C O M M U N I C A T I O N S

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

Series C: Biology

VOLUME: 31 **Number: 1** **YEAR: 2022**

Faculty of Sciences, Ankara University
06100 Beşevler, Ankara-Turkey
ISSN 1303-5991 e-ISSN 2618-6470

C O M M U N I C A T I O N S

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

Series C: Biology

Volume: 31 **Number: 1** **Year: 2022**

This Journal is published two issues in a year by the Faculty of Sciences, University of Ankara. Articles and any other material published in this journal represent the opinions of the author(s) and should not be construed to reflect the opinions of the Editor(s) and the Publisher(s).

Correspondence Address:
COMMUNICATIONS EDITORIAL OFFICE
Ankara University, Faculty of Sciences,
06100 Tandoğan, ANKARA – TURKEY
Tel: (90) 312-2126720 Fax: (90) 312-2235000
e-mail: commun@science.ankara.edu.tr
http://communications.science.ankara.edu.tr/index.php?series=A1

Print:
Ankara University Press
İncitaş Sokak No:10 06510 Beşevler
ANKARA – TURKEY
Tel: (90) 312-2136655

C O M M U N I C A T I O N S

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

DE LA FACULTE DES SCIENCESDE
L'UNIVERSITE D'ANKARA

Series C: Biology

Volume: 31 **Number: 1** **Year: 2022**

Research Articles

http://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 1, Pages 1-25 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1056557

Research Article; Received: January 11, 2022; Accepted: February 24, 2022

DETAILED CHARACTERIZATION OF LECTIN GENES IN COMMON BEAN USING BIOINFORMATIC TOOLS

AYBÜKE OKAY[1,2], E. SÜMER ARAS[1], İLKER BÜYÜK[1]

[1]Department of Biology, Faculty of Science, Ankara University, Ankara, Turkey
[2]Department of Vaccine Technology, Vaccine Institute, Hacettepe University, Ankara, Turkey

ABSTRACT. Because of differences in molecular structure, biochemical properties, and carbohydrate binding specificity, lectins are considered a complex and heterogeneous group of proteins found in all organisms. Plant lectins are important proteins in terms of their benefits in cancer treatments, biomedical applications, and many medical uses due to their numerous biological roles such as intercellular interactions, defense mechanisms formation, immunomodulation, and anticarcinogenic activity. Despite the discovery of significant amounts of lectin proteins in different plant species, many questions about their potential biological role remain unanswered in *P. vulgaris* L. In this study, using bioinformatics tools, 52 *Pvul-LEC* genes were identified in the *P. vulgaris* genome and these genes were clustered into three subgroups based on phylogenetic analysis. The majority of Pvul-LEC proteins in the same subfamily of phylogenetic tree shared similar motifs and gene structures. Eight pairs of segmental duplications were discovered based on genome wide duplication analysis. Pvul-LEC proteins' three-dimensional structure and functions were also predicted. Simultaneously, gene expression levels of *Pvul-LEC* genes against drought and salt stress in leaf tissues were evaluated based on publicly available RNAseq data. As a result, it is anticipated that the data obtained in the current study will be beneficial to literature and following studies related to lectin genes.

1. INTRODUCTION

Lectins are proteins that bind reversibly to carbohydrate epitopes on polysaccharides, glycoproteins and glycolipids. They can be found in animals, plants, fungi, bacteria and viruses [1]. Because of variances in molecular structure, biochemical characteristics and carbohydrate binding selectivity, plant lectins are thought to constitute a complex and heterogeneous group of proteins.

Keywords. Lectin, in-silico analysis, bioinformatics, common bean, RNAseq

okay.a13@hotmail.com; aras@science.ankara.edu.tr; ilker.buyuk@ankara.edu.tr; -Corresponding author;

0000-0002-6772-4316; 0000-0003-3474-9493; 0000-0002-0843-8299

Plants contain lectins in a variety of parts, including seeds, leaves, bark, roots, tubers and fruits [2]. Many plant lectins linked to cell motility, cell-cell interaction, embryogenesis and organ formation have been identified as secretory proteins, meaning they enter the secretory system and accumulate in vacuoles, cell walls, or intercellular spaces [3-6].

Plants use lectins to protect themselves from insects and fungus, as well as to transport and store sugar [7]. To defend themselves against invading diseases, plants have evolved an innate immune system [8]. One of the major hormones regulating the pathogen-induced immune response in plants is salicylic acid (SA). The activation of a local sensitivity reaction that inhibits the biotrophic pathogen's proliferation is typical of this response [9]. According to the previous studies, lectin genes play a crucial role in SA biosynthesis. Using *Pseudomonas syringae* pv. avirulence strains on tomato, Armijo et al. (2013) found that increased expression levels of *SAI-LLP* genes triggered cells to avoid leaf death, suggesting that lectin genes are components of SA-mediated defensive pathways [10]. Luo et al. (2017) showed that *LecRK* genes have a role in plant immunological signaling and SA buildup [11]. Furthermore, some lectins are critical for nitrogen fixation in the atmosphere [7]. Lectins operate as immunomodulatory molecules in addition to having antinutritional effects [12-14]. They can adhere to the surface of epithelial cells in the digestive system and generate harmful responses in intestinal permeability because this category of proteins is always resistant to destruction by temperature and digestive enzymes [15, 16].

Plant lectins have also been reported to have biological and medical applications, including the isolation of glycoconjugates from cells, microorganism recognition, monitoring of changes in carbohydrate expression on living cells, mitogenic simulation, anti-proliferative effects, anti-tumor, and drug targeting to the gastrointestinal tract [17-20]. Lectins are one of the most studied protein groups because to their extraordinary biological roles [18]. They are found in legumes, which are an essential food source for both humans and animals. *Phaseolus vulgaris*, a legume, is grown for direct human consumption in a number of countries. Protein (22-27 percent of seed weight) and carbs (39-47 percent of seed weight) are abundant in *P. vulgaris* L., making it an excellent food. Although lectins have been discovered in large quantities in *P. vulgaris*, many issues about their biological function remain unanswered [21, 22].

Plant lectins, specifically *P. vulgaris* lectins, have been found to inhibit leukemia viruses, acquired immunodeficiency syndrome (AIDS) virus, and coronaviruses potently and selectively [23, 24]. As a result, *P. vulgaris* lectins exceptional anti-HIV effectiveness has become a major research topic in recent years. Total parenteral nutrition (TPN) is a valuable medical treatment option that satisfies all of the body's nutritional requirements. Because they can bind to diverse areas of the gastrointestinal tract and induce epithelial proliferation, *P. vulgaris* lectins have recently been proposed as medicines to prevent mucosal atrophy and related

difficulties when patients receive TPN [25]. Furthermore, in recent years, lectin-mediated drug delivery methods have spawned fascinating fields of research in terms of lectin binding to receptor-like structures of the cell membrane and epithelial cells and initiating active transport of medicinal components [26].

Consumption of *P. vulgaris* lectins is critical due to multiple health hazards and major pharmacological effects in humans, however not all of the *Pvul-LEC* genes have been found and characterized so far. For this reason, this study will be used to strategically accompany future studies by discovering *Pvul-LEC* genes utilizing bioinformatics methods.

2. MATERIALS AND METHODS

2.1. Identification of lectin proteins in *P. vulgaris* genome

P. vulgaris lectin family sequences were obtained from NCBI (http https://www.ncbi.nlm.nih.gov/) and Pfam databases [27]. Putative *P. vulgaris* lectin proteins were used for query in blastp (NCBI) for characterization of hypothetical proteins. The physicochemical properties of lectin proteins were calculated using ProtParam Tool (http:web.expasy.org/protparam) and detection of domains was performed using HMMER (http:www.ebi.ac.uk/Tools/hmmer/).

2.2. Structure and physical locations of lectin genes and conserved motifs

'Gene Structure Display Server v2.0' (GSDS, http:// gsds.gao-lab.org) was used to illustrate the exon–intron structure of *Pvul-LEC* genes [28]. The lectin genes have been mapped on *P. vulgaris* chromosomes using the MapChart program [29]. Multiple Expectation Maximizations for Motif Elicitation tool (EM) was used to categorize additional conserved motifs for Pvul-LEC proteins (MEME 4.11.1; http://meme-suite.org/) [30].

2.3. Phylogenetic analysis and sequence alignment

The ClustalW was used to align various sequences of lectin proteins [31]. For the creation of phylogenetic trees with a bootstrap value of 1000 replicates (MEGA11), the Neighbor-joining (NJ) method was used, and the tree was constructed using an Interactive Life Tree (iTOL; http://itol.embl.de/index.shtml) [32].

2.4. Promoter analysis of lectin genes

The PlantCARE database was used to do a cis element scan on the 5′ upstream

regions (2 kb of DNA sequence from each *Pvul-LEC* gene) using the Phytozome database v11 (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

2.5. In-silico prediction of miRNA targets in lectin genes

MiRBase v21.0. has been used to download all known miRNA plant sequences (http://www.mirbase.org). The default miRNA prediction parameters were applied using the psRNA Target Server (http://plantgrn.noble.org/psRNATarget) [33]. BLASTX was used to search in-silico predicted miRNA targets against common bean Expressed Sequenced Tags (ESTs) in the NCBI database with a $1e^{-10}$ threshold.

2.6. Detection of gene duplication events and prediction of synonymous and non-synonymous substitution rates

The Plant Genome Duplication Database service (http://chibba.agtec.uga.edu/duplication/index/locus) was used to examine duplicated gene pairs with a display range of 2000 kb. The amino acid sequences of duplicated lectin genes were predicted using the CLUSTALW program. To estimate synonymous (Ks) and non-synonymous (Ka) substitution rates, the PAML (PAL2NAL) CODEML program (http://www.bork.embl.de/pal2nal) was used [34]. The following formula was used to compute the duplication period (Million Years Ago, Mya) and divergence of each lectin gene: $T = Ks/2\lambda$ ($\lambda=6.56E^{-9}$) [35].

2.7. Identification of expression level of lectin genes through transcriptome data

Illumina RNA-seq data was collected from the Sequence Read Archive (SRA) to measure the *Pvul-LEC* gene expression levels. For this reason, the accession numbers SRR957667 (control leaf) and SRR957668 (salt-treated leaf) were used as defined by Büyük et al. (2016) [36, 37]. The heat maps of hierarchical clustering were eventually built using the CIMMiner algorithm (http://discover.nci.nih.gov/cimminer).

2.8. Homology modeling of lectin proteins

BLASTP (with default parameters) was used to search all lectin proteins against the Protein Data Bank (PDB) to identify the best template(s) with identical sequence and three-dimensional structure [38]. The data was loaded into Phyre2 (Protein Homology/AnalogY Recognition Engine; http://www.sbg.bio.ic.ac.uk/phyre2), which used homology modeling in 'intense' mode to predict protein structure [39].

3. RESULTS AND DISCUSSION

3.1. Determination of *Pvul-LEC* genes in the genome of *P. vulgaris*

The analyses resulted in the discovery of 52 lectin genes encoded in the *P. vulgaris* genome. The order of chromosome placement was taken into account when naming the genes, and all lectin genes were categorized into two groups based on their domain types: Legume lectin domain (Lectin legB) and L-Type Lectin. According to this, starting from *Pvul-LLEC-1* to *Pvul-LLEC-30*, and starting from *Pvul-BLEC-1* to *Pvul-BLEC-22*, the naming process was carried out using the Latin name of the bean plant (*Phaseolus vulgaris*).

According to the studies, lectin legB plays a critical role in biotic and abiotic stress responses. For example, Jiang et al. (2010) discovered that lectin *legB* genes revealed a wide range of expression profiles, especially under salt, cold and drought stress and that they may operate as a regulator of environmental conditions. With their work on *Arabidopsis*, it was also hypothesized that lectin legB and the kinase domains, a structurally conserved protein domain that carries out the phosphorylation process common to all living things, may have duplicated to protect against stressors during the evolutionary process [40].

L-type lectins are mostly present in the seeds of leguminous plants, and they are delivered to the vacuole where they are condensed by specific vesicles after being generated during seed development a few weeks after blooming. L-type lectins remain stable during the drying process of the seeds and can do so indefinitely until the seeds germinate. They are typically referred to as storage proteins because they are one of several types of proteins that are kept in high concentrations in seeds [41, 42]. L-type lectins, which have structural motifs seen in glycan-binding proteins, are also found in the bark of some legume trees and to a lesser extent in other vegetal tissues of legumes (GBPs). Most of their structures have been determined, and numerous L-type lectins have been reported to be employed in a variety of biological and analytical techniques [43]. L-type lectins also play a part in plant-nitrogen-fixing bacteria symbiosis, such as Rhizobium-legumes symbiosis, however the exact role of lectins in this system is unknown. However, investigations demonstrate that the seed lectin is a lipoxygenase with the activity required to start the plant defense pathway [7, 44].

So far, lectin genes have been identified in several plant species. The number of lectin genes (n=52) in *P. vulgaris* in this study is higher than the number identified in *Cucumis sativus* (n=46) [45] however less than the number identified in *Arabidopsis thaliana* (n=72) [46], *Setaria italica* (n=120), *Zea mays* (n=126), *Oryza sativa* (n=153), *Sorghum bicolor* (n=153), *Brachypodium distachyon* (n=204) and *Saccharum spontaneum* (n=429) [47].

Protein length (aa), the isoelectric point (pI), molecular weight (Da), instability index, aliphatic index and GRAVY value were calculated for all identified Pvul-LEC proteins. Accordingly, the lengths of Pvul-LEC proteins ranged from 259 to 699 amino acids. Additionaly, 10 Pvul-LEC proteins were found to have basic and 42 Pvul-LEC proteins have acidic properties (Table 1). The molecular weights of Pvul-LEC proteins were between 26.96 to 78.50 kDa and 5 Pvul-LEC proteins were found to be unstable according to instability index scores. The most of the Pvul-LEC proteins were found to be hydrophilic according to the GRAVY values which ranged between -0.294 to 0.229 (Table 1).

The aliphatic index value, defined as the relative volume occupied by the aliphatic side chains (alanine, valine, isoleucine and leucine) can be considered to be a positive factor for enhancing global protein thermostability [48]. These values ranged from 73.71 to 96.06 for Pvul-LECs, suggesting the strong thermostability of these proteins.

All *Pvul-LEC* genes were found to be unevenly distributed on 10 out 11 chromosomes of *P. vulgaris* with the exception of chromosome 9. chromosome 1 contained the most *Pvul-LEC* genes (eleven), whereas chromosome 11 contained only one (Figure 1). Surprisingly, chromosome 4 contained only *Pvul-BLEC* genes while chromosome 5 and 6 contained only *Pvul-LLEC* genes. Dang et al. (2016) discovered that the lectin gene was found in all chromosomes (7 chromosomes) of *C. sativus* and at different rates, similar to *Pvul-LEC* genes [49]. In another study on the mungbean plant, Singh et al. (2021) classified lectin genes into three types: G-type, L-type, and C-type, and 59 of 73 *VrLecRLK* were randomly distributed on 11 chromosomes. Furthermore, the remaining *VrLecRLK* genes in the study were discovered to be unattached to any specific chromosome [50].

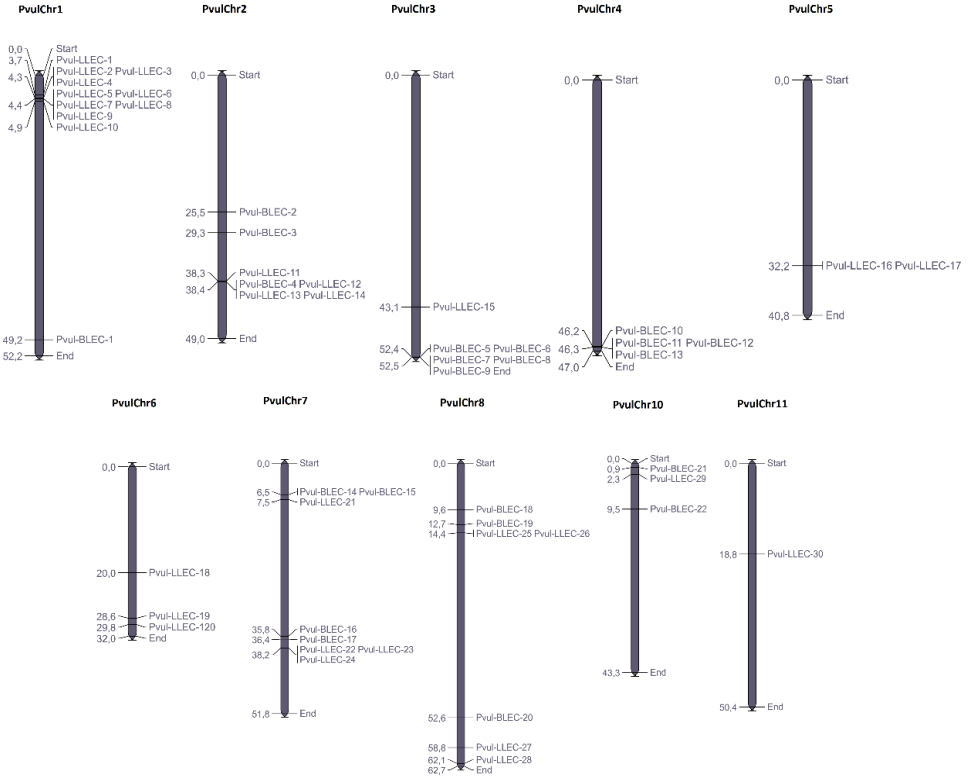

FIGURE 1. Distribution of *Pvul-LEC* genes on *P. vulgaris* chromosomes

TABLE 1. Detailed descriptive information for Pvul-LLEC proteins

ID	*Phaseolus vulgaris* Genomic Database Identifier	Physical position on *P. vulgaris* genome			Protein length (aa)	pI	Molecular weight (kDa)	Instability index	Aliphatic index	GRAVY	NCBI Accession No.
		Chr.	Start position (bp)	End Position (bp)							
Pvul-LLEC-1	Phvul.001G045400.1.p	1	3.677.580	3.680.026	667	6.04	75.27	32.38	87.06	-0.176	XP_007161136.1
Pvul-LLEC-2	Phvul.001G040800.1.p	1	4.327.634	4.329.496	620	5.68	68.75	27.71	90.05	-0.085	XP_007161076.1
Pvul-LLEC-3	Phvul.001G040700.1.p	1	4.336.106	4.338.606	672	5.77	74.99	28.89	83.11	-0.189	XP_007161075.1
Pvul-LLEC-4	Phvul.001G040600.1.p	1	4.339.156	4.340.979	607	6.01	67.9	31.81	94.43	-0.064	XP_007161074.1
Pvul-LLEC-5	Phvul.001G040500.1.p	1	4.359.201	4.361.618	661	5.73	73.92	25.07	89.05	-0.14	XP_007161073.1
Pvul-LLEC-6	Phvul.001G040400.1.p	1	4.370.066	4.372.118	636	5.62	70.87	34.63	91.89	-0.142	XP_007161072.1
Pvul-LLEC-7	Phvul.001G040300.1.p	1	4.379.952	4.382.583	664	5.63	73.98	25.57	87.91	-0.161	XP_007161071.1
Pvul-LLEC-8	Phvul.001G040100.1.p	1	4.394.207	4.396.266	636	5.62	71.1	34.65	90.66	-0.154	XP_007161068.1
Pvul-LLEC-9	Phvul.001G040000.1.p	1	4.399.520	4.401.949	666	5.83	74.15	25.7	88.11	-0.129	XP_007161067.1
Pvul-LLEC-10	Phvul.001G234200.1.p	1	4.880.986	48.811.980	664	8.68	73.85	33.83	92.3	-0.104	XP_007163432.1
Pvul-LLEC-11	Phvul.002G214900.1.p	2	38.345.248	38.347.335	695	5.85	77.3	32.79	87.8	-0.128	XP_007159169.1
Pvul-LLEC-12	Phvul.002G215200.1.p	2	38.371.310	38.373.253	647	7.31	72.85	30.71	85.09	-0.229	XP_007159173.1
Pvul-LLEC-13	Phvul.002G215300.1.p	2	38.383.003	38.384.994	663	7.32	74.17	31.47	90.89	-0.201	XP_007159174.1
Pvul-LLEC-14	Phvul.002G215400.1.p	2	38.393.780	38.395.786	668	6.75	75.53	36.9	91.44	-0.147	XP_007159175.1
Pvul-LLEC-15	Phvul.003G204500.1.p	3	43.061.296	43.063.308	670	5.11	73.7	42.72	86.1	-0.074	XP_007155476.1
Pvul-LLEC-16	Phvul.005G103200.1.p	5	32.195.113	32.197.596	691	5.99	77.7	41.04	86.43	-0.115	XP_007149844.1
Pvul-LLEC-17	Phvul.005G103300.1.p	5	32.198.665	32.200.731	688	6.34	77.07	34.86	91.89	-0.078	XP_007149845.1
Pvul-LLEC-18	Phvul.006G087700.1.p	6	19.974.245	19.976.281	678	5.75	72.71	36.68	85.27	-0.04	XP_007146994.1
Pvul-LLEC-19	Phvul.006G185000.1.p	6	28.613.191	28.616.354	692	5.51	78.5	36.34	89.22	-0.143	XP_007148157.1
Pvul-LLEC-20	Phvul.006G200800.1.p	6	29.750.251	29.752.428	639	6.96	72.04	36.62	88.17	-0.155	XP_007148350.1
Pvul-LLEC-21	Phvul.007G078200.1.p	7	7.489.146	7.491.182	678	6.24	75.42	41.56	84.1	-0.087	XP_007143518.1
Pvul-LLEC-22	Phvul.007G260300.1.p	7	38.169.156	38.172.057	670	6.25	74.86	36.79	84.51	-0.263	XP_007145693.1
Pvul-LLEC-23	Phvul.007G260400.2.p	7	38.176.633	38.178.637	450	7.61	50.66	39.48	84.6	-0.294	XP_007145694.1
Pvul-LLEC-24	Phvul.007G260500.1.p	7	38.182.876	38.185.141	657	6.43	72.94	37.51	88.23	-0.212	XP_007145695.1
Pvul-LLEC-25	Phvul.008G117700.1.p	8	14.407.039	14.409.057	672	7.01	74.2	34.28	99.33	-0.066	XP_007140498.1
Pvul-LLEC-26	Phvul.008G117800.1.p	8	14.436.075	14.438.400	668	7.32	73.97	33.83	94.03	-0.115	XP_007140499.1
Pvul-LLEC-27	Phvul.008G239600.1.p	8	58.823.983	58.826.861	699	5.59	74.93	33.42	87.78	-0.018	XP_007141948.1
Pvul-LLEC-28	Phvul.008G279300.1.p	8	62.071.477	62.073.771	662	6.64	72.8	38.34	86.56	-0.003	XP_007142422.1
Pvul-LLEC-29	Phvul.010G015800.1.p	10	2.345.298	2.347.358	686	5.74	75.22	36.4	93.76	-0.068	XP_007134058.1
Pvul-LLEC-30	Phvul.011G119200.1.p	11	18.774.057	18.775.652	531	5.98	59.06	34.97	94.11	-0.007	XP_007132721.1

