Antioxidant activity and total phenolic contents of *Galanthus woronowii* 
(Amaryllidaceae)

*Galanthus woronowii* (Amaryllidaceae)'s antioxidant activities and total phenolic contents were evaluated on *Galanthus woronowii* (Amaryllidaceae). Turk J Biod 2(1): 1-5

1. INTRODUCTION

Plants have been used for treatment of ailment since ancient times (Aksit et al., 2014; Demirtas et al., 2013; Topçu et al., 1999; Yaglioglu et al., 2013). They included secondary metabolites revealing a great many biological activities (Elmastas et al., 2016; Erenler, Pabuccu, et al., 2016; Erenler, Sen, Yaglioglu, et al., 2016; Erenler, Sen, Yildiz, et al., 2016). A large number of medicines include natural products as main active ingredients (Erenler, Demirts, et al., 2017; Erenler et al., 2018; Karan & Erenler 2017, 2018; Karan et al., 2018). Due to the including of bioactive compounds, isolation and identification of corresponding compounds have gained the great interest lately. Since the natural compounds have a little or lack of side effect as a drug, they inspired to synthetic chemists to synthesize natural compounds for pharmaceutical industry (Erenler, Meral, et al., 2017; Karan et al., 2017).

**ABSTRACT**

Plants have been used for medicinal purpose since ancient times. Due to the including bioactive secondary metabolites, plants have gained the great interest for drug discovery and development process. In this work, *Galanthus woronowii* was extracted with hexane, dichloromethane and ethyl acetate sequentially. After removing of the solvent by rotary evaporator, crude extracts were yielded. Antioxidant activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and reducing power assays were executed on corresponding extracts. In addition, total phenolic content was presented. Ethyl acetate extract included the most phenolic compounds and also it revealed the most antioxidant activity. Hence, this plant could be considered as a promising antioxidant agent.

**ÖZ**

The *Galanthus* L. genus (Amaryllidaceae family) is represented by 14 species (15 taxa) and 1 hybrid. This genus known as snowdrops is perennial onion petal liliopsida. *Galanthus woronowii* Losinsk. is distributed through north-eastern part of Turkey (Davis, 2006). Amaryllidaceae family is one of the significant alkaloid containing plant family in the world. A great many alkaloids were isolated and identified from *Galanthus* species. Hence *Galanthus* genus can be accepted as a rich source of chemically diverse alkaloids (Jin, 2013).

Previous phytochemical investigations were reported that alkaloids such as galanthusine, lycorine, galanthine, demethylhomocoryline were isolated and identified from *G. woronowii* (Kintsurashvili & Vachnadze, 2007). The alkaloids, narwedine, O-methylleucotamine, sternbergine, and sanguinine isolated from *G. woronowii* displayed the considerable acetylcholinesterase (AChE) inhibitory activity (Sarikaya et al., 2013). *G. woronowii* has been used in homeopathy and has been prepared from fresh whole flowering plants (Bokov & Samylina, 2016). Galanthamine is an alkaloid isolated from *G. woronowii* (snowdrop). It is a licensed medicine for the treatment of Alzheimer’s disease. Inhibition of AChE selectively and competitively is considered the principal mode of action of galanthamine and it increased nicotinic acetylcholine receptor function (Howes & Perry, 2011).

Reactive oxygen species such as superoxide (O$_2^-$•), peroxyl (ROO•), peroxinitrite (ONOO•), hydroxyl (OH•) and nitric oxide (NO•) are free radicals that yielded during oxidative function within the body (Koysu et al., 2018). The human body has some protection systems against oxidative stress, including antioxidant enzymes and chemical compounds. Exposure to various environmental pollutants such as smoking, bad living conditions, malnutrition, ultra violet radiation leads to natural antioxidant to be inadequate. Therefore, the excess free radicals can harm to cell membrane during the metabolism leading to degenerative illness and conditions such as Alzheimer’s disease, cardiovascular disease, liver toxicity, ageing process, nephroblast, diabetes, inflammation and DNA injury leading to carcinoogenesis. Antioxidants play a significant role in quenching singlet oxygen, deactivating free radicals, inhibiting peroxidation, releasing hydrogen, and chelating metal ion (Erenler et al., 2015). Therefore, many antioxidant based medicines have been applied to prevent and treat the corresponding diseases. Recently, considerable studies have been carried out in discovering natural antioxidants for use in food, cosmetics, and active ingredient for medicine to replace synthetic antioxidants which have been prohibited due to their carcinogenic effects (Sasaki et al., 2002). Herein, it was investigated the antioxidant activities of *G. woronowii* extracts using DPPH*, ABTS**, and reducing power assays.

