EFFECTS OF DRYING METHODS ON AMOUNTS OF PHENOLIC AND FLAVONOID COMPOUNDS AND ANTIOXIDANT CAPACITY OF PLANTAGO LANCEOLATA L.

F. Ergün

Kırşehir Ahi Evran University, Faculty of Health Sciences Kırşehir/Türkiye
Corresponding author’s E.mail: fatma.ergun@ahievran.edu.tr

ABSTRACT

In this study, the effects of some drying methods on the total amount of phenolic and flavonoid substances and antioxidant activity of the Plantago lanceolata L. plant were investigated. The plant samples were divided into three groups; the first group dried in sunlight (P1), the second group dried in a thermostatic oven at 55 °C (P2) and the third group dried in the shade (P3). After drying process, the total phenolic and flavonoid substance amounts, antioxidant activities and 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical removal and reducing power capacities of the extracts obtained from the samples belonging to the groups were determined. In this study, the total phenolic substance amounts were determined as 15.65 ±2.25 mg GAE/g, 15.20 ±1.48 mg GAE/g, 6.97 ±0.26 mg GAE/g in experimental groups, respectively. The flavonoid substances were determined as 83.85 ±3.49 mg QE/g, 57.37 ±7.04 mg QE/g and 53.65 ±3.61 mg QE/g, in experimental groups, respectively. In addition, the highest DPPH radical removal activity and the highest Fe³⁺-Fe²⁺ reducing capacity were found in P3 at all concentrations. To conclude, shade-drying is more preferable than the others with respect to phytochemical content of dried samples of P. lanceolata plant, suggesting that there is a need for new studies on how phytochemical contents are affected by such applications.

Keywords: P. lanceolata; storage; drying; antioxidant.

INTRODUCTION

There is a close relationship between nutrition and health. Recently, the increase in nutrition-related diseases has led to examined of the consumed foods. This situation has increased the interest in plants that grow naturally and have nutritive and functional properties. Such plants have positive effects on health as well as their nutritional properties. Among the bioactive compounds found in these plants, especially phenolic and flavonoid compounds are important structures for health. It has been reported that these compounds are effective against many diseases for example cancer, cholesterol and diabetes (Thangapazham et al., 2006; Erlund et al., 2008; Riaz et al., 2021).

P. lanceolata, belonging to the spreading over a wide area Plantaginaceae genus, is a species with these features (figure 1). It grows wild and is a perennial herb (Budzianowska et al., 2004). It being consumed as fresh and dried nutrient and baby diet formula as well as is used as a traditional medicine in skin infections such as boils and acne, and in colds (Samuelsen, 2000). Generally, this species and other species are used to treat anti-inflammatory, antitumoral, antifungal, antibacterial, antispasmodic, analgesic, antiviral, liver protective, diuretic, insect bites, sunburn, skin diseases, eye irritation, mouth and throat inflammation wounds. In addition, it has been known to be effective in the treatment of diseases such as cold, cough, hoarseness, asthma, emphysema, bronchitis, fever, gastritis, ulcer, bladder problems, kidney stones, intestinal complaints, irregular menstruation, hypertension, rheumatism and hay fever (Chiang et al., 2002; Ghedira et al., 2008). The dried form is used as a sedative, eye cream and for the treatment of diarrhea and dysentery or intestinal parasites in children (Koçak, 2011).

Figure 1. Plantago lanceolata L. Plant.

Harvesting initiates rapid enzymatic degradation and microbial degradation in green vegetables and fruits. Therefore, edible, cultivated or naturally grown plants must be consumed within a short period of time when harvested. In order to eliminate this negativity, various
methods are applied to the plants at the end of harvest. Drying is one of the most important methods applied on plants for this purpose (Acar et al., 2015). This method is based on the principle of removing the water in the structure of the plant from the environment. In this way, enzymatic and bacterial destruction is prevented and the nutritive feature of the plant is preserved for a long time (Adhikari et al., 2016). In addition, this method provides the advantage of storage and transportation due to weight reduction. For the drying of vegetable materials, oven drying (Alara et al., 2019), sun drying (Adhikari et al., 2016), freeze drying (Branisa et al., 2017), vacuum drying (Shonte et al., 2020), microwave drying (Mashkani et al., 2018) and infrared drying (Zhou et al., 2016) are used.