TABLE 2. Detailed descriptive information for Pvul-BLEC proteins

ID	*Phaseolus vulgaris* Genomic Database Identifier	Physical position on *P. vulgaris* genome			Protein length (aa)	pI	Molecular weight (Da)	Instability index	Aliphatic index	GRAVY	NCBI Accession No.
		Chr.	Start position (bp)	End Position (bp)							
Pvul-BLEC-1	Phvul.001G239200.1.p	1	49.244.673	49.246.082	345	5.28	37.51	28.22	89.77	-0.126	XP_007163496.1
Pvul-BLEC-2	Phvul.002G119900.1.p	2	25.542.601	25.545.814	693	6.16	76.67	33.71	90.71	-0.127	XP_007158049.1
Pvul-BLEC-3	Phvul.002G144200.1.p	2	29.269.216	29.270.007	263	4.72	28.25	34.77	87.07	0.102	XP_007158339.1
Pvul-BLEC-4	Phvul.002G215000.1.p	2	38.350.955	38.353.003	664	6.01	73.69	35.44	88.09	-0.133	XP_007159169.1
Pvul-BLEC-5	Phvul.003G286700.1.p	3	52.442.001	52.442.780	259	4.87	27.66	32.2	88.84	0.214	XP_007156448.1
Pvul-BLEC-6	Phvul.003G286800.1.p	3	52.444.624	52.445.439	271	4.93	29.7	30.56	87.05	0.049	XP_007156449.1
Pvul-BLEC-7	Phvul.003G286900.1.p	3	52.450.066	52.450.845	259	6.21	27.85	36.21	93.4	0.223	XP_007156450.1
Pvul-BLEC-8	Phvul.003G287000.1.p	3	52.452.303	52.453.082	259	4.69	27.72	32.79	88.84	0.229	XP_007156451.1
Pvul-BLEC-9	Phvul.003G287101.1.p	3	52.459.994	52.462.334	449	6.35	49.81	36.53	96.06	-0.032	XP_007156452.1
Pvul-BLEC-10	Phvul.004G158000.1.p	4	46.239.426	46.240.567	277	4.73	29.97	24.31	89.03	-0.02	XP_007152769.1
Pvul-BLEC-11	Phvul.004G158100.1.p	4	46.258.583	46.259.432	244	5.03	26.96	33.15	83.85	-0.22	XP_007152770.1
Pvul-BLEC-12	Phvul.004G158200.1.p	4	46.261.905	46.262.865	279	5.15	30.26	21.46	95.05	0.031	XP_007152771.1
Pvul-BLEC-13	Phvul.004G158300.1.p	4	46.267.391	46.268.336	272	4.92	29.55	26.45	89.56	-0.041	XP_007152772.1
Pvul-BLEC-14	Phvul.007G070100.1.p	7	6.474.389	6.475.216	275	5.37	30.64	31.26	73.71	-0.29	XP_007143413.1
Pvul-BLEC-15	Phvul.007G070300.1.p	7	6.481.954	6.483.109	299	5.3	32.91	27.95	79.57	-0.223	XP_007143415.1
Pvul-BLEC-16	Phvul.007G234400.1.p	7	35.840.909	35.843.746	643	7.96	70.63	41.18	84.07	-0.189	XP_007145382.1
Pvul-BLEC-17	Phvul.007G239800.1.p	7	36.355.865	36.356.814	274	5.53	29.36	34.33	91.5	0.039	XP_007145445.1
Pvul-BLEC-18	Phvul.008G094500.1.p	8	9.626.760	9.628.781	673	5.25	74.54	34.81	88.78	-0.156	XP_007140223.1
Pvul-BLEC-19	Phvul.008G111500.1.p	8	12.658.909	12.659.979	356	9.28	39.36	34.84	82.33	0.057	XP_007140433.1
Pvul-BLEC-20	Phvul.008G188700.1.p	8	52.631.783	52.634.655	677	7.28	75.82	38.29	85.64	-0.18	XP_007141356.1
Pvul-BLEC-21	Phvul.010G006300.1.p	10	923.471	924.954	348	9.2	38.09	32.36	85.34	0.063	XP_007133954.1
Pvul-BLEC-22	Phvul.010G060800.1.p	10	9.503.176	9.505.775	662	5.47	73.7	45.83	89.08	-0.096	XP_007134605.1

In order to expand and diversify, genes engage in a dynamic replication process which is referred to as gene duplication. Specialized genes or new gene functions can arise as a result of these evolutionary processes. For this reason, the location of *Pvul-LEC* genes on *P. vulgaris* chromosomes was investigated, as well as their duplications. While no tandem duplication events were discovered, 8 pairs of segmental duplication events were detected in *Pvul-LEC* genes. The duplicated genes homologous (Ks) and non-homologous (Ka) exchange rates were calculated (Table 3). Accordingly, the calculated mean Ka/Ks ratios were found to be 0.12 and hence this is a purifying selection since Ka / Ks <1 [51]. In addition, it was determined that the average time of duplications was 76.62 MYA (Table 3).

TABLE 3. Information regarding segmental duplication events between *Pvul-LEC* genes (Ka: non-synonymous divergence value; Ks: synonymous divergence value; Ka/Ks: rate of change; MYA: Million years ago)

Gene IDs	Gene IDs	Ka	Ks	Ka/Ks	MYA
Pvul-BLEC-3	*Pvul-BLEC-5*	46.1974	0.4735	0.0102	355,36
Pvul-LLEC-10	*Pvul-LLEC-25*	4.0418	0.2910	0.0720	31,09
Pvul-LLEC-16	*Pvul-LLEC-19*	3.0242	0.3787	0.1252	23,26
Pvul-LLEC-16	*Pvul-LLEC-30*	2.6967	0.3044	0.1129	20,74
Pvul-BLEC-18	*Pvul-LLEC-29*	11.3547	0.6572	0.0579	87,34
Pvul-LLEC-18	*Pvul-LLEC-27*	1.1548	0.1411	0.1222	88,83
Pvul-BLEC-19	*Pvul-BLEC-21*	0.7361	0.2274	0.3089	5,66
Pvul-LLEC-25	*Pvul-LLEC-29*	0.6383	0.1574	0.2465	4,91

A comparison was made between the 52 *Pvul-LEC* genes identified in the *P. vulgaris* genome and the lectin genes of *A. thaliana* and *G. max*. (Supplementary Table 1). As a result, 11 orthologous gene pairs were found to be shared between *Phaseolus vulgaris* and *Arabidopsis thaliana*, and 8 orthologous pairs were shared between *Phaseolus vulgaris* and *Glycine max*. The average Ka/Ks ratios were 0.07 and 0.028, respectively (Supplementary table 1).

When orthologous lectin genes between *P. vulgaris* and *G. max* were analyzed, it was discovered that approximately 88% of them were *Pvul-BLEC* genes. This situation has increased the possibility that the lectin *legB* genes were duplicated in the evolutionary process in parallel with the findings of Jiang et al. (2010) [40]. The number of *Pvul-LLEC* genes was compared to the total number of protein-coding genes in different genomes. Accordingly, *G. max, A. thaliana, T. aestivum* and *O. sativa* had rates of 60/56044 (0.107%), 42/27416 (0.153%), 84/99386 (0.085%), and 72/22273 (0.323%), respectively. In this study, the

number of protein-coding genes in *P. vulgaris* was determined to be 85,167, while the number of L-type genes was discovered to be 30, yielding a 30/85167 (0.035%) ratio. Various *LLEC* gene growth rates in different genomes, as well as changes in genome size, could explain differences in *Pvul-LLEC* gene copy counts. Furthermore, it was discovered that only *A. thaliana*, which included *Pvul-LEC* genes, increased the L-type genes to a larger extent than the other types (55-57).

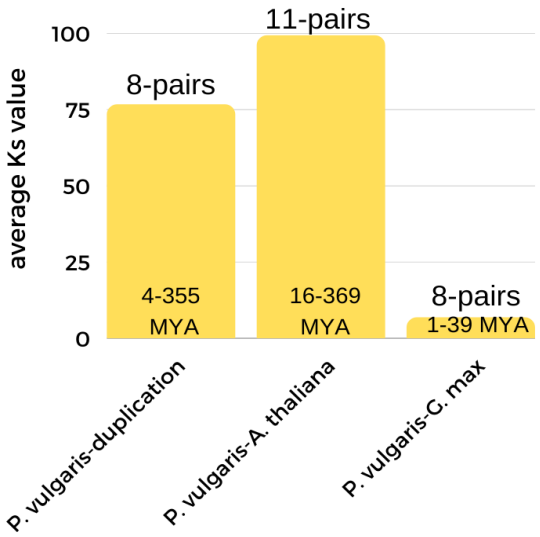

FIGURE 2. Time of duplication and divergence (MYA) based on synonymous substitituon rate (Ks).

3.2. Structure of *Pvul-LEC* genes, phylogenetic tree analysis, determination of preserved motifs, homology modeling and promoter analysis

The structure of *Pvul-LEC* genes was investigated using exon and intron regions. Exon and intron regions play a significant role in modulating gene expression and regulating genes differently [52, 53]. There were no introns discovered in 42 of the *Pvul-LEC* genes. The *Pvul-BLEC-16* gene was discovered to be the only one with multiple intron regions among all the genes examined. Furthermore, this gene has been found to have the most recent orthologous relationship with Glyma.20G072500, a *Glycine max LEC* gene (MYA=1.66). In a study on *Vigna radiata* (mung bean) [50], Singh et al. (2021) discovered that 27 *VrLecRLK* genes (out of 73 total) do not have introns. In soybean, Liu et al. (2018) discovered that nearly 70% of 60 L-type *GmLec* genes do not have intron regions [54]. The presence or lack of introns in many genomes has evolutionary

advantages for a gene's protein output by changing numerous characteristics, such as reverse transcriptase activity or mRNA stability [55].

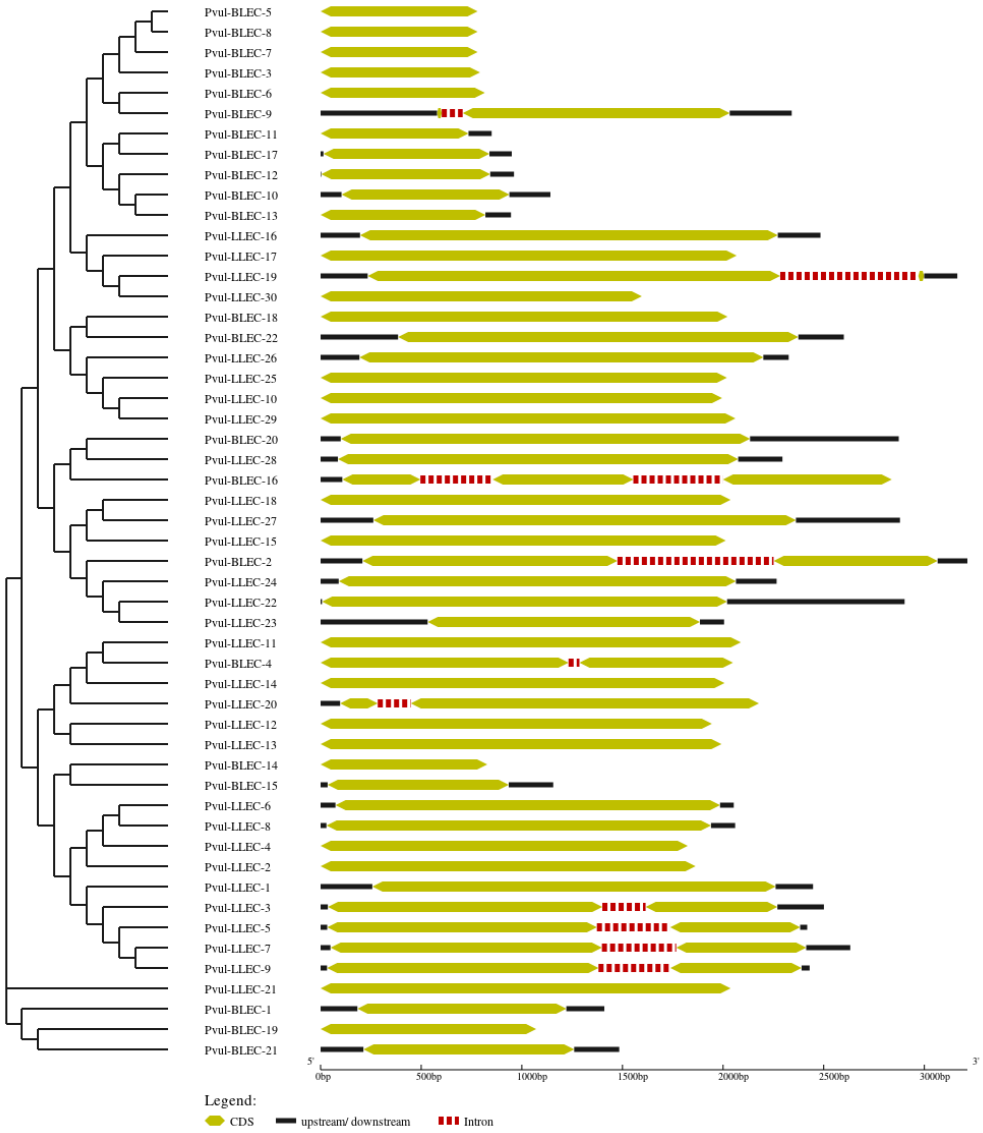

FIGURE 3. Gene structures of lectin family members from *P. vulgaris* with clustering based on NJ phylogenetic tree. Introns are presented by lines. UTR and CDS are indicated by filled black and green boxes, respectively.

A phylogenetic tree was drawn using the neighbor joining method using the evolutionary processes of different organisms (*P. vulgaris*, *A. thaliana* and *G. max*) to examine the kinship for lectin genes. The examined phylogenetic tree with 196 members was found to be divided into three major groups.

According to this, the smallest group was ‘A’ with 3 genes and the largest group was ‘D’ with 106 genes (D1-30/D2-76). Group ‘B’ and ‘C’ was found to contain 45 and 42 genes, respectively. The all lectin genes in group ‘B’ belong to *Pvul-BLEC* family.

Groups C and D contained all of the intron-containing genes. Genes with identical motif content weree clustered in the same groups of the phylogenetic tree; *Pvul-BLEC-5*, *Pvul-BLEC-8*, *Pvul-BLEC-11*, *Pvul-BLEC-13*, *Pvul-BLEC-15* and *Pvul-BLEC-17* in group B, *Pvul-LLEC-1*, *Pvul-LLEC-2*, *Pvul-LLEC-5*, *Pvul-LLEC-6*, *Pvul-LLEC-7*, *Pvul-LLEC-8*, *Pvul-LLEC-9* and *Pvul-LLEC-14* were in group *C*, while *Pvul-LLEC-17* and *Pvul-LLEC-19* were in group D2.

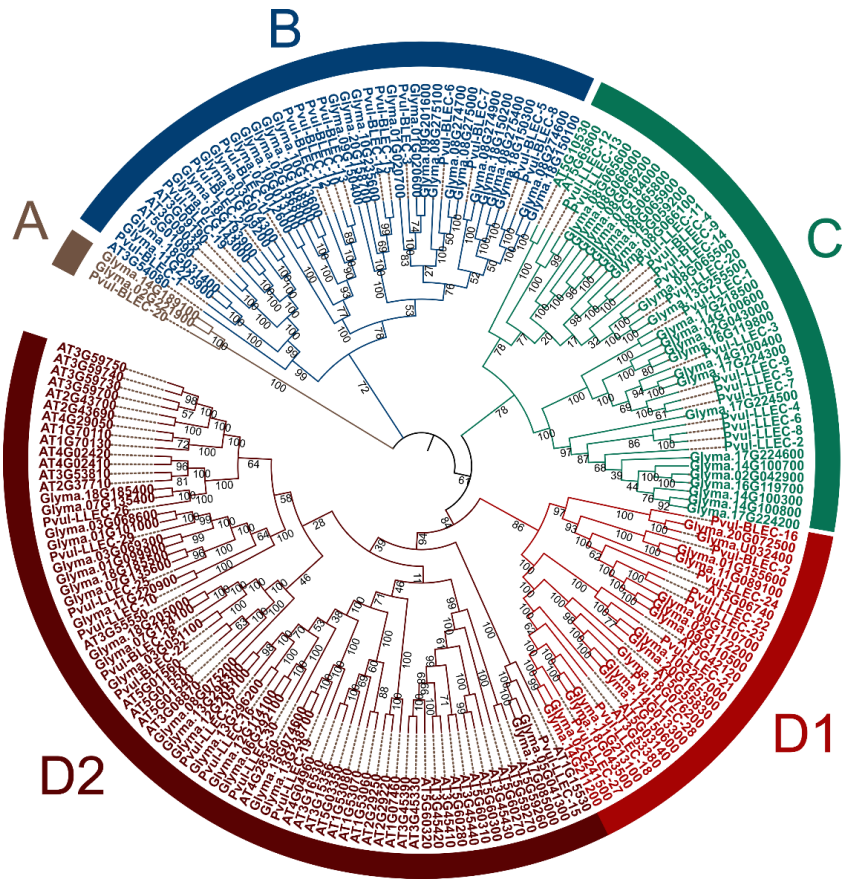

FIGURE 4. Phylogenetic analyses of lectin proteins from three plant species. The phylogenetic tree was constructed using the NJ method. The identifier names of lectin proteins of *Phaseolus vulgaris* L., *Arabidopsis thaliana* and *Glycine max* start with ‘Pvul’, ‘AT’ and ‘Glyma’, respectively.

In genome investigations, the detection of conserved motifs is critical for protein identification and categorization, as well as the identification of functional areas and particular binding sites [56]. In this context, we evaluated the conserved motif structures of lectin proteins in the *P. vulgaris* genome, and the results were reported in Supplementary Table 2. Accordingly, twenty conserved motif patterns were found in 52 Pvul-LEC proteins. As a result, all *Pvul-LLEC* genes were found to contain Motifs 1, 2, 3, 5, and 9, while all *Pvul-BLEC* genes contained Motif 14.

In this study, homology was determined using modeling of Pvul-LEC proteins with a confidence level of 90% or higher in order to obtain information about the protein structures. As a result, all of the proteins were found to be 90% or more reliable belong to the Pvul-BLEC family. Legume lectin loops have a jelly roll subtopology, also known as a superfold [57]. This fold is thought to have had multiple evolutionary origins rather than a single common ancestor [58]. The fact that approximately 88 percent of the existing orthologous genes located between *Pvul-LEC* and *G. max* are *Pvul-BLEC* genes supports the findings.

The hydrogen bond pattern between adjacent strands is broken in two places, resulting in the formation of two four-stranded sheets. Because adjacent strips appear in different layers, both layers are completely anti-parallel, with the exception of the fourth and fifth strands, which appear in the same layer. This results in a structure with a single hairpin and arched - junctions [59]. When Pvul-BLEC proteins were examined, anti-parallel – structures could be found in all of them. In addition to these structures, almost all Pvul-BLEC proteins had alpha structures (Figure 5).

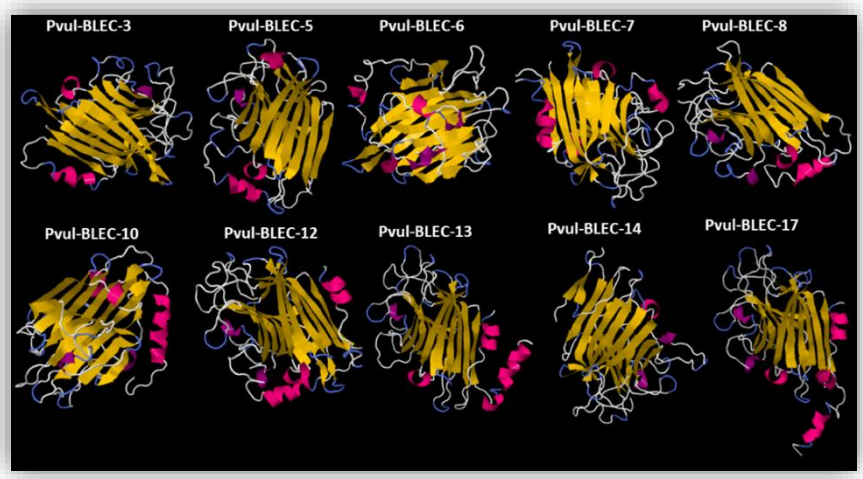

FIGURE 5. Predicted 3D models of common bean Pvul-BLEC proteins. Models were generated by using Phyre2 server. The secondary structure elements: α-helices (pink), β-sheets (yellow), and coils (blue-white) are indicated for the predicted 3D structures of Pvul-BLECs.

Cis-acting regulatory sequence elements are involved in the transcriptional regulation of gene activities, which control many biological processes, particularly abiotic stress responses, hormones, and development [60]. In this study, in silico promoter analysis was done in order to better understand the regulatory mechanisms of *Pvul-LEC* genes. The discovered cis-acting elements were classified into following eight categories: development, environmental stress, hormone, light, promoter, site binding, biotic stress, and others (Supplementary Table 3).

