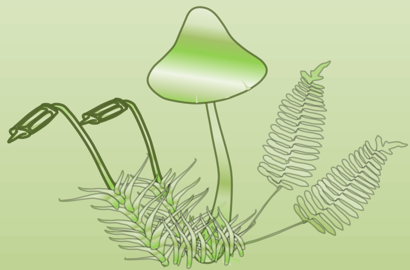
# Anatolian Journal of Botany



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# The comparative investigation of the antioxidant activities of some species belonging to the Lamiaceae and Poaceae families

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# Lamiaceae ve Poaceae familyalarına ait bazı türlerin antiksidan savunma kapasitelerinin karşılaştırmalı olarak incelenmesi

**Abstract:** The antioxidant compounds of plants have widely been investigated for the purpose of medical and industrial uses due to their aroma, color, smell and protective properties. In the study, 16 species from Lamiaceae and 14 species from Poaceae were collected from the Northeastern Mediterranean region and then they were analysed. The dry weight and water conditions of the plant samples were identified. It was identified that there wasn't a significant difference between these two families in respect to their values of chlorophyll and carotene. The mean xanthophyll content was higher in the Lamiaceae species (236 µg $g^{-1}$ FW) than in the Poacea species (142 µg $g^{-1}$ FW) and total antioxidative capacity was higher in Lamiacea species (5.19 mg $g^{-1}$ FW) than Poaceae species (3.49 mg $g^{-1}$ FW). On the other hand, mean soluble phenolics were measured as 1.83 mg $g^{-1}$ FW in Poaceae species and 1.67 mg $g^{-1}$ FW in Lamiaceae species. Superoxide dismutase enzyme activity was higher in Poaceae species. The findings revealed that significant differences could exist among the families and further comparative studies should be performed for the determination of the biochemical resources.

**Key words:** carotenoids; Mediterranean vegetation; phenolics; pigments; superoxide dismutase

**Özet:** Bitkilerin antioksidan bileşikleri aroma, renk, koku ve koruyucu özellikleri nedeniyle tıbbi ve endüstriyel kullanım amacıyla yoğun olarak araştırılmaktadır. Bu çalışmada, Lamiaceae'den 16 tür ve Poaceae'den 14 tür, Kuzeydoğu Akdeniz bölgesinden toplandı ve analiz edildi. Bitki örneklerinin kuru ağırlık ve su durumları belirlendi. Bu iki familyanın üyeleri arasında klorofil ve karoten değerleri bakımından önemli bir fark olmadığı belirlendi. Lamiaceae türlerinde ortalama ksantofil içeriği (236 µg $g^{-1}$ FW) Poacea türlerinden (142 µg $g^{-1}$ FW ) ve total antioksidatif kapasite Lamiacea türlerinde (5.19 mg $g^{-1}$ FW) Poaceae türlerinden (3.49 mg $g^{-1}$ FW) daha yüksek bulundu. Buna karşın toplam çözünür fenolikler Poaceae türlerinde ortalama 1.83 mg $g^{-1}$ FW, Lamiaceae türlerinde 1.67 mg $g^{-1}$ FW olarak ölçüldü. Süperoksit dismutaz enzim aktivitesi Poaceae türlerinde daha yüksek bulundu. Bulgular iki familya arasında önemli farklılıklar bulunabileceğini ve biyokimyasal kaynakların belirlenmesi bakımından karşılaştırmalı çalışmaların çoğaltılması gerektiğini ortaya koymaktadır.

**Anahtar Kelimeler:** Akdeniz vejetasyonu. fenolikler, karotenoidler, pigmentler, süperoksit dismutaz

## 1. Introduction

Biological systems have been protected from the destructive effects of oxygen by antioxidant defense mechanisms (Alscher and Hess, 1993). The mechanisms of tolerance or avoidance of oxidative stress differ among plant groups (Cai et al., 2004). The analysis of oxidative defense components can make a contribution for the determination of the differences among plant families. The primary elements serving for oxidative protection in plants are phenolic compounds of which antioxidants effects have been known and which have large diversity (Wojdylo et al., 2007; Rice-Evans et al., 1996). The strong water-soluble antioxidants such as glutathione and ascorbic acid are quite common in plant families. The levels of the oil-soluble antioxidants such as carotenoids and tocopherol differ among plant groups as well. The similar differences could be observed in antioxidant enzyme activities such as SOD, GR, catalase and peroxidases (Oncel et al., 2004).

Increasing reactive oxygen species in plants under stress are the harmful oxygen forms with higher chemical reactivity as compared with the oxygen molecules (Van Breusegem and Dat, 2006). Free radicals are high-powered, unstable compounds containing the electrons that not having constituted one or more pairs, in their outer atomic orbitals. Free radicals cause a damage by receiving electrons from many biological materials such as proteins, lipids, nucleic acids and coenzymes (Mitler, 2002).The studies evaluating the plant families with different biochemical characteristics in respect to their antioxidant capacities, reported significant differences (Cai et al., 2004; Wojdylo et al., 2007). There are evidences implying that the oxidative stress protection mechanisms of the Lamiaceae and Poaceae families, both of which have different biochemical characteristics, are also different (Öncel et al., 2004).

Lamiaceae's family has been represented with approximately 250 genus and 7000 species in the world (Kahraman et al., 2009). Members of this family range intensively in Mediterranean countries primarily, South West Asia and South America. Turkey is one of the important gene centers of Lamiaceae. This family has been represented with 574 species within 45 genus in Turkey (Güner et al., 2000). Most of the members of the Lamiaceae have a great importance in the fields of medical, pharmaceutical, food, cosmetic and perfumery because of being rich in essential oils, aromatic compounds, secondary metabolites (Başer, 1993). On the other hand, the use of ethnobotany is quite common among the members of the family (Matkowski et al., 2008; De Marino et al., 2012). Because of their high polyphenol content, Lamiaceae's plants were investigated as natural antioxidant resources (Rice-Evans et al., 1996).

The members of the Poaceae's family usually in the form of annual or perennial herbaceous plant, rarely in the form of bush or tree. Their roots are in the type of fibrous root and they contain some rhizome. Their stem is vertical, ascendant, leaning and creeping. Since their stem doesn't grow in width, the inner of their stem is empty, excluding nodiums. Their seeds are rich in starch. This cosmopolite family contains about 650 genus and species of much more than 9000. It has 512 species belonging to 142 genus in Turkey (Guner, 2000). It's many genus have economical value as being cultivated. In addition, it's species with sugar and oil contents are also available. They are feed and food plants which are rich in antioxidant phenolic acids and xylooligosaccharides (Reddy and Krishnan, 2013).

The species belonging to the Poaceae and Lamiaceae have been the subject of many studies in respect to their antioxidative capacities (Cai et al., 2004; Markowskaya et al., 2012). In this study, it was intended to perform comparative investigation of the antioxidative capacities of Poaceae and Lamiaceae families and determination of whether any difference exist in respect to their antioxidative capacities. The findings of this study can make a useful contribution for the determination of the systematical, physiological and biochemical characteristics of both families. Lamiaceae and Poaceae's species, which exist commonly in the Eastern Mediterranean region, were collected from different localities and analysed in laboratory in respect to their content of antioxidant featured substances and enzymes.It should not purely be a review of the subject area, and should not contain the findings or the conclusions.

## 2. Material and Methods

### 1.1. *Plants*

The plants which are the subject of the study were collected in July and August 2011 from the Çamlıyayla (Gopter-Çuvalgı), Kazanlı, Apsun, Bükdeğirmeni, Kuyuluk, Aydıncık, Değirmençay, Işıklı ve Gözne districts, which situated within the borders of Mersin province. This area is located in C4 square according to the grid system specified in Turkey's flora.16 of the species of 30 plant investigated belong to the Lamiacea, 14 of them belong to the Poaceae. Only fresh leaves of Lamiacea's samples, both leaves and stems of Poaceae's samples were cut out and stored in a freezer. The samples were taken from three different region for each species, the results of the analysis were given in average values of these samples.

### 1.2. *Dry Weight and Relative Water Content (RWC)*

Leaf dry matter content were determined as the difference between fresh weight and dry weight after drying at 110°C for 24 hours in an oven. Measurements of relative water content (RWC) were taken from segments of leaves. After floating on distilled water that allowed the leaf segments to rehydrate for 2 h at 20°C, they were blotted dry and weight. The same segments were dried overnight at 110°C and weight again. RWC of leaves was calculated according to formula: RWC=100 x [(fw-dw) / (turgid w-dw)].

### 1.3. *Chlorophyll Content*

Chlorophyll extraction from fresh leaf material was carried out with 80% acetone (buffered to pH 7.8 with phosphate buffer). The chlorophyll a, chlorophyll b and total chlorophyll measurements were done with spectrophotometer. Chlorophyll contents were calculated according to Porra et al. (1989) and chlorophyll a/b ratios were determined.

### 1.4. *Carotenoid Content*

Fresh leaf material (0.5 g) were ground in pre-chilled mortar in 5 ml aceton containing 200 mg Na2SO4 and then fitered through glass fiber disks (Whatmann GF/A). The volume of the aceton extracts was reduced in rotary evaporator and then resuspended in 1 ml chloroform. Fifty microliters of the extracts and standarts were applied to slica gel TLC plates (20x20, 0.25 mm thickness). The chromatograms were developed with hexane, dietil eter, aceton, 60:30:20, v:v:v) (Moore, 1974). Xantophyll and β-caroten spots were scraped from the TLC plates and centrifuged in 5 ml aceton for 5 min at 5000g. The absorbance of supernatants was determined at a wavelength of 450 nm by a spectrofootometer, against β-caroten and lutein standarts.

### 1.5. *Total Antioxidant Capacity*

0.5 g plant sample was crushed in porcelain mortar with 5 ml methanol (96%) for determination of total antioxidative capacity. The extract was centrifuged for 5 minutes at 5000 g and supernatant was taken. A reactive containing 6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was prepared.150 µl supernatant was mixed with the reactive in a test tube so that last volume would be 3 ml. The tubes was maintained at 95 ℃ for 90 min. and then cooled until room temperature and their absorbances were measured at 695 mm. Total antioxidative capacity was calculated as the equivalent of ascorbic acid (Prieto et al., 1999).

### 1.6. *Soluble Phenolic Content*

The frozen leaf samples (0.5 g) were rapidly plunged in 20 ml of 80% aqueous ethanol and boiled for 5 min. After filtration through Whatmann no. 1 filter paper, ethanol was eliminated from the filtrate by evaporation in vacuum. Total soluble phenolics in the remaining water phase were determined spectrophotometrically with the Folin-Ciocalteu reagent (prepared by 1:1 dilution with distilled water), against the chlorogenic acid standard (Ferraris et al., 1987).

### 1.7. *Superoxide Dismutase Activity*

Frozen leaf material (0.5 g) were homogenized in 6 mL 0.1 M potassium phosphate extraction buffer (pH 7, containing 100 mg insoluble PVP and 0.1 mM EDTA) with Ultra Turrax. The homogenate was centrifuged for 5 min at 6000Xg and 4°C. The supernatant was fıltered through a Whatman GF/A glass fiber disc with a vacuum filtration system and stored at -70°C (Schöner and Krause, 1990).

SOD activity was determined according to Beyer and Fridovich (1987). The reaction mixture (3 mL) contained potassium phosphate buffer (pH 8, 0.025% Triton X-100 and 0.1 mM EDTA), enzyme extract, 12 mM L-methionine, 75 µM nitroblue tetrazolium chloride (NBT) and 2 μM riboflavin. The reaction mixture was kept under flourescent light for ten minutes at 25°C. One SOD unit was described as the amount of enzyme where the NBT reduction ratio was 50%. NBT reduction ratios were measured with a spectrophotometer adjusted to 550 nm.

1.8. *Statistical Processing*

All analyses and measurements were made repetitively at least 3 times. The importance levels of the differences between the species belonging to the Lamiaceae and Poaceae families were determined through t-test for each parameter. The averages, standard deviations and t-test results were stated in the tables.

**3. Results**

Average dry weight was determined as 29.5%, average RWC determined as 81.3% at Lamiaceae. The dry weight percentage varies between 15.5% (*Salvia verticillata*) and 46.8% (*Phlomis leucophracta*) at this family (Table 1). The RWC values vary between 55.5% (*Phlomis leucophracta*) and 96.3% (*Salvia verticillata*). Average dry weight was determined as 31.9 %, average relative water content was determined as 74.0 % at Poaceae. The dry weight percentage varies between 15.4 % (*Cynodon* sp.) and 61.3% (*Aegilops speltoides*) at Poaceae. The RWC values vary between 29.3 % (*Aegilops speltoides*) and 97.6 % (*Echinochloa colonum*) at this family. It was observed that the dry weight percentages and the RWC values varied in a wide range in both families. The difference between the families is not statistically significant.

The chlorophyll a, b and total amounts of chlorophyll were analysed at the species belonging to both families. The average amount of chlorophyll a was determined as 1159 µg $g^{-1}$ FW, the average amount of chlorophyll b was determined as 544 µg $g^{-1}$ FW and total average amount of chlorophyll was determined as 1704 µg $g^{-1}$ FW at Lamiaceae. Highest amount of chlorophyll a was identified at *Phlomis leucophracta* species of Lamiaceae, highest amount of chlorophyll b was identified at *Sideritis rubriflora* species of the same family (Table 1). The average amount of chlorophyl a was determined as 1103 µg $g^{-1}$ FW, the average amount of chlorophyl b was determined as 483 µg $g^{-1}$ FW and total average amount of chlorophyl was determined as 1586 µg $g^{-1}$ FW at Poaceae. The greatest amount of chlorophyll a was identified at *Polypogon mospeliensis* species of Poaceae, the highest amount of chlorophyll b was identified at *Phalaris aquatica* species of the same family. The differences identified in respect to the chlorophyl contents and chlorophyll a/b ratio of these two families aren't statistically significant (Table 1, Figure 1).

Having separated with thin layer chromatography, the carotenoids were analysed by using β-carotene and their total xanthophyl content was analysed by using spectrophotometer. The average β-carotene was identified as 103 µg g-1 FW at Lamiaceae, 84 µg $g^{-1}$ FW at Poaceae. Any statistically significant difference in respect to β-carotene content wasn't determined between these two families. Total average values of the xanthophyl content was measured as 236 µg $g^{-1}$ FW at Lamiaceae, 142 µg $g^{-1}$ FW at Poaceae. While total values of the xanthophyl content vary between 88-382 µg $g^{-1}$ FW at Lamiaceae, vary between 44-304 µg $g^{-1}$ FW at Poaceae. There is a statistically significant difference (P≤0.01) between the total values of the xanthophyl content of both families.

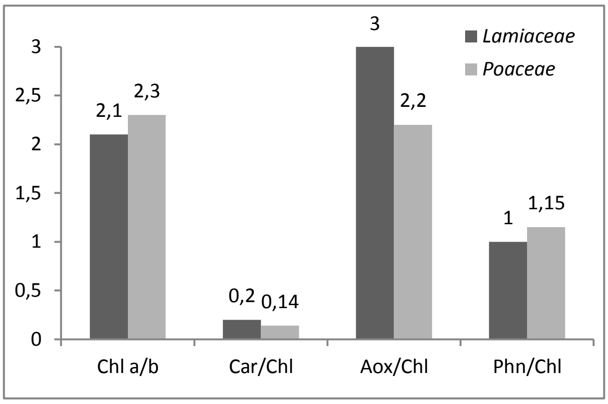


**Fig. 1.** Avarage values chlorophyll a/b, carotenoids/chlorophyll, total antioxidants/chlorophyll and soluble phenolics/chlorophyll in Lamiaceae and Poaceae families.

Total average antioxidative capacity was measured as 5.19 mg $g^{-1}$ FW at Lamiaceae, 3,49 mg $g^{-1}$ FW at Poaceae (Table 2). It was identified that Lamiaceae has higher antioxidant capacity than Poaceae has and this difference is statistically significant at P≤0.05 level (Table 2). The species with the highest antioxidative capacity at Poaceae is *Phleum pratense*, the species with the highest antioxidant capacity at Lamiaceae is *Sideritis perfoliata*. Antioxidant/chlorophyll ratio higher at Lamiaceae (Figure 1). The highest antioxidant capacity on the dry weight basis was identified at *Lamium garganicum* species belonging to the Lamiacea (Figure 2).

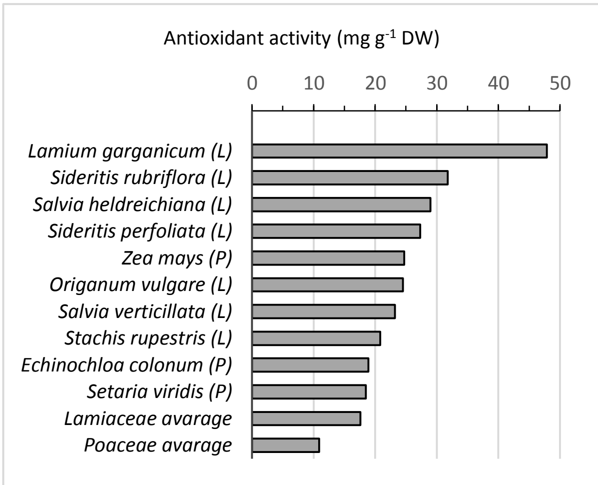


**Fig. 2.** Species with high antioxidant activity based on dry weight in Lamiaceae (L) and Poaceae (P) families.

While the phenolic content was identified as 1,67 mg $g^{-1}$ at Lamiaceae, it was identified as 1,83 mg $g^{-1}$ at Poaceae. The sample of Lamiacea family with the highest phenolic content was identified as *Sideritis rubriflora* (5,74 mg $g^{-1}$), the sample of Poaceae family with the highest phenolic content was identified as *Zea mays* (3,82 mg $g^{-1}$) (Table 2). The amount of total phenolic substance is higher at Poaceae as compared with Lamiaceae, however, this difference is not statistically significant. The species with the highest soluble phenolic content on the dry weight basis were identified as *Sideritis rubriflora* and *Zea mays* (Figure 3).

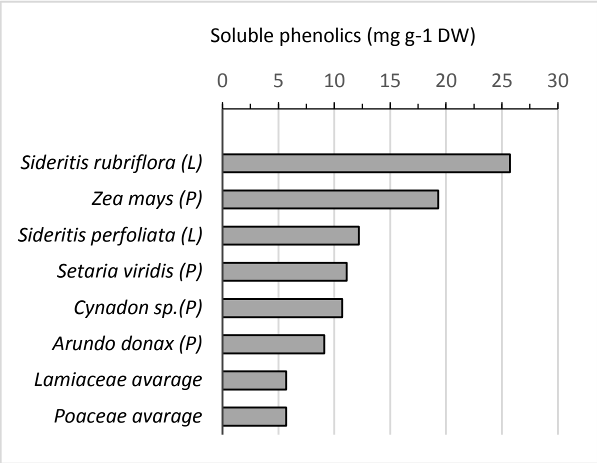


**Fig. 3**. Species with high soluble phenolics based on dry weight in Lamiaceae (L) and Poaceae (P) families,

The average amount of SOD was measured as 225 unit $g^{-1}$ FW at Poaceae, as 213 unit $g^{-1}$ FW at Lamiacea. The highest amount of SOD was identified at the *Phlaris pratense* from Poaceae, at the species of *Lamium amplexicaul*e from Lamiacea. Even though SOD content is higher at Poaceae than Lamiacea, but this difference is not statistically significant.

**4. Discussions**

The samples belonging to both families were collected in similar ecological conditions and in the same vegetative period. The average dry weight values of species of Lamiaceae and Poaceae families were identified as approximate to each other. However, significant differences were determined in respect to dry weight values among the members of the same family. These differences can be explained with soil, water conditions and microclimate differences (Oncel et al., 2004). That relative water content is higher at Lamiaceae's species can be associated with better water accession and better water conservation. It was reported in the study intending to determine the relationship between soil saltiness and RWC in the species of 6 turf (Poaceae) that the more soil saltiness increases, reduces RWC rates (Uddin et al., 2012).

Though the chlorophyl content significantly changes among the members of the same family, the difference between Lamiaceae and Poaceae is not significant. However, that the values of chlorophyl b is higher at Lamiaceae leads to the ratio of a/b to be increased in favor of Poaceae (Figure 1). Castrillo et al. (2001) investigated the contents of chlorophyl of 11 species belonging to Lamiaceae. They identified the ratios of a/b above 2.0 at two species belonging to Nepetoidae subfamily, below 2.0 (1.41-1.76) at other species as well. The contents of chlorophyl of 11 species belonging to Poaceae were analyzed and it was identified that the ratios of chlorophyl a/b varied between 3.5 and 2.0 (Uddin et al., 2012). The chlorophyl content and ratio of chlorophyl a/b is associated with receive sunlight condition of plants. The lower ratio of chlorophyl a/b is expected in plants growing in the shade.

According to the findings of this study, the amounts of both β-carotene and xanthophyl of Lamiaceae are higher than those of Poaceae (Table 2). The ratio of carotenoids per chlorophyl was found as 0.2 at Lamiaceae 0.14 at Poaceae (Figure 1). Carotenoids can be asserted to have a more important role in protection of photosynthetic system at the members of Lamiaceae. These findings are more different than those of Oncel et. al. (2004) who identified low-carotenoid contents at the species of Lamiaceae growing at stepe. Rather low amount of carotenoids (0.34 µg $g^{-1}$ DW β-carotene and 1.3 µg $g^{-1}$ DW xanthophyl ) was identified through HPLC analysis in the edible parts of the *Thymus vulgaris* plant from Lamiaceae. However, it was identified in the same study that the reference values of this species related carotenoids were 29 µg $g^{-1}$ β-carotene and 19 µg $g^{-1}$ xanthophyl (El-Qudah, 2014). In a comparative study, the contents of carotenoid were identified as 260-430 µg $g^{-1}$ FW at 10 species of Poaceae family growing at tundra (Markowskaya et al., 2012). These high values of carotenoid can be resulted in due to tundra conditions.