2. MATERIAL AND METHOD

2.1. General experimental procedures

Hitachi U-290 UV–VIS spectrophotometer was used for UV analysis. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trelox, aminonacetate (NH$_2$CO$_2$CH$_3$), potassium persulphate (K$_2$S$_2$O$_8$) were bought from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). The solvents with analytical grade, potassium dihydrogen phosphate (KH$_2$PO$_4$), potassium hydroxide (KOH), copper (II) chloride (CuCl$_2$), Folin-Ciocalteu reagent, gallic acid were supplied from E. Merck (Darmstadt, Germany).

2.2. Plant material

*Galanthus woronowii* was collected from Arhavi, Artvin, Turkey during the flowering period in July 2018 and identified by Prof. Dr. Özgür Eminagaoglu, specialists of Plant Taxonomy (ARTH 13436).

2.3. Extraction

*G. woronowii* (200 g) was extracted with hexane (3 × 200 ml), dichloromethane (3 × 200 ml) and ethyl acetate (3 × 200 ml) sequentially for 3 days. After removing of solvent from each extract solution under reduced pressure, the crude extracts were yielded. The each extract was kept in fridge (+4 °C) for antioxidant analysis (Erenler et al., 2014).

2.4. Total phenolic determination

Total phenolic content of hexane-, dicloromethane-, and ethyl acetate extracts of *Galanthus woronowii* was carried out by Folin-Ciocalteu reagent using gallic acid as a standard (Singleton & Slinkard, 1977). The treatment of extract solution (1.0 ml, containing 1.0 mg sample) with Folin-Ciocalteu reagent (0.1 mL) in distilled water (4.6 mL) was carried out in a volumetric flask. Sodium carbonate (Na$_2$CO$_3$) solution was added to the reaction flask then incubated for 2 h at room temperature. The absorbance
measurement was executed at 765 nm by a spectrophotometer (Hitachi U-2900). The standard curve was calculated using gallic acid and the results were presented as gallic acid equivalents per mg of extract (Elmastas et al., 2015). All experiments were carried out in triplicate.

2.5. DPPH free radical scavenging assay

DPPH free radical scavenging effects of hexane-, dichloromethane- and ethyl acetate extracts of G. woronowii were executed (Blois, 1958). Different concentrations of each extract (3 mL) were treated with DPPH• (1.0 mL, 0.26 mM) for 15 min at room temperature. The absorbance was recorded at 517 nm. The experiment was repeated for three times. BHA, BHT and Trolox were used as standard controls. IC₅₀ values indicate the concentration of sample that scavenges 50% of DPPH free radical. The DPPH• scavenging activity was calculated using the equation:

\[ \text{DPPH}^* \text{ scavenging effect} \% = \left( \frac{A_1 - A_2}{A_1} \right) \times 100 \]

Ac is the absorbance of the control and As is the absorbance of the sample (Elmastas et al., 2004). The results were calculated as IC₅₀.

2.6. ABTS radical cation scavenging assay

Antioxidant activity of extracts were determined using the trolox equivalent antioxidant capacity assay with the radical cation ABTS•⁺ as reported previously (Re et al., 1999). ABTS•⁺ stock solution was prepared by the treatment of ABTS (2 mM) with potassium persulfate (2.45 mM). Afterward, it was stored for 6 h in dark at room temperature. ABTS•⁺ solution (1.0 mL) treated with each sample solution (3.0 mL). The inhibition was calculated for each concentration comparative to a blank absorbance. The absorbance was determined at 734 nm. The capacity of ABTS•⁺ was calculated by the equation: ABTS•⁺ scavenging effect (%) = [(A₁ – A₂) / A₁] × 100 in which, A₁ is ABTS•⁺ initial concentration and A₂ is ABTS•⁺ remaining concentration in the sample. The results were calculated as IC₅₀.

