A Pharmacognostical Comparative Investigation on *Valeriana alliariifolia* Adams

**Burcu Sen-Utsukarci**<sup>1</sup>,<sup>*</sup>, Merve Cimsit<sup>2</sup>, Turgut Taskin<sup>3</sup>, Bahar Gurdal<sup>4</sup>, Oguzhan Baci<sup>5</sup>, Suleyman Bilgin<sup>6</sup>

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey  
<sup>2</sup>Faculty of Pharmacy, Istanbul University, Istanbul, Turkey  
<sup>3</sup>Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Turkey  
<sup>4</sup>Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey  
<sup>5</sup>Doga Pharmacy, Arakli, Trabzon, Turkey  
<sup>6</sup>Bilgin Tarım Ürünleri San. Tic. A. S., Surmene, Trabzon, Turkey

**Abstract:** Free radicals are shown as the main reason of many chronic and degenerative diseases. Because of the toxicity and undesirable effects of synthetic antioxidants, finding of new antioxidant natural agents is very important, especially edible plants. As an edible plant, valerian is used for various aims. Also, *Valeriana* species are known with their antioxidative compounds. In our previous study, some biological activities of *Valeriana alliariifolia* Adams roots extracts, collected in 2012, and the chemical compositions of active samples were determined. For this study, after 5 years collected plant materials were investigated again to determine and compare the antioxidant activities, the total phenolic contents and the chemical composition profile of the extracts from different plant-parts and to compare obtained results with the previous data. While RW1, RWM1 and RWM1residue were found most active by DPPH method and AHM1 by FRAP method, REM1 showed the highest activity by CUPRAC method. The high activity of AHM1 is parallel to its phenolic content. It can be thought that the difference between the results of our two studies, is due to the change of plant content from year to year and various environmental factors. This is important for achieving standardization in the production of its preparations.

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**1. INTRODUCTION**

Free radicals are shown as the main reason of many chronic and degenerative diseases, including coronary heart disease, stroke, inflammation, aging, cancer and diabetes mellitus. Reactive oxygen species (ROS), free radicals such as hydroxyl radical singlet oxygen, superoxide anion and hydrogen peroxide, can cause initiate peroxidation of polyunsaturated fatty acids and cellular injuries, which include DNA damage, protein damage, and important enzymes in human body. In this case, various free–radical-related diseases can be occured consequently. Therefore, investigations for finding of antioxidant agents were began to protect...
cells and organisms from oxidative damage and to reduce the risk of degenerative diseases arising from oxidative stress. The antioxidants can be classified in two groups: primary and secondary antioxidants. The primary antioxidants neutralize free radicals by a single electron transfer mechanism or by hydrogen atom transfer mechanism, and the secondary antioxidants passivate and deactivate prooxidant catalysts, including chelators of prooxidant metal ions (iron and copper etc.), exemplified by ethylenediaminetetraacetic acid (EDTA) and citric acid (CA) or reactive species such as singlet oxygen (beta-carotene etc.). Synthetic antioxidants -butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertbutylhydroquinone (TBHQ)- have been preferred as primary antioxidants against free radicals, oxidation and off-flavour development. They have been widely used in the food industry for the prevention of the oxidative deterioration, but they have also the toxicity and undesirable effects e.g. carcinogenic effects. For example, BHA and BHT are held responsible for liver damage and carcinogenesis. The consumers concern about foods without/with lower levels of chemical preservatives because of their harmful effects, and demand for the long shelf-life of food and for absence of risk of causing foodborne diseases. Because of these reasons, the researchers and also food industry focused on finding new antioxidant agents from natural sources such as plants, especially edible plants, to prevent or reduce the harmful effects of oxidative stress on cells. After the exploration of important roles of bioactive compounds in free radical chain reactions to exhibit the antioxidant activity, the investigations on these compounds, especially food phenolics, and plant extracts which are considered potential antioxidants, increased dramatically in the past 25 years. On the contrary, very few antioxidants from natural sources took place in the market because of various problems. Nevertheless, there are some commercial products of antioxidant plants or their phenolic compounds in the market, such as rosemary extract, green tea and mixture of tocopherols. The phenolic compounds, which have antioxidant activity, can be also added into wine from the wood barrels used in storage and aging [1-3].