Müller, (2007) stated in his study that drying is the most appropriate method especially for the preservation of medicinal and aromatic plants. In addition, the drying process provides ease of pharmaceutical use as well as the storage of medicinal and aromatic plants (Lorenzi and Matos, 2002). However, the bioactive components and pharmacological properties affect from drying process (Zhu et al., 2014). The study, it was aimed to detected the effects of three different drying techniques to be applied to naturally self-growing P. lanceolata plant samples on phenolic and flavonoid substance amounts and antioxidant capacity.

**MATERIALS AND METHODS**

**Collecting samples and forming groups:** P. lanceolata plant specimens was collected from three different locations (39°18'27"N'34°08'57"E 1199 m /39°05'14"N'34°15'08"E 1160 m / 39°08'36"N'34°05'51"E 1176 m) in Kırşehir Province of Turkey in May 2021. The above-ground parts of the plant were used. In the collection process, plant samples were ensured to be of similar size, taking into account the vegetation period. Samples collected from three sites were blended. The physical dirt on them was removed with the help of distilled water. The samples were then divided into 3 groups. Three groups were formed in the study and one of the drying processes in the sun, shade and oven was applied to each group.

**I. Group (P1):** Drying in the sun was applied. The samples were dried as a whole on a glass tray. The drying process took 2 day (day length 12 hours) under direct sunlight at temperatures between 18 °C -21 °C (Quispe-Fuentes et al., 2018).

**II. Group (P2):** Oven drying process was applied. The samples were dried in a thermostatic oven (Electro-Mag M 420 BP) at 55 °C for 12 hours in a glass tray (Jafari et al., 2016; Kamel et al., 2013).

**III. Group (P3):** Drying in the shade was applied. Drying was carried out on a glass tray in the shade with natural air flow and ambient temperature (21 ± 5 °C) for 72 hours (Pirbalouti et al., 2017).

The drying period in this study were determined as the moment when the samples reached constant weight. After drying, the samples were stored in polyethylene containers at +4 °C in the dark until the working day.

**Preparation of the extracts:** Merck and Sigma brand chemicals were used in all phases of the study. The plant samples were, firstly, physically ground in a grinder to prepare the extracts. 10 g of each fragmented sample was placed separately in three closed flasks. 200 mL of methanol was added to them and stirred in a magnetic stirrer. The resulting methanol extracts were filtered. This process was repeated several times. Methanol in the filtered extracts was removed by evaporator at 45°C. Extracts were kept at + 4 ° C for later use.

**Preparation of stock solutions:** Stock solutions were prepared separately for each drying method. Stock solutions were prepared from the extracts of groups by using methanol at a concentration of 1000 ppm. These solutions were stored at +4°C for this study.

**Determination of phenolic substance:** Phenolic substance determination of the groups was made according to the Folin-Ciocalteau method (Slinkard and Singleton, 1977). Standard graph was prepared using gallic acid. A 0.05 mL of the prepared stock solutions were taken, and their volume was completed to 1.84 mL with pure water. Folin-Ciocalteau reagent (FCR) was added to mixtures, separately. After waiting for 3 minutes at ambient temperature, 2% (w/v) Na2CO3 solution was added to them. They were kept for 2 h at ambient temperature. The absorbances of the samples were measured at 760 nm against a blank (Optima SP-30009). The measurements were performed in triplicate. The phenolic compounds of the extracts were detected as milligram of gallic acid equivalent.

**Determination of flavonoid substance:** The flavonoid compounds of the groups were detected by using the aluminum nitrate method (Nieva Moreno et al., 2000). Quercetin was used to prepare standard graphic. 0.05 mL of the stock solutions were taken and the volume was completed to 1.92 mL with methanol. 0.04 mL of KCH3COO (1 M) was added and after one minute 0.04 mL of Al(NO3)3 (10%) was added. Absorbances were measured at 415 nm (Optima SP-3000) after an incubation of 40 minutes. The measurements were performed in triplicate. The flavonoid compounds of the extracts were detected as milligram of quercetin equivalent using the standard quercetin graph equation.