2.7. Cupric ion reducing power assay

Cupric ion reducing power test was carried out on hexane-, dichloromethane- and ethyl acetate extracts of G. woronowii (Elmastas et al., 2018). Antioxidant extract reduced the Cu²⁺ to Cu⁺. Yellow color complex formed after addition of CuCl₂ to the reaction medium. Afterward the absorbance of corresponding complex was recorded. In this assay, each extract (40–160 µg/mL, 1.0 mL) was mixed with CuCl₂ (0.01 M, 1.0 mL), neocuproine (1.0 mL, 7.5 × 10⁻³ M), and acetate tampon (1.0 mL, 1.0 M) solution. Neocuproine and CuCl₂ were dissolved in EtOH and water respectively. After incubation of reaction mixture for 30 min, the absorbance was recorded at 450 nm (Apak et al., 2004). The result was expressed according to the Trolox equivalent (µmol/g sample).

3. RESULTS

Phenolic compounds are a large class of plant secondary metabolites and important for quality of plant based foods (Erenler, Adak et al., 2017). Aerial part of the G. woronowii was extracted with hexane, dichloromethane and ethyl acetate sequentially to yield the crude extracts. Antioxidant activity assays including DPPH• scavenging activity, ABTS•⁺ scavenging effect and cupric ion reducing power activity were executed on corresponding extracts. In addition, total phenolic contents were presented. It was observed that there was a correlation between phenolic content and antioxidant activity. Total phenolic content of hexane-, dichloromethane- and ethyl acetate extracts were found as 51.05 mg GA/g extract, 63.81 mg GA/g extract, 83.33 mg GA/g extract respectively (Table 1).

Table 1. Antioxidant activity of Galanthus woronowii extracts

<table>
<thead>
<tr>
<th>Extracts and standards</th>
<th>Total phenolic contents (mg GAE/g extract)</th>
<th>DPPH• scavenging [IC₅₀ (µg/mL)]</th>
<th>CUPRAC (µmol TE/mg extract)</th>
<th>ABTS•⁺ Scavenging [IC₅₀ (µg/mL)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane ext</td>
<td>51.05±5.86</td>
<td>69.07±0.42</td>
<td>0.49±0.03</td>
<td>28.51±1.27</td>
</tr>
<tr>
<td>CH₃Cl₂ ext</td>
<td>63.81±4.12</td>
<td>34.63±0.21</td>
<td>0.98±0.17</td>
<td>16.84±0.49</td>
</tr>
<tr>
<td>EtOAc ext</td>
<td>83.33±3.60</td>
<td>28.14±0.40</td>
<td>0.72±0.01</td>
<td>13.09±0.20</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>9.92±0.23</td>
<td>3.63±0.18</td>
<td>5.38±0.18</td>
</tr>
<tr>
<td>BHA</td>
<td>-</td>
<td>5.37±0.21</td>
<td>2.87±0.18</td>
<td>8.80±0.06</td>
</tr>
<tr>
<td>Trolox</td>
<td>-</td>
<td>5.77±0.12</td>
<td>-</td>
<td>5.57±0.09</td>
</tr>
</tbody>
</table>

In term of activity, ethyl acetate extract (IC$_{50}$ 28.14 µg/mL) revealed the most DPPH free radical scavenging effect among the extract. But this value indicated the moderate activity in comparison to BHT (IC$_{50}$ 9.92 µg/mL). Ethyl acetate extract was also exhibited the most ABTS$^+$ scavenging effect with the value of 13.09 (IC$_{50}$, µg/mL) than that of the hexane extract (IC$_{50}$ 28.51 µg/mL) and dichloromethane extract (IC$_{50}$ 16.84 µg/mL). Ethyl acetate extracts of herbal plants consist of flavonoids displaying significant antioxidant activity (Chhikara et al., 2018; Guzel et al., 2017). This could be due to the hydrogen or electron releasing ability of corresponding flavonoids (Erenler, Sen, Aksit, et al., 2016). In reducing power assay, dichloromethan extract exhibited the considerable effect (0.98 µmol TE/mg extract) among the hexane (0.49 µmol TE/mg extract) extract and ethyl acetate extract (0.72 µmol TE/mg extract). Interestingly, dichloromethane extract displayed the more reducing power effect than ethyl acetate extract. Although, dichloromethane extract included the less phenolic compounds than that of the ethyl acetate extract. This incident could be explained either the synergic effect of the compounds found in the extract or a compound in the extract reduced the copper ion selectively.

4. CONCLUSIONS
Secondary metabolites of *G. woronowii* extracts should be isolated and identified to display the exact mechanism of activity. In addition, *G. woronowii* has a potency to be used in pharmaceutical and food industry as a natural antioxidant. The cultivation of *G. woronowii* should be carried out in a large scale. In addition, the isolated compounds as well as plant extracts of *G. woronowii* should be investigated for various biological effects.

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REFERENCES


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