The antioxidative capacity of the species of Lamiaceae investigated in this study was identified higher than that of the species of Poaceae (Table 2). The antioxidant capacity per chlorophyl is significantly high at Lamiaceae (Figure 1). The total antioxidative capacity on the dry weight basis is significantly high at *Lamium garganicum* species (Figure 2). Matkowski et al. (2008) identified a high antioxidative activity at the leaves of three species of *Salvia* (*S. mitiorrhiza, S. przewalskii* and *S. verticillata*). The authors offered the species of *Salvia* as source of potential antioxidant. But it was determined in the current study that the species of *Sideritis* have greater antioxidative capacity than the species of *Salvia* have. It was reported that 4 species of Lamiaceae growing naturally in Turkey, have significantly high antioxidative capacity and this situation is associated especially with content of polyphenol (Erdemoglu et al. 2006). The high values were identified in the study in which, antioxidative activities of the species of plant being known as *Salvia* and used as a drink in Turkey such that gallic acid equivalent was 130 mg g-1 at *Salvia fruticosa*; 154 mg $g^{-1}$ at *Sideritis congesta*, 120 mg $g^{-1}$ at *Sideritis pisidica* (Erdoğan et al., 2010).

While total soluble phenolic contents of the species of Lamiaceae family distribute in a wide range, the phenolic values of the species of Poaceae were identified as approximate to each other (Table 2). A small difference in favor of Poaceae was identified with regard to soluble phenolic compounds per chlorophyl (Figure 1). It was revealed in a study which subject was antioxidative and antimicrobial activities of the species of *Echinochloa colona* belonging to Poaceae that this species showed high level of antioxidant activity. Total phenolic content of this species was stated as 734 mg $ml^{-1}$ (Ajaib et al., 2013). It was stated that the antioxidative activity of corn silk (*Zea* sp.) was resulted from high contents of phenol and flavonoid and its total phenolic content was identified as 2.78 mg $g^{-1}$ (Ebrahimzadehet et al., 2008). The species of poaceae family with high total contents of soluble phenolic were identified as *Zea mays* and *Arundo donax* (respectively 3.82 and 3.01 mg $g^{-1}$ FW) in our study. The content of phenolic on the dry weight basis was identified significantly high at the species of *Zea mays*. These values are approximate to those identified by Ebrahimzadeh et al. 2008.

It was reported in the studies investigating antioxidant activity of the species of *Salvia* growing in Turkey that the species with high total content of phenol have high antioxidant activity (Albayrak et al., 2008; Erdemoglu et al., 2006). Especially high values of the phenolic substances such as carsonic acid, rosmarinic acid, salvianolic acid are responsible for the antioxidative activity at the species of *Salvia* (Lu and Foo, 2002; Triantaphyllou et al., 2001).

**Table 1.** List of species from Lamiaceae and Poeceae, it's localities and their dry weight, RWC and chlorophyll values (*P* values were not shown any significant difference).

| ***Lamiaceae species and locality*** | **Dry Weight (%)** | **RWC (%)** | **Chl-a (µg g$^{-1}$ FW)** | **Chl-b (µg g$^{-1}$ FW)** | **Total Chl. (µg g$^{-1}$ FW)** |
|---|---|---|---|---|---|
| *Calamintha nepeta* (Apsun, Gözne) | 24.6±3.3 | 74.3±6.1 | 1000±35 | 516±30 | 1517 |
| *Lamium amplexicaule* (Bükdeğirmeni) | 19.1±1.8 | 95.7±3.9 | 776±48 | 379±22 | 1155 |
| *Lamium garganicum* (Değirmençay) | 16.7±3.0 | 88.0±5.2 | 1402±41 | 646±27 | 2048 |
| *Marrubium vulgare* (Aydıncık, Bükdeğirmeni, Apsun) | 27.4±2.2 | 64.8±2.9 | 1290±57 | 585±35 | 1876 |
| *Micromeria myrtifolia* (Kuyuluk, Değirmençay) | 33.9±0.7 | 85.1±3.4 | 990±18 | 493±27 | 1484 |
| *Origanum vulgare* (Kuyuluk, Bükdeğirmeni) | 29.0±1.3 | 85.4±3.2 | 701±64 | 365±38 | 1080 |
| *Phlomis leucophracta* (Bükdeğirmeni) | 46.8±2.5 | 55.5±3.8 | 2055±67 | 866±41 | 2921 |
| *Salvia frigida* (Çamlıyayla- Çuvalgı) | 35.0±2.8 | 89.4±4.1 | 1285±30 | 591±24 | 1876 |
| *Salvia heldreichiana* (Apsun) | 23.8±1.3 | 89.3±1.7 | 579±36 | 307±22 | 885 |
| *Salvia verticillata* (Kuyuluk) | 15.5±1.4 | 96.3±3.2 | 941±27 | 471±24 | 1413 |
| *Salvia virgata* (Apsun) | 17.8±1.0 | 83.2±3.1 | 882±31 | 460±26 | 1342 |
| *Sideritis perfoliata* (Kuyuluk) | 43.3±0.5 | 93.5±3.7 | 1774±45 | 560±31 | 2335 |
| *Sideritis rubriflora* (Işıklı) | 22.3±0.5 | 84.8±3.5 | 1952±34 | 924±15 | 2876 |
| *Stachys rupestris* (Apsun) | 38.9±1.9 | 62.9±4.4 | 1243±31 | 591±47 | 1834 |
| *Teucrium chamaedrys* (Çamlıyayla- G.) | 32.0±0.9 | 85.6±1.9 | 906±27 | 524±38 | 1430 |
| *Teucrium polium* (Aydıncık, Bükdeğirmeni, Çamlıyayla) | 45.8±3.4 | 67.4±3.0 | 762±54 | 428±37 | 1190 |
| **Lamiaceae Avarage** | **29.5±10.1** | **81.3±12.1** | **1159±443** | **544±160** | **1704±583** |
| ***Poaceae species and locality*** | | | | | |
| *Aegilops speltoides* (Çamlıyayla) | 61.3±0.8 | 29.3±0.9 | 555± 48 | 319±27 | 874 |
| *Arundo donax* ( Kazanlı) | 32.9±1.3 | 87.9±2.9 | 584±115 | 261±73 | 845 |
| *Avena sterilis* (Çamlıyayla) | 42.8±3.0 | 49.2±1.5 | 617±122 | 308±80 | 925 |
| *Brachypodium pinnatum* (Çamlıyayla- G.) | 40.9±2.7 | 78.2±2.5 | 1316±44 | 574±36 | 1890 |
| *Brachypodium sylvaticum* (Çamlıyayla) | 39.9±1.9 | 79.0±1.1 | 606±80 | 336±86 | 942 |
| *Cynodon dactylon* ( Kazanlı) | 19.3±2.6 | 84.7±2.2 | 1443±54 | 637±41 | 2080 |
| *Cynodon sp.* ( Kazanlı) | 15.4±1.1 | 94.2±0.7 | 1006±45 | 434±52 | 1440 |
| *Echinochloa colonum* (Kazanlı) | 19.0±0.6 | 97.6±3.3 | 1078±68 | 489±43 | 1567 |
| *Echinochloa crusgalli* ( Kazanlı) | 19.0±0.9 | 93.2±3.2 | 1045±45 | 437±37 | 1482 |
| *Phalaris aquatica* (Kazanlı) | 37.2±3.0 | 56.3±2.0 | 1456±56 | 727±41 | 2183 |
| *Phleum pratense* (Çamlıyayla) | 54.6±4.2 | 44.6±2.9 | 1006±77 | 481±45 | 1487 |
| *Polypogon monspeliensis* (Çamlıyayla) | 27.2±2.5 | 77.6±3.7 | 1893±82 | 713±76 | 2606 |
| *Setaria viridis* (Yenişehir) | 17.8±1.5 | 80.6±5.5 | 1670±57 | 617±52 | 2287 |
| *Zea mays* ( Kazanlı) | 19.8±1.7 | 93.2±3.2 | 1169±48 | 429±35 | 1598 |
| **Poaceae Avarage** | **31.9±14.2** | **74.0±20.0** | **1103±406** | **483±146** | **1586±546** |
| **Statistics (T-test, *P* values)** | **0.60** | **0.24** | **0.73** | **0.30** | **0,58** |

**Table 2.** Antioxidant compounds content and SOD activities of species from Lamiaceae and Poeceae families (*significant at P ≤ 0.05 and ** significant P≤ 0.01).

| *Lamiaceae* | β-Caroten (µg $g^{-1}$ FW) | Xanthophyll (µg $g^{-1}$ FW) | Antioxidant capacity (mg $g^{-1}$ FW) | Soluble phenolics (mg $g^{-1}$ FW) | Total SOD (Unit $g^{-1}$ FW) |
|---|---|---|---|---|---|
| *Calamintha nepeta* (Apsun, Gözne) | 82±6.4 | 290±21.0 | 4.1±0.43 | 1,26 ± 0,07 | 197±32 |
| *Lamium amplexicaule* (Bükdeğirmeni) | 88±8.7 | 186±12.3 | 2.6±0.41 | 1,07 ± 0,08 | 296±20 |
| *Lamium garganicum* (Değirmençay) | 164±14.4 | 336±16.8 | 8.0±0.22 | 0,75 ± 0,05 | 186±26 |
| *Marrubium vulgare* (Aydıncık, Bükdeğirmeni, Apsun) | 114±15.2 | 265±24.0 | 4.1±0.71 | 1,26 ± 0,05 | 193±45 |
| *Micromeria myrtifolia* (Kuyuluk, Değirmençay) | 160±15.0 | 232±18.1 | 2.8±0.19 | 1,27 ± 0,05 | 193±23 |
| *Origanum vulgare* (Kuyuluk, Bükdeğirmeni) | 89±11.0 | 124±19.2 | 7.1±0.27 | 0,98 ± 0,05 | 185±45 |
| *Phlomis leucophracta* (Bükdeğirmeni) | 116±22.1 | 368±19.8 | 3.0±0.25 | 3,2 ± 0,07 | 175±14 |
| *Salvia frigida* (Çamlıyayla- Çuvalgı) | 124±11.4 | 294±32.8 | 3.6±0.91 | 0,55 ± 0,07 | 181±16 |
| *Salvia heldreichiana* (Apsun) | 86±6.6 | 165±13.5 | 6.9±0.33 | 1,45 ± 0,01 | 235±45 |
| *Salvia verticillata* (Kuyuluk) | 136±13.6 | 382±22.5 | 3.6±0.54 | 0,39 ± 0,08 | 227±30 |
| *Salvia virgata* (Apsun) | 66±8.0 | 187±23.9 | 2.2±0.57 | 0,14 ± 0,09 | 266±37 |
| *Sideritis perfoliata* (Kuyuluk) | 148±12.5 | 340±26.0 | 11.8±0.59 | 5,3 ± 0,07 | 188±35 |
| *Sideritis rubriflora* (Işıklı) | 92±8.4 | 304±22.4 | 7.1±0.25 | 5,74 ± 0,07 | 221±40 |
| *Stachys rupestris* (Apsun) | 44±8.5 | 92±15.2 | 8.1±0.50 | 0,9 ± 0,08 | 204±22 |
| *Teucrium chamaedrys* (Çamlıyayla- G.) | 82±7.6 | 157±13.5 | 2.3±0.08 | 0,23 ± 0,1 | 258±40 |
| *Teucrium polium* (Aydıncık, Bükdeğirmeni, Çamlıyayla) | 63±9.2 | 88±7.5 | 5.4±0.24 | 2,17 ± 0,08 | 197±24 |
| **Lamiaceae Avarage** | **103±35** | **236±95** | **5.19±2.62** | **1.67±1.63** | **213±34** |
| ***Poaceae*** | | | | | |
| *Aegilops speltoides* (Çamlıyayla) | 92±12.7 | 127±7.1 | 4.8±0.07 | 1,7 ± 0,05 | 209±14 |
| *Arundo donax* ( Kazanlı) | 24±11.3 | 72±6.9 | 3.4±0.58 | 3,01 ± 0,14 | 234±74 |
| *Avena sterilis* (Çamlıyayla) | 68±7.6 | 84±8.0 | 4.1±0.27 | 1,94 ± 0,08 | 229±28 |
| *Brachypodium pinnatum* (Çamlıyayla- G.) | 84±15.9 | 52±7.1 | 1.7±0.30 | 1,48 ± 0,05 | 188±62 |
| *Brachypodium sylvaticum* (Çamlıyayla) | 36±6.4 | 115±9.5 | 4.8±0.99 | 1,58 ± 0,25 | 214±54 |
| *Cynodon dactylon* ( Kazanlı) | 128±18.6 | 232±8.0 | 2.9±0.57 | 1,35 ± 0,01 | 223±39 |
| *Cynodon sp.* ( Kazanlı) | 88±8.1 | 132±10.2 | 2.2±0.40 | 1,65 ± 0,33 | 161±24 |
| *Echinochloa colonum* (Kazanlı) | 132±16.7 | 146±4.7 | 3.6±0.44 | 1,43 ± 0,13 | 210±22 |
| *Echinochloa crusgalli* ( Kazanlı) | 60±12.1 | 97±5.2 | 2.2±0.46 | 1,43 ± 0,06 | 206±60 |
| *Phalaris aquatica* (Kazanlı) | 88±10.3 | 304±26.8 | 1.9±0.18 | 1,71 ± 0,07 | 295±62 |
| *Phleum pratense* (Çamlıyayla) | 52±10.8 | 148±10.4 | 5.8±0.43 | 1,33 ± 0,08 | 296±49 |
| *Polypogon monspeliensis* (Çamlıyayla) | 104±7.9 | 294±11.8 | 3.4±0.44 | 1,16 ± 0,15 | 196±34 |
| *Setaria viridis* (Yenişehir) | 180±7.9 | 158±15.1 | 3.3±0.43 | 1,98 ± 0,04 | 241±28 |
| *Zea mays* ( Kazanlı) | 55±17.1 | 44±7.7 | 4.9±0.37 | 3,82 ± 0,14 | 253±24 |
| **Poaceae Avarage** | **84±42** | **142±84** | **3.49±1.22** | **1.83±0.70** | **225±36** |
| **Statistics (T-test, *P* values)** | **0.23** | **0.006**** | **0.04*** | **0.74** | **0.34** |

The amounts of total phenolic were determined as Micromeria croatia 13,6 %, M. juliana 10,8%, M. thymfolia 9,7% in the analyse performed with dried plant samples at the species of Lamiaceae growing in Croatia. It was stated that there was a strong positive correlation between phenolic compounds and antioxidative activity (Knezevic et al. 2011). High content of substance such as 13.4 mg $g^{-1}$ FW was identified at *Lavandula angustifolia*, in a study in which total content of phenolic of four species of Lamiaceae were investigated (Swedan, 2013). The reason why total content of phenolic of the species of *Salvia* have been identified as high in the literature is that extraction was performed with acid hydrolysis. Since acid hydrolysis wasn't used in our study, phenolic values with low solubility at the species of *Salvia*, high solubility at the species of *Sideritis* were identified. The highest values of soluble phenolic at dry weight basis were identified at *Sideritis rubriflora* (Figure 3). The average total amount of soluble phenolic is higher at Poacea than Lamiaceae. But the highest values of soluble phenolic were identified at two species of *Sideritis*. The high values of phenolic existing at some species of Lamiaceae may lead these species to be used as food, syrup and tea. The reasons why the species of Lamiaceae have been intensely investigated are phenolic compounds cause the inhibition of the production of fungal enzyme and of the enzymes produced by pathogens and removal of free radicals.

Unsuitable environmental conditions cause oxidative stress in plants. SOD enzyme has a critical role in protection of plants against oxidative stress (Keleş and Everest, 2008). Cross tolerance occurs in the plants being affected simultaneously from a great number of stress factors in natural conditions. One of the important components of cross tolerance is an increase in SOD activity (Bowler et al., 1992). In the study in which effects of both temperature and water stress at two species of wheat were investigated, the highest SOD activities were identified when temperature and water stress affect together (Keleş and Öncel, 2000). In the study in which the plants adapted to high mountains and steppe conditions were compared in regard to their SOD activities; while the average of SOD activity was determined as 213 units in high mountain plants, it was determined as 134 units in steppe plants (Öncel et al., 2004). While SOD activity was identified as high at the species of Poaceae invested in the same study, it was identified as quite changeable at the species of Lamiaceae. The SOD activity was identified as 225 units at the species of Poaceae and 213 units at the species of Lamiaceae in the current study. The findings indicate that The SOD activity has been affected from habitat features of plants rather than their genetic features.

The antioxidants showing directly or indirectly radical scavenging effect, highly exist at the species of Poaceae that can be made use of feed plant, at the Sideritis species of Lamiaceae that can be made use of drink as well.

Moreover, although the species of Stachys hasn’t widely used and investigated, it is among the species containing the highest antioxidant activity. The members of Lamiaceae, which are valuable in aromatic compounds and contents of volatile oil, have been intensively investigated by the science environment. It was concluded that the species of Poaceae are especially rich in soluble phenolic compounds and they should be further investigated.

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# Cell growth inhibitory potential of *Craterellus cornucopioides (L.)* Pers. together with antioxidant and antimicrobial properties

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# Antioksidan ve antimiktobiyal özellikleri ile birlikte *Craterellus cornucopioides (L.)* Pers. türünün hücre büyümesi baskılama potansiyeli

**Abstract:** *Craterellus cornucopioides (L.)* Pers which is also known as trumpet of death or horn of plenty, is a wild edible macrofungus. This study was conducted to elucidate the potential health beneficial properties of *C. cornucopioides*. Bioactive ingredients (phenolics, flavonoids, β-carotene and lycopene) and DPPH radical scavenging activities were determined. Additionally, cell growth inhibitory effects on HepG2 cells together with some bacteria were evaluated. Accordingly, water and methanol extracts contains 37.71±1.42 µg/mg and 13.78±1.60 µg/mg phenolic contents, respectively. Similarly, methanolic extracts have higher β-caroten and lycopene content as compared to aqueous extracts. In parallel with these antioxidants, methanolic extracts have also higher DPPH scavenging activity ($IC_{50}$: 5.26±0.67 mg/ml). Besides, water extracts have higher flavonoid contents (2.13±0.06 µg/mg) then the methanolic extracts. *C. cornucopioides* has also an important cell growth inhibitory effects on HepG2 cell ($IC_{50}$: 18.41±1.10 mg/ml for aqueous extracts and $IC_{50}$: 3.14±1.07 mg/ml for methanolic extracts). Moreover, both extracts were effective on six different bacteria tested. As a result, this study indicates that *C. cornucopioides* could reduce the cellular oxidative stress because of its high antioxidant ingredients, inhibit the growth of pathogen microrganisms and have some degree of cell growth inhibitory potential at least to the HepG2 cells.

**Key words:** *Craterellus cornucopioides*, antioxidant, antibacterial, cytotoxicity, HepG2

**Özet:** Ölüm trompeti veya bolluk boynuzu olarak da bilinen *Craterellus cornucopioides (L.)* Pers, yenilebilir bir makrofungustur. Bu çalışma *Craterellus cornucopioides (L.)* Pers mantarının sağlık açısından yararlı özelliklerini açığa çıkarmak amacıyla yapılmıştır. Çalışmada ilgili mantarın biyoaktif içerikleri (fenolikler, flavonoidler, β-karoten ve likopen) ve DPPH radikal süpürücü aktiviteleri belirlenmiştir. Ek olarak, HepG2 hücreleri ve bazı bakteri türleri üzrine hücre büyümesini baskılayıcı etkileri değerlendirilmiştir. Buna göre, su ve metanol ekstraktları sırasıyla 37.71±1.42 µg/mg ve 13.78±1.60 µg/mg fenolik içeriğe sahiptir. Benzer şekilde, metanol ekstraktları sulu ekstrelere kıyasla daha yüksek β-karoten ve likopen içeriğine sahiptir. Bu antioksidanlara paralel olarak, metanol ekstraktları da DPPH süpürme aktivitesine daha fazla sahiptir (ICso: 5.26±0.67 mg/ml). Ayrıca su ekstraktları, metanol ekstraklarına göre daha yüksek flavonoid içeriğine (2.13±0.06 µg/mg) sahiptir. *C. cornucopioides*, HepG2 hücresi üzerinde de önemli bir hücre büyümesi engelleyici etkiye sahiptir ($IC_{50}$: su ekstratları için 18.41±1.10 mg/ml ve metanol ektraktları için $IC_{50}$: 3.14±1.07 mg/ml). Buna ek olarak, her iki ekstrakt da test edilen altı farklı bakteri türü üzeine etkili olmuştur. Sonuç olarak, bu çalışma *C. cornucopioides*'in yüksek antioksidan bileşenleri nedeniyle hücresel oksidatif stresi azaltabildiğini, patojen mikroorganizmalarının büyümesini inhibe edebileceğini ve en azından HepG2 hücrelerine bir miktar hücre büyümesi inhibitör potansiyeline sahip olduğunu göstermektedir.

**Anahtar Kelimeler:** *Craterellus cornucopioides*, antioksidan, antibakteriyel, sitotoksisite, HepG2

## 1. Introduction

Fungi are eukaryotic and heterotrofic organisms that are composed of tubular filamentous cells, free chlorophyll and create spores. They could not produce their own foods thus which live as saprophyts, mycorrhizal and parasite. Edible mushrooms contain macro-molecules, which are normally 19 to 35% protein, and all protein content almost comprises essential amino-acids. They also include polyunsaturated fatty acids (72-85%) and carbohydrates (51-88%) according to dry or fresh weight (Chang et al., 1996). Medicinal mushrooms have important therapeutic features and, due to these features, they have been used against many kinds of disease for treatment in traditional medicine. For example, many genre mushroom such as *Agaricus, Aleurodiscus, Clitocybe, Coprinus, Daedalea, Ganoderma, Lentinula, Merulius, Pleurotus, Polyporus, Poria, Psathyrella* and *Tricholoma* rise in value due to their major properties such as anti-microbial (Chang et al., 2012), anti-viral (Pan et al. 2013), anti-oxidant (Palacios et al., *2011*), anti-cancer (Mattila et al., 2000).

Free radicals have been generated by many biological pathways or infections in organisms, damaging the cellular components such as organelles (Yıldız et al., 2015, Manivannan et al., 2011) and they are quenched by antioxidant molecules or related antioxidant enzymes. Mushrooms gain importance according to rich bioactive compounds such as polyphenols, polysaccharides, vitamins, carotenoids and minerals (Kozarski *et al*. 2015, Cheung et al., 2002). Studies have shown that antioxidant rich foods might play an important role in reducing the risk of disease, such as cardiovascular diseases, stroke and cancer (Gan et al., 2013, Barros et al., 2007; Jagadish et al. 2009). Therefore, antioxidants in edible mushrooms might act against reactive oxygen species and contribute to create antioxidant responses. Macrofungi also gain importance in cancer inhibition and treatment with the secondary metabolites found in their structure. Moreover, they also could be used against microbial infections. Some metabolites might prevent the growth of certain bacterial and fungal pathogens (Alves et al., 2012).