The Valeriana genus (Caprifoliaceae), represented by more than 350 species worldwide, comprises about 17 species, 4 of which are endemic, in Turkey. In Turkish traditional medicine, Valeriana officinalis L. is preferred for the treatment of hysteria, neurasthenia, nervous insomnia and palpitations, and its infusions for the treatment of wounds [4-9]. Valeriana species contain over 150 chemical constituents such as sesquiterpenes, iridoids, flavonoids, alkaloids and other compounds (pinoresinol and its analogs). Many of the iridoids of Valeriana species were investigated for their biological activity e.g. the antispasmodic, sedative, antimycobacterial, antiviral, cytotoxic and anxiolytic effects. They contain valepotriates, which have an important place among the iridoids. Beside of this, 8-hydroxy pinoresinol and prinsepiol exhibited powerful antioxidant activity in Trolox equivalent antioxidant activity (TEAC) and chemiluminescence (CL) tests [10-12]. Due to the knowledge of good antioxidant activity of Valeriana species and the valepotriates, there are numerous studies on antioxidant activity of Valeriana species exist in the literature [13]. In recent years, the investigations concentrated especially on the antioxidant activity of the extracts and its compounds, and also their effect mechanisms [13-19]. The usages of V. officinalis were recorded for its analgesic and sedative effects, and its infusion to treat neural diseases and for tranquilizer effect, in Turkey [20, 21]. Also, V. alliariifolia roots are in use traditionally. Its infusion is preferred with the sedative and antispasmodic purposes, in Turkish traditional medicine [22]. With the previous studies in Turkey, the contents of the essential oils of various Valeriana species were determined [20, 21, 23-25]. Also, the investigations on the chemical composition of the essential oil from V. alliariifolia exist in literature. 68 constituents were identified in the essential oil from the subterranean parts of V. alliariifolia by using capillary Gas Chromatography (GC) and GC/MS. Isovaleric acid (28.6 %), δ-guaiane (7.2 %), α-humulene (4.7 %), hexadecanoic acid (4.3 %), valeric acid (3.7 %) and humulene epoxide-II (3.6 %) were found as the major compounds [26].
Further this study, the extracts of *V. alliariifolia*, *V. sisymbriifolia* Vahl. and *V. officinalis*, collected in Azerbaijan were analysed for their valepotriates content by the isolation of the extracts from different plant parts through using TLC and UV-spectrophotometry [27]. Along with the identification the compounds of *V. sisymbriifolia*, *V. alliariifolia* and *V. officinalis* from Iran, limonene (3.53%) was found in *V. alliariifolia* as the main component [28]. In another study on the chemical composition of the *V. alliariifolia* essential oil, trans-caryophyllene (38.96 %) was the major compound, by following β-pinene (12.06 %), α-pinene (9.94 %), α-terpinene (9.49 %), isoterpinolene (7.15 %), 1,8-cineole (6.76 %) [7].

In light of these data, the plants, especially essential oil containing plants, have different contents according to the different collecting localities or time, and different contents also reveal different biological activities. As seen in previous studies, these content differences occurred in *V. alliariifolia*. In a previous study of our group, the antioxidant, cytotoxic and insecticidal activities and the chemical composition of the active samples were investigated. Besides of the identification of the compounds in active samples, their antioxidant activities by DPPH and ABTS methods and total phenolic contents were determined. Two methanol extracts, prepared with gradient and non-gradient maceration, exhibited higher antioxidant activity and total phenolic contents than other samples. It was appeared that, there is a correlation between the antioxidant activities and total phenolic contents, the polar compounds in EM1 have a role in the antioxidant activity, and finally the compounds with high polarity and also moderate polarity in this *Valeriana* species can have a role in the antioxidant activity [29]. 5 years after this study, the aerial parts and roots of *V. alliariifolia* were collected again in Trabzon-Turkey for the present study. This present study aims to determine the antioxidant activities of the extracts from two different plant parts, the total phenolic contents and the chemical composition profile, also the presence of the valerenic acid and its derivatives, and to compare the results about the content and antioxidant activity with the previous data.

2. MATERIAL and METHODS
2.1. Plant Material
The aerial parts and roots of *Valeriana alliariifolia* were collected from Trabzon (Turkey), in July 2017 and the voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 115520).