**Determination of free radical removal activity:** The free radical removal activities of the groups were detected
using the method of Blois (1958). 1,1-diphenyl-2-picrylhydrazil (DPPH•) (0.1 mM) solution was used as the free radical. In addition butylated hydroxytoluene (BHT) (1000 ppm) was used as a standard. 20-100 μL of P1, P2, P3 and BHT stock solutions were taken. Methanol was added to make volume of 0.4 mL. Then, 1.6 mL of DPPH• solution was added. Control was prepared using methanol under the same conditions. After 30 min incubation of the prepared solutions in the dark at ambient temperature, the absorbance changes at 517 nm were measured against methanol (Optima SP-3000). Lower absorbance indicates high free radical removal activity.

The activity DPPH• radical scavenge was calculated by the following equation:

\[
\text{DPPH}•\text{ removal activity (％)=} \frac{([A_0- A_f]/A_0)\times100}{A_0}\]

\(A_0: \text{Absorbance of control reaction}\)

\(A_f: \text{Absorbance of plant extracts and standard solutions}\)

**Determination of reducing power:** The determination of the Fe^3+ reducing power of the groups were made using the method of Oyaizu (1986). BHT and stock solutions were taken into a flask with a concentration of 10-50 μg/mL. Pure water was added to make volume of 1.0 mL. On top of these solutions, 2.5 mL of phosphate buffer (0.2 M and pH 6.6) and K₃Fe(CN)₆ (1%) solution were added and kept at 50°C and 20 minutes. After, trichloroacetic acid (10%) was added and mixed. 2.5 mL pure water and 0.5 mL FeCl₃ (0.1%) was added to 2.5 mL sample from mixed flask and the absorbance was measured at 700 nm. (Optima SP-3000). Results were calculated as ascorbic acid equivalent (μg AAE/mL).

**Statistical analysis:** Windows of version of SPSS 15.0 statistical software was used in the study. The study was planned as three groups with three replications. In the present study, which was planned as three experimental groups. Drying method was the factor. One-way analysis of variance was applied in the study and the DUNCAN test was used to compare groups. All results were given as mean ± standard deviation.

**RESULTS AND DISCUSSION**

*P. lanceolata* is generally consumed fresh, cooked or dried as food. In addition, it is a very valuable natural plant in terms of health and phytochemicals (Beara et al., 2012). It has been used to heal wounds since the ancient times (Kovac et al., 2014). Its active pharmaceutical components are in the structure of phenylethanoid glycoside and iridoid glycoside (Gonda et al., 2013). The plant are rich in catalpol, aucubin and acteoside (Kalantari et al., 2017).

The study, the total amount of phenolic and flavonoid compounds, DPPH• removal activities and reducing power capacities were determined in the samples belonging to P1, P2, P3 groups.

It was detected as gallic acid equivalent by using the Folin-Ciocalteau method in the determination of the amount of phenolic substance. P1 and P3 groups were similar among themselves in terms of phenolic substance amounts, and the difference between them and the P2 group was significant (P<0.05). The amounts of phenolic substances were determined as 15.65 ±2.25 mg GAE/g in P1, 15.20±1.48 mg GAE/g in P3, and 6.97 ±0.26 mg GAE/g in P2, respectively (Table 1). While the values we found in the study were similar to the values found by Dalar et al., (2012) (19.0±0.7 mg GAE/g), they were lower than the values found by Bahadori et al. (2020) (45±1 mg GAE/g). It is higher than the values found by Nizioł-Lukszewska et al. (2019) (1.66 ± 0.38 - 4.80 ± 0.43 mg GAE/g).

Total flavonoid substance amounts were calculated as equivalent to quercetin. In terms of total flavonoid substance amounts, P2 and P3 were similar among themselves, but the differences with P1 were significant (P<0.05). It was determined that the highest value was 83.85 ±3.49 mg QE/g in P1, and the lowest value was 53.65 ±3.61 mg QE/g in P2 (Table 1). Similarly, in studies conducted on *P. lanceolata*, the total amount of flavonoids was reported, at 5.31 to 13.10 mg QE/g by Beara et al. (2009) and, as 9.6 ± 0.03 mg QE/g by Bahadori et al. (2020). The present value we found is higher than these values.