Plants require a variety of signaling chemicals, including SA (Salicyclic acid), JA (Jasmonic acid), ABA (Abscisic acid), and ET (Ethylene) to respond to stress. MYCs, which are the major regulators of the JA signaling pathway and are categorized in the environmental stress category, are found in roughly 55% (29 genes) of *Pvul-LEC* genes. MYB, which is categorized in the biotic stress category and is known to have a role in the regulation of auxins as well as drought, salt, and abscisic acid, was discovered to be present in around 53% of *Pvul-LEC* genes (28 genes). In the promoters of 20, 19, and 11 *Pvul-LEC* genes, ABA-sensitive elements (ABREs), MeJA-responsive elements (CGTCA-pattern), and SA-responsive elements (TCA-elements) were found respectively. Additionaly, *Pvul-LLEC-12* and *Pvul-BLEC-6* genes, which both have ABRE, CGTCA motif, TCA-element and WUN-motifs, were found to reveal almost same expression patterns according to the heatmaps drawn in this study (Figure 6 and 7).

3.3. Detection of miRNAs targeting *Pvul-LEC* genes

Plants exposed to abiotic stress factors develop response mechanisms at the transcriptome level. MiRNAs, in addition to TF in restoring metabolism, are involved in the regulation of gene expression [61, 62]. Under abiotic stress conditions, plants can increase or decrease the efficiency of miRNAs or synthesize new miRNAs [62). MiRNAs and associated *Pvul-LEC* genes were identified in this study (Supplementary Table 4). A total of 8226 targets were examined, and it was discovered that the most targeted gene was *Pvul-LLEC-14* among all *Pvul-LLEC* genes, and the most targeted gene was *Pvul-BLEC-4* among all *Pvul-BLEC* genes. It was worth noting that both genes were clustered together in the phylogenetic tree's group C (Figure 4).

MiRNAs targeting *Pvul-LLEC-14* gene, which was most targeted *Pvul-LLEC* gene, were studied based on the targeting frequency. Among these, miR169 is a miRNA which involves in early flowering. Additionaly, in a study to grow resistant tobacco varieties, Jianyu et al. (2019) discovered that miRNA169 played a role in response to low temperature stress. In the same study, LTR elements were detected in the promoter region of the genes targeted by miR169 in tobacco [63]. Similarly, an LTR element was also found in the promoter region of the *Pvul-LLEC-14* gene. Based on this information, it is believed that the

miR169 and LTR element may interact to reveal stress response in *P. vulgaris* under stress conditions.

MiR395, is an another miRNA targeted *Pvul-LLEC-14* gene, has been identified as a regulatory component of the sulfate assimilation network [64]. The expression of miR395 was also found to change in response to environmental stimuli [65]. MiR5241 was found to be significantly increased in a study by Cao et al (2018) that examined the responses of miRNAs to salt and alkali stress in *Medicago truncatula* [66].

At the same time, the frequency of miRNA targeting of the *Pvul-BLEC-4* gene was investigated. Plants have a set of defensive responses to prevent pathogen invasion. Some of these defense mechanisms are built into the plant, while others can be triggered by pathogen detection. According to our findings, miR160 is involved in the local defense mechanism, and Natarjan et al. (2018) discovered an increased susceptibility to infection in potato associated with miR160 regulation [67].

We hypothesize that the interaction of *Pvul-BLEC-4* - miR160 - SA may have an antagonistic effect against infections because lectin genes are known to play a central role in SA biosynthesis. According to the literature, miR172 confers plant tolerance, particularly against salt stress and water deficiency, but increases ABA sensitivity. In a study on Arabidopsis, it was discovered that overexpression of miR172 kept the plant alive [68].

3.4. Tissue-specific mRNA levels of the *Pvul-LEC* genes

The expression data of *Pvul-LEC* genes in various tissues (flower buds, flowers, leaves, stem 10, young pods, stem 19, young trifoliats, root 10, root 19, green mature pods and nodules) was obtained from the Phytosome v12 database and a heat map was plotted (Figure 6). Accordingly, *Pvul-BLEC-1*, *Pvul-LLEC-15*, *Pvul-LLEC-16*, *Pvul-LLEC-18* and *Pvul-LLEC-27* genes were found to be highly expressed in all tissues in comparison to the other *Pvul-LEC* genes (Figure 6). Genes with similar expression levels were surprisingly found to be located in the same phylogenetic groups (Figure 4 and Figure 6). For example, *Pvul-LLEC-15*, *Pvul-LLEC-16*, *Pvul-LLEC-18* and *Pvul-LLEC-27* genes with similar expression levels were found to be located in D1-D2 groups according to the phylogenetic tree.

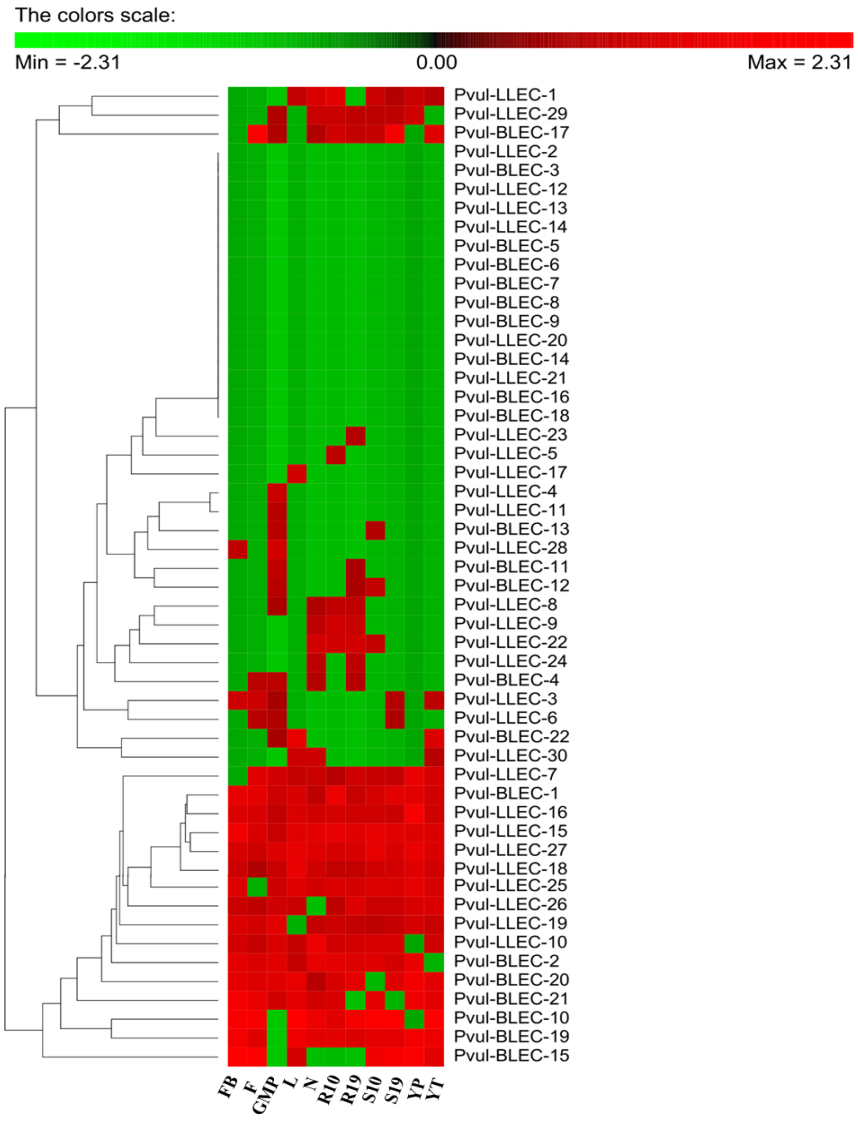

FIGURE 6. Heat map image for tissue specific mRNA levels of *Pvul-LEC* genes – from left to right; FB: Flower Buds, F: Flowers, GMP: Green Mature Pods, L: Leaves, N: Nodules, R10: Root 10, R19: Root 19, S10: Stem 10, S19: Stem 19, YP: Young Pods, YT: Young Trifoliates Tempo-spatial expression patterns of *Pvul-LEC* genes in different organs in *P. vulgaris*. The log10-transformation of the average of expression values were used to generate the heat map with PermutMatrix software. Red and green in the color scale indicate high and low transcript expression, respectively. Root 19: 19 days after planting; Root 10: 10 days after planting; Stem 19: 19 days after planting; Stem 10: 10 days after planting.

3.5. Responses of *Pvul-LEC* genes to salt stress and drought stress through RNAseq analysis

Plants are grown best in environments that are ideal for them. Exposure to an unexpected condition may result in diseases or physiological changes that affect the development and survival of the organism [69]. The leading environmental factors threatening agricultural production are salinity and drought [70]. In this regard, it is critical to understand plant defense mechanisms against these stress conditions. Accordingly, the heat map analysis was done in order to examine the responses of *Pvul-LEC* genes against salt and drought stress using RNAseq data obtained from GenBank (Figure 7). Upon salt stress treatment, the expression levels of *Pvul-BLEC-9, Pvul-LLEC-9, Pvul-LLEC-19, Pvul-LLEC-22,* and *Pvul-LLEC-29* were decreased, whereas *Pvul-BLEC-12, Pvul-BLEC-15,* and *Pvul-LLEC-23* expression levels were found to be increased in leaves. On the other hand, the expression levels of *Pvul-BLEC-1, Pvul-BLEC-3, Pvul-LLEC-9, Pvul-LLEC-17, Pvul-LLEC-18, Pvul-LLEC-19, Pvul-LLEC-27* and *Pvul-LLEC-28* genes were found to be increased whereas *Pvul-BLEC-11* expression level was decreased under drought stress conditions.

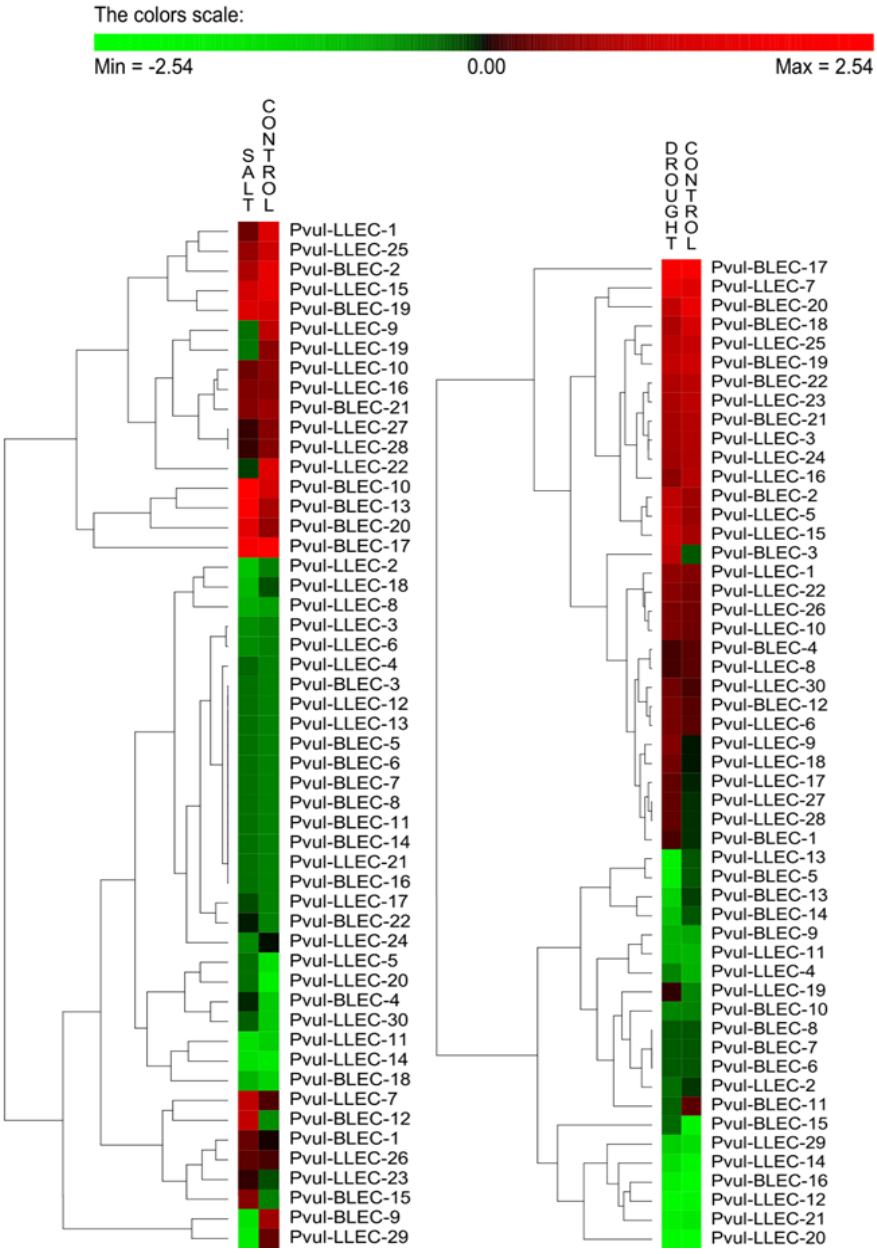

FIGURE 7. Heatmaps of *Pvul-LEC* genes differentially expressed under salt-control / drought- control stress conditions derived from RNAseq analysis. The log10-transformation of the average of expression values were used to generate the heat map with PermutMatrix software. Red and green in the color scale indicate high and low transcript expression, respectively.

4. CONCLUSIONS

The lectin gene family encoded in the *P. vulgaris* genome was identified and characterized for the first time in this study. Bioinformatics analyses were used to determine the properties of 52 newly identified *Pvul-LEC* genes. RNAseq data was also used to assess the expression levels of *Pvul-LEC* genes under salt and drought stress conditions. Since *LEC* genes were first discovered in *P. vulgaris*, we believe that our findings will open up new avenues for future research on *LEC* genes in other plant species.

Author Contribution Statements AO- data collection, management and manuscript writing. SA- project development, manuscript editing. IB- project development, data analysis, manuscript writing and manuscript editing. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

REFERENCES

[1] Sharon, N., Lis, H., History of lectins: from hemagglutinins to biological recognition molecules, *Glycobiology*, 14 (2004), 53R-62R. https://doi.org/10.1093/glycob/cwh122.

[2] Nareddy, P.K., Bobbili, K.B., Swamy, M.J., Purification, physico-chemical characterization and thermodynamics of chitooligosaccharide binding to cucumber (*Cucumis sativus*) phloem lectin, *International Journal of Biological Macromolecules*, 95 (2017), 910-919. https://doi.org/10.1016/j.ijbiomac.2016.10.078.

[3] Ogawa, H., Date, K., The "white kidney bean incident" in Japan, Lectins, Springer, (2014), 39-45.

[4] Vasconcelos, I.M., Oliveira, J.T.A., Antinutritional properties of plant lectins, *Toxicon*, 44 (2004), 385-403. https://doi.org/10.1016/j.toxicon.2004.05.005.

[5] Van Holle, S., Van Damme, E.J., Signaling through plant lectins: modulation of plant immunity and beyond, *Biochemical Society Transactions*, 46 (2018), 217-233. https://doi.org/10.1042/BST20170371.

[6] Brown, G.D., Willment, J.A., Whitehead, L., C-type lectins in immunity and homeostasis, *Nature Reviews Immunology*, 18 (2018), 374-389. https://doi.org/10.1038/s41577-018-0004-8

[7] Rüdiger, H., Gabius, H.-J., Plant lectins: occurrence, biochemistry, functions and applications, *Glycoconjugate journal*, 18 (2001), 589-613. https://doi.org/10.1023/A:1020687518999.

[8] Bittel, P., Robatzek, S., Microbe-associated molecular patterns (MAMPs) probe plant immunity, *Current Opinion in Plant Biology*, 10 (2007), 335-341. https://doi.org/10.1016/j.pbi.2007.04.021.

[9] Vlot, A.C., Dempsey, M. A., Klessig, D.F., Salicylic acid, a multifaceted hormone to combat disease, *Annual Review of Phytopathology*, 47 (2009), 177-206. https://doi.org/10.1146/annurev.phyto.050908.135202.

[10] Armijo, G., Salinas, P., Monteoliva, M.I., Seguel, A., García, C., Villarroel-Candia, E., Song W., van der Krol, A.R., Álvarez, M.E., Holuigue, L., A salicylic acid–induced lectin-like protein plays a positive role in the effector-triggered immunity response of *Arabidopsis thaliana* to *Pseudomonas syringae* Avr-Rpm1, Molecular *Plant-Microbe Interactions*, 26 (2013), 1395-1406. https://doi.org/10.1094/MPMI-02-13-0044-R.

[11] Luo, X., Xu, N., Huang, J., Gao, F., Zou,H., Boudsocq, M., Coaker, G., Liu, J., A lectin receptor-like kinase mediates pattern-triggered salicylic acid signaling, *Plant Physiology*, 174 (2017), 2501-2514. https://doi.org/10.1104/pp.17.00404.

[12] He, S., Shi, J., Walid, E. , Zhang, H., Ma, Y., Xue, S.J., Reverse micellar extraction of lectin from black turtle bean (*Phaseolus vulgaris*): Optimisation of extraction conditions by response surface methodology, *Food Chemistry*, 166 (2015), 93-100. https://doi.org/10.1016/j.foodchem.2014.05.156.

[13] Mazalovska, M., Kouokam, J.C., Lectins as promising therapeutics for the prevention and treatment of HIV and other potential coinfections, *Biomed Research International*, (2018). https://doi.org/10.1155/2018/3750646.

[14] Fitches, E., Woodhouse, S.D., Edwards, J.P., Gatehouse, J.A., In vitro and in vivo binding of snowdrop (*Galanthus nivalis* agglutinin; GNA) and jackbean (Canavalia ensiformis; Con A) lectins within tomato moth (Lacanobia oleracea) larvae; mechanisms of insecticidal action, *Journal of Insect Physiology*, 47 (2001), 777-787. https://doi.org/10.1016/S0022-1910(01)00068-3

[15] Miyake, K., Tanaka,T., McNeil, P.L., Lectin-based food poisoning: a new mechanism of protein toxicity, *PloS One*, 2 (2007), e687. https://doi.org/10.1371/journal.pone.0000687

[16] Menard, S., Cerf-Bensussan, N., Heyman, M., Multiple facets of intestinal permeability and epithelial handling of dietary antigens, *Mucosal Immunology*, 3 (2010), 247-259. https://doi.org/10.1038/mi.2010.5

[17] Lehr, C.-M., Bioadhesion technologies for the delivery of peptide and protein drugs to the gastrointestinal tract, *Critical Reviews In Therapeutic Drug Carrier Systems*, 11 (1994), 119-160.

[18] Zhang, J., Shi, J., Ilic, S., Xue, Jun., Kakuda, Y., Biological properties and characterization of lectin from red kidney bean (*Phaseolus vulgaris*), *Food Reviews International*, 25 (2008), 12-27. https://doi.org/10.1080/87559120802458115

[19] Gabius, H.-J., André, S., Jiménez-Barbero, J., Romero, A., Solís, D., From lectin structure to functional glycomics: principles of the sugar code, *Trends in biochemical sciences*, 36 (2011), 298-313. https://doi.org/10.1016/j.tibs.2011.01.005

[20] De Hoff, P.L., Brill, L.M., Hirsch, A.M., Plant lectins: the ties that bind in root symbiosis and plant defense, *Molecular Genetics and Genomics*, 282 (2009), 1-15. https://doi.org/10.1007/s00438-009-0460-8

[21] Van der Poel, A., Effect of processing on antinutritional factors and protein nutritional value of dry beans (*Phaseolus vulgaris* L.). A review, *Animal Feed Science and Technology*, 29 (1990), 179-208.

[22] Büyük, İ., Okay, A. Aksoy, T., Aras, S., The NIN-like Protein (NLP) Family in Common Bean: Genome-Wide Identificationn Evolution and Expression Analysis, *Communications Faculty of Sciences University of Ankara Series C Biology*, 30 (2021), 58-84. https://dergipark.org.tr/en/pub/communc/issue/58411/869501.

[23] Hirsch, M., Phillips, S., Solnik, C., Black, P., Schwartz, R., Carpenter, C., Activation of leukemia viruses by graft-versus-host and mixed lymphocyte reactions in vitro, *Proceedings of the National Academy of Sciences*, 69 (1972), 1069-1072.

[24] Koyanagi, Y., Miles, S., Mitsuyasu, R.T., Merrill, J.E., Vinters, H.V., Chen, I., Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms, *Science*, 236 (1987), 819-822.

[25] Lajolo, F.M., Genovese M.I., Nutritional significance of lectins and enzyme inhibitors from legumes, *Journal of Agricultural and Food Chemistry*, 50 (2002), 6592-6598. https://doi.org/10.1021/jf020191k.

[26] Woodley, J.F., Lectins for gastrointestinal targeting–15 years on, *Journal of Drug Targeting*, 7 (1999), 325-333. https://doi.org/10.3109/10611869909085515.

[27] Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes,R.D., Fazo, J., Mitros,T., Dirks, W., Hellsten,U., Putnam, N., Phytozome: a comparative platform for green plant genomics, *Nucleic Acids Research*, 40 (2012), D1178-D1186. https://doi.org/10.1093/nar/gkr944.