For example, applanoxidic acid A isolated from *Ganoderma annulare* (Fr.) Gilbn. has been showed to be effective against *Trichophyton mentagrophytes*. Moreover, 5a-ergosta- 7,22-dien-3b-ol 5,8-epidioxy-5a,8a-ergosta-

6,22-dien-3b-ol isolated from *Ganoderma applanatum* (Pers.) Pat., have been shown to affect several gram-positive and gram-negative microorganisms (Smania et al., 1999, Smania et al., 2003). In this study, *C. cornucopioides* was investigated for its antimicrobial, antioxidant, and cytotoxic properties to contribute the studies that are done in pharmaceutical area.

## 2. Materials and Method

### 2.1. Preparation of Fungal Extracts

*Craterellus cornucopioides* (L.) Pers that was used in this study was collected from Trabzon province with a voucher number of Yuzun 1852. The samples are kept at Karamanoglu Mehmetbey University, Kamil Özdağ Science Faculty, Department of Biology. Water and methanol extracts were prepared to examine cytotoxic, antioxidant and antimicrobial effects. Ten grams of entire mushrooms were homogenized by using liquid nitrogen, mortar and pestle and exposed to extraction in 300 ml methanol or distilled water with Soxhlet extraction apparatus. Then, extracts were concentrated in a rotary evaporator, lyophilized and stored at +4 ° C for further use.

### 2.2. Determination of Total Phenolic Content

Folin-Ciocalteu method was used for the determination of total phenolic content (Taga et al., 1984). Gallic acid (0.02 to 1.00 mM) was used as standard. Fungal extracts (10 mg/ml) and standards (0.02-1.00 mM) were placed in 20 µl microplate wells. Afterwards, 20 µl of Folin reagent (2N) was added and mixed by pipetting. After incubation for 3 min in the dark, 20 µl of 35% (w/v) sodium carbonate and 140 µl of $dH_2O$ were added to the plate and incubated for 10 min in the dark. The absorbance values were recorded against the blank tube at 725 nm and the amount of total phenolic content in one mg extract was calculated using a standard calibration curve generated with gallic acid.

### 2.3. Determination of Total Flavonoid Content

Total flavonoids of water and methanol extracts were determined according to method (Pal et al., 2010) with slight modifications. A volume of 50 µl of extracts (10 mg/ml) were mixed with 215 µl of ethyl alcohol (80% v/v), 5 µl of aluminum nitrate (10% w/v) and 5 µl potassium acetate (1 M) in microtiter plates and incubated for 40 min at room temperature. After reading at 415 nm, total flavonoid contents were calculated according to following equation:

Total flavonoid contents (µg/mg extract) = $(A_{415} + 0.01089)/0.002108$

### 2.4. Determination of β-carotene and Lycopene Contents

Different extracts with water and methanol that were obtained from *C. cornucopioides* were reextracted with 10 ml of acetone:hexane (4:6) mixture and filtered through Whatman No. 4 filter paper to determine β-carotene and lycopene contents. After filtration, absorbance of the filtrates was measured at 453, 505 and 663 nm. β-carotene and lycopene contents were calculated according to following equations.

β-carotene content (mg/100 mg) $=0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$

Lycopene content (mg/100 mg) $= -0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

### 2.5. Identification and Quantification of Main Phenolic Compounds by HPLC

Phenolic substance identification was carried out with HPLC (Shimadzu LC-20AD system, Japan). Data was processed using LC Solution software (Shimadzu, Japan). As mobile phase; (A) 0.1% (v/v) formic acid and (B) acetonitrile mixtures were used as gradient. Gradient elution conditions are as follows: Starting 20% B; 0-10 min 20% -30% B; 10-40 min 30-40% B; 40-60 min 40% -560% B; 60-80 min 60-80% B; and finally, 90 min from 80% -20% B. The flow rate was set to 1 ml/min, the column temperature was fixed at 30°C. Phenolic compounds were identified by using standards that were in known concentration. Gallic acid, catechin, epicatechin, epigallocatechin gallate, syringic acid, p-coumaric acid, rosmarinic acid, t-resveratrol and quercetin standard curves were constructed and the amount of these phenolics in *C. cornucopioides* extracts were quantitatively determined. At least three applications were performed for each sample (Standard or sample) and sample absorbances were monitored at 271 nm, 280 nm and 309 nm.

### 2.6. Determination of Reducing Power

After the slight modifications that were made to adopt the method to microplate measurement, reducing powers of different extracts were determined according to the previously prescribed protocol (Sadi et al., 2015). Gallic acid (0.01–0.10 mM) was used as a standard antioxidant. Briefly, in a total volume of 200 µl, various concentrations of 50 µl mushroom extracts (2, 4, 6, 8, 10 mg/ml) were mixed with 75 µl phosphate buffer (0.2 M pH: 6.6),75 µl potassium ferricyanide (1% w/v) and incubated at 50°C for 20 min. After adding 75 µl trichloroacetic acid (10% w/v), samples were centrifuged for 10 min at 1000 *g* and supernatants (75 µl) were transferred to another microtiter plate. Then, they were mixed with 75 µl distilled water and 15 µl iron (III) chloride (0.1% w/v). Spectrophotometric measurements were employed at 700 nm and the effective concentrations ($EC_{50}$) at which the absorbance was 0.500 for the reducing power was calculated.

### 2.7. Determination of DPPH Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *C. cornucopioides* was measured (Türkoglu et al., 2007) to determine antioxidant power. Accordingly, different concentration *C. cornucopioides* extracts (0.25 to 10 mg/ml) and gallic acid (0.005 to 0.2 mM) were prepared for measuring the elimination activity of DPPH radical. From *C. cornucopioides* extracts and standards, 20 µl was added to each microplate well and 180 µl DPPH (0.06 mM in methanol) was added. After incubation for 60 min in dark, the reduction of DPPH radical was followed by the absorbance values that were measured at 517 nm. Free radical capturing activities were calculated according to the following formula. The DPPH radical scavenging activity (RSA) was calculated as $IC_{50}$ values for each sample.

$$\text{RSA (\%)} = \frac{\text{DPPH absorbance} - \text{DPPH and extract absorbance}}{\text{DPPH absorbance}}$$

### 2.8. Determination of cell growth inhibitory potential

#### 2.8.1. Antimicrobial properties

Six test bacteria; *Bacillus subtilis, Enterococcus faecalis, Bacillus licheniformis, Staphylococcus aureus* (ATCC 2921) as Gram Positive; *Agrobacterium tumefaciens* and *Escherichia coli* (0157: H7 ATCC 43895) as Gram negative were grown in liquid Mueller Hinton Broth overnight in a shaker incubator. The concentrations of the microorganisms were adjusted to be equal to 0.5 McFarland standard ($1.5x10^{+8}$ CFU/ml). The empty discs were loaded with 20 µl of stock 200 mg/ml *C. cornucopioides* extracts and placed on petri plates for disk diffusion method. Gentamicin was used as the standard antibiotic. Then *A. tumefaciens* (28°C) and other microorganisms (35°C) were incubated overnight to examine the antimicrobial activity in the petri dishes. At the end of this period, the zones of inhibition around the discs were measured by means of a digital ruler.

#### 2.8.2. Cytotoxic effects on HepG2 cells

HepG2 (human hepatocellular cancer) cells were used to investigate of cytotoxic effect of aqueous and methanol extracts of *C. cornucopioides.* Growth medium (RPMI with L-glutamine) was heated to 37°C and cells were added. Then, they were grown in a 5% $CO_2$ incubator (Sanyo, USA) at 37 °C with 95% humidity. One day after, the cells were washed with PBS and detached by trypsinization when 80-90% saturation was reached. Passaged cells were grown again at 37 °C in 5% $CO_2$ (Sanyo MCO 17AIC, USA) until confluency of 90%. The cytotoxic effects of *C. cornucopioides* extracts were determined in accordance with the manufacturer's protocol with the XTT cell proliferation assay kit (Biological Industries, Israel). For this, 50 µl of activated XTT (Cell Proliferation Assay Kit) was added to the cells which were preincubated with different concentration of *C. cornucopioides* extracts for 48 hours at 37 ° C, 5% $CO_2$. At the end of the 5 h XTT incubation, absorbance values at 450 nm was measured with a microplate reader (Multiskan™ GO, Thermo Scientific, USA) and $IC_{50}$ values were calculated.

### 2.9. Statistical Analyses

All the assays were carried out at least in triplicate measurements. The results are expressed as mean values and standard error of mean (SEM). Antioxidant, antibacterial and cytotoxicity activities were analyzed using Student t-test and values with $P < 0.05$ were considered as statistically significant. $IC_{50}$ and $EC_{50}$ values were calculated with non-lineer regression analysis. For all statistical calculations Statistical Package for Social Sciences (SPSS®, version 21.0) were utilized.

## 3. Results

Health and nutrition problems are getting increase due to world population which is irregularly growing. Nowadays, unconscious consumption of natural resources and economic difficulties obligates the use of natural resources and this increases the importance of macrofungi in diet. In addition to the nutritional properties, their biologically active substances have gained reputation in the pharmaceutical area.

In studies of antioxidant, antimicrobial and cytotoxic activities of macrofungi and other medical effects, important data have been obtained. In this study, antioxidant, antimicrobial and cytotoxic activities of *Craterellus cornucopioides* (L.) Pers. is researched.

Amount of bioactive compounds present in aqueous and methanolic extracts of *C. cornucopioides* are summarized in Table 1. Results demonstrated that water extracts of *C. cornucopioides* has very high amount of total phenolics (37.71±1.42 µg/mg) which is also in parallel with the main phenolics; gallic acid and p-coumaric acid determined with HPLC analysis. Similarly, total flavonoid contents (2.13±0.06 µg/mg) were also higher as compared with methanolic extracts (1.83±0.02 µg/mg). On the other hand, methanolic extracts have higher content of the β-carotene and lycopene. Additionally, the phenolic compounds such as gallic acid, catechin, epicatechin, epigallocatechin gallate, syringic acid, p-coumaric acid, rosmarinic acid, t-resveratrol and quercetin in *C. cornucopioides* extracts were quantitatively determined by HPLC but, the amount of other phenolic compounds, except p-coumaric acid and gallic acid was either under HPLC limit or had no in *C. cornucopioides* extracts.

**Table 1:** Antioxidant activity, reducing power and bioactive ingredients of *C. cornucopioides* extracts.

| **Bioactive Ingredients** | ***C. cornucopioides* extract** | **Content** |
|---|---|---|
| **DPPH scavenging ($IC_{50}$ : mg/ml)** | Water | 12.01±1.72 |
| | MeOH | 5.26±0.67 |
| **Reducing power ($EC_{50}$: mg/ml)** | Water | 4.54±0.61 |
| | MeOH | 6.52±1.53 |
| **Phenolics (µg//mg)** | Water | 37.71±1.42 |
| | MeOH | 13.78±1.59 |
| **ß-carotene (µg/mg)** | Water | 3.89±0.06 |
| | MeOH | 6.34±0.08 |
| **Lycopene (µg/mg)** | Water | 2.49±0.01 |
| | MeOH | 5.55±0.08 |
| **Flavonoid (µg/mg)** | Water | 2.13± 0.06 |
| | MeOH | 1.83±0.02 |
| **Gallic Acid (µg/mg)** | Water | 0.55 ± 0.02 |
| | MeOH | 0.29 ± 0.02 |
| **p-coumaric acid (µg/mg)** | Water | 3.73 ± 0.01 |
| | MeOH | 1.76 ± 0.01 |

DPPH radical scavenging activities were measured with different concentrations of *C. cornucopioides* up to 10 mg/ml of extracts and scavenging activities enhanced with elevated concentrations (Fig. 1). The best radical scavenging was obtained with the methanolic extracts of *C. cornucopioides* in 10 mg/ml concentration, as over than 75% DPPH reduction takes place. Aqueous extracts of the same mushrooms also possessed 40% inhibition at highest concentration tested. According to $IC_{50}$ values, methanolic extracts of *C. cornucopioides* ($IC_{50}$: 5.26±0.67 mg/ml) are more efficient than its aqueous extracts ($IC_{50}$: 12.01±1.72 mg/ml).

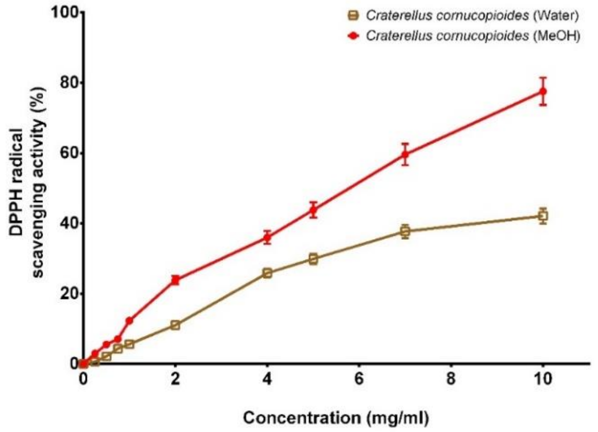


**Figure 1:** DPPH radical scavenging activities of aqueous and methanolic extracts of *C. cornucopioides.*

Reducing power of a pharmaceutical generally strongly correlates well with the antioxidant capacity. Therefore, $EC_{50}$ values were determined to describe the extract concentration yielding an absorbance value of 0.500. According to the results, aqueous extracts showed higher reducing activity than methanolic extracts in general view (Fig. 2). Aqueous extract of *C. cornucopioides* had the highest reducing activity with the lowest $EC_{50}$ value (4.54±0.61 mg/ml).

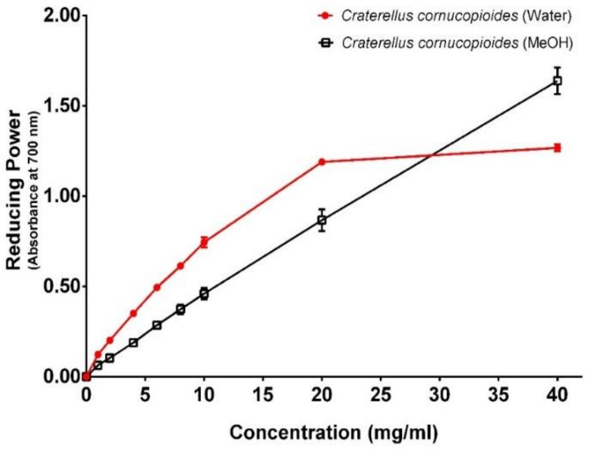


**Figure 2:** Reducing powers of aqueous and methanolic extracts from *C. cornucopioides*.

Cell growth inhibitory potentials of *C. cornucopioides* extracts on HepG2 cells were also inspected in this study, which was not reported previously. Results indicated that extracts have some degree of cytotoxicity over HepG2 cells and cytotoxic effects of the extracts increased with elevated concentrations (Fig. 3). Methanolic extracts of *C. cornucopioides* had the lowest $IC_{50}$ values (3.14±1.07 mg/ml). Considering the water extracts, $IC_{50}$ values of 18.41±1.10 mg/ml were obtained showing that methanolic extracts has higher cell growth inhibitory potential. As a result, *C. cornucopioides* might play a role in cancer and related researches in cytotoxic effect studies.

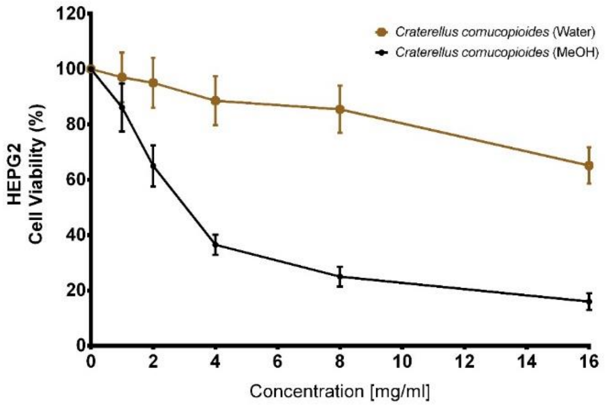


**Figure 3:** Cytotoxicity of aqueous and methanolic extracts from *C. cornucopioides* over HepG2 cells after 48 hours exposure time.

In antibacterial studies, it was revealed that extracts of *C. cornucopioides* have high antimicrobial potential. As it can be seen clearly in Table 2, *C. cornucopioides* exerted antibacterial activity on all tested microorganisms; *B. subtilis, E. faecalis, B. licheniformis, A tumefaciens, E. coli* and *S. aureus*. With its both water and methanolic extracts, *C. cornucopioides* inhibited microbial growth with inhibitory zone (IZ) values ranging 6-8 mm length.

**Table 2:** Antibacterial effects of *C. cornucopioides* on six different tested microorganisms.

| Tested Bacteria | Extract | *C. cornucopioides* IZ: mm | Gentamicin IZ: cm |
|---|---|---|---|
| ***E. coli*** | Water | 6 | 2.3 |
| | MeOH | 7 | |
| ***S. aureus*** | Water | 6 | 2.4 |
| | MeOH | 6 | |
| ***B. subtilis*** | Water | 6 | 2.5 |
| | MeOH | 8 | |
| ***B. licheniformis*** | Water | 6 | 2.4 |
| | MeOH | 7 | |
| ***A. tumefaciens*** | Water | 6 | 2.7 |
| | MeOH | 7 | |
| ***E. faecalis*** | Water | 6 | 2.5 |
| | MeOH | 7 | |

In conclusion, in the near feature mashrooms having nutritional and economical values will be more used in medicine, pharmacy and industrial area due to their high antioxidant, antimicrobial, and cytotoxic features. *C. cornucopioides* shows noticeable activities with its antioxidant, antibacterial and cell growth inhibitory potential together with high gallic acid and p-coumaric acid content. It might be utilized as a promising source of therapeutics since it might provide an appropriate source of antioxidant, antibacterial and cytotoxic natural compounds and also could be searched as potent antibacterial drugs against infectious diseases.

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# New additions to Turkish *Tricholomataceae*

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# Türkiye *Tricholomataceae*'lerine yeni ilaveler

**Abstract:** Four macrofungi species, *Arrhenia lilacinicolor* (Bon) P.-A. Moreau & Courtec., *Cellypha goldbachii* (Weinm.) Donk, *Resupinatus taxi* (Lév.) Thorn, Moncalvo & Redhead and *Rimbachia neckerae* (Fr.) Redhead, within the family *Tricholomataceae* were recorded for the first time from Turkey. Short descriptions of the species are provided together with the photographs related to their macro and micromorphologies.

**Key words:** Macrofungi, new record, Gaziantep, Turkey

**Özet:** *Tricholomataceae* familyası içinde yer alan dört makromantar türü, *Arrhenia lilacinicolor* (Bon) P.-A. Moreau & Courtec., *Cellypha goldbachii* (Weinm.) Donk, *Resupinatus taxi* (Lév.) Thorn, Moncalvo & Redhead ve *Rimbachia neckerae* (Fr.) Redhead Türkiye'den ilk kez kaydedilmiştir. Türlere ait kısa betimlemeler, türlerin makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

**Anahtar Kelimeler:** Makromantar, yeni kayıt, Gaziantep, Türkiye

## 1. Introduction

*Tricholomataceae* Lotsy is the largest family of the *Agaricales* Underw. with more than 100 genera. The family is usually known as not having any definite distinguishing characters from other families. But white or pale flesh, white to pale pink or lilac spore print and attached gills can be assumed as some common properties of *Tricholomataceae*. The genera *Arrhenia* Fr., *Cellypha* Donk, *Resupinatus* Nees ex Gray and *Rimbachia* Pat. are also regarded as the members of *Tricholomataceae* (Kirk et al., 2008).

Though more then 750 European species of *Tricholomataceae* have been determined, only 171 members belonging to 20 genera have been recorded from Turkey (Sesli and Denchev, 2014; Solak et al., 2015). Of the determined 171 taxa, 10 species belong to *Arrhenia* (Pilát, 1933; Aktaş et al., 2003; Doğan et al., 2000; Türkekul, 2003; Kaya, 2006; Kaya et al., 2008; Alkan et al., 2010; Demirel et al., 2010; Solak et al., 2015), three species belong to *Resupinatus* (Sümer, 1982; Kaşık et al., 2003) and one species belong to *Rimbachia* (Kaya et al., 2013).

In this paper, one member of each of the genera *Arrhenia*, *Cellypha*, *Resupinatus* and *Rimbachia*, which were collected from Gaziantep and determined as *Arrhenia lilacinicolor* (Bon) P.-A. Moreau & Courtec., *Cellypha goldbachii* (Weinm.) Donk, *Resupinatus taxi* (Lév.) Thorn, Moncalvo & Redhead and *Rimbachia neckerae* (Fr.) Redhead, and are reported as new records for Turkey, since they have not been reported from Turkey before (Sesli and Denchev, 2014; Solak et al., 2015; Demirel et al., 2016; Sesli et al., 2016; Acar and Uzun 2017; Akata and Uzun, 2017; Allı et al., 2017; Demirel et al., 2017; Işık and Türkekul, 2017; Kaşık et al., 2017; Öztürk et al., 2017; Sesli and Sesli, 2017; Türkekul, 2017; Uzun and Demirel, 2017; Uzun et al., 2017a; 2017b).

The study aims to make a contribution to the mycobiota of Turkey.

## 2. Materials and Method

Macrofungi samples were collected from suitable habitats within the boundaries of Gaziantep province between 2014 and 2015. During field studies necessary ecological and morphological characteristics of the samples were recorded and they were photographed in their natural habitats. The collected specimens were transferred to the lab within paper bags. Studies related to micromorphology were carried out under Nikon Eclipse Ci trinocular light microscope. Identification were performed with the help of Moser (1983), Courtecuisse and Duhem (1995), Gerault (2005), Gibson (2007), Winkel (2009) and Gonou-Zagou et al. (2011). The samples are kept at Karamanoğlu Mehmetbey University, Kâmil Özdağ Science Faculty, Department of Biology.

## 3. Results

The systematics of the taxa are given in accordance with Cannon and Kirk (2007), Kirk et al. (2008), and the Index Fungorum (www.indexfungorum.org; accessed 15 April 2017). The taxa are presented in alphabetical order with a brief description, habitat, locality, collection date, and accession numbers.

***Basidiomycota*** R.T. Moore
***Agaricales*** Underw.
***Tricholomataceae*** Lotsy
***Arrhenia*** Fr
***Arrhenia lilacinicolor*** (Bon) P.-A. Moreau & Courtec.