2.2. Preliminary Qualitative Phytochemical Analysis
2.2.1. Preparation of the Plant Material for the Preliminary Analysis
From 5 g of each air-dried and powdered plant parts, the infusions were prepared with 100 boiled water in 30 min. After cooling, the infusions were filtered. With these filtrates, following tests were performed for qualitative detection of phytochemicals e.g. flavonoids, anthracenes, saponins, tannins (catechic tannin and gallic tannin). In the tests alkaloids, the dried aerial parts and roots were used separately [30]. The results are expressed in the presence (+) and absence (−) of the phytochemicals.

2.2.2. Test for flavonoids
Over 5 mL infusion, 5 mL Shibata Reagent (1 part conc. HCL + 1 part water + 1 part ethanol) and a piece of Magnesium were added and it has been observed whether orange, red or purple color has occurred (Shibata Reaction = Cyanidin Reaction)

2.2.3. Test for Anthracene Compounds
Over 10 mL infusion, 5 drops concentrated H₂SO₄ was added. The mixture was warmed in boiling water for 15 min. and extracted with 5 mL toluene after cooling. Over the toluene...
phase, 3 mL NH$_3$ solution (10%) was added and it has been observed whether rose pink to red color has occurred.

2.2.4. Test for Saponins

10 mL infusion was shaken vigorously in a graduated cylinder for 30 sec. It has been observed after 15 min. whether minimum 1 cm foam layer has occurred.

2.2.5. Test for Tannins

2.2.5.1. Gelatin Test (for the General Determination of Tannins)

Over 5 mL infusion, 2 mL Gelatin-salt Reagent (Gelatin solution (1%) saturated with NaCl) was added. It has been observed whether cream-beige precipitate has occurred.

2.2.5.2. Separation of The Type of Tannins

1. step: Ferric Chloride Test: Over 10 mL infusion, 3 drops FeCl$_3$ (5%) was added. It has been observed whether blue-black colour (gallic tannin) or dark olive green (catechic tannin) has occurred.

2. step: Stiasny Reaction (for the separation of tannin types): Over 10 mL infusion, 5 mL Stiasny Reagent (formol in water (30%) + conc. HCl) was added. The mixture was warmed in water (80°C) for 30 min. When the material contains catechic tannin, precipitates in potions appeared. After cooling of the mixture, it was filtered. 3mL filtrate was saturated with sodium acetate. After adding of 3 drops diluted FeCl$_3$ solution, it has been observed whether blue-black precipitate or colour has occurred.

2.2.6. Test for Alkaloids

1 g of each air-dried and powdered plant parts were extracted with 10mL H$_2$SO$_4$ solution (3%) in hot water. They were cooled and filtered. After adding 5 mL NH$_3$ solution (10%), it was stirred with 10 mL ether. The layers were allowed to separate. The etheric phase was evaporated to dryness. The residue was dissolved in 10 mL H$_2$SO$_4$ solution (3%). The alkaloid control reactions were made on this solution in 3 portions.

1. After adding of the Mayer Reagent (mercuric chloride + potassium iodide + water), it has been observed whether milk-white precipitate has occurred.

2. After adding of the Bouchardat Reagent (iodine + potassium iodide + water), it has been observed whether dark red precipitate has occurred.

3. After adding of the Dragendorff Reagent (bismuth carbonate + potassium iodide + water), it has been observed whether orange-red precipitate has occurred.

2.3. Determination of Total Moisture (Loss on Drying) and Total Ash Content

2.3.1. Total Moisture (Loss on Drying) Content

For the determination of the moisture content, two different methods in European and Turkish pharmacopoeia were used.

Method in European pharmacopoeia: Air-dried and powdered plant parts (weight: A) were put into a pre-dried and weighed (Wa1) crucible. The samples were dried in an oven at 100-105 °C for 2 hours and weighed (Wa2). The percent loss of weight of air-dried sample was calculated by equation: % = (Wa2-Wa1) x100 / drug weight

Method in Turkish pharmacopoeia: Air-dried and powdered plant parts (weight: A) were put into a pre-dried and weighed (Wb1) crucible. The samples were dried in an oven at 100-105 °C for 3 hours and reweighed (Wb2). The percent loss of weight of air-dried sample was calculated by equation: % = (Wb2-Wb1) x100 / drug weight
2.3.2. Total Ash Content