[28] Guo, A., Zhu, Q., Chen, X., Luo, J., GSDS: a gene structure display server, *Yi chuan= Hereditas*, 29 (2007), 1023-1026. https://doi.org/10.1360/yc-007-1023

[29] Voorrips, R., MapChart: software for the graphical presentation of linkage maps and QTLs, *Journal of Heredity*, 93 (2002), 77-78. https://doi.org/10.1093/jhered/93.1.77

[30] Bailey, T.L., Williams, N., Misleh. C., Li,W.W., MEME: discovering and analyzing DNA and protein sequence motifs, *Nucleic Acids Research*, 34 (2006), W369-W373. https://doi.org/10.1093/nar/gkl198

[31] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Molecular Biology and Evolution*, 28 (2011), 2731-2739. https://doi.org/10.1093/molbev/msr121

[32] Letunic, I., Bork, P., Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy, *Nucleic Acids Research*, 39 (2011), W475-W478. https://doi.org/10.1093/nar/gkr201

[33] Zhang Y., miRU: an automated plant miRNA target prediction server, Nucleic acids research, 33 (2005), W701-W704. https://doi.org/10.1093/nar/gki383

[34] Suyama, M., Torrents, D., Bork, P., PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments, *Nucleic Acids Research*, 34 (2006), W609-W612. https://doi.org/10.1093/nar/gkl315

[35] Yang, Z., Nielsen, R., Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models, *Molecular Biology and Evolution*, 17 (2000), 32-43. https://doi.org/10.1093/oxfordjournals.molbev.a026236

[36] Hiz, M.C., Canher, B., Niron, H., Turet, M., Transcriptome analysis of salt tolerant common bean (*Phaseolus vulgaris* L.) under saline conditions, *PloS One*, 9 (2014), e92598. https://doi.org/10.1371/journal.pone.0092598

[37] Büyük, İ., Inal, B., Ilhan, E., Tanriseven, M., Aras, S., Erayman, M., Genome-wide identification of salinity responsive HSP70 s in common bean, *Molecular Biology Reports*, 43 (2016), 1251-1266. https://doi.org/10.1007/s11033-016-4057-0

[38] Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., The protein data bank, *Nucleic Acids Research*, 28 (2000), 235-242. https://doi.org/10.1093/nar/28.1.235

[39] Kelley, L.A., Sternberg, M.J., Protein structure prediction on the Web: a case study using the Phyre server, *Nature Protocols*, 4 (2009), 363. https://doi.org/10.1038/nprot.2009.2.

[40] Jiang, S.-Y., Ma, Z., Ramachandran, S., Evolutionary history and stress regulation of the lectin superfamily in higher plants, *BMC Evolutionary Biology*, 10 (2010), 1-24. https://doi.org/10.1186/1471-2148-10-79.

[41] Kouno, T., Watanabe, N., Sakai, N., Nakamura, T., Nabeshima, Y., Morita, M., Mizuguchi, M., Aizawa, T., Demura, M., Imanaka, T., The structure of *Physarum polycephalum* hemagglutinin I suggests a minimal carbohydrate recognition domain of legume lectin fold, *Journal of Molecular Biology*, 405 (2011), 560-569. https://doi.org/10.1016/j.jmb.2010.11.024

[42] Bouwmeester,K., Govers, F., *Arabidopsis* L-type lectin receptor kinases: phylogeny, classification, and expression profiles, *Journal of Experimental Botany*, 60 (2009), 4383-4396. https://doi.org/10.1093/jxb/erp277

[43] Lis, H., Sharon, N., Lectins: carbohydrate-specific proteins that mediate cellular recognition, *Chemical Reviews*, 98 (1998), 637-674. https://doi.org/10.1021/cr940413g .

[44] Gegg, C.V., Roberts, D.D., Segel,I.H., Etzler, M.E., Characterization of the adenine binding sites of two Dolichos biflorus lectins, *Biochemistry*, 31 (1992), 6938-6942. https://doi.org/10.1021/bi00145a011

[45] Lv, D., Wang, G., Xiong, L.-R., Sun, J.-X., Chen, Y., Guo, C.-L., Yu, Y., He, H.-L., Cai, R., Pan, J.-S., Genome-wide identification and characterization of lectin receptor-like kinase gene family in cucumber and expression profiling analysis under different treatments, *Genes*, 11 (2020), 1032. https://doi.org/10.3390/genes11091032

[46] Vaid, N., Pandey, P.K., Tuteja, N., Genome-wide analysis of lectin receptor-like kinase family from Arabidopsis and rice, *Plant Molecular Biology*, 80 (2012), 365-388. https://doi.org/10.1007/s11103-012-9952-8

[47] Wang, Z., Ren, H., Xu, F., Lu, G., Cheng, W., Que, Y., Xu, L., Genome-Wide Characterization of Lectin Receptor Kinases in *Saccharum spontaneum* L. and Their Responses to Stagonospora tainanensis Infection, *Plants*, 10 (2021), 322.

[48] Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M.R., Appel R.D., Bairoch, A., Protein identification and analysis tools on the ExPASy server. *The proteomics protocols handbook*, (2005), 571-607. https://doi.org/10.1385/1-59259-890-0:571

[49] Dang, L., Van Damme, E.J., Genome-wide identification and domain organization of lectin domains in cucumber, *Plant Physiology and Biochemistry*, 108 (2016), 165-176. https://doi.org/10.1016/j.plaphy.2016.07.009

[50] Singh, P., Mishra A.K., Singh C.M., Genome-wide identification and characterization of Lectin receptor-like kinase (LecRLK) genes in mungbean (*Vigna radiata* L. Wilczek), Journal of Applied Genetics, (2021), 1-12. https://doi.org/10.1007/s13353-021-00613-8

[51] Wang, Y., Wang, X., Paterson, A.H., Genome and gene duplications and gene expression divergence: a view from plants, *Annals of the New York Academy of Sciences*, 1256 (2012), 1-14. https://doi.org/10.1111/j.1749-6632.2011.06384.x

[52] Le Hir, H., Nott, A., Moore, M.J., How introns influence and enhance eukaryotic gene expression, *Trends in Biochemical Sciences*, 28 (2003), 215-220. https://doi.org/10.1016/S0968-0004(03)00052-5

[53] Rose, A., Intron-mediated regulation of gene expression, *Nuclear pre-mRNA Processing in Plants*, (2008), 277-290. https://doi.org/10.1007/978-3-540-76776-3_15

[54] Liu, P.-L., Huang, Y., Shi, P.-H.,Yu,M., Xie, J.-B., Xie, L., Duplication and diversification of lectin receptor-like kinases (LecRLK) genes in soybean, Scientific Reports, 8 (2018), 1-14. https://doi.org/10.1038/s41598-018-24266-6

[55] Carmel, L., Wolf, Y.I., Rogozin, I.B., Koonin, E.V., Three distinct modes of intron dynamics in the evolution of eukaryotes, *Genome Research*, 17 (2007), 1034-1044. http://www.genome.org/cgi/doi/10.1101/gr.6438607

[56] Fierro-Monti, I., Mathews, M.B., Proteins binding to duplexed RNA: one motif, multiple functions, *Trends in Biochemical Sciences*, 25 (2000), 241-246. https://doi.org/10.1016/S0968-0004(00)01580-2

[57] Richardson, J.S., Protein anatomy, Adv. *Protein Chem*, 34 (1981), 339. https://doi.org/10.1016/S0065-3233(08)60520-3

[58] Orengo, C.A., Jones, D.T., Thornton, J.M., Protein superfamilles and domain superfolds, *Nature*, 372 (1994), 631-634. https://doi.org/10.1038/372631a0

[59] Williams, A., Westhead, D., Sequence relationships in the legume lectin fold and other jelly rolls, *Protein Engineering*, 15 (2002), 771-774. https://doi.org/10.1093/protein/15.10.771

[60] Yamaguchi-Shinozaki, K., Shinozaki, K., Organization of cis-acting regulatory elements in osmotic-and cold-stress-responsive promoters, *Trends in Plant Science*, 10 (2005), 88-94. https://doi.org/10.1016/j.tplants.2004.12.012

[61] Lu, X.-Y., Huang, X.-L., Plant miRNAs and abiotic stress responses, *Biochemical and Biophysical Research Communications*, 368 (2008), 458-462. https://doi.org/10.1016/j.bbrc.2008.02.007

[62] Khraiwesh, B., Zhu, J.-K., Zhu, J., Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants, *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, 1819 (2012), 137-148. https://doi.org/10.1016/j.bbagrm.2011.05.001

[63] Tang, F., Chu, L., Shu, W., He, X., Wang, L., Lu, M., Selection and validation of reference genes for quantitative expression analysis of miRNAs and mRNAs in Poplar, *Plant Methods*, 41 (2019), 28-37. https://doi.org/10.1186/s13007-019-0420-1

[64] Matthewman, C.A., Kawashima, C.G., Húska, D., Csorba, T., Dalmay, T., Kopriva, S., miR395 is a general component of the sulfate assimilation regulatory network in *Arabidopsis*, *FEBS Letters*, 586 (2012), 3242-3248. https://doi.org/10.1016/j.febslet.2012.06.044

[65] Jones-Rhoades, M.W., Bartel, D.P., Computational identification of plant microRNAs and their targets, including a stress-induced miRNA, *Molecular Cell*, 14 (2004), 787-799. https://doi.org/10.1016/j.molcel.2004.05.027

[66] Cao, C., Long, R., Zhang, T., Kang, J., Wang, Z., Wang, P., Sun, H., Yu, J., Yang, Q., Genome-wide identification of microRNAs in response to salt/alkali stress in *Medicago truncatula* through high-throughput sequencing, *International Journal of Molecular Sciences*, 19 (2018), 4076. https://doi.org/10.3390/ijms19124076

[67] Natarajan, B., Kalsi, H.S., Godbole, P., Malankar, N., Thiagarayaselvam,A., Siddappa, S., Thulasiram, H.V., Chakrabarti, S.K., Banerjee, A.K., MiRNA160 is associated with local defense and systemic acquired resistance against *Phytophthora infestans* infection in potato, *Journal of Experimental Botany*, 69 (2018), 2023-2036. https://doi.org/10.1093/jxb/ery025

[68] Li, W., Wang, T., Zhang, Y., Li, Y., Overexpression of soybean miR172c confers tolerance to water deficit and salt stress, but increases ABA sensitivity in transgenic *Arabidopsis thaliana*, *Journal of Experimental Botany*, 67 (2016), 175-194. https://doi.org/10.1093/jxb/erv450

[69] Shao, H.-B., Chu, L.-Y., Jaleel, C.A., Zhao, C.-X., Water-deficit stress-induced anatomical changes in higher plants, *Comptes Rendus Biologies*, 331 (2008), 215-225. https://doi.org/10.1016/j.crvi.2008.01.002

[70] Zapata, P.J., Serrano, M., Pretel, M.T., Botella, M.A., Changes in free polyamine concentration induced by salt stress in seedlings of different species, *Plant Growth Regulation*, 56 (2008), 167-177. https://doi.org/10.1007/s10725-008-9298-z

https://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 1, Pages 26-38 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1069705

Received by the Editors: February 7, 2022; Accepted: April 23, 2022

ANTIOXIDANT ACTIVITIES OF TURKISH EXTRA VIRGIN OLIVE OILS

SAKOU SEYDOU[1], GÜL KOCA[1], OKAN ONAR[2], ÖZLEM YILDIRIM[2]

[1]Ankara University, Graduate School of Natural and Applied Sciences, Department of Biology, Ankara 06100, TURKEY
[2]Ankara University, Faculty of Science, Department of Biology, Ankara 06100, TURKEY

ABSTRACT. Extra virgin olive oil is the highest grade of virgin olive oil derived by cold mechanical extraction without the use of solvents or refining methods. These olive oils are known for their composition in phenolic compounds that have antioxidant properties. This study aims to determine the total phenolic and flavonoid compounds and antioxidant activities of four Turkish extra virgin olive oil samples: Kilis yağlık, İzmir sofralık, Ayvalık, and Tavşan yüreği. The highest sample concentration used for the experiments was 4 mg/ml while 1 mg/mL was used for ABTS radical scavenging assay. The lowest total phenolic and flavonoid content was observed in Tavşan yüreği sample. All extra virgin olive oil samples showed scavenging activity against DPPH and ABTS free radicals. Extra virgin olive oil samples with high phenolic and flavonoid content presented more effective radical scavenging activity with low IC_{50} values. This study provides information about the phenolic content and antioxidant activities of four important Turkish olive oil samples.

1. INTRODUCTION

Initially consumed in particular by Mediterranean populations, olive oil is increasingly gaining ground on the world market because of its beneficial effects on health. These beneficial effects on health mean that outside of Mediterranean countries, olive oil is also consumed in other regions of the world [1]. Mediterranean countries represent 65% of world olive oil production and 43% of world consumption [2]. Turkey occupies a significant place among the world's major

Keywords. *Extra virgin olive oil, total phenolic content, total flavonoid, antioxidant activity*

seydsak@gmail.com; gulkoca1108@gmail.com; okanonar@gmail.com; yildirim@science.ankara.edu.tr -Corresponding author
0000-0003-3485-2845; 0000-0001-8724-8954; 0000-0003-4197-527X; 0000-0003-1018-0335

producers. Turkey is the fifth-largest olive oil producer in the world after Spain, Italy, Greece, and Tunisia [3]. In 2018, olive oil production in Turkey amounted to 206,300 tonnes [4]. Turkey is very rich in olive oil diversity. Most of Turkey's olive oil varieties are found in the Aegean region, which is responsible for around 80% of production [5]. Ayvalık and Memecik varieties, which are of great economic importance, are also found in this region.

Olive oil is recognized for its composition rich in bioactive substances such as phenolic compounds. This richness in phenolic compounds and unsaturated fatty acids distinguishes it from other oils and gives it beneficial effects for health [6]. It has been determined that various polyphenolic compounds in virgin oil are responsible for the beneficial effects. These compounds are phenolic alcohols (hydroxytyrosol and tyrosol), phenolic acids (vanillic acid and p-cumaric acid), secoiridoids (oleuropein aglycones, ligstroside aglycone, and oleanolic acid, etc), and flavonoids (luteolin and apigenin), and other minor compounds [7-8]. Extra virgin olive oil is also rich in oleic acid, linoleic acid, and palmitoleic acid, which are believed to be associated with the prevention of cardiovascular disease and various types of cancer. The most important of these monounsaturated fatty acids is oleic acid, its content varies between 68 and 82% of the fatty acids contained in olive oil [9-10]. Oleic acid plays also a significant role in the biological effects of olive oil.

The phenolic compounds in extra virgin oil have antioxidant properties. Antioxidants prevent the oxidation of macromolecules (DNA, RNA, proteins, and lipids) that play an important role in cell metabolism. The phenolic compounds and fatty acids contained in extra virgin olive oil can modulate the secretion of pro-inflammatory cytokines and certain markers of inflammation [11-12]. Extra virgin olive oil due to its composition rich in antioxidant compounds may prevent the occurrence of various types of cancers such as intestinal cancer, prostate cancer, breast cancer, and various neurological disorders as well as cardiovascular diseases [13-14-15-16].

The composition of extra virgin oil can be influenced by various factors such as geographic location, soil types, climate, rainfall, extraction method but also storage [17-18-19]. The stage of ripening is also another factor, which can affect extra virgin olive oil composition. Olive oil extracted from immature and moderately mature fruits contains more phenolic compounds than oil obtained from ripe fruits [20-21]. During the ripening, a large proportion of fatty acids depends on the degree of hydrolysis of triacylglycerol. The ripening of the fruits causes a decrease in the level

of hydroxytyrosol, total phenolic compounds and flavonoids, and the rate of rutin thus influencing the biological activities of extra virgin olive oil [22].

To our knowledge, there is not enough data on Turkish extra virgin olive oil's chemical characterization and antioxidant properties. Sarı Hasebi, Gemlik, and Halhali olive oils, which are cultivated in the Mediterranean region of Turkey have been studied and showed that the type of variety has a significant effect on the composition of fatty acids, sterols, and total phenolics [23]. Therefore, this study aims to determine the total phenolics and flavonoids content and the antioxidant activities of Izmir sofralık, Ayvalık, Tavşan yüreği and Kilis yağlık extra virgin olive oil.

2. MATERIALS AND METHODS

2.1. Extra virgin olive oil samples

Extra virgin olive oil samples were obtained from different regions of Turkey**.** The Ayvalık sample was obtained from Buta Assos (Ayvalık), Izmir sofralık from Hedef Ziraat (Izmir), Kilis yağlık from Fersis (Kilis) and Tavşan yüreği from Zeytin Akademi (Antalya).

2.2. Extraction of phenolic fraction

The extraction of phenolic fractions was obtained following the procedure of Reboredo-Rodríguez et al. [24]. Briefly, 10 g of each olive oil sample was weighed into a 15 ml tube, and 5 mL of MeOH : H_2O (80:20, v/v) was added and shaken vigorously for 3 minutes. Finally, the tube was centrifuged at 4800 rpm for 25 minutes, and the MeOH: H_2O phase was collected. This process was repeated three times for each sample. Then three methanolic phases were collected and evaporated using a rotary evaporator. The dry extracts obtained after evaporation were weighed and dissolved in methanol. The resulting stock concentrations were stored at -20 °C until later use.

2.3. Total phenolic content

The total phenolic content (TPC) of the olive oil extracts was determined using the Folin-Ciocalteau method [25]. Briefly, 100 µL of extra virgin olive oil sample was added to 100 µL of Folin-Ciocalteau reagent, the mixture was vortexed for 3 minutes. Then 500 µl of 6% (v/v) sodium carbonate was added. After 5 minutes, the reaction mixture was stirred and diluted to 2 ml with distilled water. The absorbance of the mixture was measured after 2 hours of incubation at 725 nm. Total phenolic compounds found in extra virgin olive oil samples were determined using the

calibration curve of standard gallic acid. The results obtained are expressed as mg gallic acid equivalent per gram (mg GAE/g).

2.4. Total flavonoid content

The total flavonoid content (TFC) was determined according to the method described by Huang et al. [22]. Briefly, 100 µL of $NaNO_2$ (5%) and 100 µL of $AlCl_3$ (10%) were added to the 100 µL of virgin olive oil sample or quercetin standard at different concentrations. After 10 minutes of incubation, 2 mL of NaOH (4%) was added to the mixture. The solution was mixed and absorbance was measured at 415 nm against the prepared blank reagent. Total flavonoid contents are expressed as mg quercetin equivalent per gram of dry extract (mg QE/g).

2.5. DPPH radical scavenging activity

The radical scavenging activity of extra virgin olive oil was determined using the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay method [26]. The DPPH stock solution was diluted with ethanol to an absorbance of 1.400 at 517 nm before analysis. In each test tube, 100 µL of different concentrations of the sample or gallic acid standard solution were mixed with 1.4 mL of DPPH and shaken for 1 minute. After 30 minutes of incubation, the absorbance of each solution was measured at 517 nm. The experiments were repeated three times. The radical scavenging activity (RSA) was calculated according to the following formula.

$$\% \text{ RSA} = [(A_{control}-A_{sample})/A_{control}]*100$$

2.6. ABTS radical scavenging activity

ABTS radical scavenging activity was determined according to the method described by Wu et al. [27]. ABTS was mixed with ammonium persulfate solution in a 1:1 volume ratio and incubated at room temperature for 12~16 hours in the dark to form a radical cation stock solution (ABTS +). ABTS + stock solution was diluted with ethanol to 0.700 (± 0.020) absorbance at 734 nm before analysis. Next, 20 µL of extra virgin olive oil sample of different concentrations was mixed with 980 µL of ABTS + stock solution, and absorbance at 734 nm will be recorded after 10 minutes. Trolox was used as standard. The experiments were repeated three times. The radical scavenging activity (RSA) was calculated according to the following formula.

$$\% \text{ RSA} = [(A_{control}-A_{sample})/A_{control}]*100$$

2.7. Statistical analysis

All the results were expressed as mean ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) and Tukey tests were performed to compare mean values ($p < 0.05$). Each experiment was repeated at least three times ($n \geq 3$). Statistical analyzes were conducted using GraphPad Prism 8.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoids content

Phenolic compounds are known to be a major part of vegetable oils after fatty acids components. The type of olive oil variety or the stage of ripening can influence the total phenolic content [21]. These phenolic compounds apart from their antioxidant properties are responsible for the flavor of olive oils. The determination of total phenolic compounds from olive oil samples revealed a significant difference between varieties. The differences observed in the composition in total phenolic compounds of the different varieties of extra virgin olive oil can be due to the genetic profile of the variety of extra olive oil and to agro-environmental factors [28-29]. Other factors such as the stage of ripening and method of extraction can affect total phenolic content in extra virgin olive oil [30]. Moreover, other studies have shown that phenolic compounds are a real index in the discrimination of olive oil varieties obtained from different regions. Turkish olive oil varieties are generally rich in phenolic compounds. A study taken out on Arbequina, a variety of olive oil native to the Aegean region showed that the Turkish variety is richer in phenolic compounds than the Arbequina variety grown in Tunisia, Italy, and Spain [31]. Thus, the same variety cultivated under different agronomic conditions may present differences in terms of phenolic compounds. In our study, the highest values of total phenolic compounds were observed in Kilis yağlık and Izmir sofralık samples, while Ayvalık and Tavşan yüreği contain relatively low levels of total phenolics (Table 1). TPC were calculated as 180.12 and 157.76 mg GAE/g for Kilis yağlık and Izmir sofralık, respectively. Ayvalık and Tavşan yüreği were found to contain fewer phenolic compounds than the other two samples. The Izmir sofralık and Ayvalık extra virgin olive oil samples originate from the Aegean region. However, Izmir sofralık contains more total phenolic compounds than the Ayvalık, which originates from the north side of the Aegean region. According to a study carried out on the Ayvalık olive oil, the ripening period and the altitude can influence the chemical composition of this variety in particular its content of total phenolic compounds [32]. Therefore, within the same region factors such as the nature of the soil, precipitation can influence the composition of extra virgin olive oil. The Tavşan yüreği originating

from the Mediterranean region showed the lowest values in terms of total phenolic and flavonoid content. The stage of maturation strongly influences the content of phenolic or flavonoids. In fact, within the same variety, the content of phenolic and flavonoid compounds can differ depending on the stage of maturation [33]. A previous study carried out on the Cobrançosa and Picual olive oil cultivars showed that the green olives, presented total phenolic values of 50.1 and 43.5 mg/kg respectively, for the semi-mature of 35.3 and 28.8 mg/kg and mature fruits values of 34.6 and 31.4 mg/kg [34].