In addition, in similar studies conducted on different species belonging to the Plantaginaceae genus, total phenolic and flavonoid substance amounts were reported as 17.18±2.25 mg GAE/g in *Plantago major* L. and 28.91±3.12 mg GAE/g in *Plantago media* L. (Lukova et al., 2017). Similarly, in another study conducted on *Plantago argentea* Chaix., *Plantago holosteam Scop., P. major L., Plantago maritima L. and P. media* L. species, these values were found to be in the range of 38.43-70.97 mg GAE/g and 5.31-13.10 mg QE/g (Oladoja, 2015). This situation proves that the phytochemical structures differ between species.

**Table 1. The amount of phenolic and flavonoid substance belonging to the groups (P<0.05).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>The amount of phenolic (mg GAE/g)</th>
<th>The amount of flavonoid (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>15.65 ±2.25^a</td>
<td>83.85 ±3.49^a</td>
</tr>
<tr>
<td>P2</td>
<td>6.97 ±0.26^b</td>
<td>53.65 ±3.61^b</td>
</tr>
<tr>
<td>P3</td>
<td>15.20 ±1.48^a</td>
<td>57.37 ±7.04^b</td>
</tr>
</tbody>
</table>

In the studies on different plants, it was reported the highest amount of phenolic and flavonoid substances
was detected in vacuum drying on *Vitex agnus-caitus* (Vuong et al., 2015), in vacuum drying at 90°C in *Citrus limon* (Papoutsis et al., 2017), in *Kappaphycus alvarezii* in Oven-dried 40 °C (Ling et al., 2015), in *Aristotelia chilensis* fruits (Quispe-Fuentes et al., 2018) and in freeze drying in *Chokeberries* (Samoticha et al., 2016).

DPPH• free radical and BHT as a standard were used to find the free radical removal activity of the groups. DPPH• radical removal activities of the extracts and BHT were calculated (%) (Table 2). Parallel to the increase in concentration (20–100 μg/mL), it was observed an increase in DPPH• radical removal activities in groups and BHT. The differences between the groups were significant at the P<0.05 level. The highest activity value was detected in P3 at all concentrations.

Table 2. DPPH radical removal (%) activities of groups and BHT (P<0.05).

<table>
<thead>
<tr>
<th>Extracts/standard</th>
<th>20 μg/mL</th>
<th>40 μg/mL</th>
<th>60 μg/mL</th>
<th>80 μg/mL</th>
<th>100 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>8.17±1.53b</td>
<td>13.58±1.79b</td>
<td>18.40±1.62c</td>
<td>24.57±0.61c</td>
<td>28.95±0.54c</td>
</tr>
<tr>
<td>P2</td>
<td>0.30±0.10c</td>
<td>1.69±0.55c</td>
<td>2.43±0.30d</td>
<td>2.96±0.64d</td>
<td>3.58±0.44d</td>
</tr>
<tr>
<td>P3</td>
<td>9.64±2.76ab</td>
<td>15.95±1.60b</td>
<td>31.17±2.67b</td>
<td>38.77±4.75b</td>
<td>47.54±1.11b</td>
</tr>
<tr>
<td>BHT</td>
<td>12.45±1.21a</td>
<td>38.27±1.22a</td>
<td>56.07±2.51a</td>
<td>65.98±2.12a</td>
<td>75.15±1.98a</td>
</tr>
</tbody>
</table>

Selamoglu et al. (2017) reported the % DPPH radical removal activities in *P. lanceolata* as, 50.50 at 0.20 mg/mL, 67.75 at 0.40 mg/mL, 72.25 at 0.60 mg/mL, 77.75 at 0.80 mg/mL and 84.25 at 1 mg/mL. In addition, these activities in *P. major* were as, 69.75 at 0.20 mg/mL, 74.75 at 0.40 mg/mL, 80.00 at 0.60 mg/mL, 84.50 at 0.80 mg/mL, and 90.25 at 1 mg/mL, respectively.