Air-dried and powdered plant parts (weight: A) were put into a pre-dried and weighed (Wc1) crucible. The samples were ignited gradually in an electrical muffle in 600 °C for 30 min. It was cooled in desiccators and reweighed (Wc2). Total ash content was calculated as in equation: \% = (Wc2 – Wc1) x 100 / A

2.4. Preparation of Extracts

The dried and powdered roots, used in traditional medicine, were successively macerated with hexane (AHM1 for the aerial parts extract and RHM1 for the roots extract), chloroform (ACM1 for the aerial parts extract and RCM1 for the roots extract), and ethanol (AEM1 for the aerial parts extract and REM1 for the roots extract) and water (AWM1 for the aerial parts extract and RWM1 for the roots extract), with stirring for a day. Furthermore, two portions of the aerial parts were individually macerated with ethanol (AEM2 for the aerial parts extract and REM2 for the roots extract) and water (AWM2 for the aerial parts extract and RWM2 for the roots extract), with stirring for a day. An infusion was prepared from another portion with boiled water (AWI for the aerial parts infusion and RWI for the roots infusion) as in its traditional use. The organic extracts were evaporated to dryness and the aqueous extracts were lyophilized. The extracts were stored at ± 4°C till further used.

Due to the occurrence of precipitation in the AWM1 and RWM1 during the storage at ± 4°C, the extracts were filtered, and the filtrates (AWM1filtrate and RWM1filtrate) and residues (AWM1residue and RWM1residue) were studied separately.

2.5. Extract Yield Percentage

The extraction yield is a measure of the solvents efficiency to extract specific components from the aerial parts and roots. The percentage yield was calculated with: \((A2 - A1 / A0) ×100\)

(A0 = weight of the initial dried, used plant part; A1 = weight of container; A2 = weight of container + extract)

2.6. Chromatographical Methods of Chemical Composition

2.6.1. Thin Layer Chromatography (TLC)

In order to estimate the chemical profile of the extract, thin layer chromatographic analyses were performed. As stationary phase, silica gel 60 F254 aluminium plates. For the development used mobile phases can be seen in the Table 1. Chromatograms were visualised by exposing to UV at 254 and 366 nm (Camag UV lamp) or by using derivatization agent, Anisaldehyde reagent (105°C; 5 min).

Anisaldehyde reagent:

Stock solution: 10 mL anisaldehyde + 90 mL ethanol
Dilution of stock: 20 mL stock solution + 2 mL conc. H2SO4

2.7. Qualitative Analyse of the Valepotriates

The qualitative analyses of two plant parts were performed by using the method in the investigation of Hassan et al. [31]. In this study, it was recorded that, the absorbance measurement at 212 nm (hydroxyvalerenic acid), 217 nm (acetoxyvalerenic acid) and 218 nm (valerenic acid). The evaluation of the present results were realized according of this absorbance values.

2.8. Determination of Total Phenolic Contents in Extract

Total phenolic content of plant extracts was determined with the Folin-Ciocalteu reagent (FCR) method [32]. Briefly, 0.1 mL of the extract was put in a plate and 4.5 mL of water was
added. Then, 0.1 mL of FCR (diluted with distilled water to the ratio 1:3) and 0.3 mL of 2 % sodium carbonate solution were added to the mixture. The mixture was left at room temperature for 2 h, and then absorbance was measured against the reference at 760 nm. Total phenolic content was expressed as mg of gallic acid equivalents per g of the extract.

Table 1. TLC mobile phases

<table>
<thead>
<tr>
<th>System No.</th>
<th>Mobile phases</th>
<th>Solvent ratios</th>
<th>Aim</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>toluene : ethyl acetate : methyl ethyl ketone</td>
<td>80 : 15 : 5</td>
<td>To determine and compare the low, moderate and high polarity</td>
</tr>
<tr>
<td>2</td>
<td>hexane : ethyl acetate : glacial acetic acid</td>
<td>65 : 35 : 0.5</td>
<td>To determine and compare the low, moderate and high polarity</td>
</tr>
<tr>
<td>3</td>
<td>chloroform : methanol</td>
<td>20 : 1</td>
<td>To determine and compare the moderate and high polarity</td>
</tr>
<tr>
<td>4</td>
<td>chloroform : methanol</td>
<td>9 : 1</td>
<td>To determine and compare the moderate and high polarity</td>
</tr>
<tr>
<td>5</td>
<td>chloroform : methanol</td>
<td>1 : 20</td>
<td>To determine and compare the moderate and high polarity</td>
</tr>
</tbody>
</table>

2.9. Antioxidant Assays

2.9.1. Chemicals and Reagent for Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH•), Folin-Ciocalteu's phenol reagent 2N, gallic acid, and ascorbic acid, all of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MO, US).