The flavonoid content can also be affected by factors such as temperature, precipitation, but also by the stage of maturity. It has been reported that the flavonoid content is found to be high in olive oil obtained from ripe fruits [35]. The content of individual flavonoids such as apigenin and luteolin increases with the degree of maturity [36-37]. It has also been shown that the highest flavonoid content is usually obtained from olives in regions with high rainfall [35]. Our results showed that the total flavonoid content of the extra virgin olive oil samples were also different from each other (Table 1). The observed differences are due to the type of olive oil variety and agro-ecological conditions [38]. Kilis yağlık showed the highest flavonoid content with 273.06 mg QE/g. Tavşan yüreği variety contains the lowest total flavonoid content. Izmir sofralık showed a good result in terms of total phenolic content than Ayvalık, while the contrary is observed in the result of total flavonoid content. This could be due to the advanced stage of maturity of the Ayvalık extra virgin olive sample resulting in a decrease in the content of total phenolic compounds and an increase in its content of flavonoids. According to other studies, the content of phenolic compounds may depend on the level of glucosides and the activity of β-glucosidase in olive fruits. This enzyme is believed to be responsible for the hydrolysis of phenolic glucosides and the oxidation of phenolic compounds [39-40].

TABLE 1. Total phenolic and flavonoid contents of extra virgin olive oils samples

Olive oil	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Ayvalık	39.60 ± 1.10	176.82 ± 10.14
Izmir sofralık	180.12 ± 1.04	128.29 ± 6.64
Tavşan yüreği	33.13 ± 5.88	45.44 ± 5.02
Kilis yağlık	157.76 ± 1.3	273.06 ± 5.22

TPC was calculated using linear regression of gallic acid ($R^2 = 0.9998$) and expressed as gallic acid equivalent in milligram per gram of dry sample weight (mg GAE/g of dry sample weight). TFC was calculated using linear regression of quercetin (R^2 =

0.9978) and expressed as quercetin equivalent in milligram per gram of dry sample weight (mg QE/g of dry sample weight).

3.2. Radical scavenging activities of extra virgin olive oils

Extra virgin olive oil is known for its richness in phenolic compounds and it is credited with many virtues, in particular its antioxidant properties. DPPH and ABTS radical reducing activity is a widely used method to assess the antioxidant activity of foods. These methods were used to evaluate the antioxidant capacity of the extra virgin olive oil samples. All four extra virgin olive oil samples showed effective DPPH and ABTS radical scavenging activity. At the maximum concentration of 4 mg/mL, the samples Kilis yağlık, Izmir sofralık, Ayvalık, and Tavşan yüreği showed percentages of inhibition of the DPPH radical respectively of 94%, 92%, 91%, and 28% while the standard gallic acid solution showed 93% (Figure 1). It was observed that the radical scavenging activity of extra virgin olive oils samples is dose-dependent. Tavşan yüreği extra virgin olive oil sample showed very low antioxidant activity compared to the others. The antioxidant activity of olive oils depends on the type of variety, location, or degree of ripening [41]. As shown in table 2, Kilis yağlık showed a good DPPH and ABTS radıcals scavenging activity compared to the three other samples. At maximum concentration, the ABTS radical scavenging activity was 81%, 68%, 50% and 36% respectively for Kilis yağlık, Izmir sofralık, Tavşan yüreği and Ayvalık samples (Figure 2). Taken together, Tavşan yüreği showed the weakest DPPH radical scavenging activity among extra virgin olive oil samples while on the ABTS radical scavenging activity the lowest value was obtained with Ayvalık sample. On the other hand, a small concentration range (0.125-1 mg/mL) is sufficient to scavenge the ABTS radical, while it is necessary to use a higher concentration range (0.25-4 mg/mL) for the DPPH radical. This implies that the ABTS radical is more sensitive and easily reducible by the antioxidant compounds contained in different extra virgin olive oils. At identical concentration, the values of ABTS radical scavenging is higher than those of the DPPH radical. Similar results have been obtained in studies on Halhali ve Nizip yağlık olive oils [20-42]. In addition, all varieties having exhibited high content of phenolic compounds and total flavonoids showed high scavenging radical activities, therefore, there is a correlation between the antioxidant activities and the content of phenolic compounds [20-41-43].

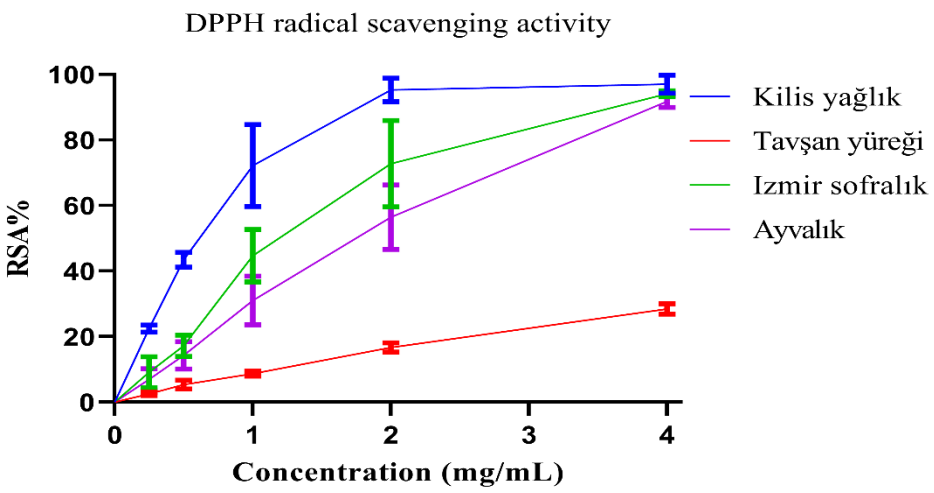

FIGURE 1. DPPH free radical scavenging activity of extra virgin olive oil samples.

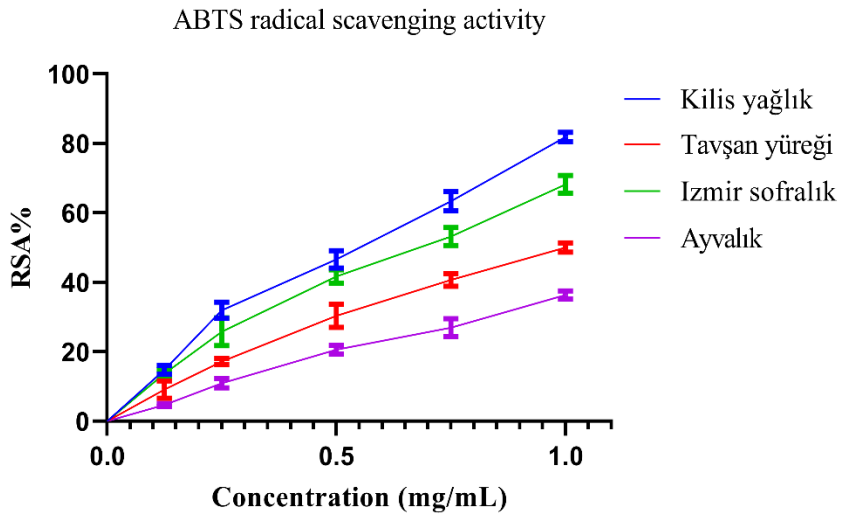

FIGURE 2. ABTS free radical scavenging activity of extra virgin olive oil samples.

TABLE 2. IC_{50} (mg/ml) values of DPPH and ABTS radical scavenging activity of extra virgin olive oil samples

	Kilis yağlık	**Tavşan yüreği**	**Izmir sofralık**	**Ayvalık**	**Gallic acid**	**Trolox**
DPPH	0.82 ±0.03	7.07 ± 0.41	1.20 ± 0.08	1.83 ± 0.04	0.009 ± 0.06	-
ABTS	0.56 ± 0.01	0.94 ±0.01	0.69 ± 0.03	1.40 ± 0.05	-	0.002 ± 0.01

4. CONCLUSION

This study focused on the determination of the total phenolic and flavonoid content as well as the antioxidant activities of extra virgin olive oils from Turkey. There is a significant difference between the total phenolic and flavonoid content of the extra virgin olive oil samples. Kilis yağlık sample, which was rich in total phenolic and flavonoid content (157.76 mg GAE/g and 273.06 mg QE/g respectively), also showed the highest DPPH and ABTS radical scavenging activity (94% and 81% respectively). Therefore, the type or the location of extra virgin olive oil affects their phenolic content and antioxidant activity. Our study provided preliminary information on the phenolic content and antioxidant properties of these different extra virgin olive oils. Further studies including the determination of phenolic composition using chromatographic methods and the stage of maturity could help to better understand the variation of phenolic compounds and the antioxidant properties of these four extra virgin olive oils.

Acknowledgements This work was financially supported by the Coordination of Scientific Research Projects of Ankara University (Research Project No: 21L0430007) in Turkey.

Author Contribution Statement SS- data collection, management and manuscript writing. GK- data collection, project development. OO- data analysis, manuscript editing. ÖY- project development, manuscript writing and manuscript editing. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest.

REFERENCES

[1] Dag, C., Demirtas, I., Ozdemir, I., Bekiroglu, S., Ertas, E., Biochemical characterization of turkish extra virgin olive oils from six different olive varieties of identical growing conditions. *JAOCS*, 92 (2015), 1349–1356. https://doi 10.1007/s11746-015-2691-7

[2] International Olive Oil Council (IOOC)., Trade standard applying to olive oil and olive-pomace oil. *COI/T15/NC No 3* (2015), http://www.internationaloliveoil.org/ Rev. 8 February

[3] Arslan, D., Schreiner, M., Chemical characteristics and antioxidant activity of olive oils from turkish varieties grown in Hatay province, *Sci. Hortic*, 144 (2012), 141–152. https://doi.org/10.1016/j.scienta.2012.07.006

[4] FAO. FAOSTAT Database. Available from: http://www.fao.org/faostat/en/#data (2018). Accessed: Aug. 12, 2018.

[5] Dağdelen, A., Identifying antioxidant and antimicrobial activities of the phenolic extracts and mineral contents of virgin olive oils (Olea europaea L. cv. Edincik Su) from Different Regions in Turkey, *Journal of Chemistry*, 9589763 (2016), 1-11. http://dx.doi.org/10.1155/2016/9589763

[6] Reboredo-Rodríguez, P., Figueiredo-González, M., González-Barreiro, C., Simal-Gándara, J., María D, S., Cancho-Grande, B., Giuseppe, F., State of the art on functional virgin olive oils enriched with bioactive compounds and their properties, *Int. J. Mol. Sci*, 18 (2017), 668. https://doi.org/10.3390/ijms18030668

[7] Servili, M., Sordini, B., Esposto, S., Urbani, S., Veneziani, G., Di Maio, I., Selvaggini, R.; Taticchi, A. Biological activities of phenolic compounds of extra virgin olive oil. *Antioxidants*, 3(2014), 1–23. https://doi.org/10.3390/antiox3010001

[8] Chiara, F., Susanna D, P., Alessandra, V., Laura, D, Marina, R,, Tommasangelo, P., Luigi, M., Laura de, G., Extraction, analysis, and antioxidant activity evaluation of phenolic compounds in different italian extra-virgin olive oils, *Molecules*, 23 (2018), 3249. https://doi.org/10.3390/molecules23123249

[9] Parkinson, L., Cicerale, S., The health benefiting mechanisms of virgin olive oil phenolic compounds, *Molecules*, 21 (2016), 1734. https://doi.org/10.3390/molecules21121734

[10] Lerma-Garcia, M.J. Characterization and authentication of olive and other vegetable oils: New analytical methods. Doctoral thesis, Universitat de Valencia, Spain, (2012), Chapter 1, p. 2. https://doi.org/10.1007/978-3-642-31418-8

[11] Casas, R., Sacanella, E., Urpí-Sardà, M., Chiva-Blanch, G., Ros, E., Martínez-González, M.A., Covas, M.I., Salas-Salvadó, J., Fiol, M., Arós, F., The effects of the mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease, *A randomized trial. PLoS ONE*, 9 (2014), e100084. https://doi.org/10.1371/journal.pone.0100084

[12] Schwingshackl, L., Christoph, M., Hoffmann, G., Effects of olive oil on markers of inflammation and endothelial function-a systematic review and meta-analysis, *Nutrients*, 7 (2015), 7651–7675. https://doi.org/10.3390/nu7095356

[13] Fuentes, E., Paucar, F., Tapia, F., Ortiz, J., Jimenez, P., Effect of the composition of extra virgin olive oils on the differentiation and antioxidant capacities of twelve

monovarietals, *Food Chemistry*, 243 (2018), 285–294. https://doi.org/10.1016/j.foodchem.2017.09.130

[14] Lozano-sanchez, J., segura-carretero, A., Menendez, J., Oliveras-ferraros C., Lorenzo C., Fernandez- Gutierrez, A., Prediction of extra virgin olive oil varieties through their phenolic profile. potential cytotoxic activity against human breast cancer Cells, *J. Agric. Food Chem*, 58(2010), 9942–9955. https://doi.org/10.1021/jf101502q

[15] Carolina E. Storniolo., Martínez-Hovelman, N., Martínez-Huelamo, M., Rosa, M. Lamuela-Raventos., Juan J. Moren, Extra virgin olive oil minor compounds modulate mitogenic action of oleic acid on colon cancer cell line, *J. Agric. Food Chem*, 67(2019), 11420−11427. https://doi.org/10.1021/acs.jafc.9b04816

[16] Roman, Jackson G, C., Reis, J., Roman, A.N .,Toledo, J,B ., Toledo, E., Extra-virgin olive oil for potential prevention of Alzheimer disease, *Revue Neurologique*, 175(2019) 705-723. https://doi.org/10.1016/j.neurol.2019.07.017

[17] Ghanbari, S, E., Sivri, O, D., Taha O, M., Ustune, N, F. Changes occurring in chemical composition and oxidative stability of virgin olive oil during storage, *Oilseeds & fats Crops and Lipids (OCL)*, 25(6) (2018), A602. https://doi.org/10.1051/ocl/2018052

[18] Deiana, P., Santona, M., Dettori, S., Culeddu, N., Dore, A., Molinu, M.G., Multivariate approach to assess the chemical composition of Italian virgin olive oils as a function of variety and harvest period, *Food Chemistry*, 300 (2019), 125243. https://doi.org/10.1016/j.foodchem.2019.125243

[19] Fuentes, E., Paucar, F., Tapia, F., Ortiz, J., Jimenez, P., Effect of the composition of extra virgin olive oils on the differentiation and antioxidant capacities of twelve monovarietals, *Food Chemistry*, 243 (2018), 285–294. https://doi.org/10.1016/j.foodchem.2017.09.130

[20] Amanpour, A., Kelebek, H., Selli, S., LC- DAD-ESI-MS/MS–based phenolic profiling and antioxidant activity in Turkish cv. Nizip Yaglik olive oils from different maturity olives, *J Mass Spectrom*. 54 (2019), 227–238. https://doi.org/10.1002/jms.4326

[21] Le Yu., Yongjin, W., Gangcheng Wu., Jun, J., Qingzhe, J., Xingguo, W., Chemical and volatile characteristics of olive oils extracted from four varieties grown in southwest of China, *Food Research International*, 140 (2021), 109987. https://doi.org/10.1016/j.foodres.2020.109987

[22] Huang, S., Wang, Q., Yaosong, W., Ruifeng, Y., Gongjian, F., Huang, M., Mary, A., and Physicochemical characterization and antioxidant activities of Chongqing virgin olive oil: effects of variety and ripening stage, *Journal of Food Measurement and Characterization* 14 (2020), 2010–2020. https://doi.org/10.1007/s11694-020-00447-8

[23] Yorulmaz, H, O., Konuskan, D, B., Antioxidant activity, sterol and fatty acid compositions of Turkish olive oils as an indicator of variety and ripening degree, *J Food Sci Technol (November 2017)* 54 (12) (2017), 4067–4077. https://doi.org/10.1007/s13197-017-2879-y

[24] Reboredo-Rodríguez, P., onzález-Barreiro, C., Cancho-Grande, B., Forbes-Hernández,T,Y., Gasparrini, M., Afrin, S., Cianciosi, D., Carrasco-Pancorbo, A., Simal-Gándara, J., Giampieri, F., Battino, M., Characterization of phenolic extracts from Brava extra virgin olive oils and their cytotoxic effects on MCF-7 breast cancer

cells, *Food and Chemical Toxicology*, 119 (2018), 73–85. https://doi.org/10.1016/j.fct.2018.05.026

[25] Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Enol. Vitic*, 16 (1965), 44–158.

[26] Blois, M.S. Antioxidant determinations by the use of a stable free radical, *Nature*, 181(1958), 1199- 1200. https://doi.org/10.1038/1811199a0

[27] Wu, Y., Wang, X., Xue, J., Enguo, F., Plant phenolics extraction from flos chrysanthemi: response surface methodology based optimization and the correlation between extracts and free radical scavenging activity, *Journal of Food Science,* Vol. 82 (2017), 11, 2017. https://doi.org/10.1111/1750-3841.13916

[28] Rotondi A., Magli M., Licausi E., Alfei B., Pannelli G Influence of genetic matrix on chemical and sensory profiles of Italian monovarietal olive oils, *Acta Hort (ISHS,)* 924 (2011), 401–406. https://doi.org/10.17660/ActaHortic.2011.924.50

[29] Baiano, A., Terracone, C., Viggiani, I., Alessandro, M., Nobile, D., Effects of cultivars and location on quality, phenolic content and antioxidant activity of extra-virgin olive oils, *J Am Oil Chem Soc*, 90 (2013), 103–111. https://doi.org/10.1007/s11746-012-2141-8

[30] López-Yerena, A., Ninot, A., Jiménez-Ruiz, N., Lozano-Castellón, J., Pérez, M., Escribano-Ferrer, E., Romero-Aroca, A., Rosa M., Lamuela-Raventós., Vallverdú-Queralt A. Influence of the ripening stage and extraction conditions on the phenolic fingerprint of 'corbella' extra-virgin olive oil, *Antioxidants*, 10 (2021), 877. https://doi.org/10.3390/antiox10060877

[31] Uluata, S., Altuntas, U., Beraat, Ö., Biochemical characterization of arbequina extra virgin olive oil produced in Turkey, *J Am Oil Chem Soc*, 93 (2016). 617–626. https://doi.org/10.1007/s11746-016-2811-z

[32] Usanmaz, S., öztürkler, F., Helvaci, M., Alas, T., Kahramanoğlu, I.. Aşkin, M, A., Effects of periods and altitudes on the phenolic compounds and oil contents of olives, cv. ayvalik. I*nternational Journal of Agriculture, Forestry and Life Science*, 2 (2) (2018), 32-39.

[33] Pedan, V., Martin P., Rohn, S., Nyfeler, M., Bongartz, A., Characterization of phenolic compounds and their contribution to sensory properties of olive oil, *Molecules*, 24 (2019), 2041. https://doi.org/10.3390/molecules24112041

[34] Gouvinhas, I., Machado, N., Sobreira, C., Domínguez-Perles, R., Gomes, S., Rosa, E., Ana I. R. N. A. Barros., Critical review on the significance of olive phytochemicals in plant physiology and human health, *Molecules*, 22 (2017), 1986. https://doi.org/10.3390/molecules22111986

[35] De Torres, A., Espínola, F., Moya, M., Alcalá, S., Alfonso, M. Vidal., Castro, E., Assessment of phenolic compounds in virgin olive oil by response surface methodology with particular focus on flavonoids and lignans, *WT - Food Science and Technology*, 90 (2018), 22–30. https://doi.org/10.1016/j.lwt.2017.12.003

[36] Benganan M., Bakhouche, A., Lozano-Sánchez, J., Amir, Y., Youyou, A., Segura-Carretero, A., Fernández-Gutiérrez, A., Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil, *Food Research International*, 54 (2013), 1868–1875. https://doi.org/10.1016/j.foodres.2013.08.037

[37] Lukic, I., Z˘anetic, M., Maja Jukic´ Špika., Lukic´, M., Koprivnjak, O., Bubola, K, B., Complex interactive effects of ripening degree, malaxation duration and temperature on Oblica cv. virgin olive oil phenols, volatiles and sensory quality, *Food Chemistry* 232 (2017), 610–620. https://doi.org/10.1016/j.foodchem.2017.04.047

[38] Rózanska, A., Russo, M., Cacciol, F., Salafia, F., Polkowska, Z., Dugo, P., Mondello, L., Concentration of potentially bioactive compounds in italian extra virgin olive oils from various sources by using lc-ms and multivariate data analysis, *Foods*, 9 (2020), 1120. https://doi.org/10.3390/foods9081120

[39] Hbaieb, R, H., Kotti, F., García-Rodríguez, R., Gargouri, M., Sanz, C., Ana G. Pérez., Monitoring endogenous enzymes during olive fruit ripening and storage: Correlation with virgin olive oil phenolic profiles, *Food Chemistry*, 174 (2015), 240–247. https://doi.org/10.1016/j.foodchem.2014.11.033

[40] Hbaieba R, H., Kotti, F., Valli, E., Bendini, A., Toschi, T., Gargouri, G., Effect of Tunisian olive ripeness on endogenous enzymes and virgin olive oil phenolic composition, *Journal of Food Composition and Analysis*, 62 (2017), 43–50. https://doi.org/10.1016/j.jfca.2017.04.016

[41] Yıldız, G., Uylaşer, V., Profile and total content of phenolics and antioxidant activity of commercial table olives from Turkey, *Quality Assurance and Safety of Crops & Foods*, 7 (5) (2015), 635-642. https://doi.org/10.3920/QAS2014.0430

[42] Kesen, S., Kelebek,H., Selli, S., Characterization of the volatile, phenolic and antioxidant properties of monovarietal olive oil obtained from cv. Halhali, *J Am Oil Chem Soc*, 90 (2013), 1685–1696. https://doi.org/10.1007/s11746-013-2327-8

[43] Kelebek, H., Kesen, S., Selli S., Comparative study of bioactive constituents in Turkish olive oils by LC-ESI/MS/MS, *Int J Food Prop*, 18 (10) (2015), 2231-2245. https://doi.org/10.1080/10942912.2014.968788

https://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 1, Pages 39-52 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1062833

Received by the Editors: January 25, 2022; Accepted: June 4, 2022

AN INVESTIGATION OF RABIES VIRUS EXISTENCE ON RODENTS BY USING NEW PCR PRIMER PAIRS

TUĞÇE GUNKAN[1], MEHMET ALI ÖKTEM[1], FERHAT MATUR[2]

[1] Zonguldak Bülent Ecevit University, Faculty of Arts and Science, Department of Biology, İncivez, Zonguldak, TURKEY
[2] Dokuz Eylül University, Faculty of Medicine, Department of Microbiology, Balçova, Izmir, TURKEY
[3] Dokuz Eylül University, Faculty of Science, Department of Biology, Buca, Izmir, TURKEY

ABSTRACT. Zoonotic diseases are the infections to be carried between human and other vertebrates. Rhabdoviruses belong to a virus family which could infect a wide range of host organism. It is important to find new molecular diagnostic tools and primers for the identification of the viruses to be able to make the molecular identification process faster and reveal new forms of the virus. Rodents are the primary mammals group that can uncontrollably go in and out from quarantine regions. Therefore, in this study, 242 *Apodemus* spp. (wood mouse) and *Myodes glareolus* (bank vole) specimens collected from 16 localities in province Zonguldak, Çaycuma district were used to scan brain tissues to determine RABV using hemi-nested PCR. Also, to determine RABV a new primer pairs were designed using already published sequences. According to results, eight specimens were showed positive bands for RABV. Those eight sequences blasted. But the sequences did not match according to the Blast result. The designed primer pairs provide positive bands on electrophoresis for positive control so that the primer pairs are new and can be used for following studies. With this study it was also tested whether rodents are the potential carriers for RABV since they are primarily prey source for carnivores, and domestic animals.