The concentration of extract and standard substance was determined as IC$_{50}$, which inhibited 50% of DPPH• radical removal. The IC$_{50}$ values was detected as P2 1402.79±75.61 μg/mL, in P1 179.23±5.18 μg/mL, in P3 103.59±4.94 μg/mL, and in BHT 60.57±1.74 μg/mL (figure 2). A low IC$_{50}$ value means a high radical removal activity. According to these results, the antioxidant activities of the groups were determined as BHT>P3>P1>P2.

Figure 2. IC$_{50}$ values of groups and standard (P<0.05).

Similarly, in a study by Akbalik et al., in 2021, it was reported that the IC$_{50}$ (μg/mL) values of *P. lanceolata* plants collected from different regions of Turkey were 3.12 in Tatvan region, 12.13 in Bitlis region, 22.43 in Adilcevaz region and 89.52 in Mutki region (Akbalik et al., 2021). In addition, in a similar study conducted with *Kappaphycus alvarezii* plant by applying different drying techniques, it was reported that the lowest IC$_{50}$ value was 12.80±0.92 in oven-drying at 40 °C and the highest IC$_{50}$ value was 56.93±8.40 in the Sun-dried group (Ling et al., 2015).
The Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing capacity of P1, P2 and P3 extracts and BHT was determined using the method of Oyaizu (1986). In the measurements of Fe<sup>3+</sup> reducing capacity, absorbances at 700 nm were determined. Increasing absorbance values in figure 3 indicates increased reducing power.

**Figure 3. Comparison of the Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing capacity of the extracts of the groups and BHT (20-50 μg/mL).**

In addition, the Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing power of the groups was calculated as ascorbic acid equivalent (μg AAE/mL) (Table 3). The similarity between P3 and P1 and the differences with other groups were found statistically significant (P<0.05). Respectively, it was detected as 1048.03±27.89 μg AAE/mL in BHT, 130.28±5.63 μg AAE/mL in P3, 118.15±2.13 μg AAE/mL in P1 and P2 65.03±4.14 μg AAE/mL. In a similar study conducted by Beara et al., in 2012, it was reported that 66.70±3.33 μg AAE/mL in Plantago altissima and 109.80±6.57 μg AAE/mL in P. lanceolata.

**Table 3. Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing capacity of groups and BHT (P<0.05).**

<table>
<thead>
<tr>
<th>Extracts and standard</th>
<th>μg AAE/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>118.15±2.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P2</td>
<td>65.03±4.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P3</td>
<td>130.28±5.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT</td>
<td>1048.03±27.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Conclusion:** Although the previous studies on P. lanceolata were about its active compounds and healing properties, there has been a little information about the phytochemical structure of this plant. In addition, there has been no information on how the phytochemicals of the P. lanceolata plant, which has a high antioxidant capacity, are affected by the drying process.

It is one of the most recent research topics that as well as their nutritional properties the phytochemical and pharmaceutical structures of fresh vegetables and fruits may be affected during and as a result of the drying process. Because the effects of processes such as drying on foods are great. The drying process to be applied may cause the release of some phenolic acids and flavonides in the bound form in the structure of the plants (Hayat et al., 2010). The applied process can reduce the activation of the enzyme polyphenol oxidase, which is responsible for the oxidation of polyphenols (Krapfenbauer et al., 2006). In addition, drying process may cause degradation of compounds with phytochemical properties (Lou et al., 2015).

The study, it was determined that the phytochemical content of P. lanceolata plant was affected by drying techniques. Sun drying method was advantageous in terms of total phenolic and flavonoid compounds while shade drying was advantageous in terms of DPPH radical removal activities, antioxidant activity and Fe<sup>3+</sup>-Fe<sup>2+</sup> reducing capacity.

In conclusion, there was no correlation between the levels of phenolic and flavonoid contents and their antioxidant activities. It was observed that the antioxidant activity of the P. lanceolata plant, which is easily accessible and has natural antioxidant potential, was less affected by shade drying compared to the other drying methods. Finally, it can be suggested that there is a need for new studies on how bioactive compounds are affected by such applications.

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