2.9.2. DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picryl-hydrazil) radical scavenging activity of different extracts were measured by the DPPH• method proposed by Fu et al. [33]. Briefly, 240 µL of DPPH solution (0.1 mM) was added to 10 µL of extracts. Then the mixture was allowed to stand at room temperature for 30 min. The absorbance of the mixture was measured against the reference using a micro plate reader at 517 nm.

2.9.3. Ferric Reducing Antioxidant Power Activity (FRAP)

The FRAP reagent was prepared by mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of the TPTZ solution (10 mM TPTZ in 40 mM HCl) and 2.5 mL of 20 mM FeCl₃.6H₂O. Then the FRAP reagent was kept at 37°C for 30 minutes in incubator device (Nuve). 190 µL of the FRAP reagent was mixed with 10 µL of extract and after 4 minutes the absorbance of the mixture was measured against the reference at 593 nm. FRAP values of the extracts were expressed as mM Fe²⁺/mg extract [34].

2.9.4. Cupric Reducing Antioxidant Capacity (CUPRAC)

Cupric reducing antioxidant capacity assay was carried out according to the method of Apak et al. [35]. 60 µL Cu(II)₂H₂O, 60 µL neocuproine and 60 µL, NH₄Ac (1 M) were mixed. Then 60 µL of the extract and 10 µL of ethanol were added to the mixture. After the duration time of 60 min, the mixture absorbance was spectrophotometrically measured at 450 nm. CUPRAC values of the extracts were given as mM trolox/mg extract.
2.10. Statistical Analysis

All the experiments were done in triplicates. The results of the antioxidant experiments were demonstrated as mean ± SD. All the data was analysed by the Graphpad Prism 5 program.

3. RESULTS and DISCUSSION

3.1. Preliminary Qualitative Phytochemical Analysis

The results of the preliminary qualitative phytochemical analysis on aerial parts and roots of *V. alliariifolia* are shown in Table 2.

Table 2. The preliminary qualitative phytochemical analysis results to determine the contents of *V. alliariifolia* aerial parts and roots.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Aerial Parts</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bouchardat Reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dragendorff Reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mayer Reaction</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anthracene analogs</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Flavones (Light orange)</td>
<td>Flavonols (Light red)</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Gallic tannin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Catechic tannin</td>
<td>+</td>
</tr>
</tbody>
</table>

3.2. Determination of Total Moisture (loss on drying) and Total Ash Content

3.2.1. Total Moisture (loss on drying) Content

The total moisture contents of the aerial parts and roots according to the European pharmacopoeia (EP) and Turkish pharmacopoeia (TP) methods are represented in Table 3.

Table 3. The values of the total moisture contents according to the European pharmacopoeia (EP) and Turkish pharmacopoeia (TP) methods.

<table>
<thead>
<tr>
<th>Total Moisture Contents</th>
<th>Aerial Parts</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>with EP method (%)</td>
<td>14.44</td>
<td>10.85</td>
</tr>
<tr>
<td>with TP method (%)</td>
<td>11.62</td>
<td>11.28</td>
</tr>
</tbody>
</table>

3.2.2. Total Ash Content

The total ash contents according to the European pharmacopoeia (EP) and Turkish pharmacopoeia (TP) methods are given in Table 4.

Table 4. The values of the total ash contents.

<table>
<thead>
<tr>
<th>Total Ash Contents (%)</th>
<th>Aerial Parts</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial Parts</td>
<td>6.88</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>4.43</td>
<td></td>
</tr>
</tbody>
</table>
Both of the loss on drying values and total ash content of valerian roots should not exceed 12.0% (w/w) according to TP and EP. Since these values were below the maximum acceptable limit of TP and EP, the roots of *V. alliariifolia* were found to be suitable for TP and EP.