1. INTRODUCTION

Zoonotic diseases are naturally transmitted infections and diseases between humans and other vertebrates. The reservoir host animal acts as a source of infection because it carries the pathogen virus. Important zoonotic diseases include, Crimean-Congo Hemorrhagic Fever, Hantavirus infections, Rabies and Tularemia diseases [1]. There

Keyword and phrases. Rodents, rabies, heminested PCR, sequence analysis, Apodemus, Myodes, Turkey

tugceeceylan@gmail.com; mali.oktem@gmail.com;ferhat.matur@gmail.com-Corresponding author
0000-0003-1698-6620, 0000-0002-3185-8355, 0000-0001-9488-1408

are many animal groups with zoonotic agents including rodents. Rodents are extremely important in terms of zoonotic diseases because of their frequent contact with people and having the highest number of groups in mammals. For this reason, knowing the spread areas, the viruses they carry, the relationships with the living things around them and the diseases they spread are the primary topics for understanding of human infectious diseases.

Rhabdoviruses (Figure 1) are a large family of viruses that can infect a wide range of hosts [2,3,4]. More than 160 Rhabdovirus species have been isolated from plants, invertebrates and vertebrates to date with the discovery of new species [3,5,6,7]. Vesiculovirus, Lyssavirus and Ephemerrovirus species of rhabdoviruses cause various diseases in humans and animals [3,8]. Rabies is a disease with a history of 5000 years [4,9] showing a widespread distribution worldwide except Australia and some island countries [1,10]. Despite significant scientific developments, it still exists as a global disease [11]. There are two epidemiological forms of the disease, usually the urban form seen in domestic animals and the sylvatic form seen in wildlife [12]. It has been reported that 54% of animal rabies are caused by dogs, 42% by terrestrial wild animals and 4% by bats [13].

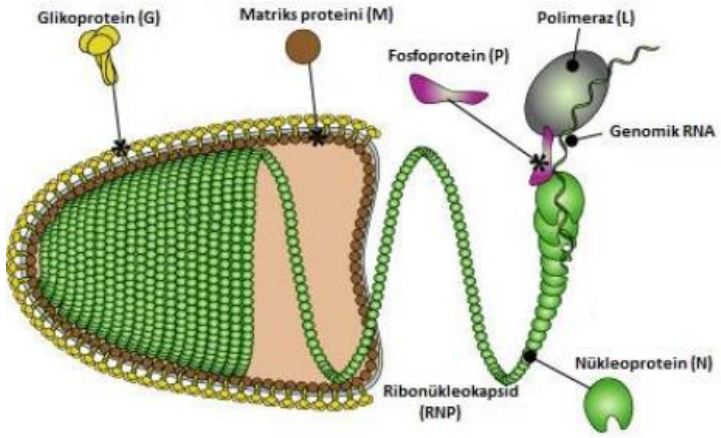

FIGURE 1. Schematic diagram of a Rhabdovirus (©ViralZone 2008, Swiss Institute of Bionformatics).

The screening and detection of viruses by molecular tools has been accepted and highly differentiated. Similarly, detection of rabies viruses can be done in different ways [14]. Each one has differences from the other. One of the most used is the real time PCR technique. In addition, considering that the endemic forms are quite high,

each primer designed for the diagnosis made by Real Time PCR will be important. Although this technique is reduced the time and the one of the most sensitive methods, false positives can also be observed due to the possibility of mixing with the genome of the host [14]. Serological assays are not suitable for routine testing used as identification. The test may lack sensitivity and specificity, and the interpretation of the test results may be difficult as the host response to infection varies substantially between individuals. As such, the negative predictive value of serological tests for rabies diagnosis is considered poor [14].

Small rodents, such as squirrels and mice, are rarely known to be natural reservoirs or vectors that cause human rabies [13,15]. Although rabies does not have a single host, the virus is spreading rapidly from one infected animal to another by inter-species interaction. Most mammalian species can be infected with rabies virus. According to Rabies surveillance data of the USA and Puerto Rico for the period 2005–2010 [16], 6,153 cases of rabies in animals and 2 in humans were reported [17,18]. Wild animals accounted for 92 % of reported cases in this study. Racoons were the most frequently reported rabid wildlife species (2,246 raccoons, 36.5 % of all rabid animals during 2010), followed by 1,448 skunks (23.5 %), 1,430 bats (23.2 %), 429 foxes (6.9 %), 303 cats (4.9 %), 71 cattle (1.1 %), and 69 dogs (1.1 %). Other wild animals included rodents and lagomorphs (1.8 %) [17]. Small rodents (squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, and mice) and lagomorphs (rabbits and hares) are rarely infected with rabies [16].

In another study, only one of the 57 *Apodemus agrarius* samples collected from the Zhejiang region of China was found positive for rabies [19] In addition, Özsoy et al. [20] with the suspicious animal bite Refik Saydam Hygiene Center of the rabies vaccination station, 92 (6%) of the patients reported to have applied to this unit [20].

A number of rabies cases have been seen with a high frequency in many villages in the province of Zonguldak Çaycuma district and they are originated from wild animals according to data from Zonguldak Provincial Directorate of Health. The Directorate have decided to take this region under quarantine several times and carnivores such as wolves and jackals were given as a source of rabies in the quarantine area. However, uncontrolled rodents' group in quarantine areas may have higher potential to be the bearer of many viruses due to their ease of travel between areas and also, they are primary food sources for carnivores. The existence of this uncontrolled group/animal might cause management studies fail.

The aim of this study is to prove that the rodents are the host for rabies and they should not be ignored during quarantine times, by screening for rabies virus on rodents and design new primer pairs to detect rabies in a host.

2. Materials and Methods

In this study, a total of 242 rodent specimens (197 *Apodemus* sp, 45 *Myodes* sp.) were collected from Çaycuma, Zonguldak province (Figure 2, Table 1). The individuals were anesthetized with ether. The research permission was taken from General Directorate of Nature Conservation and National Parks (01/12/2011- 83-703). After they were knocked out, they were killed quickly by breaking neck. For viral RNA study, the whole brain tissue of each animal is carefully removed from the foramen magnum with a pasteur pipette without damaging the skull [21]. Brain tissues were taken into the RNA preservation solution and stored at -80 ° C. RNA extraction was performed with Acide-guanidium-phenol-chloroform method [22].

Table 1. Distribution of species by locality.

	Species		
Locality	***Myodes glareolus***	***Apodemus* sp.**	**Total**
Akpınar	2	17	19
Temenler	13	7	20
Türkali	-	12	12
Derecikören	7	14	21
Çömlekçi	7	6	13
Yeşilköy	2	6	8
Çomranlı	-	1	1
Yeşilyayla	-	4	4
Y. İhsaniye	3	9	12
A. İhsaniye	1	5	6
Sazköy	2	18	20
Sarmaşık	-	27	27
Sarmasık countrysice	6	29	35
Olukyanı Village	-	11	11
Filyos	2	31	33
Total	45	197	242

FIGURE 2. Sample collected localities.

Two forward primers were designed, and Heaton et al. [23] reference primers were used (Table 2).

TABLO 2. Rabies (RABV) and rabies like viruses primers for (RRV) hnRT-PCR [23].

Primer	Sequence	Sense	Positon
JW12	ATGTAACACC(C/T)CTACAATTG	M	55-73
JW6 (DPL)	CAATTCGCACACATTTTGTG	G	660-641
JW6 (E)	CAGTTGGCACACATCTTGTG	G	660-641
JW6 (M)	CAGTTAGCGCACATCTTATG	G	660-641
JW10 (DLE2)	GTCATCAAAGTGTG(A/G)TGCTC	G	636-617
JW10 (ME1)	GTCATCAATGTGTG(A/G)TGTTC	G	636-617
JW10 (P)	GTCATTAGAGTATGGTGTTC	G	636-617
SB1	GATCA(A/G)TATGAGTACAAGTACCCTGC	M	140-165
SB2	GATCAATATGAATATAAATATCCCGC	M	140-165

Already published RABV sequences that derived from different taxon were downloaded from Genebank (Suppl. Table 8). The sequences were aligned by MEGA v.5.2.1 [24].

The forward and reverse primers were selected from the conserved region of the whole genome, the N gene, to be replicated to 300 nucleotide regions. To determine the suitability of the selected primers, we uploaded to the web page of Integrated DNA Technologies (http://eu.idtdna.com/) to review the melting temperature, GC percentage, self-dimer and hair-pin states of the primers, as well as BLAST (http: //blast.ncbi.nlm.nih.gov/Blast.cgi). The software was used to check sequence and organism matches.

2.1. cDNA Synthesis

In order to replicate the DNA of RABV RNA virus by PCR, first of all, cDNA synthesis was performed. cDNA synthesis was performed according to the recommendations of the manufacturer (Fermentas #EP0352) Details were provided in the additional file.

2.2. PCR optimization with designed primers for RABV

In the optimization process of the designed primers, the sensitivity of the polymerase chain reaction was maximized and the lowest amount of RNA found in the tissue was targeted (Table 3). For this reason, positive control dilutions with a known initial amount (104 copies/ml) were used.

Reaction mixture concentrations, loops, and annealing temperatures were determined with Hemi-nested and Touchdown PCR. The most appropriate PCR mixtures and reaction conditions are given in the additional files to search the samples for both designed and reference primers.

TABLE 3. In this study the designed primers for the N gene of RABV for PCR.

Primer	Sequence	Polarity	Positon	Number of Nucleotide	%GC	Tm	Hairpin Tm	Self-dimer (kcal/mole)
RABV-F	5'-ATGGATGCYG AYAAGATTG-3'	Sense	1-19	19	% 42.1	50.0 °C	-	-
RABV-R1	5'-GTC ART TCC AWG CCT CCT G-3' Reverse: 5'- CAG GAG GCW TGG AAY TGA C -3'	Antisense	368-386	19	% 55.3	54.6 °C	-	-
RABV-R2	5'- ACG YTT TAT BTC YAC CAG AGA -3' Reverse: 5'- TCT CTG GTR GAV ATA AAR CGT -3'	Antisense	319-339	21	% 41.3	52.5 °C	-	-

2.3. Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed to view the PCR products. 2 µL of 10xDNA loading buffer was mixed into 10 µL of PCR product and loaded into wells drilled in the gel. A "ladder" was loaded in one of the wells. The electrophoresis power supply was set to 120V and the gel was run for 40 minutes. The gel was visualized in medium-wave UV (280-340nm) in a transluminator and photographed with the (Vilber Lourtmat Infinity-1000/26MX) device. The displayed band sizes were compared with the "ladder".

2.4. Sequence Analysis

Samples with positive bands were sent to Macrogen company for sequence analysis. After a second PCR of the purificated PCR products was performed, the sequences were created with the ABI device. the Sequences are controlled with Seqscape and Bioedit programs and aligned with MEGA and Bioedit programs; aligned sequences were blast-analyzed with sequences in GenBank.

3. Results and Discussion

In this study, RABV screening was performed with Hemi-nested and Touchdown PCR methods in 242 samples collected from 16 localities in Çaycuma district of Zonguldak province (Figure 3, Table 4).

Table 4. Positive samples and information.

Sample no	Species	Locality
6511	*Apodemus* sp.	Filyos
6517	*Apodemus* sp.	Türkali
6536	*Myodes glareolus*	Çömlekçi
6810	*Myodes glareolus*	Filyos
6686	*Apodemus* sp.	Yukarı İhsaniye
6867	*Apodemus* sp.	Olukyanı
6582	*Myodes glareolus*	Temenler
6677	*Myodes glareolus*	Yeşilköy

FIGURE 3. The distribution of Positive samples.

It was observed that the band size of the R + control was the same as expected (Figure 4). RNA extraction from the brain tissue of the specimens and PCR screening performed as a result of cDNA synthesis revealed a positive RABV in 8 samples of 8 localities. The result of PCR scanning with the reference primers is shown in Figure 5. Table 4 shows the information about the localities and samples where the positively identified samples were collected.

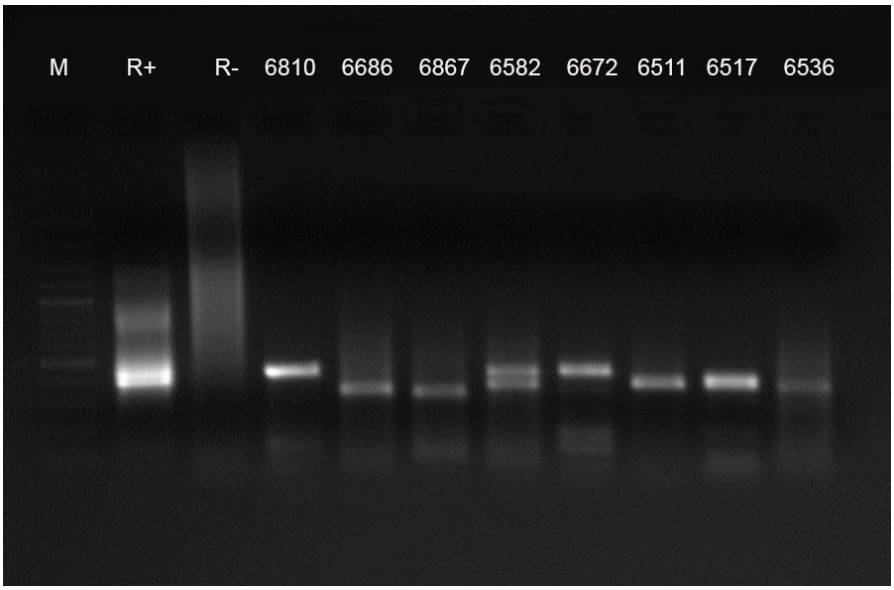

FIGURE 4. Gel images of positive samples from PCR results of the designed primers.

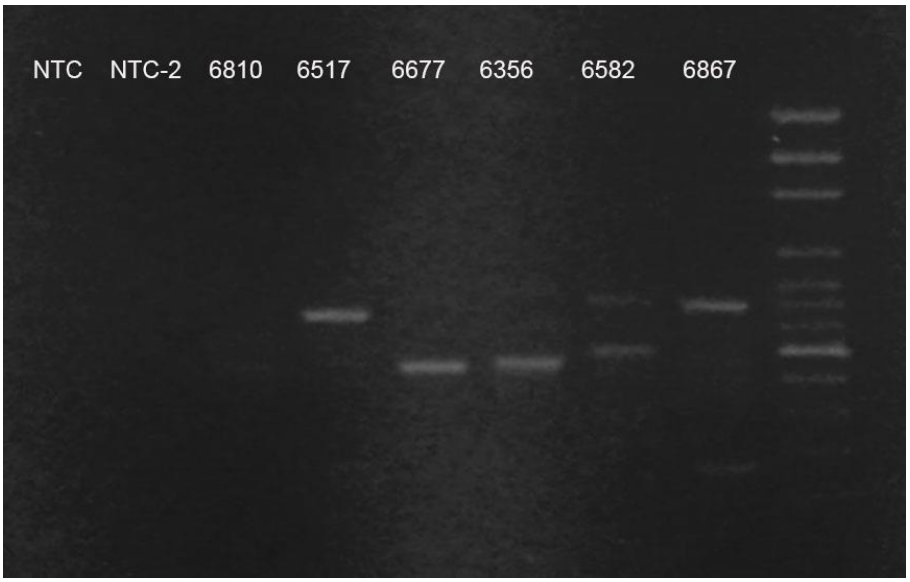

FIGURE 5. Gel image of the PCR result performed as a service delivery with the reference primers.

However, since these findings need to be verified at a higher DNA sequence level, PCR products of positive samples were sent to Macrogen for DNA sequence analysis. Sequences were obtained from 8 samples with an average of 340 bp. However, in blast analyzes, it was found that only two samples showed a low degree of similarity with the sequences of published Rabies viruses.

Because rabies has not a single host, the virus rapidly spreads from one infected animal to another by inter-species interaction. The existence of rabies in a host or in a specific area should be double check by investing among prey species that move faster, quicker than their predators. In the diets of carnivores, rodents have been assumed as an important member of their diets [25]. Due to ecological niches, rodents were the first place in the investigation of presence of rabies. In developed countries, rabies was a disease usually seen in wild animals and transmitted from these hosts to pets and humans [11, 26]. Human rabies suspected animal bites and rabies was still an important health problem in Turkey [27] and as seen as intense in these cases where the quarantine zone was formed. When we look at the study area, the distribution of the carnivores in the area was fragmented due to the fragmentation of the settlements and it was uncertain that they might spread throughout the area. From this point of view, it was likely that rodents might be a source that spread the rabies virus. Lei et al. [19] showed the presence of rabies virus in *Apodemus agrarius* and emphasized that they could play a role in human rabies. Winkler, Schneider & Jennings [27] described rabies rodent specimens at 31 out of 50 states in the United States between 1953-1970. It was also reported that rodent rabies were as common as other species [27]. Nel & Markotter [28] stated that rodents could be considered as carriers of rabies virus. However, in the Middle East, only squirrels were found among the rodents as carriers of the rabies [28]. Mukherjee et al [25] stated that rodents constitute a significant amount of carnivores and feline nutrients. In this study, positive band was obtained from 8 samples among 242 samples when the sequence results were examined, with a 23 bp similarity was found with the rabies virus in the sequences of only two out of the eight samples. According to these results, it can be stated that rodents in the study area were likely to carry rabies among fragmented habitats since carnivores could not travel among the habitats.

As a result of the rabies protection and control guidelines issued by the Ministry of Health in 2005, vaccination was applied as a result of contact with any species that was likely to be exposed to rabies [29]. However, as stated in the directive, no vaccination was performed for those who was bitten and contacted by other small rodents such as mice [27]. Lei et al. [19] reported that one of 57 *Apodemus agrarius* specimens collected from the Zhejiang region of China was positive for rabies. In addition, according to Özsoy et al. [20], 92 (6 %) patients who came to Refik Saydam Hıfzısıhha Center Rabies Vaccine Station with a suspicious animal bite applied to

this unit due to rat bites. However, we also suggest a final test to check each specimen using recent molecular diagnostic tool listed in Duong et al. [30].

In order to obtain a host of information about missing data for the study of rabies in Turkey, our study is based on investigating the presence of rabies virus in rodents in a quarantine zone was the first study. Even the study might be considered as a preliminary study We discovered some clue that rodents could carry the virus among fragmented area since carnivores couldn't travel among habitats so that during fighting back an epidemic disease, the screening of rabies virus on rodents might be examined by a bigger sampling. Our secondary finding was new primer pairs for detecting rodents. To design new primer pairs against a group which has highly mutational genome and capability to do would be crucial for further studies. Recent molecular diagnostic tools have a potential to give interesting results especially in quarantine site.

Acknowledgement. The authors stated that there is no conflict of interest to declare, The results covered some part of Tuğçe Ceylan's Master thesis, and This study was supported by Bülent Ecevit University Scientific Research Projects Unit (Project No: 2012- 10- 06- 02) and TUBITAK TBAG (Project No: 112T070). We also thank the anonymous referees who contributed to the final version of the article.

Author Contribution Statement TG and FM - specimen collection, wet lab works Virus identification (TG), data analysis, manuscript writing. MAÖ-Virus identification, data analysis, manuscript writing.

Declaration of Competing Interests The authors declare no conflict of interest.

References

[1] Us, D., Moleküler, Klinik ve Tanısal Viroloji, Güneş Tıp Kitapevi, Ankara, 2008.
[2] Van Regenmortel, M. H. V., Fauquet C. M., Division M. S. V., Bishop, D. H. L., Virus Taxonomy: Classification and Nomenclature of Viruses: Seventh Report of the International Committee on Taxonomy of Viruses, Academic Press, 2000.
[3] Fu, Z. F., The World of Rhabdoviruses, Springer Verlag, Berlin, 2005.
[4] Fu, Z. F., Rabies and rabies research: past, present and future. *Vaccine*, 15 (1997), 20-24. https://doi.org/10.1016/s0264-410x(96)00312-x
[5] Wagner R.R., The Rhabdoviruses, Plenum Press, New York, 1987
[6] Travassos Da Rosa, A. P. A., Mather, T. N., Takeda, T., Whitehouse, C. A., Shope, R. E., Popov, V. L., Guzman, H., Coffey, L., Araujo, T. P., Tesh, R. B., Two new rhabdoviruses (Rhabdoviridae) isolated from birds during surveillance for arboviral, *Emerging Infection Disease*, 8 (6) (2002), 616–618.

https://doi.org/10.3201/eid0806.010384

[7] Kuzmin, I. V., Orciari, L. A., Arai, Y. T., Smith, J. S., Hanlon, C. A., Kameoka, Y., Rupprecht, C. E., Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences, *Virus research* 97 (2) (2003), 65-79. https://doi.org/10.1016/s0168-1702(03)00217-x

[8] Gönen, İ., Türkiye'deki Hekimlerin Kuduz Hastalığıyla İlgili Bilgi ve Tutumlarınının Değerlendirilmesi. Uzmanlık Tezi, Sağlık Bilimleri Enstitüsü Çocuk Sağlığı ve Hastalıkları Anabilim Dalı İstanbul. (2010).