**Syn:** [*Omphalia galericolor* Romagn., *Omphalina galericolor* (Romagn.) Bon, *Omphalina galericolor* (Romagn.) Bon, var. *galericolor*, *Omphalina galericolor* var. *lilacinicolor* (Bon) Kuyper, *Omphalina lilacinicolor* Bon].

**Macroscopic and microscopic features:** Basidiocarp 5-20 mm in diameter, slightly infundibuliform, surface smooth, pinkish, pinkish ocher, lilac beige, cream ocher when dry, sometimes slightly grooved towards the thinner and crenulate margin. Flesh thin, concolorous. Lamellae decurrent (Figure 1a), sometimes interveined, concolorous beige. Stipe 10-40 × 2-4 mm, cylindrical, concolorous with the pileus or paler, somehow woolly towards the base. Basidia cylindrical, tapered toward the base, four spored (Figure 1b). Basidiospores 6,5-8 × 6-7 µm, elliptical, smooth (Figure 1c).

**Specimen examined:** Gaziantep–İslahiye, Hanağzı village, roadside, among mosses, 37°03'N-36°36'E, 625 m, 08.11.2014, K.10523.

***Cellypha*** Donk
***Cellypha goldbachii*** (Weinm.) Donk.

**Syn:** [*Calyptella goldbachii* (Weinm.) Quél., *Calyptella lactea* (Bres.) Quél., *Calyptella ochroleuca (*Berk. & Broome) Bigeard & H. Guill., *Cellypha lactea* (Bres.) W.B. Cooke, *Chaetocypha lactea* (Bres.) Kuntze, *Chaetocypha ochroleuca* (Berk. & Broome) Kuntze, *Chaetocypha rubi* (Fuckel) Kuntze, *Cyphella goldbachii* Weinm., *Cyphella lactea* Bres., *Cyphella lactea* Bres. var. *lactea*, *Cyphella lactea* var. *rubi* (Fuckel) Pilát, *Cyphella ochroleuca* Berk. & Broome, *Cyphella rubi* Fuckel, *Phaeocyphella ochroleuca* (Berk. & Broome) Rea, *Phaeoglabrotricha rubi* (Fuckel) W.B. Cooke].

**Macroscopic and microscopic features:** Basidiocarp 3-5 mm in diameter, stands stalkless on herbaceous stems, irregularly cup shaped, white, coated with clavate hairs. Hymenial surface smooth, concolorous with the outer surface (Figure 2a). Flesh white, membranous. Basidia 30-35 × 7-8 µm, cylindrical to clavate, with 2-4 sterigmata (Figure 2b). Basidiospores 12-16 × 3.3-5 µm, ellipsoid or elongated club-shaped, some slighthy curved (Figure 2c).

**Specimen examined:** Gaziantep–Nurdağı, Hamidiye village, roadside, on decaying herbs, 37°05'N-36°53'E, 810 m, 12.12.2014, K.11017.


**Figure 1.** *Arrhenia lilacinicolor*: a. basidiocarps b. badisia and cystydia c. basidiospores

***Resupinatus*** Nees ex Gray
***Resupinatus taxi*** (Lév.) Thorn, Moncalvo & Redhead.

**Syn:** [*Chaetocypha taxi* (Lév.) Kuntze, *Cyphella taxi* Lév., *Stigmatolemma taxi* (Lév.].

**Macroscopic and microscopic features:** Basidiocarp 0.5-1 mm high and 0.5-1.2 mm wide, irregular cup shaped, whitish to cream. Hymenial surface smooth. Flesh thin, whitish. Stipe absent or greatly reduced (Figure 3a). Basidia 18-25 × 4-6 µm, cylindrical, tapering towards the base, with 2-4 sterigmata (Figure 3b). Basidiospores 4.7-5.7 × 4.5-5.2 µm, elliptical, smooth (Figure 3c).

**Specimen examined:** Gaziantep–Nurdağı, Hamidiye village, pine forest, on dead plant remains with moss, 37°05'N-36°53'E, 810 m, 12.12.2014, K.11040.

***Rimbachia*** Pat
***Rimbachia neckerae*** (Fr.) Redhead

**Syn:** [*Chaetocypha neckerae* (Fr.) Kuntze, *Cyphella muscicola* d *neckerae* Fr., *Cyphella neckerae* (Fr.) Fr., *Leptoglossum candidum* D.A. Reid, *Mniopetalum megalosporum* Singer, *Rimbachia neckerae* subsp. *megalospora* (Singer) Redhead, *Rimbachia neckerae* (Fr.) Redhead, subsp. *neckerae*].

**Macroscopic and microscopic features:** Basidiocarp 3-12 mm in diameter, urceolate, margin wavy, surface whitish to cream, sparsely pubescent or glabrous. Flesh thin, whitish to cream. Hymenium smooth, concolorous with the outer surface (Figure 4a). Stipe absent or with a rudimentary pseudostem. Basidia four spored. Basidiospores 8-12 × 4-7 µm, elliptical to almond shaped, smooth (Figure 4b).

**Specimen examined:** Gaziantep–İslahiye, Altınüzüm village, mixed forest clearing, on moss, 36°54'N-36°33'E, 480 m, 21.03.2015, K.11457.

## 4. Discussions

With this study *Arrhenia lilacinicolor* (Bon) P.A. Moreau & Courtec., *Cellypha goldbachii* (Weinm.) Donk, *Resupinatus taxi* (Lév.) Thorn, Moncalvo & Redhead and *Rimbachia neckerae* (Fr.) Redhead were added as new records for Turkey. Among them, *Cellypha goldbachii* is the first member of the genus *Cellypha* in Turkey.

Though the fruiting bodies of most of the members of *Cellypha*, *Resupinatus* and *Rimbachia* resemble the species of discomycetes, they bear their spores on basidia. Due to their disc-, tube-, or cup-shaped basidiocarps, they are referred as cyphelloid fungi and the family name *Cyphellaceae* Lotsy was used to keep most of such segregate genera together. This *Ascoymycete* look-alike *Basidiomycete* group, also known as false discomycetes, is quite an artficial group of basidiomycete fungi from different sections, having in common their cupulate and hairy fruitbodies without gills.

The external appearances of *Rimbachia neckerae* and *R. bryophila* (Pers.) Redhead resemble each other. But the two species can easily be distinguished from each other with the presence of lamellar structure on the hymenial surface of *R. bryophila*. Besides its smooth hymenium, *R. neckerae* also have slightly bigger basidiospores.

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**Figure 2.** *Cellypha goldbachii*: a. basidiocarps b. badisia and cystydia c. basidiospores


**Figure 3.** *Resupinatus taxi*: a. basidiocarps b. badisia and cystydia c. basidiospores


**Figure 4.** *Rimbachia neckerae*: a. basidiocarps, b. basidiospores

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# Endemic plants of Lake Van Basin

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# Van Havzası endemik bitkileri

**Abstract:** This study was carried out on endemic plants collected from the Lake Van basin between 1994 and 2002. As a result, 98 endemic taxa, 23 of which grow only in the Lake Van basin, were determined. Including the endemic taxa reported before from the region, a list of 259 endemic taxa belonging to 34 families and 117 genera were compiled. Twenty-three of 259 taxa listed, are unique to the Lake Van Basin. There are 249 endemic taxa in the VANF Herbarium. The authors collected 98 of them. The other 151 endemic taxa were determined by other researchers. Ten endemic taxa were recorded from the literature. In addition to the features in the Flora of Turkey, new features and variations of nine endemic taxa identified. Total 612 images and 200 pictures belonging to 98 endemic taxa scanned and transferred to computer. All images were uploaded to the web page, Virtual Herbarium of the Lake Van Basin, which is also a part of VANF Herbarium.

**Key words:** Endemic plants, taxonomy, IUCN, Turkey

**Özet:** Bu çalışma 1994-2002 yılları arasında Van Gölü havzasından toplanan endemik bitkiler üzerinde gerçekleştirilmiştir. Sonuçta, yirmi üç tanesi sadece Van Gölü havzasında yetişen, 98 endemik takson belirlenmiştir. Bölgeden daha önceden rapor edilen taksonların da dahil edilmesiyle, 34 familya ve 117 cins içinde yer alan 259 endemik taksondan oluşan bir liste oluşturulmuştur. Listelenen 259 taksondan 23 tanesi sadece Van Gölü Havzasına özgüdür. VANF Herbaryumu'nda 249 endemik takson vardır. Bunlardan 98 tanesi yazarlar tarafından toplanmıtır. Kalan 151 endemik takson ise diğer araştırmacılar tarafından belirlenmiştir. On endemik takson da literatürden kaydedilmiştir. Türkiye Florasındaki özelliklere ek olarak, dokuz endemik taksonun yeni özellikleri ve varyasyonları belirlenmiştir. Doksan sekiz endemik taksona ait 612 görüntü ve 200 resim camlı tarayıcıdan taranarak bilgisayar ortamına aktarılmıştır. Bütün görüntüler VANF Herbaryum'unun bir parçası da olan web sayfasına, Van Gölü Havzası'nın Sanal Herbaryumu'na, yüklenmiştir.

**Anahtar kelimeler**: Endemik bitkiler, taksonomi, IUCN, Türkiye

## 1. Introduction

Floristic studies indicate that Turkey has a very rich species diversity. This is because the country is located in the transition area of three, Irano-Turanien, Mediterranean and Euro-Siberian, phytogeographical regions (Bulut and Yılmaz, 2010). About 12.000 seed plant taxa have been identified in Turkey while this number is about 13.000 in whole of Europe (Erik ve Tarıkahya, 2004; Ekim, 2005). Turkey is also among the most important countries in terms of endemic plant species (Davis et al., 1988; Ekim, 2005; Özhatay et al., 2009). More than 3000 endemic plant taxa, most of which are threatened, have so far been determined within the boundaries of Turkey (Atalay, 1997; Ekim et al., 2000) and the localities of almost 2900 endemic taxa have been mapped (Şenkul and Kaya, 2017).

The Lake Van is situated on the high plateaus of Eastern Anatolia, between 38°18′ - 39°00′N latitudes and 42°17′-43°39′E longitudes. The lake surface stands at 1648 m above sea level, with a surface area of 3574 km$^2$ (Degens et al., 1984). The drainage basin of Lake Van covers an area of about 20.000 km$^2$ (Çiftçi et al., 2008) within the boundaries of Bitlis and Van provinces and falls in B9 according to P.H. Davis' grid square system. The basin is surrounded by high volcanic mountains. The long term annual precipitation is 387.2 mm and annual average temperature is 9.4 °C (mgm, 2018). In general the basin is characterized by steppe vegetation, and forest and shrubby areas are generally dominated by *Quercus* sp.

The study aims to present a complete list of endemic plant taxa growing in Van Lake basin.

## 2. Materials and Method

Endemic plant samples, which were collected by the authors from the field, existing in Yüzüncü Yıl University Herbarium (VANF) herbarium or those, obtained from the literature make the material of this study.

Endemic plant samples were collected from Lake Van basin (Fig 1) between the years 1994 and 2002. During field studies, colour slides were taken and required ecological data were recorded. Then the plant samples were transferred to the herbarium and prepared as herbarium materials. Identification was performed with the help of Flora of Turkey and the East Agean Islands (Davis, 1965-1982). Later on, the existing plant samples in VANF herbarium were traced and endemic taxa a belonging to Van Lake basin were listed together with the necessary information about the taxa. Meanwhile the related literature (Behçet, 2001; Doğan and Akaydın, 2004; Özgökçe et al., 2005; Sutorý, 2005; Akan et al., 2008; Karabacak and Behçet, 2009; Dirmenci et al., 2010; Eker and Babaç, 2010; Yıldırım et al., 2011; Yıldız et al., 2011; İlçim et al., 2013; Vitek et al., 2014; Yıldırırm, 2014; Doğan et. al., 2015; Tekşen and Karaman, 2015; Fırat and Yıldız, 2016; Gültepe et al., 2016; Fırat, 2017a,b; Pınar, 2017) concerning the endemic plant taxa of Van Lake basin were also traced, and an endemic plant list with 259 taxa was created. Collection localities are given in Table 1. The collected samples are kept in VANF.

The validity of Endemic plants names was checked mainly from The Plant List Version 1.1 (2018). If required The

Euro + Med Plant Base (2018) and The International Plant Names Index (IPNI) were used as secondary or tertiary sources.

### 3. Results

The compiled list of endemic plant taxa determined in the Lake Van basin is listed as cited in Flora of Turkey, together with their habitats, locality numbers, collection dates, voucher number(s), IUCN Red Data categories (if known) and the variations from the description given in Flora of Turkey (if determined). IUCN Red Data Categories and the abbreviations are in accordance with Ekim et al. (2000).

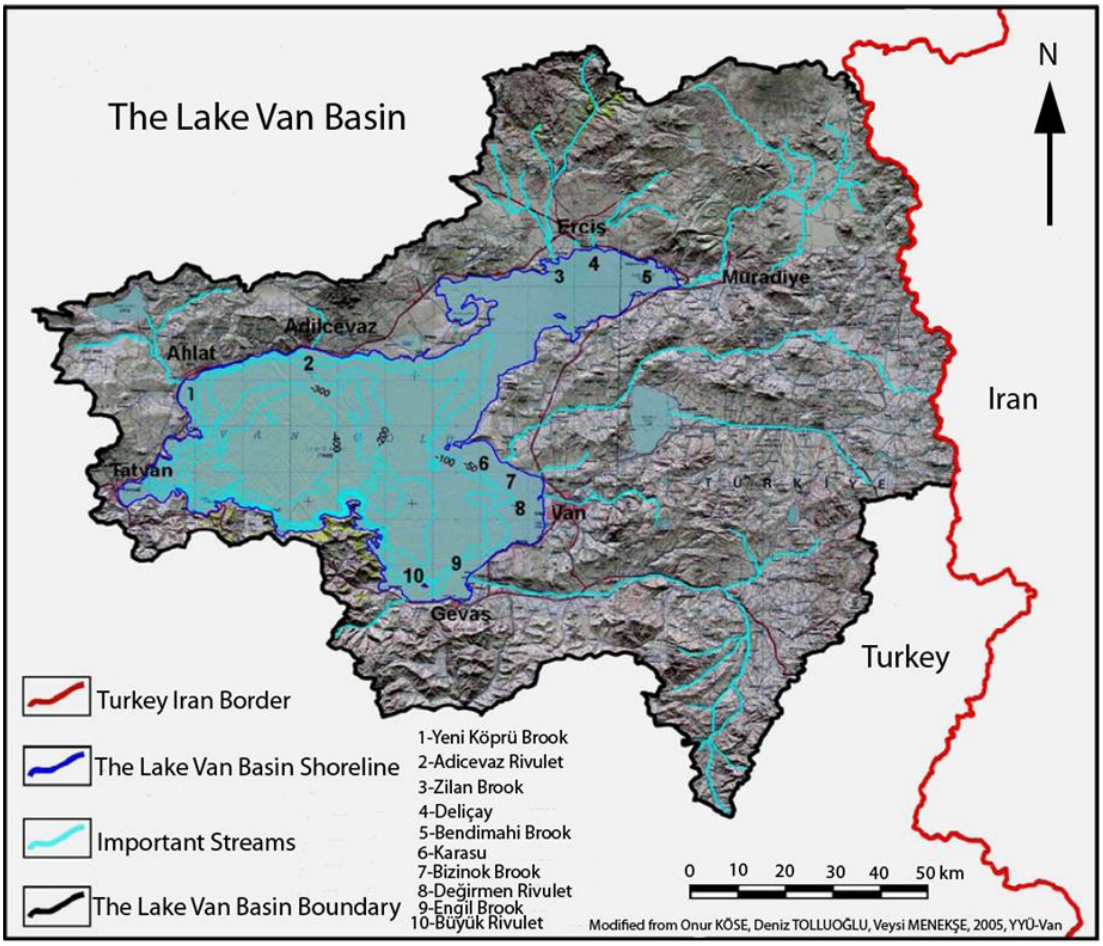


**Figure 1.** The hydrological border and the main drainage system of the Lake Van basin. (Modified from Çiftçi et al., 2008)

For The Citation Of The Voucher Numbers, Names Of The Collectors Are Abbreviated As Fallows: A.Altiok (Aa); Bayram Yildiz (Y); Davut Avlamaz (Da); Doğan & Akaydin (D&A); Ismail Eker (E); Fevzi Özgökçe (Fö); Hasan Özçelik (Hö); Hüseyin Eroğlu (He); H.Yildirim (Hy); Ibrahim Demir (Id); L.Behçet & D. Avlamaz (B&A); Lütfü Behçet (B); M. Gültep (Mg); Metin Armağan (Ma); Mehmet Firat (Mf); Mehmet Koyuncu (Mk); Mesut Pinar (Mp); Muzaffer Mükemre (Mm); M.Tekşen (Mt); Nasip Demirkuş (Nd); Murat Ünal (Mü); Tuncay Dirmenci (Td); Osman Kara Karabacak (Ok); Ömer Bingöl (Öb); Turan Çelik (Tç).

IUCN Red Data categories were cited with the following abbreviations: Critically endangered (cr); Data deficient (dd); Endangered (en); Extinct (ex); Extinct in the wild (ew); Lower risk (lr); Least concern (lc); Conservation dependent (cd); Near threatened (nt); Not evaluated (ne); Vulnerable (vu) (Ekim et al. 2000). The taxa collected by the authors are marked with an asterisk (∗).

**Table 1.** Collection localities of the endemic within Van Lake basin.

| No | Locality | Altitude |
|---|---|---|
| 1 | B9 Van: Değirmen Village, Erek Mountain | 2000-2500 m |
| 2 | B9 Van: Değirmen Village, Erek Mountain | 2000-2300 m |
| 3 | B9 Van: Gürpınar, Kurubaş pass | 1900 m |
| 4 | B9 Van: Hoşap, Güzeldere pass, around guardhouse | 2600-2730 m |
| 5 | B9 Van: Tevekli and Alaköy meadows | 1850 m |
| 6 | B9 Van: Hoşap, Güzeldere pass from fountain to gourdhouse | 2600-2730 m |
| 7 | B9 Van: Van to Erciş 35. Km | 1850 m |
| 8 | B9 Van: Hoşap, Güzeldere pass | 2250 m |
| 9 | B9 Van: Hoşap, Güzeldere pass | 2500 m |

| | | |
|---|---|---|
| 10 | B9 Van: Hoşap, Güzeldere pass | 2750 m |
| 11 | B9 Van: Gevaş, Artos Mountain, west slopes | 2000-2350 m |
| 12 | B9 Van: Artos Mountain, north side | 2200-2500 m |
| 13 | B9 Van: Hoşap, Güzeldere pass | 2600-2730 m |
| 14 | B9 Van: From Van to Çatak road 70. km | 1750-2000 m |
| 15 | B9 Van: from Gürpınar to Çatak 35. km | 2200-2500 m |
| 16 | B9 Van: Çaldıran, Tendürek Mountains | 2650 m |
| 17 | B9 Van: Erek Mountain meadows of Değirmen Village | 2600 m |
| 18 | B9 Van: Değirmen Village, Erek Mountain | 2400 m |
| 19 | B9 Van: Muradiye, meadows of Yumaklı Village | 2250 m |
| 20 | B9 Van: Hoşap, Güzeldere pass, around FedaiTaşı | 2780-2900 m |
| 21 | B9 Van: Hoşap, Güzeldere pass | 2650 m |
| 22 | B9 Van: Bahçesaray, from Müküs Water Cavern to Sündüs plateau | 1700-2000 m |
| 23 | B9 Van: Erek Mountain, Şuşanıs Village steppe slopes | 2300 m |
| 24 | B9 Van: Hoşap, Güzeldere pass | 2400- 2700 m |
| 25 | B9 Van: Erek Mountain, Şuşanıs Village slopes | 2400- 2500 m |
| 26 | B9 Van: Around Ayanıs Villages | 1750 m |
| 27 | B9 Van: Gürpınar, Kurubaş pass | 2000 m |
| 28 | B9 Van: Zernek Dam (60-62. Km) north slopes | 2000 m |
| 29 | B9 Van: Hoşap, Güzeldere pass, around highway station | 2750 m |
| 30 | B9 Van: Gürpınar, Kurubaş pass | 1800-2000 m |
| 31 | B9 Van: Hoşap, Güzeldere pass, around sentry | 2750 m |
| 32 | B9 Van: Hoşap, Güzeldere pass, around highway station | 2790 m |
| 33 | B9 Van: Özalp road 41. Km | 1970 m |
| 34 | B9 Van: Kopanis Village, Erek Mountain | 2150-2700 m |
| 35 | B9 Van: Kopannis Village, Erek Mountain | 2500-2700 m |
| 36 | B9 Van: Özalp road 20. Km, slopes | 2000 m |
| 37 | B9 Van: Hoşap, Güzeldere pass, around fountain | 2500-2650 m |
| 38 | B9 Van: Hoşap, Güzeldere pass, around FedaiTaşı | 2750-2900 m |
| 39 | B9 Van: Bahçesaray, Sündüz plateau, valley slopes | 2100 m |
| 40 | B9 Van: Bahçesaray, from Krapet pass to Liçan Village | 2700 m |
| 41 | B9 Van: Hoşap, Güzeldere pass, around sentry, | 2750-2800 m |
| 42 | B9 Van: Bahçesaray, around Liçan Village | 1900-2000 m |
| 43 | B9 Van: Muradiye, Suphan Lake, meadows of Adaklı Village | 2350 -2500 m |
| 44 | B9 Van: Around Muradiye Waterfall | 2000 m |
| 45 | B9 Van: Muradiye, 3 Km to Çaldıran | 2150 m |
| 46 | B9 Van: Muradiye 2 Km to Çaldıran, Ayrancılar Village | 2150-2200 m |
| 47 | B9 Van: Hoşap, Güzeldere pass | 2500-2750 m |
| 48 | B9 Van: Cross of Çatak and Bahçesaray roads | 2000 m |
| 49 | B9 Van: From Gürpınar to Çatak, before Görentaş Village | 2000 m |
| 50 | B9 Van: Bahçesaray, from krapet pass to Liçan Village | 2500-2800 m |
| 51 | B9 Van: Bahçesaray, around Müküs Water Cavern | 1800-1850 m |
| 52 | B9 Van: Bahçesaray, from Liçan Village to Krapet pass | 1800-2500 m |
| 53 | B9 Van: Kopannis Village, Erek Mountain | 3000-3200 m |
| 54 | B9 Van: Kopannis Village, Erek Mountain | 2500-3000 m |
| 55 | B9 Van: from Gürpınar to Hoşap 10. Km, around road | 1900 m |
| 56 | B9 Van: from Van to Tatvan | 2500 m |
| 57 | B9 Van: Hoşap, Güzeldere pass, around fountain | 2650 m |
| 58 | B9 Van:Erciş District, northeast of Aksakal village, Kırdekar hill | 2692m |
| 59 | C9 Van: Çatak, Konalga village, Tanrıverdi hamlet, around Zevviçal | 2372 m |
| 60 | B9 Van: Miks (Bahçesaray) district, Çıravis Mountains (Çiyayê Çirawîs) | 3343 m |
| 61 | B9 Van: Ahta Dağ, southeast of Kalecik village near Özalp | 2500–2700 m |
| 62 | B9 Van: Bahçesaray, Karabel (Kirapit) pass | 3200–3400 m |
| 63 | B9 Van: Çatak, North of Çatak Valley, surroundings of Bilgi Village | 1723 m |
| 64 | B9 Bitlis: Adilcevaz, Suphan Mountain north of Kıçkılı village | 2500 m |
| 65 | B9 Van: ,Bahçesaray, from Altındere village to Kavushhahap Mount. | 2600m |
| 66 | B9 Van, Bahçesaray, Serkani location | 1800 m |
| 67 | B9 Van: Bahçearay, Arnos Mountain, | 2200m |
| 68 | B9 Van: between Çatak and Bahçesaray, north of Yukarınarlı village | 2200- 2400 m |
| 69 | B9 Van: between Başkale and Van | 2052 m |
| 70 | B9 Van: Gevaş, Deveboynu peninsula, above İnköy | 2100 m |
| 71 | B9 Van: Başkale, Çaldıran village | 2000–2050 m |
| 72 | B9 Van: Muradiye above the Adaklı, village | 2300m |
| 73 | B9 Van: from Van to Muradiye, Bendi mahi region | 1659 m |
| 74 | B9 Van: Çatak, Kavuşşahap Mountain, Karapet pass | 2750 m |
| 75 | B10 Van:Van- Hakkari road, around Başkale | 2100 m |
| 76 | B9 Van: Gevaş, Artos Mountain | 2500 m |