### 3.3. Extracts Yields Percentages

The yields of the extracts are shown in Table 5 and summarized in Figure 1.

**Table 5.** Extracts yields by prepared with different solvents and different methods from the aerial parts and roots of *V. alliariifolia*.

<table>
<thead>
<tr>
<th>Yields (%)</th>
<th>Aerial Parts</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane extr. – gradient maceration (GM)</td>
<td>1.786</td>
<td>2.626</td>
</tr>
<tr>
<td>Chloroform extr. – GM</td>
<td>1.41</td>
<td>4.066</td>
</tr>
<tr>
<td>Ethanol extr. – GM</td>
<td>3.196</td>
<td>4.362</td>
</tr>
<tr>
<td>Water extr. filt. – GM</td>
<td>5.542</td>
<td>3.6</td>
</tr>
<tr>
<td>Water extr. residue – GM</td>
<td>2.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Ethanol extr. – standard maceration (SM; maceration for 24h)</td>
<td>2.314</td>
<td>4.82</td>
</tr>
<tr>
<td>Water extr. – SM</td>
<td>16.680</td>
<td>17</td>
</tr>
<tr>
<td>Infusion</td>
<td>12.748</td>
<td>9.5</td>
</tr>
</tbody>
</table>

![Figure 1. Summary of the extracts yields.](image)

### 3.4. Chromatographical Methods of Chemical Composition

The mobile phases, mentioned mostly in literature, were used in thin layer chromatography (TLC) methods. However, when the chromatograms were examined, a different mobile phase (System 5) was also needed beside of the mobile phases System 3 and 4. Therefore, the extracts were applied on TLC additionally with the System 5. By comparing the chromatograms, the system 5 was found the best mobile phase for examination of the alcohol and water extracts. For the separation of the compounds in nonpolar extracts, it was seen that the systems 1 and 2 were more useful than other mobile phases. In various studies, the Rf values of valerenic acid analogs were found similar to slightly different depending on the polarity of TLC mobile phases. In a study on the quantification of valerenic acid and its derivatives in certain *Valeriana* species, valerenic acid appeared at Rf 0.49 on TLC chromatogram developed with hexane:ethyl acetate:glacial acetic acid (65:35:0.5) beside of the presence of acetoxyvalerenic and hydroxyvalerenic acids [31]. In a reference book, it was recorded that, the spots of valerenic, acetoxyvalerenic and hydroxyvalerenic acids could be monitored as violet after derivatization with anisaldehyde–sulphuric acid reagent by TLC.
[hexane:ethyl acetate:glacial acetic acid (65:35:0.5); Rf values: 0.55, 0.34, 0.1]. [36]. Additionally, Caudal et al. used a mixture of cyclohexane, ethyl acetate and acetic acid (60:38:2) as mobile phase and designated acetoxyvalerenic acid at Rf 0.43 beside of valerenic and hydroxyvalerenic acids (Rf values: 0.61 and 0.25, respectively) [37]. Similarly in extracts prepared from the aerial parts (especially AEM2 and AEM1), the valerenic acid analogs (valerenic, acetoxyvalerenic and hydroxyvalerenic acids) showed a fluorescence quenching at 254 nm on the chromatogram (Mobile Phase System 2), which were seen as violet spots after derivatization with anisaldehyde–sulphuric acid reagent. Thus, the presences of valerenic, acetoxyvalerenic and hydroxyvalerenic acids were determined as like as in System 1 and 3. From the TLC chromatograms developed with mobile phase systems 1 and 3, the valerenic acid analogs content in root extracts were also indicated. Especially RCM1 contains higher amount of these compounds than other extracts prepared from the roots.

3.5. Qualitative Analyse of the Valerenic Acid and Its Derivatives

Following the method described by Hassan et al., the extrcats were prepared from the aerial parts and roots of V. alliariifolia and measured between the ranges of 190-1500 by UV-spectrophotometry. The absorbance values (Table 6) were evaluated in comparison to the absorbance values, which were specified for the valerenic acid analogs (valerenic, acetoxyvalerenic and hydroxyvalerenic acids) in the study of Hassan et al. [31]. The valerenic, acetoxyvalerenic and hydroxyvalerenic acids were detected in these extracts in parallel to the results of TLC.