[9] Lackay, S. N., Kuang, Y., Fu, Z. F., Rabies in small animals. Veterinary Clinics of North America, *Small Animal Practice*, 38 (4) (2008), 851-861. https://doi.org/10.1016/j.cvsm.2008.03.003

[10] Buzgan, T., Irmak, H., Yılmaz, G., Torunoğlu, M. A., Safran, A., Epidemiology of human rabies in Turkey: 1992-2007, *Turkish Journal of Medical Science*, 39 (4) (2009), 591-597. https://doi.org/10.3906/sag-0901-6

[11] Meslin, F., Fishbein, D., Matter, H., Rationale and prospects for rabies elimination in developing countries, *Current topics microbiology and immunology*, 187 (1993), 1-26. https://doi.org/110.1007/978-3-642-78490-3_1

[12] Özkütük, N., İnsan Diploid Hücresi Kuduz Aşısı (HCDV) İle İkinci Kuşak Hücre Kültürü Kuduz Aşılarının Etkinliklerinin Karşılaştırılması. Doktora Tezi, Sağlık Bilimleri Enstitüsü Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı Manisa, (2002).

[13] Mandell, G. L., Bennett, J. E., Dolin, R., Principles and Practice of Infectious Diseases. *Elsevier Churchill*, Livingstone, (2009).

[14] Fooks, A. R., Johnson, N., Freuling, C. M., Wakeley, P. R., Banyard, A. C., McElhinney, L. M., Marston, D.A., Dastjerdi, A., Wright, E., Weiss, R. A., Müller, T., Emerging Technologies for the Detection of Rabies Virus: Challenges and Hopes in the 21st Century, *PLOS Neglected Tropical Diseases*, 3(9) (2009), e530. https://doi.org/10.1371/journal.pntd.0000530

[15] WHO. WHO: Mediterranean Zoonoses Control Centre Information Circular, 33 (1994) 7-9.

[16] Gang, S. R., Rabies in Man and Animals. Springer Verlag, India, 2014.

[17] Blanton, J. D., Palmer, D., Dyer, J., Rupprecht, C. E., Rabies surveillance in the United States during 2010, *Journal of American Veterinary Medicine Association*, 239, (2011), 773–783. https://doi.org/10.2460/javma.239.6.773

[18] CDC. The burden of rabies. Centers for Disease Control and Prevention. http://www.cdc.gov/Features/ dsRabies/. Accessed 8 Apr 2012.

[19] Lei, Y., Wang, X., Li, H., Chen, X., Ye, B., Liu, F., Lan, J., Ye, X., Mei, J., Tang, Q., [New animal hosts of rabies virus in mountain areas in Zhejiang province]. Zhonghua liu xing bing xue za zhi. *Zhonghua liuxingbingxue zazh*, 30 (4) (2009), 344-347.

[20] Özsoy, M., Yakıştıran, S., Özkan, E., 2000 Yılında Kuduz Aşı Merkezine Başvuran Hastaların Değerlendirilmesi, *Türk Hijyen ve Deneysel Biyoloji Dergisi*, 59 (1) (2002), 1-6.

[21] Iamamoto, K., Quadros, J., Queiroz, L., Use of aspiration method for collecting brain samples for rabies diagnosis in small wild animals, *Zoonoses and Public Health*, 58 (1)

(2011), 28-31. https://doi.org/10.1111/j.1863-2378.2009.01282.x

[22] Chomczynski, P., Sacchi, N., The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on, *Nature protocols,* 1 (2) (2006), 581-585.

[23] Heaton, P. R., Johnston, E. P., McElhinney, L. M., Cowley, R., O'Sullivan, E., Whitby, J. E., Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses, *Journal of Clinical Microbiology,* 35 (11) (1997), 2762-2766. https://doi.org/10.1128/jcm.35.11.2762-2766.1997

[24] Kumar, S., Nei, M., Dudley, J., Tamura, K., MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences, *Briefings in Bioinformatics,* 9 (4) (2008), 299-306. https://doi.org/10.1093/bib/bbn017

[25] Mukherjee, S., Goyal, S., Johnsingh, A., Leite Pitman, M. R. P., The importance of rodents in the diet of jungle cat (*Felis chaus*), caracal (*Caracal caracal*) and golden jackal (*Canis aureus*) in Sariska Tiger Reserve, Rajasthan, India, *Journal of Zoology,* 262 (4) (2004), 405-411. https://doi.org/10.1017/S0952836903004783

[26] Kilic, B., Unal, B., Semin, S., Konakci, S. K., An important public health problem: rabies suspected bites and post-exposure prophylaxis in a health district in Turkey, *International journal of infectious diseases*, 10 (3) (1997), 248-254. https://doi.org/10.1016/j.ijid.2005.05.010

[27] Winkler, W. G., Schneider, N. J., Jennings, W. L., Experimental rabies infection in wild rodents, *Journal of wildlife diseases,* 8 (1) (1972), 99-103. https://doi.org/10.7589/0090-3558-8.1.99

[28] Nel, L. H., Markotter, W., Lyssaviruses, *Critical Reviews in Microbiology*, 33 (4) (2007), 301-324. https://doi.org/10.1080/10408410701647602

[29] URL.http://www.saglik.gov.tr/TR/belge/1-15918/kuduz-korunma-ve-kontrol-yonergesi.html. (2013).

[30] Duong, V., Tarantola, A., Ong, S., Mey, C., Choeung, R., Ly, S., Bourhy, H., Dussart, P., Buchy, P., Laboratory diagnostics in dog-mediated rabies: an overview of performance and a proposed strategy for various settings, *International Journal of Infectious Diseases,* 46 (2016), 107–114. https://doi.org/10.1016/j.ijid.2016.03.016

https://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 1, Pages 53-64 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOİ: 10.53447/communc.1122558

Received by the Editors: May 30, 2022; Accepted: June 10, 2022

DETERMINATION OF CHEMICAL CONTENT OF *LEMNA MINOR* L. BY GC-MS AND INVESTIGATION OF ANTIOXIDANT ACTIVITY

SEMA YIYIT DOGAN[1,2], SIBEL ATASAGUN[1], MEHMET BORGA ERGONUL[1]

[1]Department of Biology, Faculty of Science, Ankara University Ankara, TURKEY
[2] Department of Chemical and Chemical Processing Technologies, Technical Sciences Vocational School, Gazi University, Ankara, TURKEY

ABSTRACT. *Lemna minor* L. has been traditionally used for a long time for its analgesic, antipyretic, vitamin C supplement, astringent, antipruritic effects. Although there are many heavy metal removals using *L. minor*, unfortunately, biological activity studies are very limited. In this study, the chemical content and total phenol content, DPPH removal, metal chelation (Fe^{2+}) and β-Carotene-lycopene methods of the *L. minor* macrophyte we obtained from Turkey were determined by GC-MS. The results of the study showed that our plant contains 25 different essential oils and has a high phenol content. In addition, 72% DPPH removal of *L.minor* was determined when it had 71% iron chelating ability. As a result of our study, it has been revealed that the *L. minor* we use is an effective antioxidant. It is thought that its usability in the fields of food and medicine can be investigated with further studies.

1. INTRODUCTION

Free radicals are high-energy, unstable molecules that carry one or more unpaired electrons in their final orbitals. These unpaired electrons give free radicals great reactivity, causing them to damage many biological materials such as proteins, lipids, DNA and coenzymes. Under normal conditions, there is a balance between free radicals and antioxidant defense system in our body. However, an increase in reactive oxygen species and/or a deficiency in the defense systems cause the antioxidant balance in the body to deteriorate and "oxidative stress" conditions occur. Recent studies have shown that this oxidative damage caused by free radicals can be the cause

Keywords. Lemna minor, antioxidants, DPPH, total phenol, GC-MS

semadogan@gazi.edu.tr-Corresponding author; yigit@science.ankara.edu.tr; borga@science.ankara.edu.tr
0000-0003-4292-2233; 0000-0001-8507-0961; 0000-0002-0263-9129

of many important diseases such as diabetes [1], cancer [2], atherosclerosis [3], immune system [4] and cardiovascular diseases [3-4-5]. Antioxidants are natural or synthetic substances that play an important role in preventing cancer formation by neutralizing free radicals, which are toxic by-products of normal cell metabolism [6-7-8].

Plants have a high antioxidant activity due to the secondary metabolites they contain, and due to these properties, they have been used both in folk medicine and in the pharmaceutical industry for years. Flavonoids, cinnamic acid derivatives, cumarins, tocopherols and phenolic acids are the most common herbal antioxidants. Studies have shown that plants increase antioxidant enzyme activity and reduce lipid oxidation thanks to these phenolics they have [6-9-10].

Lemna minor (Duckweed) belongs to the Lemnaceae family and is a perennial, simple, small plant that can be found floating or submerged in fresh waters. Although there are limited scientific studies, *L. minor* is a herb that has been used frequently in traditional medicine and homeopathy for many years. It is known that it is used externally as an antipyretic, diuretic, anti-inflammatory in upper respiratory tract and chronic rheumatic diseases, as well as in eczema, acne, wound healing and insect bites [11]. There are many commercial drugs prepared with *L. minor* extract for use in allergic asthma, rhinitis and nasal congestion problems. The fact that Lemna species contain phenolic compounds such as gallic acid, tannins, flavonoids, anthocyanins, quercetin, and compounds such as thiol and terpene known as steroids suggest that they may have antimicrobial, antioxidant and even anticarcinogenic properties. Studies have shown that they have antimicrobial activity against the pathogens *Bacillus subtilis, B. cereus, Staphylococus aureus, S. saprophyticus, S. warneri, Proteus vulgaris, Citrobacter freundii, C. koseri, Neisseria lactamica, Micrococus luteus* and *Streptococus pneumoniae* [12-13]. Gülçin et al. showed that the *Lemna minor* has antibacterial, anticandidal and antioxidant effects in their study with ethanol and water extracts [13]. In the studies by Popov et al., it has been shown that lemnan isolated from *Lemna minor* macrophyte has safety and tolerability in cellular and humoral immunity, as well as ease of formulation and can be used as an adjuvant in vaccines developed for various infections [14].

Within the scope of our study, the chemical content, total phenol amount, DPPH scavenging activity, iron chelating activity and β-carotene/lycopene content of *L. minor* was determined and its usability in medicine, pharmacy and food fields was investigated.

2. Materials and Methods

2.1. Extraction of *L. minor*

L. minor used in the study was developed in the aquarium of Hydrobiology Laboratory from Ankara University. The collected plants were washed twice with distilled water and left to dry at room temperature. The dried plants were crushed to powder and stored in room conditions, out of the sun until the study was carried out.

Microwave assisted extraction method was used for extraction. Microwave extraction method is a frequently used method for obtaining plant extract in recent years. It has advantages such as less solvent consumption, shorter time and no evaporation phase compared to the classical soxhlet method. The dried and powdered plant samples were prepared in proportion to 1g/20ml volume and extracted in 60% ethanol using microwave at 850 Watt, 90 seconds conditions. The obtained extracts were centrifuged at 2500 rpm for 20 minutes, the supernatant was passed through filter paper and dried in a lyophilizer (Christ, Alpha 1-2 LD). The samples were stored at +4˚C until the study was carried out [15].

2.2. Determination of Chemical Contents by GC-MS

Gas Chromatography/Mass Spectrometer (GC-MS) method was used to identify the volatile components obtained by microdistillation, and Gas Chromatography method was used to determine their relative percentages. System was used Agilent 7890B GC 5977B Mass Selective System that consisted of Agilent HP-Innowax. The sample taken into the hexane phase was injected into the system as 1 microliter with a 10:1 split ratio. definitions were made with the help of the Wiley-9 Nist 11 Mass Spectral database (Anadolu University Plant, Medicine and Scientific Research Application and Research Center, AUBIBAM).

2.3. Determination of Total Phenolic Content

The total amount of phenolic compounds was determined spectrophotometrically by adapting the Folin-Ciocalteu method of Barros et al. to 96-well plates. 0.02 ml (concentration 1 mg / ml) was taken from the extracts, mixed with 40 µl of Folin-Ciocalteus reagent:water (50:50) and 0.2 ml of 2% sodium carbonate, and incubated for 90 minutes at room temperature. After incubation, absorbances were read at 760 nm wavelength (Epoch, BioTek) and calculations were made according to the Gallic acid standard [16].

2.4. Investigation of DPPH Scavenging

The scavenging capacity of 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical was determined by spectrophotometric method. 0.004% DPPH solution was added to the determined concentrations (25-400 µg/ml) of the extracts and incubated for 30 minutes in the dark. At the end of the incubation, the free radical scavenging effect was determined according to the following formula (2.1) by measuring spectrophotometrically (Epoch microplate reader, BioTek) at a wavelength of 517 nm [17].

$$\% \ Scavenging = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \quad (2.1)$$

2.5. Investigation of Iron (Fe^{+2}) Ion Chelating Activity

Iron ion chelating activity was determined by making some changes in Decker and Welch's method. By adding 0.05 ml 2 mM FeCl2 and 0.1 ml 5 mM ferrosine to the concentrations of the extracts (25-400 µg/ml), the samples were left for incubation at room temperature for 15 minutes After incubation, the absorbance was measured at 562 nm wavelength and the calculation was made according to the following equation (2.2)[18].

$$\% \ Chelating = [(A_{Control} - A_{Sample}) / A_{Control}] \times 100 \quad (2.2)$$

2.6. Determination of β-Carotene and Lycopene Contents

100 mg of dry extract was completely dissolved in 10 ml of acetone:hexane (6:4) mixture and passed through a 0.45 µm filter. The absorbances at 453, 505, 645 and 663 nm wavelengths were measured and calculated according to the following formulas (2.3) [19].

$$Lycopene \ (mg/100 \ ml) = -0.0458 A_{663} + 0.204 A_{645} + 0.372 A_{505} - 0.0806 A_{453}$$
$$\beta\text{-}Carotene \ (mg/100 \ ml) = 0.216 A_{663} - 1.22 A_{645} - 0.304 A_{505} + 0.452 A_{453} \quad (2.3)$$

3. RESULTS

3.1. Determination of Chemical Contents of *L. minor*

It was determined that the *L. minor* methanol extract we used in our study contained 25 different volatile compounds, and the compound with the highest rate was phytol (19,8 %) (Table 1). Phytol is an acyclic diterpene alcohol that can be used as a precursor in the production of synthetic forms of vitamin E and vitamin K. Many studies with Phytol have shown that this molecule has antimicrobial, antidiabetic, antidiuretic, anti-inflammatory and anticarcinogenic properties [20]. Moraes et al. reported that phytol, which is widely used as a food additive and in medical fields, is effective against parasites (antiscistosomal) in vitro and in vivo studies with mice [21]. Other compounds in *L. minor* were found to be hexanal (5.6 %), 2,4-Di-tert-butylphenol (2.2 %), 5-Tetradecene (5.0 %), cetene (4.5 %). 2,4-Di-tert-butylphenol is known to have antifungal and antioxidant properties. The 2,4 DTBP compound obtained from the bacterium (*Lactococcus* sp.) can be an antifungal and antimicrobial food additive that can improve food safety and also contribute positively to health [22]. Hexanal, also called hexanaldehyde or caproaldehyde, is used as a food additive due to its shelf life extension and flavoring properties. Tacheva et al., in their similar study, they stated that *L. minor* extract has 12 different antioxidant compounds (phytol, campesterol, loliolide, dihydroactinidiolide, ascorbic acid, vanillic acid, 2,3-dihydroxybenzoic acid, caffeic acid, chlorogenic acid, esculetin, esculin and fraxetin) [23]. Their content analysis is similar to our study. When the chemical contents of 0.5% relative ratio are analyzed using GC-MS, it is seen that *L. minor* contains antimicrobial and antioxidant compounds.

3.2. Determination of Total Phenolic Content

It is known that phenolic content and antioxidant capacity are parallel. Therefore, the *L. minor* total phenol content was calculated in the next step. Plants with high phenolic content show higher antioxidant and anticarcinogenic activity. As a result of our study, it was determined that the *L.minor* extract we studied contained 20.44 mg GAE/100g total phenol. There are very few antioxidant activity studies with *L. minor*. However, the total phenol results found in the study of Gülcin et al. are similar to our study. They were reported that water (WELM) and ethanol (EELM) extracts had phenolic substances between 22.0 ± 0.8 and 16.7 ± 0.0 μg GAE.

TABLE 1. Volatile compound content of *Lemna minor*.

No	Compound	%
1	Pentanal $$ n-Pentanal $$ n-Valeraldehyde $$ Valeral	5.0
2	Hexanal $$ n-Hexanal $$ Hexaldehyde $$ Caproaldehyde $$ Capronaldehyde	5.6
3	2-Pentylfuranm (2-Amylfuran)	3.8
4	1-Dodecene	2.2
5	*p*-Cymene	4.4
6	Isopropyl pentyl ketone (2-Methyl-3-octanone)	1.8
7	*Spektrum-1*	1.0
8	6-Methyl-5-hepten-2-one	1.5
9	Nonanal (CAS) $$ n-Nonanal $$ n-Nonylaldehyde $$ Nonaldehyde $$ n-Nonaldehyde	1.8
10	(*E*)-5-Tetradecene	5.0
11	Cetene (1-Hexadecene)	4.5
12	Heptadecane	4.4
13	1-Octadecene	2.0
14	*Spektrum-2* ((*E*)-Geranylacetone)	2.3
15	Neophytadiene	2.1
16	(*E*)-beta.-Ionone	2.6
17	*Spektrum-3* Neophytadiene isomer I: II	7.1
18	Pentadecanal- $$ 1-Pentadecanal $$ n-Pentadecanal	5.3
19	Hexahydrofarnesyl acetone	1.6
20	*Spektrum-4*	4.3
21	Thymol	1.3
22	*Spektrum-5*	5.8
23	2,4-Di-tert-butylphenol	2.2
24	Farnesyl acetone	1.6
25	Phytol	19.8
	Toplam	**99.0**

* ≥ % 0.5

Gülçin et al., in their study with Lemna minor, reported that water (WELM) and ethanol (EELM) extracts had phenolic substances between 22.0 ± 0.8 and 16.7 ± 0.0 µg GAE [13].

3.3. DPPH Scavenging Activity

It was observed that the DPPH scavenging effect increased in a dose-dependent manner. Although *L. minor* methanol extract had activity (18-72 %) at all concentrations studied, it showed the highest efficiency at 400 µg /ml with 72% removal (Figure 1).

L. minor is a fast growing plant with high protein content and is an important food source for aquatic animals. It is also known for its antipyretic and analgesic properties. Based on these properties, Kim et al., in their study, claiming that the substances in the environment will affect the metabolite content, the total phenol amount and indirectly the antioxidant activity, and when they added 3% sucrose and 0.5 mM proline, they found the DPPH scavenging effect to be 69.1% [24]. In our study, *L. minor* showed a similar effect and swept the DPPH radical in the environment by 62%. Antioxidant studies are among the subjects of much research today, and now more comprehensive and advanced methods are tried to increase the effectiveness of plants whose effectiveness is known. Saying that *L. minor* macrophyte contains high protein and is a good source of bioactive peptides, Tran et al. investigated the antioxidant activity of hydrolyzed proteins obtained from lean *L. minor*. It was observed that *L. minor* samples hydrolyzed with flavourzyme and alcalase removed 28.91-54.15% DPPH. The results showed that protein recovery, hydrolysis degree values and antioxidant activities were found increased with increasing enzyme concentration and hydrolysis time. They said that under the same enzymatic hydrolysis condition, samples hydrolyzed with flavourzyme had a higher inhibitory effect on ABTS and DPPH radical scavenging than samples hydrolyzed by alcalase and alkaline treatment [25]. Saritha and Saraswathi, on the other hand, studied another popular topic, nanoparticles, and looked at the DPPH scavenging effect of gold nanoparticles they synthesized using *L. minor*. As a result of their study, it was determined that the *L. minor* extract had a DPPH scavenging effect, however, they found that the DPPH scavenging activity of the gold nanoparticles synthesized with *L. minor* was higher. *L. minor* is an important food source for living creatures in the aquatic environment. In addition to its rich nutritional content and known pharmacological properties, its rapid vegetation period and easy development have attracted the attention of researchers [26]. *L. minor*, which is considered to have antioxidant properties because it contains high amounts of vitamin E, carotenoids and flavonoids, is also used as a feed and feed additive due to its high nutritional content. Iskandar et al. found the DPPH scavenging ability of *L. minor* extract, which they plan to use as a fish feed additive, as IC_{50}; 54.517 ppm. In the next stage of the study, they looked at the effect of antioxidant effect on growth and immunity in Nile tilapia fish, which they fed with ready-to-feed + 25,50,75% and 100 IC_{50} extract. As a result of the study, it was found that the group fed with 25% IC_{50} *L. minor* extract increased the daily growth rate, vitality and immune system [27].

When all these studies are examined, we can say that the *L. minor* macrophyte we use has high DPPH removal, thus it has the potential to be used as an antioxidant in many fields such as food, cosmetics, feed additives, and the pharmaceutical industry.