| | | |
|---|---|---|
| 77 | B9 Van: Gürpınar–Başkale road, around Hoşap (Güzelsu) | 2050 m |
| 78 | B9 Van: Hoşab (Güzeldere) province, Güzeldere passage | 2791 m |
| 79 | B9 Van: from Narlıca Village to Bahçesaray, Karapetpass | 2885 m |
| 80 | B9 Van: between Van-Başkale, Güzeldere pass | 2738 m |
| 81 | B9 Van: Gürpınar, Işıkpınar to Hacıköy, around the pond | 2154 m |
| 82 | B9 Van: From Vari Krapet pass to Bahçesaray | 2500-2700 m |
| 83 | B9 Van: Gürpınar, behind Koçgüden village | 2500 m |
| 84 | B9 Van: Erciş, 1. Km to the Yukarıgöze neighborhood | 2000-2200 m |
| 85 | B9 Van: Gürpınar, Hamurkesen Village, inside the valley | 2400 m |
| 86 | B10 Van: Özalp, Aşağı Koçkıran Village, towards Karatepe | 2275 m |
| 87 | C9 Van: Gürpınar, from Üçdoğan to Bükülmez Village | 2153 m |
| 88 | B9 Van: Erciş, Taşkapı village, Karakaya | 2448 m |
| 89 | B9 Van: Gürpınar, in the west of Tutmaç village | 2800m |
| 90 | B9 Van: Gürpınar, the opposite of Koçgüden village | 2300 m |
| 91 | B9 Van: Özalp, East of Kırkçalı | 1900-2050 m |
| 92 | B9 Van: Muradiye, Kemerköprü Village, Akçadağ, Neri Tepe | 2000 m |
| 93 | B9 Van: Özalp, from Y. Ayazca to Y. Mollahasan village | 2003 m |
| 94 | B10 Van: Özalp, from Aşağı Tulgalı to Y.Dönerdere Village | 2053 m |
| 95 | B9 Van: Gürpınar, behind the Koçgüden village | 2950 m |
| 96 | B9 Van: Çatak, from Çatak to the Kirazlı village, south slope | 1800 m |
| 97 | C9 Van: Gürpınar, from Topçudeğirmeni to Yalınca Village | 2269 m |
| 98 | B9 Van: Gürpınarthe behind of Tutmak village | 2600 m |
| 99 | B9 Van: Gürpınar, Kapçık village, the edge of the creek | 2134 m |
| 100 | B9 Van: Özalp, A.Sağmallı village, Pirreşit Mountain | 2500 m |
| 101 | B9 Van: Muradiye, Görecek village, around Şevlii | 2200-2500 m |
| 102 | B9 Van: Özalp, from Y.Koçkıran to A.Koçkıran village | 2300 m |
| 103 | B9 Van: Özalp, A.Akçagöl village "Pirreşit" mountain | 2293m |
| 104 | B10 Van: Özalp, Y.Balçıklı village plateau | 2300m |
| 105 | B9 Van: Erçek, Erçek Lake shores | 1800 m |
| 106 | B9 Van: Muradiye, Babacan village | 1900 m |
| 107 | B9 Van: Erciş, around of Soluhan village | 2634 m |
| 108 | B9 Van: Erciş, around of Şehirpazarı village | 1937 m |
| 109 | B9 Van: Gürpınar, from Taşdöndüren village to Belkış slope | 2300 m |
| 110 | B9 Bitlis: Tatvan, Tanrıyer village | 1700 m |
| 111 | B9 Van: Erciş, from Doluca to Gürgürbaba hill | 2912 m |
| 112 | B9 Van: Gürpınar, from Hamurkesen to Işıkpınar village | 2000 m |
| 113 | B9 Bitlis: Hizan, from Hizan to Sürmecek village | 1500 m |
| 114 | B9 Van: Muradiye, Görecek village , around Şevkii | 2350 m |
| 115 | B9 Van: Gürpınar, from Sarıyaprak to Güleçler village | 2300 m |
| 116 | B9 Van: Gürpınar, from Cevizalan to Umut Village | 2500 m |
| 117 | B9 Van: Çatak, Bilgi village, south slopes | 1900 m |
| 118 | B9 Van: Erciş, from the village of Taşkapı to the ganissipi | 2200 m |
| 119 | B9 Van: Erciş, Şehirpazarı village, Adalar plateau | 1937 m |
| 120 | B9 Van: Erciş, Şehirpazarı village, Adalar plateau , behind the spa | 1950 m |
| 121 | B9 Van: Özalp, Sugeçer and Boğazkesen villages, Ziyaret hill | 2400 m |
| 122 | B9 Van: Erciş, around Şehirpazarı village | 2293 m |
| 123 | B9 Van: Erciş, Altındere village, hara surroundings | 1845 m |
| 124 | B9 Van: Özalp, from Y.Ayazca to Mollahasan village | 2003 m |
| 125 | B9 Bitlis: around İçmeli village, | 1510 m |
| 126 | B9 Van: Muradiye Kemerköprü village, around Akçadağ | 2200 m |
| 127 | B9 Van: Erciş, Şehirpazarı village, Adalar plateau | 1950 m |
| 128 | B9 Van: Özalp, south of Y. Çavdarlık village | 2219 m |
| 129 | B9 Van: Gürpınar, from Hoşap to Zernek barrage, stream edge | 1900 m |
| 130 | B9 Van: Hoşap, Güzeldere pess | 2400 m |
| 131 | B9 Van: Özalp Ahta Mountain | 2700 m |
| 132 | B9 Van: Gürpınar, Geçerli village | 1890 m |
| 133 | B9 Van: Gürpınar, north of Yedisalkım village | 2400 m |
| 134 | B9 Van: Kurubaş pass, Doğanlar village | 1900 m |
| 135 | B9 Van:Gürpınar, Taşdöndüren village roadsides | 1900 m |
| 136 | B9 Van:Gürpınar, Taşdöndüren village roadsides | 1900 m |
| 137 | B9 Van: Erciş, around Ilıca | 1912 m |
| 138 | B9 Bitlis: Hizan, Sarpkaya village | 2000 m |
| 139 | B9 Van: Gürpınar, from Işıkpınarı village to Südis Mountain | 2350 m |
| 140 | B9 Van: from Dorutay to Aksaağaç and Yumruklu villages | 2100m |
| 141 | B9 Van: Erciş, from Taşkapı village to Hüdavendigar Mountain | 3186 m |
| 142 | B10 Van: Başkale,Darıca village road, steppe around the bridge | 1920 m |
| 143 | B9 Van: Gürpınar, above the Kochgüden village | 2800 m |

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|---|---|---|
| 144 | B9 Bitlis: Adilcevaz, the north of the Kıçıklı village | 2400-2700 m |
| 145 | B9 Van: Muradiye, Derviş Plateau, Hacı Cave, Kom stream | 2000-2200 m |
| 146 | B9 Van: Gürpınar, Sarıyaprak Plateau-Güleçler village | 2400 m |
| 147 | B9 Van: Özalp, Mollahasan village | 2380 m |
| 148 | B9 Van: Gürpınar, behind the Hamurkesen village | 2300 m |
| 149 | B9 Van: Gürpınar, southeastern slopes of the Başet mountain | 2400 m |
| 150 | B9 Van: Erciş, around Doluca village | 1965 m |
| 151 | B10 Van: Başkale, around Oğulveren village | 2250 m |
| 152 | B9 Van: Gürpınar, from Bölmeçalı to Hacıköy village | 2500 m |
| 153 | B9 Van: Erciş, the edge of Zilan Creek | 1700 m |
| 154 | B10 Van: Özalp, around Özalp | 2300 m |
| 155 | B9 Van: Özalp, Kaşıkara village surroundings | 1800 m |
| 156 | B9 Van: Muradiye, Beşparmak village surroundings | 2300 m |
| 157 | B9 Bitlis: Tatvan, Kesan brook 4.km | 1700 m |
| 158 | B9 Van: Özalp, in the West of Şehittepe village | 2435 m |
| 159 | B10 Van: Özalp, Yukaru Tulgalı village, Kel hill | 2280 m |
| 160 | B9 Van: Özalp, from Bodurağaç to Seydibey village | 2194 m |
| 161 | B9 Van: Gürpınar, from Hamurkesen to Işıkpınar village | 2000 m |
| 162 | B9 Van: Erciş, from Taşkapı village to Hüdavendigar Mountain | 2100-2800 m |
| 163 | B9 Van: Erciş, the edge of Zilan creek | 1750 m |
| 164 | B9 Van: Erçek Lake Coast | 1800 m |
| 165 | B9 Van: Muradiye, south slopes of the Pirreşit Mountain | 2000 m |
| 166 | B9 Van: Gürpınar, the back side of Yedisalkım village | 2200 m |
| 167 | B9 Van: Gürpınar, south of Hamurkesen village | 1900 m |
| 168 | B9 Van: Erciş, Doluca village, Kuzubulak lake periphery | 2535m |
| 169 | B9 Van: Özalp, east of Seydibey village | 2100 m |
| 170 | B9 Van: Gürpınar, the southern slopes of Başet Mountain | 2600 m |
| 171 | B9 Van: Muradiye, Görecek village, Şevki location | 2200-2500 m |
| 172 | B9 Van: Özalp, from A.Molahasan to Y.Molahasan village | 1800 m |
| 173 | B9 Van: Gürpınar, Zernek barrage, roadside | 1850 m |
| 174 | B9 Van: Erciş, Sabanbüken village, Çakali plateau | 2825 m |
| 175 | B9 Bitlis: Tatvan, at the 7th km of Kesan stream | 1700-1800 m |
| 176 | B9 Van: Gürpınar north slopes of Zernek Dam | 1900 m |
| 177 | B9 Van: Gürpınar, around Yurtbaşı village | 2100 m |
| 178 | B9 Bitlis: Adilcevaz , from Çanak yayla to Cihangir village, | 2300-2500 m |
| 179 | B9 Van : Gürpınar, northwest of Koçgüden village | 2950 m |
| 180 | B9 Van: Erciş, Dolucan vilage, Lake Şama, Lignite man. periphery | 2634 m |
| 181 | B9 Van: Erciş, Altındere stud farm circumference | 1845 m |
| 182 | B9 Van: Özalp, Kalecik village | 2000 m |
| 183 | B9 Van: Muradiye, Görecek village, Şevki location | 2200-2500 m |
| 184 | B9 Van: Van castle, rock crevices | 1720 m |
| 185 | B9 Van: Erciş, Doluca village, Gürgürbaba hill | 2738 m |
| 186 | B9 Van: Muradiye, slopes of Pirreşit Mountain opposite Özalp | 2000 m |
| 187 | B9 Van: Erciş, around the lake İkizçalı | 1978 m |
| 188 | B9 Bitlis: Tatvan, the Kesan brook 4. km | 1700 m |
| 189 | B9 Van: Özalp, the south of Y. Koçkıran village | 2050 m |
| 190 | B9 Bitlis: Adilcevaz, from Harmantepe to Çanakyayla village | 2200 m |
| 191 | B9 Van: Özalp, the north of Y. Yargılı village, Pirreşit Mountain | 2070 m |
| 192 | B9 Van: the west of Erek Mountain | 2500 m |
| 193 | B9 Van: Özalp, from Özalp to Dorutay village, Koyun yatağı | 2072 m |
| 194 | B9 Van: Gürpınar, opposite of Koçgüden village | 2500 m |
| 195 | B9 Van: Gürpınar, from Işıkpınar village to Hacı village | 2100 m |
| 196 | B9 Van: Erciş, around Ulupamir village, creek edge | 1811 m |
| 197 | B9 Van: Özalp, Damlacik village, wheat field | 2300 m |
| 198 | B9 Van: Muradiye, the edges of the valley behind Adaklı village | 2400 m |
| 199 | B9 Bitlis: Adilcevaz, the north of Bahçedere village | 2100 m |
| 200 | B9 Van: Özalp, Yukarı Tulgalı village, border plateau | 2250 m |
| 201 | B9 Van: Gürpınar, Sarıyaprak plateau, valley inside | 2800 m |
| 202 | B9 Van: Muradiye, along the Yumaklı valley | 2200 m |
| 203 | B9 Van: Erciş, the northwestern part of Ulupamir village | 1871 m |
| 204 | B9 Van: Gürpınar, from Zernek Dam to Üçgen vilage | 1700-1900 m |
| 205 | B10 Van: Özalp, from A. Koçkıran vilage to Iranian borderı | 2200 m |
| 206 | B9 Bitlis: Tatvan, Kesan creek 10-15.km, road side | 1550 m |
| 207 | B9 Van: Muradiye, Ünseli village, Akçadağ (Esruk) mountain | 2400 m |
| 208 | B10 Van: Başkale, Esenyamaç village 3. km north slope | 1976 m |
| 209 | B9 Van: Erciş, from Ilıca to Yeknal plateau | 2019 m |
| 210 | B9 Van: Muradiye, 3 km to the village of Görecek | 1800 m |

**I. FAM: RANUNCULACEAE**

***Delphinium carduchorum** Chowdhuri & P.H.Davis: meadows, locality 29; 19.07.1997, Nd 6191 -Mk 12755; meadows, locality 47; 13.07.1997, Nd 6179, 6191-Mk 12743, 12755; rocky slopes, locality 54; 27.07.1997, Nd 6225 -Mk 12789;locality 56; 23.07.1997, Nd 6304a - Mk 12867a**,** IUCN: nt.

***Delphinium dolichostachium** Chowdhuri & P.H.Davis: road slopes, steppe, locality 52; 16.07.1997, Nd 5945 -Mk 12509, IUCN: cd.

***Delphinium cyphoplectrum** Boiss. var. ***vanense*** (Rech. Fil) P.H. Davis: rocky slopes, locality 48 ; 15.07.1997, Nd 5883-Mk 12447; meadows, locality 47; 13.07.1997, Nd 6189-Mk 12753; rocky slopes, locality 54; 27.07.1997, Nd 6238 -Mk 12802; steppe, locality 57; 19.07.1997, Nd 6209 -Mk 12773, IUCN: cd.

***Delphinium cyphoplectrum*** Boiss. var. ***stenophyllum*** Boiss.: steppe, locality 83; 31.07.2002, Mü 7800.

***Ranunculus crateris** P.H. Davis : meadows, locality 17; 08.06.1997, Mk 11836-Nd 5266, IUCN: cd. **Variations**: Stems up to 12 flowered (stems 1-6 flowered), all sepals not reflexed (sepals reflexed).

***Ranunculus fenzlii** Boiss.: steppe, locality 21; 13.06.1997, Mk 11901, 11922 -Nd 5331, 5352, IUCN: lc.

***Ranunculus bingoeldaghensis*** A.Engin: locality 84; 19.05.2002, Ok 2931 IUCN: en.

***Ranunculus vanensis** P.H. Davis: B9 Van: steppe, locality 38; 27.06.1997, Mk 12260-Nd 5688, IUCN: cd. **Variations**: Early stage of upper leaves linear lanceolate, later divided to linear lobes, some samples include undivided and divided upper leaves. Early stage of achene semicircular, later semicircular to slender. Posterior of achene beak brownish to blackish (unknown).

***Ranunculus munzurensis** S.Erik & Yildirimli: Rocky slopes, locality 12; 26.05.1997, Nd 5211 -Mk 11747, IUCN: cd.

**II. FAM: PAPAVERACEAE**

***Papaver persicum*** Lindl. subsp**.** ***fulvum*** Kit-Tan & Sorger: steppe, locality 85; 23.07.2003, Mü 8832, IUCN: lc.

***Papaver fugax** Poiret var. **platydiscus** Cullen: steppe, locality 31; 21.06.1997, Mk 12053-Nd 5480; steppe locality 38; 27.06.1997, Mk 12224 - Nd 5652, IUCN: lc.

**III. FAM: BRASSICACEAE**

***Isatis cappadocica*** Desv. subsp. ***alyssifolia*** (Boiss.) P.H. Davis: locality 86; 05.07.1997, Fö 4767, IUCN: nt.

***Isatis erzurumica*** P.H.Davis: creek edge, locality 87; 16.06.2007, Mf 1856, IUCN: nt.

***Isatis candolleana** Boiss.: steppe, locality 41; 19.07.1997, Nd 6100, IUCN: lc.

***Isatis undulata** Aucher ex Boiss.: wet meadows, locality 35; 22.06.1997, Mk 12157-Nd5585, IUCN: en.

***Isatis aucheri** Boiss : rocky slopes, locality 34; 22.06.1997, Mk 12114, 12159 -Nd5542, 5587; meadows, locality 43; 12.07.1997, Nd 5792 -Mk 12353, IUCN: lc.

***Isatis glauca** Aucher&Boiss.
subsp. ***icaonia*** (Boiss. & Heldr.) P.H. Davis: rocky slopes, locality 26; 19.06.1997, Mk 11974 - Nd 5403; slopes, locality 36; 26.06.1997, Mk 12212 - Nd 5640, IUCN: lc.

***Isatis spatella*** P.H. Davis : sandy soil area, locality 39; 02.05.1999, Mf; 1013, IUCN: en.

***Physocardamum davisii*** Hedge : steppe, calcareous soil, locality 88; 21.06.2007, Ok 6558, IUCN: vu.

***Aethionema froedinii*** Rech. fil.: road slopes, locality 50; 15.07.1997, Mf 1162. IUCN: lc.

***Aethionema eunomioides** (Boiss.) Bornm.: rocky slopes, locality 53; 16.07.1997, Nd 7016, IUCN: lc.

***Aethionema iberideum*** (Boiss.) Boiss : steppe area, locality 89; 08.06.2003, Mü 8358.

***Aethionema caespitosum*** (Boiss.) Boiss.: steppe area, locality 89; 08.06.2003, Mü 8359., IUCN: nt.

***Bornmuellera cappadocica** (DC.) Cullen & Dudley: steppe, locality 38; 27.06.1997, Mk 12234-Nd 5662, IUCN: lc.

***Alyssum huetii*** Boiss.: steppe, locality 90; 27.05.2001, Mü4935b, IUCN: lc.

***Alyssum blepharocarpum*** Dudley&Hub.-Mor.: locality 91; 27.06.1996, Fö 2530, IUCN: nt.

***Alyssum ochroleucum*** Boiss.: steppe, locality 92; 21.06.2001, Ok 1859, IUCN: lc.

***Alyssum aurantiacum*** Boiss.: locality 93; 02.07.1997, F 5155, IUCN: cd.

***Alyssum niveum*** Dudley: steppe, locality 94; 10.07.1997, F 4916, IUCN: en.

***Alyssum pateri*** Nyár. subsp. ***prostratum*** (Nyár.) Dudley: steppe, locality 95; 31.07.2002, Mü 7798, IUCN: lc.

***Alyssum filiforme*** Nyár.: steppe, locality 96; 21.06.2003, Mp 1973, IUCN: lc.

***Alyssum haussknechtii*** Boiss.: steppe, locality 97; 26.07.2006, Mf 392, IUCN: Lr (cd)

***Draba rosularis** Boiss.: steppe, locality 10; 24.05.1997, Mk11697-Nd 5163; steppe, locality 21; 13.06.1997, Mk 11906- Nd 5336, IUCN: lc.

***Draba cappadocica** Boiss. & Bal.: steppe, locality 38; 27.06.1997, Mk 12226- Nd 5654, IUCN: lc.

***Draba orientalis*** Karabacak & Behçet: Holotype, rocky crevices, locality 58; 28.05.2007, Ok 6081.

***Arabis carduchorum*** Boiss.: steppe, locality 98; 23.07.2002 , Mü 7563, IUCN: nt.

***Sterigmostemum sulphureum*** Bornm. subsp**.** ***glandulosum*** Hub.-Mor. & Reese: the edge of the creek, locality 99; 16.06.2007, Mf 1721, IUCN: vu.

***Erysimum sintenisianum*** Bornm.: steppe, locality 100; 26.06.1997, Fö 3513, IUCN: lc.

***Erysimum echinellum*** Hand.-Mazz.: steppe, locality 142; 31.05.2008, Da 1246b, IUCN: en.

**IV. FAM: CARYOPHYLLACEAE**

***Minuartia umbellifera** (Boiss.) Mc Neill subsp. ***umbellifera*** var. ***kurdica*** McNeil: meadows, locality 29; 19.07.1997, Nd 6114 -Mk 12676; rocky slopes, locality 53; 16.07.1997, Nd 5951, 5973, 6001- Mk 12515, 12535, 12564, IUCN: nt.

