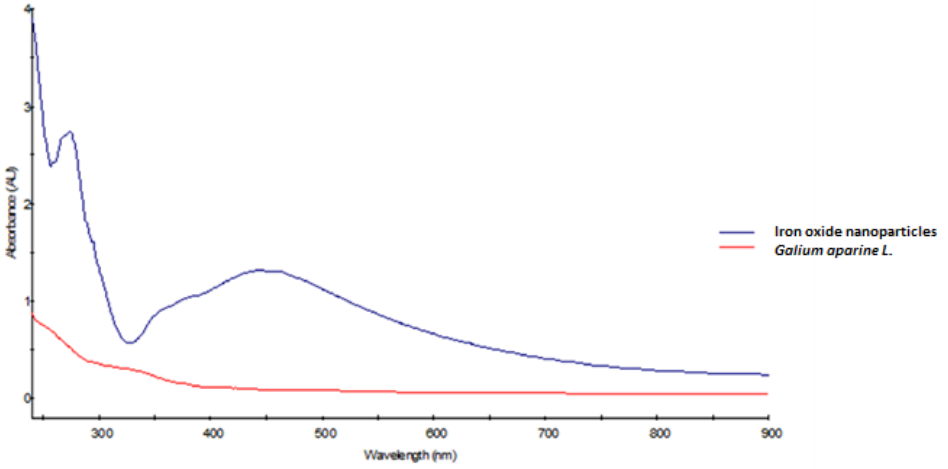

Figure 2. UV-vis spectrum of *Galium aparine L*. and iron oxide nanoparticles

3.2. Antioxidant Activity

Antioxidant activity was measured using DPPH, ABTS and DMPD radical scavenging tests. The results are shown as percent inhibition in Figure 3.

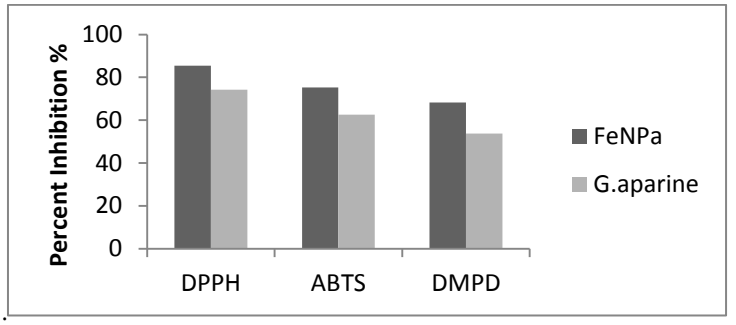

Figure 3. DPPH, ABTS, DMPD radical scavenging activity of G.aparine and FeNPs

In the DPPH test, the ability of *Galium aparine* L. to act as a donor for hydrogen atoms or electrons to reduce DPPH to DPPH-H was spectrophotometrically measured. The antioxidant activity of *Galium aparine* L. can be related to the presence of antioxidants like phenolics and flavonoids [17]. While the DPPH radical scavenging activity of the plant extracted with water was 74.24%, this inhibition rate increased to 85.43% with iron oxide nanoparticles. Iron oxide nanoparticles showed good antioxidant activity by scavenging free radicals significantly. This feature signed to electron transfer from Fe^{+2} / Fe^{+3} systems of iron oxide nanoparticles. In a study conducted by Neupane, iron oxide nanoparticles synthesized with Himalayan honey have higher antioxidant potential compared to Himalayan honey [18]. The DPPH activity of BHT(Butylated hydroxytoluen) used as a standard was 91.39%. The antioxidant activity of iron oxide nanoparticles obtained by plant extraction approached that of using BHT synthetically.

In the ABTS test, the reaction between ABTS and potassium persulfate forms the ABTS radical cation ($ABTS^{+)}$ and a blue green color is observed. When the antioxidant is present, the radical reverts to a colorless state. The ABTS radical scavenging activity of the plant was 62.59%. The synthesis of iron oxide nanoparticles increased this radical scavenging activity to 75.28%. ABTS activity of BHT(Butylated hydroxytoluen) used as a standard was 93.2%. The antioxidant activity of iron oxide nanoparticles obtained by plant extraction approached that of using BHT synthetically.

DMPD radical cation ($DMPD^{+}$) is generated through a reaction between DMPD and potassium persulfate and is subsequently reduced in the presence of hydrogen-donating antioxidants. DMPD radical scavenging activity was found to be less than other radicals. However, the synthesis of iron nanoparticles increased the plant's DMPD radical scavenging activity from 53.82% to 68.25%. The DMPD activity of BHT (Butylated hydroxytoluene) used as a standard was 63%. The antioxidant activity of iron oxide nanoparticles obtained by plant extraction was higher than that of BHT used as a synthetic antioxidant.

4. CONCLUSION

Research has been developed on the synthesis of metallic nanoparticles with plants, and the interest is gradually increasing due to their many properties.

The study concludes that iron oxide nanoparticles were obtained with *Galium aparine L.* using an eco-friendly method. FT-IR analysis showed vibrations of iron oxide which was the

proof of iron oxide nanoparticles formation. UV-Vis absorption spectrum also showed that iron oxide nanoparticle was formed. It was stated that iron oxide nanoparticles formed with plants have more antioxidant activity than plant extracts.

Producing nanoparticles from plants is environmentally friendly and inexpensive. Nanoparticles can be obtained quickly. It is emphasized that the FeNPs synthesized from the results of this study can be used in a wide variety of applications in the pharmaceutical, biomedicine or food industry.

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