Table 6. The absorbance values of the extracts from aerial parts and roots by UV-spectrophotometry.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Absorbance values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract from aerial parts</td>
<td>219, 216.5, 214</td>
</tr>
<tr>
<td>Extract from roots</td>
<td>219, 216, 214</td>
</tr>
</tbody>
</table>

3.6. Antioxidant Assays and Determination of Total Phenolic Contents

The results are obtained as in Table 7. According to the antioxidant activity results by DPPH method, RWI, RWM1 and RWM1residue were found most active (67.67±0.38; 63.91±1.13 and 63.91±0.99, respectively). The antioxidant activities of polar compounds can be determined with this method. Also, it is known that, the phenolic compounds are related to the antioxidant activity. As a matter of fact, the active extracts have quite high phenolic content. The much higher activity of REM1 than AEM1 by DPPH can be indicated that, the roots of V. alliariifolia contain more polar compounds than its aerial parts.

Beside of DPPH method, the FRAP and CUPRAC methods are very important for testing of the reductive potentials of the compounds on heavy metals. While AHM1 was most active (0.269±0.012 mM Fe^{2+}/mg extract) by FRAP method, REM1 showed the highest activity by CUPRAC method (0.891±0.034 mM trolox/mg extract). The high activity of AHM1 is parallel to its phenolic content, but the total phenolic contents of other active extracts by FRAP were found fewer than expected amounts. It can be concluded that, the nonphenolic compounds in these extracts have a role in activity of extracts. Similarly, the phenolic content of REM1 (most active extract by CUPRAC) was found less than the content of less active extracts. In FRAP tests, exhibiting the higher activities of the nonpolar extracts than other extracts can be elucidated that nonpolar compounds of this species have a role in this activity. Due to the results of CUPRAC tests, it can be seen that these process in FRAP tests has proceeded for the nonpolar extracts of the roots differently and the polar extracts of the roots showed higher activity than its nonpolar extracts. But the nonpolar extracts of the aerial parts exhibited higher activity by CUPRAC, as expected.
Table 7. The absorbance values of the extracts from aerial parts and roots by UV-spectrophotometry.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH (%) (200 µg/mL)</th>
<th>FRAP (mM Fe²⁺/mg extract)</th>
<th>CUPRAC (mM trolox/mg extract)</th>
<th>Total phenolic content (mg of GAE per g of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHM1</td>
<td>5.64±0.75</td>
<td>0.269±0.012</td>
<td>0.369±0.002</td>
<td>34±0.008</td>
</tr>
<tr>
<td>ACM1</td>
<td>4.89±1.13</td>
<td>0.210±0.028</td>
<td>0.629±0.179</td>
<td>26±0.014</td>
</tr>
<tr>
<td>AEM1</td>
<td>28.32±1.52</td>
<td>0.116±0.002</td>
<td>0.680±0.084</td>
<td>4±0.003</td>
</tr>
<tr>
<td>AWM1</td>
<td>43.61±0.75</td>
<td>0.044±0.008</td>
<td>0.464±0.042</td>
<td>11±0.002</td>
</tr>
<tr>
<td>AWM1residue</td>
<td>48.75±0.95</td>
<td>0.039±0.005</td>
<td>0.584±0.061</td>
<td>14±0.001</td>
</tr>
<tr>
<td>AEM2</td>
<td>30.08±1.59</td>
<td>0.136±0.005</td>
<td>0.571±0.007</td>
<td>15±0.009</td>
</tr>
<tr>
<td>AWM2</td>
<td>26.32±0.38</td>
<td>0.087±0.011</td>
<td>0.400±0.092</td>
<td>9±0.002</td>
</tr>
<tr>
<td>AWI</td>
<td>44.74±1.36</td>
<td>0.030±0.006</td>
<td>0.425±0.0638</td>
<td>6±0.002</td>
</tr>
<tr>
<td>RHM1</td>
<td>4.14±0.65</td>
<td>0.217±0.016</td>
<td>0.243±0.015</td>
<td>2±0.001</td>
</tr>
<tr>
<td>RCM1</td>
<td>10.53±0.99</td>
<td>0.232±0.017</td>
<td>0.223±0.002</td>
<td>10±0.005</td>
</tr>
<tr>
<td>REM1</td>
<td>53.13±1.78</td>
<td>0.070±0.002</td>
<td>0.891±0.034</td>
<td>17±0.001</td>
</tr>
<tr>
<td>RWM1</td>
<td>63.91±1.93</td>
<td>0.026±0.002</td>
<td>0.619±0.095</td>
<td>20±0.005</td>
</tr>
<tr>
<td>RWM1residue</td>
<td>63.91±0.99</td>
<td>0.044±0.004</td>
<td>0.549±0.031</td>
<td>16±0.005</td>
</tr>
<tr>
<td>REM2</td>
<td>41.48±0.78</td>
<td>0.143±0.004</td>
<td>0.720±0.030</td>
<td>14±0.002</td>
</tr>
<tr>
<td>RWM2</td>
<td>35.09±0.22</td>
<td>0.041±0.003</td>
<td>0.390±0.052</td>
<td>8±0.001</td>
</tr>
<tr>
<td>RWI</td>
<td>67.67±0.38</td>
<td>0.057±0.003</td>
<td>0.721±0.050</td>
<td>23±0.003</td>
</tr>
<tr>
<td>BHA</td>
<td>83.22±0.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BHT</td>
<td>-</td>
<td>1.1±0.12</td>
<td>5.78±0.07</td>
<td></td>
</tr>
</tbody>
</table>