***Minuartia anatolica*** (Boiss.) Woronow var. ***anatolica***: steppe, locality 143; 16.07.2002, Mü7349, IUCN: cd.

***Minuartia glandulosa** (Boiss. & Huet) Bornm.: rocky slopes, locality 54; 27.07.1997, Nd 6232 -Mk 12796, IUCN: lc.

***Cerastium araraticum** Rupr.: rocky slopes, locality 53; 16.07.1997, Nd 5968 -Mk12531, IUCN: lc.

***Cerastium gnaphalodes** Fenzl : rocky slopes, locality 53; 16.07.1997, Nd 5967, IUCN: lc.

***Dianthus lactiflorus** Fenzl : rocky slopes, locality 53; 16.07.1997, Nd 5966, IUCN: cd.

***Dianthus erythrocoleus** Boiss.: meadows locality 29; 19.07.1997, Nd 6204- Mk 12768, Nd 6059, 6101- Mk 12622, 12663; meadows , locality 46; 13.07.1997, Nd 5834 -Mk 12395; meadows, locality 47; 13.07.1997, Nd 6133 -Mk 12695; rocky slopes, locality 53; 16.07.1997,Nd 5970, 6022- Mk 12533, 12585; rocky slopes, locality 54; 27.07.1997, Nd 5765 -Mk 12327, IUCN: lc.

***Dianthus masmaneus** Boiss. var. ***glabrescens*** Boiss. : meadows, locality 43;12.07.1997, Nd 5804 -Mk 12365, IUCN: lc.

***Dianthus muschianus*** Kotschy & Boiss.: wet meadows, locality 24: 23.07.1997, Mf 1835, Nd 6884z, IUCN: lc.

***Dianthus zederbaueri** Vierh.: meadows, locality 43; 12.07. 1997, Nd 5803, IUCN: cd.

***Dianthus vanensis*** Behçet & İlçim: , steppe, locality 59; 25.06.2010, Mm 300.

***Gypsophila adenophylla** Bark.: slopes, steppe, locality 52; 16.07.1997, Nd 5971 -Mk 12534, IUCN: lc.

***Gypsophila bitlisensis** Bark.: steppe slopes, locality 27; 21.06.1997, Mk 12023-Nd 5451, IUCN: cd.

***Silene capitellata** Boiss.: meadows, locality 15; 05.06.1997, Mk 11755, IUCN: lc.

***Silene muradica** Schschkin.: steppe, locality 44; 13.07.1997, Nd 5811 -Mk 12372, IUCN: lc.

***Silene cartilaginea*** Hub.-Mor.: steppe, locality 30; 13.06.1999, Ma 1603, IUCN: cd.

***Silene lucida** Chowdh**.** subsp. ***lucida***: steppe, locality 38; 27.06.1997, Nd 5680, 6799, IUCN: lc.

***Silene sclerophylla** Chowdh.: steppe, locality 30; 13.06.1999, Nd 6598d, IUCN: lc.

***Silene miksensis*** Fırat & K.Yıldız: limestone and rocky slopes, locality 60; 10.08.2011, Mf 27345.

***Silene acaulis*** (L.) Jacq. subsp***. vanensis*** Özgökçe & Kit Tan: locality 61; 15.05.1998, Fö 5911

**V. FAM: POLYGONACEAE**

***Rumex ponticus*** E.H. L. Krause: steppe rocky place, locality 101; 08.07.2001, Ok 2076. IUCN: lc.

**VI. FAM: HYPERICACEAE**

***Hypericum thymbrifolium*** Boiss. & Noë: steppe, locality 102; 04.07.1997, Fö 4610, IUCN: cd.

**VII. FAM: LINACEAE**

***Linum unguiculatum*** Da P.H. Davis vis: steppe, locality 103; 26.06.1997, Fö 3407. IUCN: lc.

***Linum obtusatum*** (Boiss.) Stapf: steppe, locality 104; 23.5.1998, Fö 5946, IUCN: lc.

**VIII. FAM: GERANIACEAE**

***Geranium ibericum*** Cav. subsp. ***jubatum*** (Hand.-Mazz.) Da P.H. Davis: damp rocky, locality 144; 08.07.2001, B 6648, IUCN: lc.

***Erodium hakkiaricum*** P.H.Davis: steppe, locality 146; 18.07.2001, Mü 6068, IUCN: en.

***Erodium amanum*** Boiss. & Kotschy: locality 144; 09.07.1987, B 346, IUCN: lc.

**IX. FAM: RUTACEAE**

***Haplophyllum cappadocicum*** Spach: steppe, locality 147; 30.06.1998, Fö 8168, IUCN: nt.

**X. FAM: FABACEAE**

***Astragalus hirsutus*** Vahl: rocky-steppe, locality 148; 22.05.2002, Mü 6805, IUCN: lc.

***Astragalus ovatus*** DC.: steppe, locality 149; Mü 8503, 10.07.2003, IUCN: dd.

***Astragalus dasycarpus** Chamberlain: meadows, locality 29; 19.07.1997, Nd 6105 -Mk 12657, IUCN: nt.

***Astragalus sachanewii** Sirj.: rocky slopes, locality 53; 16.07.1997, Nd 6017 -Mk12580, IUCN: nt.

***Astragalus bashkalensis** Chamberlain: meadows, locality 29; 19.07.1997, Nd 6095 -Mk 12657, rocky slopes locality 34; 22.06.1997, Mk 12126-Nd 5554; steppe, locality 37; 27.06.1997, Mk 12073-Nd5502, Mk 12289-Nd 5717; steppe, locality 38 ; 27.06.1997, Nd 5693, 5704 -Mk 12265,12276; rocky slopes, locality 54; 27.07.1997, Nd 6280 -Mk 12843, IUCN: vu. **Variations**: Legume; c. 16 mm villous with long white and short black hairy, ovoid sessile to short stipitate ( legume unknown).

***Astragalus tauricolus** Boiss.: steppe, locality 7; 22.05.1997, Mk 11681, IUCN: lc.

***Astragalus pinetorum** Boiss.: steppe slopes, locality 27; 21.06.1997, Nd 5121 -Mk 11629, IUCN: lc.

***Astragalus icmadophilus** Hand.-Mazz.: rocky slopes, locality 53; 16.07.1997, Nd 5996, IUCN: lc.

***Astragalus rechingeri** Sirj.: road slopes, locality 50; 15.07.1997, Nd 5910 -Mk 12474; rocky slopes, locality 53 ; 16.07.1997, Nd 5996 -Mk 12559. IUCN: nt. **Variations**: Calyx black and white hairy (sparsely black hairy).

***Astragalus trifoliastrum** Hub.-Mor. & Matthews: steppe slopes, locality 23; 18.06.1997, Mk 11933-Nd 5362; rocky slopes, locality 26; 19.06.1997, Mk 11994-Nd 5422; locality 36; 26.06.1997, Mk 12210- Nd 5638, IUCN: nt.

***Astragalus ermineus** Matthews: rocky slopes, locality 53; 16.07.1997, Nd 5902a, 6032 -Mk 12466a, 12595, IUCN: cd.

***Astragalus halicacabus** Lam.: meadows, locality 43; 12.07. 1997 Nd 5785 -Mk 12534, IUCN: lc.

***Astragalus gymnalopecias*** Reich. fil.: locality 42; 15.06.1999, Mf 1599, IUCN: en.

***Astragalus asciocalyx** Bunge : locality 25; 18.06.1997, Mk 11931-Nd 5360; rocky slopes, locality 26; 19.06.1997, Mk 11978-Nd 5407,IUCN: lc.

***Astragalus karamasicus*** Boiss. & Balansa : steppe, locality 105; 08.06.1991 B 1731, IUCN: lc.

***Astragalus lycius*** Boiss. : steppe, locality 106; 28.05.2002, Ok 2997, IUCN: lc.

***Astragalus fumosus** Boriss. : around road, locality 28; 21.06.1997, Mk 12030-Nd 5458, IUCN: nt.

***Astragalus xerophilus*** Ledeb.: steppe, locality 107; 12.07.2007, Ok 6773, IUCN: nt.

***Astragalus bicolor*** Lam.: steppe, locality 108; 26.06.2005, Ok 3941.

***Astragalus globosus** Vahl: locality 11; 25.05.1997, Nd 6426k, IUCN: lc.

***Astragalus armeniacus*** Boiss.: steppe, locality 109; 07.06.2003, Mü 8282.

***Astragalus schizopterus*** Boiss.: steppe, locality 109; 07.06.2003, Mü 8233.

***Astragalus nitens*** Boiss. & Heldr.: steppe, locality 177; 07.06.2003, Mü 8217.

***Astragalus campylosema** Boiss. subsp. **nigripilis** Hub.-Mor. & Chamb.: steppe, locality 3; 10.08.1996, Nd 5061a, IUCN: lc.

***Astragalus cinereus** Willd.: steppe, locality 21; 13.06.1997,Mk 11923-Nd 5353; meadows, locality 29; 19.07.1997, Nd 6093 -Mk 12655; rocky slopes, locality 34; 22.06.1997, Mk 12107b, 12135-Nd 5538 b, 5563; steppe, locality 38; 27.06.1997, Mk 12222-Nd 5650; meadows, locality 47; 13.07.1997, Nd 6143 -Mk 12706; rocky crevices, locality 58; 28.05.2007, Mk 12065- Nd 5492, IUCN: lc.

***Astragalus davisii** Chamb. & Matthews: locality 1; 04.06.1996, Nd 4991; rocky slopes, locality 18; 08.06.1997, Mk 11821-Nd 5252; road slopes, locality 50; 15.07.1997, Nd 5930 -Mk 12494, IUCN: cd. **Variations**:Legume c. 20 x 9 mm white tomentose to felted hairy (unknown).

***Astragalus chaldiranicus** Kit Tan & Sorger: meadows, locality 46; 13.07.1997, Nd 5839, 5860 -Mk 12400, IUCN: vu.

****Astragalus comosoides** Chamb. & Matthews: meadows, locality 8; 24.05.1997, Nd 5155, 7065, IUCN: lc.

***Astragalus bahcesarayensis*** Akan, Fırat & Ekici: stony places, locality 62; 05.08.2004, Mf 4221.

***Cicer pinnatifidum*** Jaub. &Spach: rocky slopes , locality 110; 04.05.2003, Aa 2956.

***Vicia alpestris*** Stev. subsp. ***hypoleuca*** (Boiss) P.H. Davis: steppe , locality 111; 12.07.2007, Ok 6852. , IUCN: lc.

***Lathyrus brachypterus** Cel. var. **haussknechtii** (Sirj) D P.H. Davis: meadows, locality 19; 12.06. 1997, Mk 11855- Nd 5285, IUCN: lc.

***Lathyrus nivalis*** Hand.-Mazz.: rocky slopes, locality 82; 21.06.2000, Mf 2590, IUCN: lc.

***Trifolium longidentatum*** Nábělek: meadow, rocky, locality 201; 18.08.2002, M 7952b,

***Hedysarum vanense*** Hedge & Hub.-Mor.: steppe, locality 204; 10.06.2007, İd 443, IUCN: vu.

***Hedysarum cappadocicum** Boiss.: steppe, locality 38; 27.06.1997, Mk 12227-Nd 5655, IUCN: lc.

***Hedysarum erythroleucum** Boiss.: rocky slopes, locality 53; 16.07.1997, Nd 6026- Mk12562, IUCN: lc.

***Onobrychis stenostachya*** Freyn subsp. ***sosnowskyi*** (Grossh.) Hedge: steppe, locality 205; 26.07.1997, Fö 5386, IUCN: vu.

***Onobrychis fallax*** Freyn & Sint.: steppe meadows, locality 4; 22.07.2001, Ma.1217, IUCN: lc.

***Onobrychis huetiana*** Boiss.: meadow, locality 208; 31.05.2008, Da 1222, IUCN: nt.

**XI. FAM: ROSACEAE**

***Prunus kurdica*** Fenzl ex Fritsch: roadside, locality 206; 14.07.2001, Tç 747, IUCN: en.

***Potentilla anatolica** Peşmen: meadows, locality 29; 19.07.1997, Nd 6057- Mk 12620; meadows, locality 43; 12.07. 1997, Nd 5781- Mk 12342; rocky slopes rocky slopes, locality 54; 27.07.1997, 27.07.1997, Nd 6231 -Mk 12795, IUCN: lc.

***Potentilla armeniaca*** Siegfr. ex Th.Wolf steppe, locality 209; 05.08.2007, Ok 7533, IUCN: vu.

***Rosa pisiformis** (Christ.) D. Sosn.: around road, locality 55; 19.07.1997, Nd 7056, IUCN: nt.

**XII. FAM: LYTHRACEAE**

***Lythrum anatolicum*** Leblebici & Seçmen subsp. ***vanense*** Pınar: wet and sandy places, locality 63; 25.8.2002, Mp 1229

**XIII. FAM: CRASSULACEAE**

***Rosularia davisii*** Muirhead: rocky place, locality 112; 03.07.2003, Mü 8404a, IUCN: cd.

***Sempervivum armenum*** Boiss&Huet var. ***armenum***: steppe rocky place, locality 114; 15.07.2002, Ok 3561, IUCN: lc.

**XIV. FAM: GROSSULARIACEAE**

***Ribes anatolica*** Behçet: moving stone area, locality 64; 01.08.2013, B 318.

**XV. FAM: APIACEAE**

***Chaerophyllum hakkiaricum*** Hedge&Lamand.: steppe, locality 115; 18.07.2001, Mü 6110, IUCN: vu.

***Prangos uechtritzii** Boiss. & Hausskn.: meadows, locality 29; 19.07.1997, Nd 6102 -Mk12664, IUCN: lc.

***Cymbocarpum wiedemanii*** Boiss.: arable field place, locality 116; 21.06.2008, İd 1369, IUCN: cd.

***Diplotaenia turcica*** Pimenov & Kljuykov: moving stone area, locality 65; 01.08.2013, Mf 30469, IUCN: nt.

***Laserpitium carduchorum*** Hedge & Lemond.: arable field place, locality 116; 21.06.2008, Mp 2537.

***Malabaila lasiocarpa*** Boiss.: steppe, locality 150; 29.06.2006,Ok4791, IUCN: lc.

***Heracleum crenatifolium*** Boiss.: the slopes of the valley, locality 118; 29.06.2006, Ok 4884, IUCN: nt.

***Trigonosciadium tuberosum*** Boiss.: steppe, locality 119; 21.06.2007, Ok 6713, IUCN: dd.

***Trigonosciadium intermedium*** Freyn&Sint.: oak clearance slope, locality 120; 09.06.2012, He 1020, IUCN: en.

**XVI. FAM: VALERIANACEAE**

***Valerianella glomerata*** Boiss & Bal.: locality 121; 28.06.1997, Fö 4280, IUCN: lc.

**XVII. FAM: DIPSACACEAE**

***Cephalaria anatolica*** Shkhiyan: steppe, locality 151; 18.07.2008, Da 1314, IUCN: cr.

***Cephalaria sparsipilosa*** Matthews: meadows, locality 122; 30.07.2007, Ok 7384, IUCN: cd.

***Scabiosa rufescens*** Freyn & Sint.: meadows, locality 152; 25.06.2002, Mü 7274, IUCN: cd.

**XVIII. FAM: ASTERACEAE**

***Inula helenium L. subsp. orgyalis*** (Boiss.) Grierson: meadow, locality 123; 25.08.2007, Ok7627, IUCN: nt.

***Pulicaria armena*** Boiss. & Kotschy: the edge of Zilan Creek, locality 153; 07.08.2006, Ok5179, IUCN: lc.

***Helichrysum compactum*** Boiss.: steppe, locality 172; 07.08.1996, Fö 2575, IUCN: en.

***Helichrysum arenarium*** (L.) Moench subsp. ***aucheri*** (Boiss.) P.H. Davis & Kupicha: steppe, locality 173; 07.06.2008,İ 1185, IUCN: lc.

***Helichrysum arenarium*** (L.) Moench subsp***. erzincanicum*** P.H. Davis & Kupicha: steppe, locality 174; 16.08.2007 Ok 7580, IUCN: vu.

****Anthemis wiedemanniana*** Fish. & Mey.: meadows, locality 5; 03.06.1997, Nd 7076, IUCN: lc.

***Anacyclus anatolicus*** Behçet & Almanar: steppe, locality 175; 09.05.2004, Tç 92,

***Achillea cappadocica*** Hausskn. & Bornm.:alpine steppe, locality 178; 20.07.1988, B1317, IUCN: lc.

****Tanacetum zahlbruckneri*** (Nab.) Grierson: rocky slopes, locality 34; 22.06.1997, Mk 12121,12166-Nd 5549,5494; road slopes, locality 50; 15.07.1997, Nd 5926 -Mk 12490, IUCN: lc.

***Tanacetum heterotomum*** (Bornm.) Grierson: steppe, locality 182; 25.05.1996, Fö 393, IUCN: vu.

***Tanacetum nitens*** (Boiss. & Noë) Grierson: steppe-rocky areas, locality 183; 08.07.2001, Ok 2144, IUCN: lc.

***Tanacetum cadmeum***(Boiss.) Heywood subsp***. orientale*** Grierson: steppe fields, locality 1194; 23.07.2001, Mü 5968, IUCN: lc.

****Tripleurospermum callosum*** (Boiss. & Heldr.) E. Hossain: road slopes, locality 50; 15.07.1997, Nd 5925-12489, IUCN: lc.

****Tripleurospermum monticolum*** (Boiss. & Huet) Bornm.: meadows, locality 46; 13.07.1997, Nd 5831 -Mk 12392, IUCN: lc.

****Cousinia bicolor*** Freyn & Sint.: rocky slopes, locality 26; 19.06.1997, Mk 12016, Nd 5444, IUCN: lc.

****Cousinia eriocephala*** Boiss. & Hausskn.: road slopes, locality 50; 15.07.1997, Mk 100798, IUCN: lc.

***Cousinia nabelekii*** Bornm.: steppe, locality 6; 22.07.2001, Ma 1605, IUCN: nt.

***Cousinia hakkarica*** Hub.-Mor.: steppe, locality 179; 31.07.2002, Mü 7809, IUCN: vu.

****Cousinia vanensis*** Hub.-Mor.: meadows, locality 8; 24.05.1997, Nd 7009, IUCN: lc.

***Cirsium peshmenianum*** Yıldız, Dirmenci & Arabacı: conglomerate locality 68; 18.08. 2008, BY 16958

****Carduus lanuginosus*** Willd.: rocky slopes meadows, locality 47; 13.07.1997, Nd 6215, IUCN: lc. **Variations**: Capitula to 4x4 cm (2-3x2-3 cm), spines 1-7 mm (2-10 mm).

***Gundelia dersim*** Vitek, Yüce & Ergin: steppe areas, locality 66; 10.5.2017, Mf 33729

***Gundelia colemerikensis*** Fırat: dry steppe fields, locality 67; 10.05.2017, Mf 33744

***Jurinea cataonica*** Boiss.& Hausskn.: steppe, locality 180; 12.07.2007, Ok6790, IUCN: lc.

****Centaurea saligna*** (C.Koch) Wagenitz: creek edge steppe, locality 51; 16.07.1997, Mk 12884, IUCN: lc.

***Centaurea sessilis*** J.F.Gmel.;steppe, May 1986, Hö 1745, IUCN: lc.

***Centaurea armena*** Boiss.: steppe, locality 185; 12.07.2007, Ok6912, IUCN: lc.

***Centaurea demirizii*** Wagenitz: steppe, locality 186; 30.06.1998, Mü3468, IUCN: vu.

***Centaurea fenzlii*** Reichardt: steppe, locality 187; 29.07.2007, Ok7289, IUCN: lc.

***Centaurea lydia*** Boiss.: locality 193; 09.06.1996 Fö964, IUCN: cd.

***Echinops pungens*** Trautvt. var. ***adenoclados*** Hedge: steppe, locality 195; 01.08.2001, M6411 195, IUCN: nt.

***Echinops orientalis*** Trautv.: steppe, locality 202; 19.10.1997, M2240,.

***Scorzonera semicana*** DC.: meadows, locality 203; 26.05.2007 Ok5940, IUCN: lc.

****Scorzonera eriophora*** DC.: creek edge steppe, locality 51; 16.07.1997, Mk 12881, IUCN: lc.

***Tragopogon aureus*** Boiss.: steppe, locality 207; 11.06.2001, Ok1639, IUCN: lc.

***Tragopogon vanensis*** Gültepe, Coşkunç. & Makbul: locality 69; 08.04.2010, Mg 267.

***Taraxacum davisii*** Van Soest.: meadow area, locality 210; 5.05.2002, Ok2890, IUCN: en.

****Crepis dioritica*** Schott. & Ky. ex Boiss.: rocky slopes, locality 53; 16.07.1997, Nd 5949 -Mk 12514, IUCN: lc.

****Crepis macropus*** Boiss. & Heldr.: meadows, locality 29; Nd 6110 -Mk 12672; rocky slopes, locality 54; 27.07.1997, Nd 6222- Mk IUCN: lc.

****Crepis armena*** DC. : rocky slopes, locality 34; 22.06.1997, Mk 12110-Nd 5539, rocky slopes, locality 53; 16.07.1997, Nd 6034- Mk 12597, IUCN: lc.

***Crepis gemicii*** Yıldırım , Bingöl & Armağan: meadows and openings in *Quercus petraea subsp. pinnatiloba*, a.s.l. locality 70 ; 17.07.2002, Öb 12199.

***Psephellus vanensis*** A. Duran, Behçet & B. Doğan: steppe fields, a.s.l, locality 71; 17.06.2009, B&A 1603

**XIX. FAM: CAMPANULACEAE**

****Campanula coriacea*** P.H. Davis: basalt rocky slopes, locality 45; 13.07.1997, Mk 12415-Nd 5852, IUCN: lc.

***Campanula hedgei*** P.H.Davis: steppe, locality 154; 09.08.1998, Fö 5480, IUCN: cd.

****Campanula bornmuelleri*** Nab.: steppe, locality 25; 18.06.1997, Mk11955-Nd5385; rocky slopes, locality 48; 15.07.1997, Mk11768; rocky slopes, locality 53; 16.07.1997, Nd5948, 6016 -Mk 12512, 12579; rocky slopes, locality 54; 27.07.1997, Nd 6240- Mk 12804, IUCN: cd. **Variations**: Some samples; Leaves glabrous both sides (leaves hirsute both sides) and stem usually 2-4 flowered (usually one flowered).

