Determination of Antioxidant Capacity and Total Polyphenol Content of the Extracts from Three Different Parts of Olive Plant and the Green Production of Iron Nano Particles (GInPs)

Ulker Bakir Ogutveren, Fadime Karaer, Esra Findik, and Burcu Kagnicioglu

Abstract—In this study, three different extracts were obtained from the stems, leaves and seeds of the olive plant by ultrasonic method, and the antioxidant capacities and total polyphenol contents of these extracts were determined. Chemical and physical properties of GInPs produced using these extracts were identified.

In order to synthesize GInPs, 5 g sample was extracted in 100 mL water at 80 °C during 15 minutes in ultrasonic reactor with 45 kHz frequency. Folin-Ciocalteu method was used for determining anti-oxidant capacity of the extracts and total polyphenol contents were determined by FT-IR (SHIMADZU IRTracer-100 Model) and ATR (Pike Tech). 0.1 M FeCl₃ solution as a source of iron was mixed with these extracts using different volumetric ratio to achieve nano-sized iron particle. The size distribution and zeta potential of iron particles produced from each extracts were determined with zetasizer (Malvern 500210). The volumetric ratio of iron solution and these extracts were investigated; these results were correlated with anti-oxidant capacity and total polyphenol content. Final GInPs products image was taken by stereomicroscope (Zeiss Discovery 20).

As a result of this study, it was shown that GInPs ranged from 10 nm to 100 nm were produced easily, and the production efficiency was related anti-oxidant capacity and total polyphenol content.

Index Terms—Green synthesis, iron nanoparticles anti-oxidant capacity, total polyphenol contents.

I. INTRODUCTION

Green synthesis of nanoparticles using various plant extracts containing polyphenols was gained importance thanks to production feasibility with low cost requirement and environmentally friendly methods as an alternative to high technology synthesis methods, which contains toxic, corrosive and hazardous substances with higher cost [1]-[3].

Antioxidants are substances that react with oxygen to remove or delay the negative effects of body and food. Antioxidant substances are very useful for human health. The interest and demand for antioxidant foods has increased recently. The best sources of antioxidants are natural, unprocessed foods such as fruits and vegetables, and vitamins.

In addition, the group containing the most antioxidant compound is phenols. Antioxidant capacity were analysed via two methods: hydrogen atom transfer mechanism (HAT) and single electron transfer mechanism (ET). There are various alternatives in HAT and ET-based methods, and the Total Phenol Method with Folin-Ciocalteu separator is one of these methods [4], [5].

Dietary antioxidants such as polyphenolic compounds, vitamins E and C and carotenoids are thought to be effective nutrients in the prevention of the oxidative stress-related diseases [6]. Antioxidant activity is expressed as inhibition of ROS-mediated oxidation of the probe or as a selected reference antioxidant equivalent such as troloxacin, ascorbic acid. The oxidation of probes can be measured by various detection technologies. These include spectrophotometric, fluorometric, chemiluminescence, EPR (electron paramagnetic resonance), Fourier transform infrared spectroscopy (FT-IR), NMR (nuclear magnetic resonance) and amperometric methods [7].

In recent years, the use of green synthetic iron nanoparticles (GInPs) has been increasing in environmental remediation. Iron nanoparticles can be prepared by conventional chemical, physical and mechanical methods. However, these conventional methods involve toxic, abrasive and combustible chemical substances, require special equipment and higher costs. Biosynthesis of nanoparticles using plant extracts to solve these problems in conventional synthesis methods; low cost, simple and environmentally friendly alternative method. Various plant extracts contain abundant chemical components such as phenols, reducing sugars, ascorbic acid, which are responsible for the reduction of metal ions and the stabilization of nanoparticles [8]-[10]. It has been shown that metal nanoparticles produced by plants are more stable than those produced by other organisms and that plants (especially plant extracts), fungi and bacteria are reduced more rapidly. Moreover, nanoparticles have more features in shape and size than those produced by other organisms [11].

The purpose of this study is to examine the methods used to determine the antioxidant capacities, polyphenol profiles and antioxidant capacities of various parts (leaf, seed and stem) of olive plant. In the first step of the study, plant products were extracted and antioxidant activity and total phenol contents were determined. Folin-Ciocalteu and FT-IR methods were chosen for this purpose. In this study, three different extracts were obtained from the stems, leaves and seeds of the olive plant by ultrasonic method, and the antioxidant capacities and...
III. RESULTS

A. Size Distribution and Zeta Potential Results of Green Iron Nanoparticle

Zetasizer (Malvern 500210) size distribution results were given in Table I. According to the results of size distribution analysis, it was obtained that size of produced GnPPs ranged from 10-10 nm in the leaf extract solution of 25 % (v/v). When GnP solution was not prepared freshly and sonication was not applied, agglomeration occurred in the solution instantaneously.

B. SEM-EDX Analysis Results of Green Iron Nanoparticle

EDX spectrum of green iron nanoparticles obtained with leaf extract solution of 25 % (v/v) was given in Fig. 2. The main elements were C, Fe and Cl in EDX spectrum. Also, O, Ca, K, Mg, and Al elements found in GnP solution. EDX elemental results of green iron nanoparticles were summarized in Table II. This results showed the compounds that can be formed by main elements in EDX spectrum.

![Fig. 2. EDX spectrum of green iron nanoparticle produced with 25 % iron solution and leaf extract.](image)

The result of EDX analysis indicated that GnPps were produced as Fe₂O₃ form. Other compounds observed can be sourced from olive leaves ingredients. SEM images of green iron nanoparticle at 20 kV EHT voltage and 11 mm working distance with different magnification were given in Fig. 3.
The anti-oxidant capacity of each extract was summarized in Table III. Calibration graph was shown in Fig. 4. The results indicated anti-oxidant capacity related phenol content. Folin-Ciocalteu Method using gallic acid calibration was used. SEM images demonstrated that size distribution of GInPs ranged smaller than 1 μm. Also, it was clearly obtained from these images that GInPs were agglomerated.

### Table III: The Result of Anti-Oxidant Capacity of Each Extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>ABS</th>
<th>Gallic acid (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf extract</td>
<td>0.263</td>
<td>0.91</td>
</tr>
<tr>
<td>Seed extract</td>
<td>0.133</td>
<td>0.46</td>
</tr>
<tr>
<td>Stem extract</td>
<td>0.083</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**D. Total Polyphenol Contents Determination of the Extracts by FT-IR**

Total polyphenol contents determined by FT-IR were shown in Fig. 5.

As a result of this study, it was shown that GlnPs ranged from 10 nm to 100 nm were produced successfully using an environmentally friendly, green manufacturing process. It was observed that the nano particles produced were disposed at agglomeration. Production in an ultrasonic bath can be proposed to overcome this drawback.

Production efficiency of GlnPs was found to be related anti-oxidant capacity as gallic acid and total polyphenol content of the extracts. The results showed that the gallic acid content was the higher value of 0.91 μg and the fraction of polyphenolic compounds was nearly 25% (v/v) in leaf extract. The maximum efficiency of GlnPs production was achieved using olive leaf extract due to this highest anti-oxidant capacity and total polyphenol content comparing the other parts of the olive.

**References**

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