These values are the means of three replicates ± standard deviation. ABTS – 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; BHT – butylated hydroxytoluene; DPPH – 2,2-diphenyl-1-picrylhydrazyl; GAE – gallic acid equivalents.

In general, the total phenolic contents were not parallel to the activity results. In the study by Jugran et al., the correlation between phenolic content and the antioxidant activity of the extracts from 25 populations of *V. jatamansi* Jones. were exhibited obviously [38]. In the contrary of the data about this correlation in previous studies on *Valeriana* species, any correlation between phenolic content and antioxidant activity was found in this study as like as in our previous investigation on *V. alliariifolia* collected in 2012 [29]. In our previous study on *V. alliariifolia* roots, the activity of the extracts, evaluated in terms of DPPH radical scavenging activities, is as follows: EM1 (IC50: 17.69 µg / mL) > EM2 (IC50: 20 µg / mL) > W1 (IC50: 37 µg / mL). In this study, the DPPH radical scavenging ability of root extracts was ranked as: W1 (67.67%) > WM1=WM1residue (63.91%) > EM1 (53.13%) > EM2 (41.48%). According to these results, the activity of the EM1 and EM2 extracts was in parallel with the previous study results, but in this study, no parallelism was detected in the W1 extract and it was found that it showed higher activity than the other extracts. In comparison between the extracts from aerial parts and roots, it is seen that the root extracts exhibited higher DPPH radical scavenging activity than the extracts from aerial parts. In our previous study, it was found that the extracts, prepared from the roots, contain higher phenolic substance amounts than the total phenolic content results in this study. These differences between the results can be explained by the fact that the content of plants, especially the essential oil containing plants, can be variable depending on the collection of the plant in different locations and in different time periods. Thus, these content differences occurred also in *V. alliariifolia*, as seen in previous studies on the composition of its essential oil. From this point of view, besides polar and nonpolar compounds play a role in the activity together, phenolic substances as well as non-phenolic substances involve in eliciting antioxidant effect. Based on this, it can be concluded that both of the polar and nonpolar compounds of *V. alliariifolia* play a role in the activity together, and also its phenolic substances as well as non-phenolic substances involve in eliciting antioxidant effect.
4. CONCLUSION

When the antioxidant test results of the root extracts in our two studies were compared, it was observed that the results differed from each other. It can be thought that this difference is due to the change of plant content from year to year and various environmental factors. This is important for achieving standardization in the use of the plant as a medicine and in the production of its preparations. By increasing the studies in this direction, it can be concluded that this plant can be very useful in the treatment area by standardization in plant studies.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author(s).

Orcid

Burcu Sen-Utsukarci https://orcid.org/0000-0002-7270-9686
Merve Cimsit https://orcid.org/0000-0002-7561-5953
Turgut Taskin https://orcid.org/0000-0001-8475-6478
Bahar Gurdal https://orcid.org/0000-0003-4009-100X
Oguzhan Baci https://orcid.org/0000-0002-0972-4844
Suleyman Bilgin https://orcid.org/0000-0003-1707-0243

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