****Campanula saxonorum*** Gandoger: steppe slopes, locality 14; 05.06.1997, Mk 11772, IUCN: lc. **Variations**: Stems, 5-20 cm [(10) 15-25(30) cm].

***Asyneuma limonifolium*** (L.) Janch. subsp. ***pestalozzae*** (Boiss.) Damboldt: steppe, locality 124; 07.07.1997, Fö 5172, IUCN: lc.

***Asyneuma trichostegium*** (Boiss.) Bornm.: meadows, locality 155; 14.08.1997, Fö 1617, IUCN: dd.

***Asyneuma linifolium*** (Boiss. & Heldr.) Bornm. subsp. ***eximium*** (Rech.f.) Damboldt: steppe, locality 156; 17.07.1997, Mü 1672.

**XX. FAM: CONVOLVULACEAE**

****Convolvulus galaticus*** **Rostan** ex Choisy: valley slopes, locality 49; 15.07.1997,Nd 5861- Mk12424, IUCN: lc.

**XXI. FAM: BORAGINACEAE**

***Cynoglossum vanense*** Sutorý : locality 72; 22.07. 1998 Mü 4533

***Rochelia disperma*** (L.f.) K.Koch var. ***microcalycina*** (Bornm.) Edmondson: steppe, locality 168; 12.07.2007, Ok 6822, IUCN: lc.

***Myosotis platyphylla*** Boiss: meadows, locality 181; 25.08.2007, Ok 7662, IUCN: vu.

****Rindera caespitosa*** (A.DC.) Bunge: plateau, locality 22; 02.04.1999, Mf 1045, IUCN: lc.

***Onosma neglecta*** Riedl: meadow area, locality 188; 20.07.2005, Tç 825, IUCN: cd.

****Onosma polioxantha*** Rech. f.: rocky slopes, locality 26; 19.06.1997, Nd 5438, 6337a, IUCN: lc.

***Onosma argentata*** Hub- Mor.: steppe, locality 189; 28.06.1996, Fö 2542, IUCN: vu.

***Onosma velutina*** Boiss.: steppe, locality 190; 10.06.1987, B 710, IUCN: cd.

***Onosma procera*** Boiss: steppe, locality 191; 26.06.1997. Fö 3633, IUCN: nt.

****Onosma isauricum*** Boiss. & Heldr.: Çatak, valley slopes, locality 49; 15.07.1997,Nd 5864 -Mk 12427, IUCN: lc.

***Onosma bracteosa*** Hausskn. & Bornm.: wet meadows, locality 13; 10.06.2001, Ma1309.IUCN: lc.

****Onosma mutabile*** Boiss.: meadows, locality 47; 13.07.1997, Mk 11924- Nd 5354, IUCN: lc. **Variations**: Stems up to 8 (1-4) some basal leaves not widest (basal leaves widest).

***Onosma armena***DC.: steppe, locality 192: 19.07.1986, Hö 878, IUCN: lc.

***Onosma proballan*****thera** Rech. fil.: rocky slopes, locality 20; 23.09.200, Ma. 2129, IUCN: cd.

***Anchusa leptophylla*** Roemer & Schultz subsp. ***incana*** (Ledeb.) D. F. Chamb.: creek edge, locality 196; 17.06.2006, Ok 5440, IUCN: cd.

***Nonea karsensis*** M.Popov:: wheat field, locality 197; 02.08.1996, F 616, IUCN: dd.

***Nonea stenosolen*** Boiss. & Balansa: steppe, locality 198; 10.05.1998, M 2362, IUCN: lc.

***Alkanna tubulosa*** Boiss.: alpine meadow, locality 199; 10.05.1998, B 687, IUCN: lc.

***Alkanna froedinii*** Rech.fil.: border plateau, locality 200; 18.07.1997, Fö5870, IUCN: lc.

**XXII. FAM: SOLANACEAE**

****Lycium anatolicum*** A. Baytop & R. Mill: steppe slopes, locality 23; 18.06.1997, Mk, 11962-Nd 5392, IUCN: lc.

**XXIII. FAM: SCROPHULrIACEAE**

***Verbascum kurdicum*** Hub.-Mor.: steppe, locality 125; 25.05.2002, Aa 2342, IUCN: lc.

***Verbascum oreophilum*** C. Koch var***.*** ***oreophilum***: meadow, locality 157; 12.06.2005, Tç 589, IUCN: lc.

***Verbascum vanense*** Hub.-Mor.: steppe, locality 126; 21.06.2001, Ok1787, IUCN: cd.

***Verbascum golawanense*** Fırat: fallow fields, edge of fields, locality 73; 01.07.2011, Mf 27749.

***Rhynchocorys kurdica*** Nábělek: plateau, moist area, locality 127; 26.06.2005, B718.

****Scrophularia pulverulenta*** Boiss. & Nöe: Çatak, slopes, locality 49; 15.07.1997, Nd 5876 -Mk 12439, IUCN: lc.

****Scrophularia libanotica*** Boiss. subsp. ***libanotica*** var. ***urartuensis*** R. Mill: rocky slopes, locality 2; 03.08.1996

Nd 5001; rocky slopes, locality 18; 08.06.1997, Mk 11838-Nd 5268; steppe, locality 21; 13.06.1997, Mk 11925-Nd 5355; wet meadows, locality 24; 23.07.1997, Mk 11960-Nd 5390; rocky slopes, locality 26; 19.06.1997, Mk 1190-Nd 5418; valley slopes, locality 49; 15.07.1997, Nd 5876 -Mk12439, Nd 5962,5994- Mk 12525 , 12557, IUCN: lc.

***Scrophularia versicolor*** Boiss.: steppe, locality 128; 10.08.1996, Fö 2630 , IUCN: vu.

***Chaenorhinum minus*** (L.) Lange subsp. ***anatolicum*** P.H.Davis: sandy area, locality 129; 26.07.2007, İd 819, IUCN: lc.

***Linaria genistifolia*** (L.) Miller subsp. ***confertiflora*** (Boiss.) P.H.Davis: steppe, locality 158; 29.06.1996, Fö 2684, IUCN: lc.

***Linaria corifolia*** Desf.: steppe, locality 159; 05.10.1997, Fö 1440 , IUCN: lc.

***Veronica fridericae*** M.A. Fischer: meadows, locality 32; 21.07.2002, Ma 2874,IUCN: cd.

***Bungea trifida*** (Vahl) C.A.Meyer: steppe, locality 130; 07.06.2002, Ma 2452.

**XXIV. FAM: LAMIACEAE**

***Ajuga bombycina*** Boiss.: steppe, locality 131; 15.05.1996, Fö 1164, IUCN: nt.

****Scutellaria orientalis*** L. subsp**. *santaloides* (**Hausskn. ex Bornm.) Edmonson: locality 33; 03.07.1997, Nd 5729, IUCN: lc.

****Phlomis armeniaca*** Willd.: Özalp road 20. Km, slopes, locality 36; Mk 12201- Nd 5629; meadows, locality 47; 13.07.1997, Nd 6135- Mk12967, IUCN: lc.

****Marrubium parviflorum*** Fisch. & C. A. Mey. subsp. ***oligodon*** (Boiss.) Seybold: locality 55; Nd 6050- Mk 12613; steppe, locality 57; 19.07.1997, Nd 6211- Mk 12775, IUCN: lc.

***Marrubium vanense*** Hub.-Mor.: steppe, locality 132; 29.07.2006, Mf627, IUCN: en.

***Marrubium vulcanicum*** Hub.-Mor.: steppe, locality 133; 23.07.2003, Mü 8826, IUCN: vu.

***Stachys ramosissima*** Montbret & Aucher ex Benth.var. ***ramosissima***: steppe, locality 160; 29.06.1997, Fö 4444 , IUCN: cd.

***Lophanthus turcicus*** Dirmenci, Yıldız & Hedge: rocky and stony north slope, locality 74; 24.07.2009, Td 3707

****Micromeria cristata*** (Hampe) Griseb. subsp**. *orientalis*** P.H. Davis: creek edge steppe, locality 51; 16.07.1997, Nd 5933 -Mk 12497, IUCN: lc.

***Micromeria cremnophila*** Boiss. & Heldr. subsp**. *anatolica*** P.H.Davis: rocky cracks, locality 167; 15.07.2003, Mü8595, IUCN: lc.

****Cyclotrchium glabrescens*** (Boiss. & Kotschy ex Rech. fil.) Leblebici: creek edge steppe, locality 51; 16.07.1997, Mk 12883, Nd 5936 -Mk 12500, IUCN: cd.

****Thymus brachychilus*** Jalas: rocky slopes, locality 53; 16.07.1997, Nd 5979 -Mk 12542, IUCN: lc.

****Salvia kronenburgii*** Rech. fil.: steppe slopes, locality 27; 21.06.1997, Mk11201-Nd 5449, IUCN: vu.

***Salvia longipedicellata*** Hedge.: steppe, locality 139; 22.05.2002, Mü 7015, IUCN: nt.

***Salvia dichroantha*** Stapf.: steppe, locality 140; 09.06.1997, Fö1899, IUCN: lc.

**XXV. FAM: PLUMBAGINACEAE**

****Limonium vanense*** Kit-Tan & Sorger : meadows, locality 46; 13.07.1997, Nd 5848 -Mk 12411, IUCN: vu.

***Limoniopsis davisii*** Bokhari: rocky and stone steppe, locality 161; 03.07.2003, Mü8413, IUCN: en.

***Acantholimon venustum*** Boiss. var. ***assyriacum*** ( Boiss.) Boiss.: steppe, locality 162; 16.07.2006, Ok5007, IUCN: nt.

***Acantholimon acerosum*** (Willd.) Boiss. var. ***brachystachyum*** Boiss.: steppe, locality 170; 14.07.2001, Mü5224a, IUCN: vu.

***Acantholimon calvertii*** Boiss.: rocky steppe, locality 171; 08.07.2001, Ok2075, IUCN: lc.

****Acantholimon reflexifolium*** Bokhari :rocky slopes, locality 54; 27.07.1997, Nd 6235 -Mk 12799, IUCN: nt.

***Acantholimon bashkaleicum*** Doğan & Akaydın: steppe, locality 75; 06.07.2002, D&A 7544.

***Acantholimon artosense*** Doğan & Akaydın: steppe, locality 76; 05.07.2002, D&A 7532.

***Acantholimon hoshapicum*** Doğan & Akaydın:steppe, locality 77; D&A 7540.

**XXVI. FAM: PLANTAGINACEAE**

****Plantago anatolica*** Tutel & R. Mill.: meadows, locality 19; 12.06.1997, Mk 11850- Nd 5280, IUCN: cd.

**XXVII. FAM: EUPHORBIACEAE**

****Euphorbia grisophylla*** M. L. S. Khan: plateau, locality 22; 02.04.1999, Mf 2062, IUCN: lc.

***Euphorbia falcata*** L. subsp. ***macrostegia*** (Bornm.) O.Schwartz: steppe, locality 113; 08.09.2007, İ 848, IUCN: lc.

**XXVIII. FAM: FAGACEAE**

****Quercus petraea*** (Mattuschka) Liebl. subsp. ***pinnatiloba*** (C. Koch) Menitsky***:*** steppe, locality 42;15.06.1999,Mf 1776, 53;Mf1542, IUCN: lc.

**XXIX. FAM: RUBIACEAE**

***Galium margaceum*** Ehrend. & Schönb.-Tem. : rocky slope, locality 134; 20.06.1992, Fö 1404, IUCN: lc.

**XXX. FAM: LILIACEAE**

****Allium microspatum*** Ekberg: rocky slopes, locality 54; 27.07.1997, Mk:12785-Nd 6221, IUCN: cd.

***Allium tauricola*** Boiss.: rocky slopes, locality 166; 04.08.2001, Mü 6533, IUCN: lc.

****Allium stearnianum*** Koyuncu, Özhatay &Kollmann subsp. **vanense** Kollmann &Koyuncu : wet meadows, locality 24; 23.07.1997,Mk 12861-Nd 6298; steppe, locality 38; 27.06.1997, Mk 12280-Nd 5708; : meadows, locality 47; 13.07.1997, Mk 12685-Nd 6123; road slopes, steppe, locality 52; 16.07.1997, Mk 12508-Nd 5944; rocky slopes, locality 53; 16.07.1997, Mk 12545-Nd 5982; steppe, locality 57; 19.07.1997, Mk 12772-Nd 6208; steppe, locality 41; 19.07.1997, Mk 12769-Nd6205, IUCN: nt.

****Allium shatakiense*** Rech. fil.: wet meadows, locality 35; 22.06.1997, Mk 12132-Nd 5560, IUCN: nt.

***Allium armenum*** Boiss. & Kotschy: rocky steppe, locality 141; 02.09.2007, Ok 7833, IUCN: lc.

***Allium hoshabicum*** Fırat: Holotype, steppe and meadow, elevation, locality 78; 21.07.2012, Mf 28979.

***Puschkinia bilgineri*** Yıldırım: alpine meadows, locality 79; 19.06.2014, Hy 2695.

****Bellevalia rixi*** Wendelbo: meadows, locality 9; 24.05.1997 Mk 11704-Nd5170; steppe, locality 21; 13.06.1997, Mk 11894-Nd5324, locality 37; Mk 12069-Nd 5498: steppe, locality 38; 27.06.1997, Mk 12279-Nd 5707, IUCN: en.

****Fritillaria michailovskyi*** Fomin: steppe slopes, locality 16; 07.06.1997, Mk 11798-Nd 5229, IUCN: cd.

****Fritillaria minima*** Rix: rocky slopes, locality 12; 26.05.1997, Mk 11736-Nd 5200, steppe slopes, locality 16; 07.06.1997, Mk 11798-Nd 5229, rocky slopes, locality 53; 16.07.1997, Mk 12520- Nd 5957**,** IUCN: vu.

***Tulipa koyuncui*** Eker & Babaç: steppe, locality 80; 13.05.2010, E 1565.

***Gagea vanensis*** Tekşen & Karaman: rocky slopes, locality 81; 21.06.2000, Mt 2460

### XXXI. FAM: IRIDACEAE

****Iris sari*** Schott ex Baker: road slopes, locality 40; 16.05.1999, Mf 1292, IUCN: lc.

### XXXII. FAM: ORCIDACEAE

***Dactylorhiza osmanica*** (Klinge) P.F.Hunt & Summerh. subsp. ***osmaniaca***: the edge of Zilan creek, locality 163; 29.05.2006, Ok 4386. IUCN: lc.

### XXXIII. FAM: CYPERACEAE

***Carex distans*** L.: meadows, locality 136; 07.06.2003, Mü 8309a

### XXXIV. FAM: POACEAE

***Bromus macrocladus*** Boiss.: steppe, locality 137; 17.06.2007, Ok 631, IUCN: en.

***Festuca anatolica*** Markgr.-Dann. subsp. ***borealis*** Markgr.-Dannenb.: steppe, locality 164; 08.06.1991, B1696, IUCN: cd.

***Festuca anatolica*** Markgr.-Dann. subsp***. anatolica***: steppe, locality 165; 30.06.1998, Mü 3749, IUCN: lc.

***Eremopoa mardinensis*** R.Mill: steppe, locality 138; 30.06.1990, B 3204, IUCN: en.

## 4. Discussions

With this study, 259 endemic taxa belonging to 34 families and 117 genera were compiled in the Lake Van Basin. Twenty three of them, within 20 genera, are unique to the Lake Van Basin.

The distribution of 117 genera and 259 taxa within 34 families are as follows: *Asteraceae*- 20 genera 41 taxa; *Fabaceae*- 7 genera 40 taxa; *Brassicaceae*- 9 genera 29 taxa; *Boraginaceae*- 8 genera 20 taxa; *Caryophyllaceae*- 5 genera, 20 taxa; *Lamiaceae*- 10 genera 15 taxa; *Scrophulriaceae*- 7 genera 13 taxa; *Liliaceae*- 6 genara 12 taxa; *Apiaceae*- 8 genera 9 taxa; *Plumbaginaceae*- 3 genera, 9 taxa; *Ranunculaceae;* 2 genera 9 taxa; *Campanulaceae*- 2 genera 7 taxa; *Poaceae*- 3 genera 4 taxa; *Rosaceae*- 3 genera 4 taxa; *Dipsacaceae*- 2 genera 3 taxa; *Geraniaceae*- 2 genera 3 taxa; *Crassulaceae*- 2 genera 2 taxa; *Papaveraceae*- 2 genera 2 taxa; *Euphorbiaceae*- 1genus 2 taxa; *Linaceae*- 1genus 2 taxa; *Convolvulaceae*- 1 genus 1 taxon; *Cyperaceae*- 1 genus 1 taxon; *Fagaceae*- 1 genus 1 taxon; *Grossulariaceae*- 1 genus 1 taxon; *Hypericaceae*- 1 genus 1 taxon; *Iridaceae*-1 genus 1 taxon; *Lythraceae*-1 genus 1 taxon; *Orcidaceae*- 1 genus 1 taxon; *Plantaginaceae*- 1 genus 1 taxon; *Polygonaceae*- 1 genus 1 taxon; *Rubiaceae*- 1 genus 1 taxon; *Rutaceae*- 1 genus 1 taxon; *Solanaceae*- 1 genus 1 taxon; *Valerianaceae*- 1 genus 1 taxon.

Most of the endemic taxa have been located around Güzeldere pass (Çuh pass), Artos, Kavuşşahap and Ispiriz mountains respectively (Davis et al., 1988; Ekim, 2005; Özhatay et al., 2009).

Among the endemic taxa, *Acantholimon artosense, Acantholimon bashkaleicum, Acantholimon hoshapicum, Astragalus bahcesarayensis, Cirsium peshmenianum, Crepis gemicii, Dianthus vanensis, Draba orientalis, Fritillaria minima, Gagea vanensis, Limoniopsis davisii, Limonium vanense, Lophanthus turcicus, Lythrum anatolicum* subsp. *vanense, Psephellus vanensis, Puschkinia bilgineri, Ranunculus vanensis, Ribes anatolica, Silene acaulis* subsp. *vanensis, Silene miksensis, Tragopogon vanensis, Tulipa koyuncui* and *Verbascum golawanense* are unique to Lake Van basin.

IUCN Red Data Categories (if known) of 259 taxa in our study are as follows (Ekim et al. 2000): IUCN: Lr (lc) 118, IUCN: Lr (cd) 33, IUCN: Lr (nt) 28, IUCN: vu 20, IUCN: en 16, IUCN: dd 4, IUCN: cr 1. IUCN Red Data categories of 40 taxa are unknown.

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# *Conocybe anthracophila*, A new record for the Turkish mycobiota

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# *Conocybe anthracophila*, Türkiye mikobiyotası için bir yeni kayıt

**Abstract:** A new bolbitoid species, *Conocybe anthracophila* Maire & Kühner ex Kühner & Watling belonging to the family *Bolbitiaceae*, is given as new record for the mycobiota of Turkey from Sarıkamış Allahukeber Mountains National Park (Kars/Erzurum). A brief description of the taxon is given together with its photographs related to macro and micromorphologies.

**Key words:** *Conocybe anthracophila*, *Bolbitiaceae*, new record, Sarıkamış Allahukeber Mountains National Park, Turkey.

**Özet:** *Bolbitiaceae* familyasına ait bir bolbitoid tür olan *Conocybe anthracophila* Maire & Kühner ex Kühner & Watling Sarıkamış Allahuekber Dağları Milli Parkı'ndan (Kars/Erzurum) Türkiye mikobiyotası için yeni kayıt olarak verilmiştir. Taksonun kısa betimlemesi makro ve mikromorfolojisine ait fotoğraflarla birlikte verilmiştir.

**Anahtar Kelimeler:** *Conocybe anthracophila*, *Bolbitiaceae*, yeni kayıt, Sarıkamış Allahuekber Dağları Milli Parkı, Türkiye.

## 1. Introduction

*Conocybe* Fayod is a bolbitoid genus within the family *Bolbitiaceae* Singer (Agaricales, Agaricomycetes, Basidiomycota) and can be differentiated from the other genera of the family by lecythiform cheilocystidia with a round capitellum and pileal margin not plicate-sulcate. It is characterized by its fragile basidiocarps which are small to medium sized with conical-thimble shaped cap usually of rust-brown, yellow-brown, rarely grey or flesh-reddish colours. Spores rust-brown, smooth, with distinct germination pore (Moser, 1983; Singer, 1986; Pegler (1977; 1983; 1986; Amandeep et al., 2015).

Though Kirk et al. (2008) recognized 200 species of *Conocybe* the world over, only 28 conformed members of the genus *Conocybe* have so far been recorded from Turkey (Sesli and Denchev, 2014; Solak et al., 2015).

During routine field studies in Sarıkamış Allahuekber Mountains National Park (Kars/Erzurum) some basidiomes were collected. *Conocybe anthracophila* Maire & Kühner ex Kühner & Watling, was described as a new record according to the current checklists on Turkish macromycota (Sesli and Denchev, 2014; Solak et al., 2015) and the latest contributions to the basidiomycetous macrofungi of Turkey (Demirel et al., 2016; Akata and Sesli, 2017; Akata and Uzun, 2017; Allı et al., 2017; Demirel et al., 2017; Işık and Türkekul, 2017; Kaşık et al., 2017; Kaya and Uzun, 2017; Keleş et al., 2017; Keleş and Şelem, 2017; Özkazanç et al., 2017; Öztürk et al., 2017; Sesli and Topcu Sesli, 2017; Türkekul, 2017; Türkekul and Işık 2017; Uzun et al., 2017a,b; Işık and Türkekul, 2018a,b; Sadullahoğlu and Demirel, 2018; Sesli and Liimatainen, 2018; Uzun et al., 2018a,b; Sesli, 2018; Uzun and Acar, 2018; Uzun and Kaya, 2018a,b).

The present study aims to make a contribution to the macrofungi of Turkey.

## 2. Materials and Method

Specimens were collected from Kızılçubuk village, Sarıkamış (Kars-Turkey) at Allahuekber Mountains National Park in 2014. Morphological and ecological chracteristics of the samples were recorded during the field study and they were photographed in their natural habitats. Then, they were taken to the laboratory and microscopic investigations were carried out on them.

Microscopic investigation of the samples were done by using a Leica DM500 light microscope mounted Leica ICC50 HD camera. Reagents such as 5 % KOH and Congo red were used. Identification was performed with the aid of the relevant literature (Moser, 1983; Hausknecht et al., 2005; Knudsen and Vesterholt, 2008).

## 3. Results

***Basidiomycota*** R.T. Moore
***Agaricales*** Underw.
***Bolbitiaceae*** Singer
***Conocybe*** Fayod
***Conocybe anthracophila*** Maire & Kühner ex Kühner & Watling

**Macroscopic features:** Pileus 15-40 mm in diameter, bell-shaped at first, later convex, longitudinally grooved when moist, ocher or reddish-yellow, darker in the margins and center, margins acute, slightly wavy and sometimes upwardly curved. Flesh, whitish or pale skin color, thin, taste and odour unclear. Lamellae, adnexed, ocher when young, later reddish-brown, wider in the middle, and gently toothed edges. Stipe, 35-75 x 1,5-3 mm in size, ocher or pale brownish, darker at the base, cylindrical, sometimes slightly bulbous at the base, fragile, smooth, longitudinally silvery-white fibrillose (Fig. 1a).

**Microscopic features:** Spores 10-12 × 6-7 µm, ellipsoid to egg-shaped, reddish-brown, thick walled, smooth, with distinct germination pore and small apiculus (Fig. 1b). Basidia 15-22 × 6-8 µm, cylindrical-clavate, with 2 or 4 sterigmata and a basal clamp (Fig. 1c). Cheilocystidia lecythiform, 15-25 × 7-12 µm, with 3-5 µm wied capitula (Fig. 16).

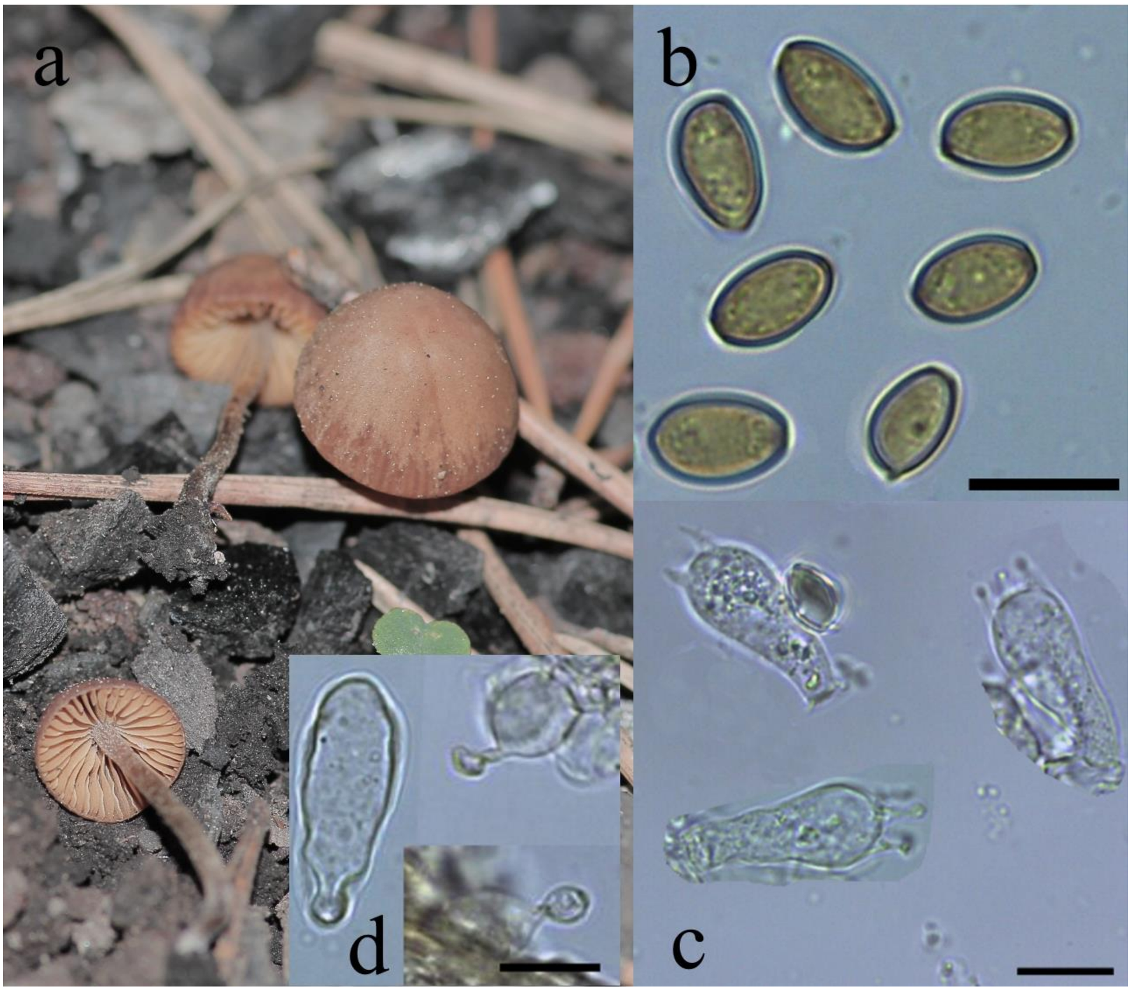


**Figure 1.** *Conocybe anthracophila*: a- basidiomata; b- basidiospores; c- basidia, d- cheliocystidia. (Bars= 10 µm)

**Specimen examined:** Kars, Sarıkamış, Allahuekber Mountains National Park, Kızılçubuk village, on burned coals, 40° 22'745"N, 42° 30'541"E, 2388 m, 13.06.2014. MEA. 826.

## 4. Discussions

*Conocybe anthracophila* is a member of the section *Pilosellae* and closely related to *C. velutipes* (Velen.) Hauskn. & Svrček and *C. siennophylla* (Berk. & Broome) Singer ex Chiari & Papetti. However, it has much larger fruiting bodies than the two taxa and differs from *C. velutipes* also by non lenticular broad-pressed spores. The spores are about as large as *C. sienophylla*, which is usually 9-12 × 5.5-7 microns, but have a different shape and are darker under the microscope with slightly thicker walls (Hausknecht et al., 2005).

The type of this species comes from North Africa, and all species that grew on burnt substrate were simply determined as *C. anthracophila* for a long time. Peintner et al. (1999) and Gminder (2003) reported the species on mineral-rich sites such as compost, fertilized meadows and garden beds. Also with the material from Finland no macro- and microscopic differences could be found between the find on fire site and that on fertilized soil. It is certainly an extremely rare species, but often fruits in large numbers of individuals under suitable conditions. (Hausknecht et al., 2005).

*Conocybe anthracophila* was added to Turkish mycobiota as a new member of the genus *Conocybe*. Macro and micromorphological properties of the newly recorded taxon agree with those described by Moser (1983) and Knudsen and Vesterholt (2008).

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# New locality records for two *Tuber* species in Turkey

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# İki *Tuber* türü için Türkiye'de yeni lokalite kayıtları

**Abstract:** New specimens of two previously reported *Tuber* taxa, *T. excavatum* and *T. puberulum,* were collected from Eastern Black Sea region, and determined for the second time from Turkey. New distribution localities and brief descriptions of the species were provided together with the photographs related to their macro and micromorphologies.

**Key words:** Biodiversity, hypogeous fungi, *Tuber,* Turkey

**Özet:** Daha önceden rapor edilmiş olan iki *Tuber* taksonu, *T. excavatum* ve *T. puberulum,* Doğu Karadeniz Bölgesinden toplanarak Türkiye'den ikinci kez belirlenmiştir. Türlerin yeni yayılış lokaliteleri ve kısa betimlemeleri, makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

**Anahtar Kelimeler:** Biyoçeşitlilik, toprakaltı mantarlar, *Tuber,* Türkiye

## 1. Introduction

*Tuber* P. Micheli ex F.H. Wigg. is a truffle genus within the family *Tuberaceae* Dumort.. Members of the genus form ectomycorrhizas with many kinds of trees within the genera *Abies* Mill., *Betula* L., *Corylus* L., *Cistus* L., *Fagus* L., *Larix* Mill., *Olea* L., *Picea* A. Dietr., *Pinus* L., *Populus* L., *Pseudotsuga* Carrière, *Quercus* L., *Taxus* L., *Tilia* L. (Hawker, 1954; Lange, 1956; Honrubia et al., 1992; Medardi, 2006; Bidaud and Van Vooren, 2008; Wang et al., 2013, Türkoğlu and Castellano, 2014). Though Kirk et al (2008) mentions about the existence of 86 members of the genus, Index fungorum (www.indexfungorum.org; accessed 22 September 2018) currently lists 138 conformed *Tuber* species.

Until the end of August 2018, nine *Tuber* species, *Tuber aestivum* (Wulfen) Spreng., *T. borchii* Vittad., *T. brumale* Vittad., *T. excavatum* Vittad., *T. ferrugineum* Vittad., *T. mesentericum* Vittad., *T. nitidum* Vittad., *T. puberulum* Berk. & Broome and *T. rufum* Pollini, have been reported from Turkey (Öztürk et al., 1997; Kaya, 2009; Castellano and Türkoğlu, 2012; Türkoğlu and Castellano, 2014; Türkoğlu et al., 2015; Elliot et al., 2016; Şen et al., 2016; Alkan et al., 2018). Here we present new localities for two of them, *T. excavatum* and *T. puberulum* Berk. & Broome.

The study aims to make a contribution to the mycobiota of Turkey by presenting new distributions of some truffles.

## 2. Materials and Method

*Tuber* samples were collected from Artvin and Trabzon provinces during routine field studies between 2014 and 2018 within the Eastern Black Sea Region of Turkey. Required characteristics of the samples were recorded and they were photographed in their natural habitat. The samples were dried in and air conditioned room and prepared as fungarium materials. Measuremental evaluations were performed in the fungarium. Micromorphological investigations were carried out under a Nikon eclipse Ci-S trinocular light microscope and the photographs related to micromorphology were taken by a DS-Fi2 digital camera aided by a Nikon DS-L3 displaying apparatus. The specimens were identified with the help of Vittadini (1831), Mattirolo (1903), Hawker (1954), Lange (1956), Honrubia et al. (1992), Pegler et al., (1993), Arroyo et al., (2005), Medardi (2006), Bidaud and Van Vooren (2008), Alvarado et al. (2012), Türkoğlu and Castellano (2014) and Elliot et al. (2016).

The specimens are deposited at Biology Department, Kamil Özdağ Science Faculty, Karamanoğlu Mehmetbey University.

## 3. Results

***Ascomycota*** Caval.-Sm

***Pezizomycetes*** O.E.Erikss. & Winka

***Pezizales*** J.Schröt.

***Tuberaceae*** Dumort.

***Tuber excavatum*** Vittad., Monogr. Tuberac. (Milano): 49 (1831) [**Syn:** *Rhizopogon excavatus* (Vittad.) Rabenh., *Tuber excavatum* Vittad. f. *excavatum, Tuber excavatum* f. *globispora* Vaček, *Tuber excavatum* Vittad. subsp. *excavatum, Tuber excavatum* subsp. *lapideum* (Mattir.) E.Fisch., *Tuber excavatum* subsp. *typicum* E.Fisch., *Tuber excavatum* var. *brevisporum* E. Fisch., *Tuber excavatum* Vittad. var. *excavatum, Tuber excavatum* var. *fulgens* G. Gross, *Tuber excavatum* var. *intermedium* G.Gross, *Tuber excavatum* var. *longisporum* E.Fisch., *Tuber excavatum* var. *sulphureum* G.Riousset & Riousset, *Tuber lapideum* Mattir.]

Ascomata 10-30(35) mm in diameter, hypogeous, subglobose to globose or slightly lobed to irregular, with a distinct cavity, smooth in appearance but minutely papillate to somewhat coarsely warted, pale yellowish brown to yellowish brown when young, reddish brown at maturity (Figure 1). Peridium 200-350 µm thick, yellowish in section. Gleba whitish to straw colored at first, greyish-brown, reddish-brown to purplish-black when mature, marbled with cream or pale yellowish, branching and radially arranged veins. Asci 90-100 × 65-80(90) µm, ellipsoid to subglobose, sessile or short-

**Figure 1.** Ascocarps of *Tuber excavatum*

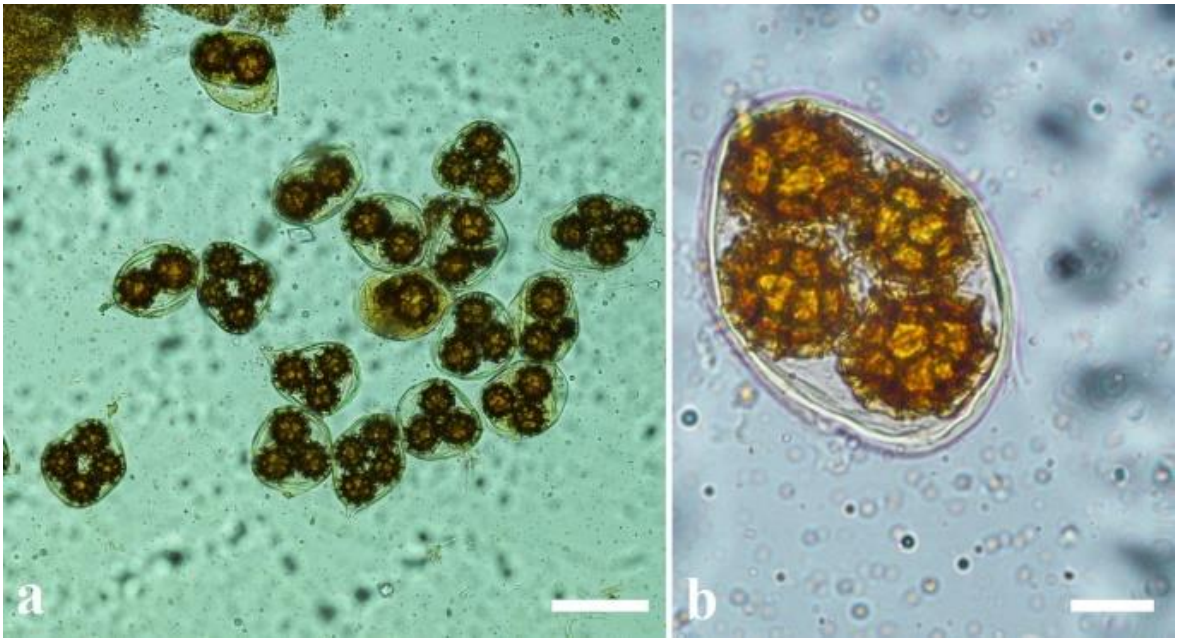


**Figure 2.** Asci (a,b) and ascospores (a,b) of *Tuber excavatum* (Bars a: 100 µm, b: 20 µm)

stalked, 1-4-spored (Figure 2a,b). Ascospores 30–40(45) × 25–35(40) µm, spore size varies depending on the number of spores in the ascus, ellipsoid, broadly ellipsoid or subglobose, yellowish-brown, ornamented with a coarse reticulum (Figure 2b).

*Tuber excavatum* grows under both coniferous and deciduous trees such as *Pinus, Fagus, Olea, Quercus* and *Taxus* species (Hawker, 1954; Pegler et al., 1993; Medardi, 2006; Türkoğlu and Castellano, 2014; Arroyo et al., 2005).

*Tuber excavatum* was reported previously from Turkey only once from one locality in Denizli province (Türkoğlu and Castellano, 2014).

**Specimen examined:** Trabzon, Maçka, Hamsiköy village, under death levaes of *Carpinus* and *Corylus* sp. under mixed stants of *Carpinus betulus* L., *Corylus* sp. and *Picea orientalis* L., 40°42′N -39°30′E, 970 m, 01.09.2018, Yuzun 6746.

***Tuber puberulum*** Berk. & Broome, Ann. Mag. nat. Hist., Ser. 1 18: 81 (1846) (1973) [**Syn:** *Tuber puberulum* var. *albidum* Bucholtz, *Tuber puberulum* var. *borchioides* G.Gross, *Tuber puberulum* var. *longisporum* Bucholtz, *Tuber puberulum* var. *michailowskjanum* Bucholtz, *Tuber puberulum* Berk. & Broome var. *puberulum*]

Ascomata 5-25 mm in diameter, hypogeous, subglobose, sometimes irregular or lobed with some slight grooves, surface smooth, initially whitish, becoming beige to greyish or yellowish-brown when mature (Figure 3). Peridium 100-200 µm thick, pseudoparenchymatous, composed of small, subglobose to subangular cells, covered with hairs in young specimens. Hairs 50-110 µm long, 4-6 µm across at the base, hyaline, some septate, tapered towards the apex (Figure 4a). Gleba firm, solid, whitish at first, then light brown, brown pink, marbled with numerous, white, branching veins. Taste and odour not distinctive. Asci 70–93 × 60–80 µm, ellipsoid, subglobose to globose to subspherical, sessile or short-stalked, 1-4-spored (Figure 4b). Ascospores 32-45- (50) × 27-35(40) µm, subglobose to spherical or broadly ellipsoid, light yellow, reddish brown at maturity, ornamented with a reticulo-alveolate, polygonal meshes (Figure 4c).

*Tuber puberulum* was reported to grow under different hardwoods and conifers such as *Fagus, Quercus, Tilia, Larix, Pinus, Pseudotsuga, Corylus, Picea,* (Lange, 1956; Honrubia et al., 1992; Bidaud and Van Vooren, 2008; Elliot et al., 2016).

*Tuber puberulum* was reported previously from Turkey only once by Elliot et al. (2016) from the localities within the boundaries of Aydın (Kuyucak), Denizli (Acıpayam, Buldan, Serinhisar), Muğla (Central district, Dalaman, Fethiye), Osmaniye (Central district) provinces.

**Figure 3.** Ascocarps of *Tuber puberulum*

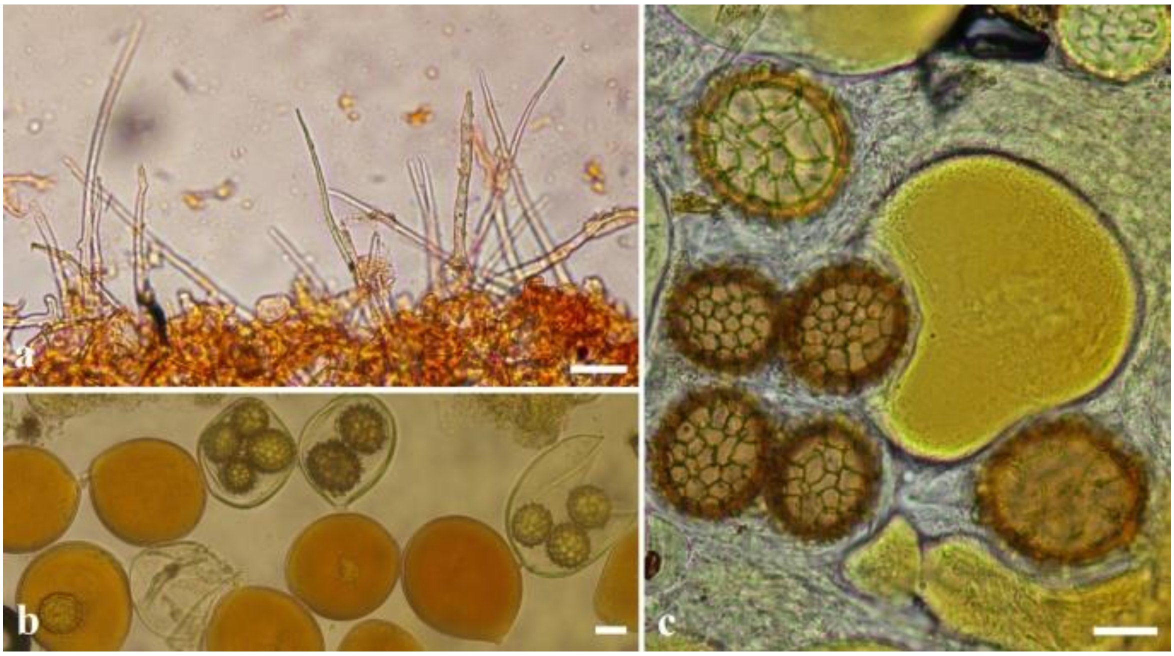


**Figure 4.** Peridial hairs (a), asci (b) and ascospores (b,c) of *Tuber puberulum* (Bars 20 µm)

**Specimen examined**: Artvin, Borçka, Kaynarca village, in soil under *Fagus orientalis* Lipsky., *Castanea sativa* Miller and *Rhododendron ponticum* L. mixed forest, 41°22′N-41°51′E, 1590 m, 09.11.2016, Yuzun 5425; Trabzon, Central district, Esenyurt village, in soil under *Castanea sativa, Fagus orientalis* and *Rhododendron ponticum* mixed forest, 40°54′N-39°45′E, 660 m, 02.09.2018, Yuzun 6755; Tonya, Erikbeli village, in soil under *Castanea sativa, Fagus orientalis, Alnus glutinosa* (L.) Gaertner and *Rhododendron ponticum* mixed forest, 40°45′N-39°14′E, 1680 m, 22.09.2015, Yuzun 4605; Yomra, Özdil village, in soil under *Castanea sativa, Fagus orientalis, Alnus glutinosa, Corylus* sp. and *Rhododendron ponticum* mixed forest, 40°50′N-39°48′E, 1210 m, 25.08.2018, Yuzun 6678.

## 4. Discussions

Both *T. excavatum* and *T. puberulum* have only been reported from Turkey once. The first Turkish record of *T. excavatum* was given by Türkoğlu and Castellano (2014) from Bozturt district of Denizli province with the samples collected under mixed *Quercus* spp. and *Pinus* spp. Our sample was collected under mixed stants of *Carpinus betulus, Corylus* sp. and *Picea orientalis.*

The presence of *T. puberulum* in Turkey was also noted by Elliot et al. (2016) based on the samples collected from Aydın, Denizli, Muğla and Osmaniye provinces, under different mixed stands of *Pinus brutia, Quercus cerris, Q. ilex, Q. coccifera, Q. ithaburensis, Q. trojana* and some other *Pinus* and *Quercus* spp. We found it under different mixed stands of *Fagus orientalis, Castanea sativa, Rhododendron ponticum Alnus glutinosa* and *Corylus* sp.

Morphologically *T. puberulum* could be confused with *T. borchii*. Both species are among the white truffles group. Young specimens of *Tuber borchii* could also have peridial hairs and globose spores, but the ascocarps of it always bigger (Honrubia et al., 1992).

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