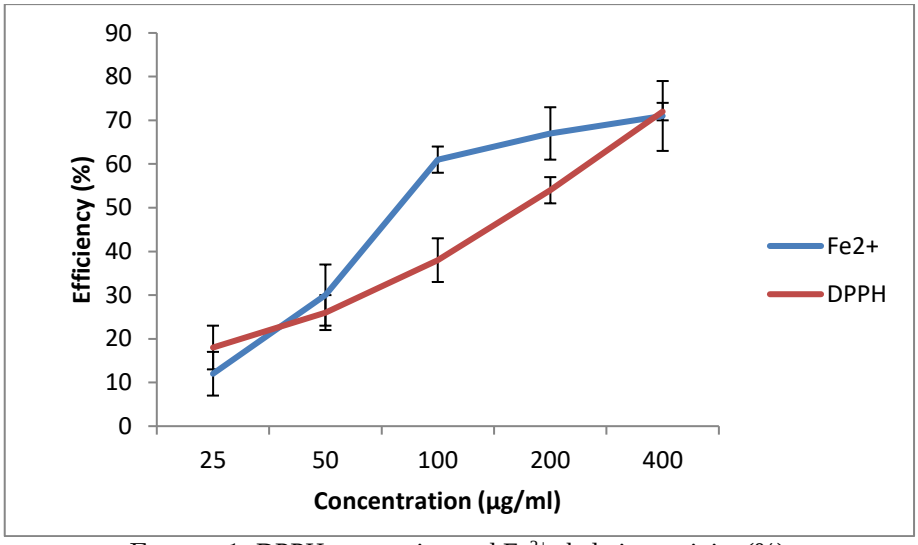

FIGURE 1. DPPH scavenging and Fe^{2+} chelating activity (%).

3.4. Determination of Metal chelating activity on ferrous ions (Fe^{2+})

Among the transition metals, iron is known as the most important pro-oxidant in lipid oxidation due to its high reactivity. At the same time, iron ions play a role in the occurrence of Fenton reactions and cause the formation of hydroxyl radicals. It is known that the hydroxyl radical, the amount of which increases in the environment, damages DNA and causes genetic mutations and cancer in advanced processes. Secondary antioxidants that come into play in this process can prevent this process, which progresses to cancer formation, by forming chelate with metal ions and inhibiting the Fenton reactions as in the equation $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^*OH + OH–$. Our study results showed that the *L. minor* we analyzed was effective at every concentration studied. The chelating efficiency increased in a dose-dependent manner (Figure 1). Our macrophyte, which has a chelating effect of 12-71%, showed the highest efficiency at a concentration of 400 µg /ml. There are a limited number of studies showing that *L. minor* have antioxidant effects and iron chelating activity. Gülçin et al., in their study with *L. minor*, stated that ethanol extract (EELM) was 61%, water extract (WELM) 63%, and the chelating effect was BHA > WELM > EELM > BHT > trolox > αtocopherol, respectively [13]. When we compare these results, we can say that *L. minor* in our study has higher activity.

3.3. Determination of β-Carotene and Lycopene Contents

β-Carotene and Lycopene are the main hydrocarbon carotenoids with apolar properties. Carotenoids are secondary plant pigments that give yellow, orange and red colors and can be synthesized by plants and some bacteria, algae and fungi. Studies have shown that β-Carotene and lycopene are powerful antioxidants and that their dietary intake is very beneficial for health. As a result of our analysis, it was determined that *Lemna minor* extract contained 0.116 mg/100 ml of β-carotene. It is known that β-carotene has antioxidant activity due to its free radical scavenging ability. In addition, β-carotene is the precursor of vitamin A, which has skin rejuvenation, visual functions, reproduction, anmmune-enhancing effects. Lycopene is not a vitamin A precursor, but is the strongest antioxidant in the carotenoids. In addition, in many scientific studies, it has been reported that lycopene induces apoptosis on many cancer cell lines such as prostate, lung, colon, and also has an effect on cardiovascular diseases, bone, skin and eye health. It was determined that the *L. minor* extract we studied contains 0.091 mg/ 100ml lycopene. As a result of the study, it was observed that the methanol extract of *L. minor* had high carotene and lycopene [28].

TABLE 1. Contents of total phenols, β-carotene, lycopene and IC_{50} (μg /ml) values of DPPH and Fe^{+2} in the methanolic extract.

Species	Total Phenol (mg/GAE g)	DPPH IC_{50}	Fe^{+2} IC_{50}	β-carotene mg/100 ml	Lycopene mg/ 100ml
L. minor	20,44±1,03	159,08±5,14	103,76±3,26	0.116±0,03	0.091±0,01

4. CONCLUSION

Within the scope of this study, the chemical content and antioxidant properties of *Lemna minor* macrophyte obtained from inland waters of Turkey were investigated. The results of the study showed that the *L. minor* extract has high phenolic content and these results were also confirmed by GC-MS analysis. At the same time, it was determined that our extract had high DPPH scavenging and iron chelating efficiency in parallel with its phenol content. Considering these results, it is thought that our L.

minor extract is a strong natural antioxidant and its usability in food and pharmacy areas can be investigated.

Acknowledgement. This work was financially supported by the Coordination of Scientific Research Projects of Ankara University (Research Project No: 21L0430004) in Turkey. We would like to thank Asst. Prof. Ayşegül H. MENDİ for her assistance.

Within the scope of this study, the chemical content and antioxidant properties of Lemna minor macrophyte obtained from inland waters of Turkey were investigated.

Author Contribution Statements MBE- Collection, identification and development of *Lemna minor*. SA- project development, manuscript editing. SYD- data analysis, manuscript writing and manuscript editing. All authors have read and approved the manuscript.

Declaration of Competing Interests The authors declare no conflict of interest

REFERENCES

[1] Yildirim, M., Degirmenci, U., Akkapulu, M., Comelekoglu, U., Balli, E., Ozcan, T. M., Yalin, S., The effect of Rheum ribes L. on oxidative stress in diabetic rats, *Journal of Basic and Clinical Physiology and Pharmacology*, 32(1) (2021). http://doi 10.1515/jbcpp-2020-0058

[2] Preci, D. P., Almeida, A., Weiler, A. L., Franciosi, M. L. M., Cardoso, A. M., Oxidative damage and antioxidants in cervical cancer, *International Journal of Gynecologic Cancer*, 31(2) (2021). http://dx.doi.org/10.1136/ijgc-2020-001587

[3] Garcia, C., Blesso, C. N., Antioxidant properties of anthocyanins and their mechanism of action in atherosclerosis, *Free Radical Biology and Medicine*, 172 (2021), 152-166. https://doi.org/10.1016/j.freeradbiomed.2021.05.040

[4] Karamalakova, Y., Stefanov, I., Georgieva, E., Nikolova, G., Pulmonary Protein Oxidation and Oxidative Stress Modulation by Lemna minor L. in Progressive Bleomycin-Induced Idiopathic Pulmonary Fibrosis. *Antioxidants*, 11(3) (2022), 523. http://doi 10.3390/antiox11030523.

[5] Zhang, P. Y., Xu, X., Li, X. C., Cardiovascular diseases: oxidative damage and antioxidant protection, *European Review for Medical and Pharmacological Sciences*, 18(20) (2014), 3091-3096.

[6] Koca, N., Karadeniz, F., Serbest radikal oluşum mekanizmaları ve vücuttaki antioksidan savunma sistemleri, *Gıda Mühendisliği Dergisi*, 16, (2003), 32-37.

[7] Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., Dhama, K., Oxidative stress, prooxidants, and antioxidants: the interplay, *BioMed Research International*, (2014):761274. https://doi.org/10.1155/2014/761264

[8] Karabulut, H., Gülay, M. Ş., Antioksidanlar. *Veterinary Journal of Mehmet Akif Ersoy University*, 1 (1) (2016), 65-76.

[9] Simsek, I., Aytekin, F., Yesilada, E., Yildirimli, Ş., An ethnobotanical survey of the Beypazari, Ayas, and Güdül district towns of Ankara Province (Turkey). *Economic botany*, 58(4) (2004), 705-720. https://doi.10.1663/0013-0001(2004)058 [0705:AESOTB]2.0.CO;2

[10] Özhatay, N., Koyuncu, M., Atay, S., Byfield, A., Türkiye'nin doğal tıbbi bitkilerinin ticareti hakkında bir çalışma, Doğal Hayatı Koruma Derneği, (1997).

[11] Al-Snai, A. E., Lemna minor: Traditional uses, chemical constituents and pharmacological effects-A review, *IOSR Journal of Pharmacy*, 9(8) (2019), 6-11.

[12] González-Renteria, M., del Carmen Monroy-Dosta, M., Guzmán-García, X., Hernández-Calderas, I., Antibacterial activity of Lemna minor extracts against Pseudomonas fluorescens and safety evaluation in a zebrafish model, *Saudi Journal of Biological Sciences*, 27(12) (2020), 3465-3473. https://doi.org/10.1016/j.sjbs.2020.09.043

[13] Gulcin, I., Kirecci, E., Akkemik, E., Topal, F., Hisar, O., Antioxidant, antibacterial, and anticandidal activities of an aquatic plant: duckweed (Lemna minor L. Lemnaceae), *Turkish Journal of Biology*, 34(2) (2010), 175-188. http://doi. 10.3906/biy-0806-7

[14] Popov, S. V., A. Günter, E., Markov, P. A., Smirnov, V. V., Khramova, D. S., Ovodov, Y. S., Adjuvant effect of lemnan, pectic polysaccharide of callus culture of Lemna minor L. at oral administration, *Immunopharmacology and Immunotoxicology*, 28(1) (2006), 141-152. https://doi.org/10.1080/08923970600626098

[15] Yağcıoğlu, P., *Farklı ekstraksiyon metotları ile adaçayı (Salvia officinalis L.) bitkisinden antioksidan ekstraksiyonunun optimizasyonu* (Doctoral dissertation, Fen Bilimleri Enstitüsü), 2015.

[16] Barros, L., Ferreira, M. J., Queiros, B., Ferreira, I. C., Baptista, P., Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities, *Food chemistry*, 103(2) (2007), 413-419. https://doi.org/10.1016/j.foodchem.2006.07.038

[17] Blois, M. S., Antioxidant determinations by the use of a stable free radical, *Nature*, 181(4617) (1958), 1199-1200. https://doi.org/10.1038/1811199a0

[18] Decker, E. A., & Welch, B., Role of ferritin as a lipid oxidation catalyst in muscle food, *Journal of Agricultural and food Chemistry*, 38(3) (1990), 674-677.

[19] Nagata, M., Yamashita, I., Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit, *Nippon Shokuhin Kogyo Gakkaishi*, 39(10) (1992), 925-928. https://doi.org/10.3136/nskkk1962.39.925

[20] Muniandy, K., Gothai, S., Tan, W. S., Kumar, S. S., Mohd Esa, N., Chandramohan, G., Arulselvan, P., In vitro wound healing potential of stem extract of Alternanthera sessilis, *Evidence-based complementary and alternative medicine*, (2018), http://doi10.1155/*2018*/3142073.

[21] Moraes, J., de Oliveira, R. N., Costa, J. P., Junior, A. L., de Sousa, D. P., Freitas, R. M., & Pinto, P. L., Phytol, a diterpene alcohol from chlorophyll, as a drug against neglected

tropical disease Schistosomiasis mansoni, *PLoS Neglected Tropical Diseases*, 8(1) (2014), e2617. https://doi.org/10.1371/journal.pntd.0002617

[22] Varsha, K. K., Devendra, L., Shilpa, G., Priya, S., Pandey, A., Nampoothiri, K. M., 2, 4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp., *International journal of food microbiology*, 211 (2015), 44-50. https://doi.org/10.1016/j.ijfoodmicro.2015.06.025

[23] Petrova-Tacheva, V., Ivanov, V., & Atanasov, A., Lemna minor L. as a source of antioxidants, *Trakia Journal of Science*, 18 (2020), 157-162. http://doi 10.15547/tjs.2020.s.01.029

[24] Kim, Y., Hyun, S. H., Park, H. E., & Choi, H. K., Metabolic profiling, free-radical scavenging and tyrosinase inhibitory activities of Lemna minor whole plants cultivated in various concentrations of proline and sucrose, *Process Biochemistry*, 47(1) (2012), 62-68. https://doi.org/10.1016/j.procbio.2011.10.010

[25] Tran, H. C., Le, H. A. T., Le, T. T., Effects of Enzyme Types and Extraction Conditions on Protein Recovery and Antioxidant Properties of Hydrolysed Proteins Derived from Defatted Lemna minor, *Applied Science and Engineering Progress*, 14(3) (2021), 360-369. https://doi.org/10.14416/j.asep.2021.05.003

[26] Saritha, K., Saraswathi, U., Antioxidant activity of gold nanoparticles synthesized using Lemna minor, *World Journal of Pharmaceutical Sciences*, (2014), 1545-1551.

[27] Iskandar, I., Andriani, Y., Rostika, R., Zidni, I., & Riyanti, N. A., Effect of using fermented Lemna sp. in fish feed on growth rate of nilem carp (Osteochilus hasselti), *World News of Natural Sciences*, 26 (2019), 157-166.

[28] Baynal, Ö. F., Likopen, Erciyes Üniversitesi Eczacılık Fakültesi, Bitirme tezi, 2012.

https://communications.science.ankara.edu.tr

Commun.Fac.Sci.Univ.Ank.Ser. C Biology
Volume 31, Number 1, Pages 65-96 (2022)
ISSN 1303-6025 E-ISSN 2651-3749
DOI: 10.53447/communc.1128722

Received by the Editors: June 10, 2022; Accepted: June 22, 2022

CONTRIBUTIONS TO THE FLORA OF BEYAĞAÇ (DENİZLİ)

MEHMET ÇİÇEK[1], OKAN ÇON[2]

[1]Pamukkale University, Faculty of Science and Arts, Department of Molecular Biology and Genetics, 20160 Denizli, TURKEY
[2]Pamukkale University, Faculty of Science and Arts, Department of Biology, 20160 Denizli, TURKEY

ABSTRACT. In this study, in order to determine the flora of Beyağaç (Denizli), 767 plant samples were collected and observed from 74 different localities by the field studies carried out in Beyağaç and its close surroundings in the years 2017 and 2018. The results of examination of the collected plant samples and evaluation of the observed plants, it was determined 362 species and totally 363 plant taxa, belonging to 63 families and 228 genera. Of 363 vascular plant taxa, 3 taxa belong to Pteridophyta division, 360 taxa to Magnoliophyta division. Of the Magnoliophyta division, 4 taxa belong to Gymnospermae subdivision and 356 taxa to Angiospermae subdivision. Of the Angiospermae subdivision, 312 taxa belong to Dicotyledonae classis and 44 taxa to Monocotyledonae classis. The phytogeographical spectrum of the flora of Beyağaç (Denizli) as follows: Mediterranean elements 115 taxa (31.6%), Irano-Turanian elements 13 taxa (3.6%), Euro-Siberian elements 20 taxa (5.5%) and multi-regional or unknown origin 216 taxa (59.3%). The largest ten families in flora of Beyağaç (Denizli) as follows: Fabaceae 43 taxa (11.8%), Asteraceae 42 taxa (11.5%), Lamiaceae 28 taxa (7.7%), Caryophyllaceae 27 taxa (% 7.4), Brassicaceae 24 taxa (6.6%), Poaceae 16 taxa (4.4%), Boraginaceae 14 taxa (3.8%), Apiaceae 13 taxa (3.6%), Asparagaceae 11 taxa (3.0%), and Plantaginaceae 10 taxa (2.7%). The largest ten families constitute 64% of the flora of Beyağaç (Denizli). The largest ten genera in the flora of Beyağaç (Denizli) as follows: *Alyssum* L. 8 taxa (2.2%), *Silene* L. 7 taxa (1.9%), *Medicago* L. 6 taxa (1.6%), *Salvia* L. 6 taxa (1.6%), *Cerastium* L. 5 taxa (1.4%), *Euphorbia* L. 5 taxa (1.4%), *Muscari* Mill. 5 taxa (1.4%), *Teucrium* L. 5 taxa (1.4%), *Veronica* L. 5 taxa (1.4%), and *Vicia* L. 5 taxa (1.4%). The number of endemic taxa in the flora of Beyağaç (Denizli) are 39 (10.7%). Their threat categories were given according to "Red Data Book of Turkish Plants". It is found 21 taxa in LC category, 4 taxa in NT category, 5 taxa in VU category, 6 taxa in EN category and 2 taxa in CR category.

Keyword and phrases. Systematics, Sandras Mountain, threat category, Denizli

mcicek@pau.edu.tr-Corresponding author; ocon@pau.edu.tr
0000-0003-4058-3717; 0000-0002-1087-1729

1. Introduction

Turkey is located in the temperate Mediterranean climate zone and the intersection point of Iran-Turanian, Euro-Siberian and Mediterranean phytogeographic regions [1]. With 9222 species, the flora of Turkey is one of the richest in region [2]. Among the main reasons for this rich plant species diversity in Turkey are climatic changes, topographical and geological diversity, different ecological environments, and the existence of several large mountain ranges in Anatolia that create effective barriers to the geographical spread of species [3,4].

The first work written on the flora of Turkey is “Flora Orientalis” [5]. Secondly, it is "Flora of Turkey and the East Aegean Islands", which was shown as one of the best and most comprehensive flora in the world at the time it was published [6]. In the following years, 10. and 11. volumes were published as the supplementary to the main work [1,2]. Finally, “Plant List of Turkey” including also taxa added after the completion of the main flora has been published [7]. According to the “Plant List of Turkey” [7], it is found 11707 taxa, 3649 (31.82%) of which are endemic to Turkey. Turkish endemic plants are seen intensively, especially in the Mediterranean region and around the Anatolian diagonal. In addition, Munzur Mountains, Van-Bitlis-Hakkari environs, Kazdağları, Uludağ and Ilgaz Mountains are among our regions that are very rich in terms of endemic plant diversity. However, some endemic species are faced with threats such as industrialization, urbanization, expansion of agricultural areas, overgrazing, collection for traditional usage, reclamation of barren areas and fires [8].

Beyağaç located in the western end of Sandras Mountain, Gölgeli (Bozdağ) Mountains and Taurus Mountains is an edge district of Denizli province. It takes place in the square of C2 according to "Davis' squaring system" and also in the Mediterranean phytogeographic region. The study area, which is mostly composed of serpentine rocks and partly limestone and sandstone rocks, has a unique vegetation. The region mainly contains the vegetation types such as maquis, forest and alpine steppe. The alpine steppes are also rich in herbaceous vegetation and many endemic plants, which are unique to medium and high lands [9].

It is known that the soils formed on the serpentine bedrock are generally composed of limeless, brown forest soils or terra rossa soils [10]. Serpentine soils are known to be quite challenging habitats for plants. Although the ecology of serpentine systems and the adaptive morphology of serpentine-specific plants are quite interesting, serpentine communities have a unique structure [11]. These plants, which grow on serpentine soils, have developed various adaptations specific to this soil structure,

such as sclerophyll structure, microphilia, and spiny stem structure [12]. Sandras Mountain is very rich in terms of endemic plants. The highest peak of Sandras Mountain, which reaches a height of 2295 m, is known as Çiçekbaba Hill. Due to its height reaching 2295 m, it is one of the highest mountains of Southwest Anatolia [13].

This study presents contributions to the flora of Beyağaç (Denizli), which has the high endemism ratito.

2. Materials and Methods

The field studies were carried out in Beyağaç (Denizli) district and its close surroundings in the flowering and the fruiting times of plants in the years 2017 and 2018. During the field studies, the plant samples were collected for identification, and also their natural appearance was photographed. A list is given in the Appendix 1 of the plant sampling localities as numbered. It was also recorded the GPS coordinates of the plant collection localities (Figure 1). The plant specimens collected were turned into herbarium material and identified under the Leica S8 Apo stereo microscope by using "Flora of Turkey and the East Aegean Islands" [1,2,6]. The voucher specimens are kept in the M. Çiçek herbarium (PAU).

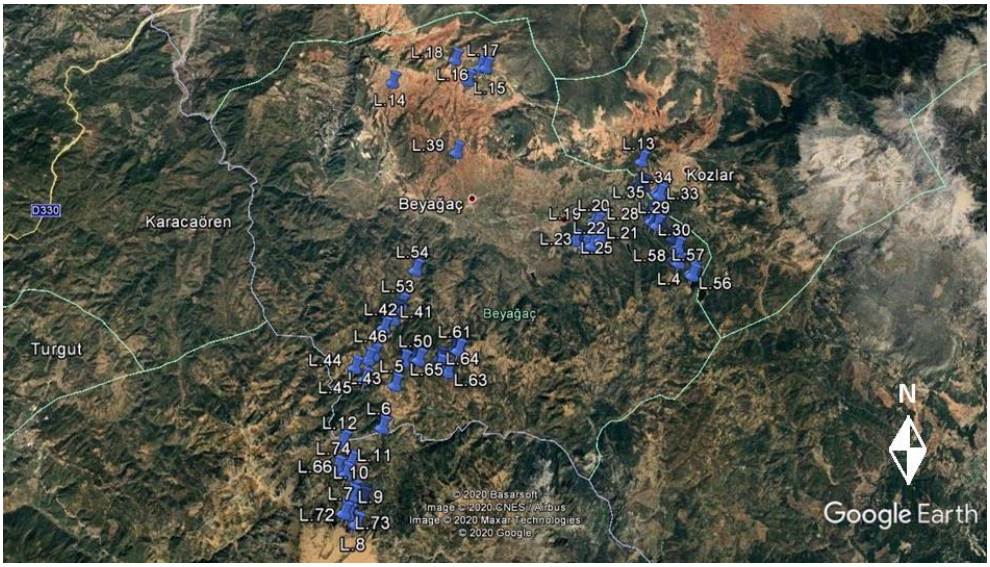

FIGURE 1. A map showing the plant sampling localities in the flora of Beyağaç (Denizli).

Taxon names were checked using “Türkiye Bitkileri Listesi (Damarlı Bitkiler)” and the online databases named “International Plant Names Index”, “The Plant List”, and “Bizim Bitkiler”, and their current taxonomic statuses were given [7,14,15,16]. The threat categories of the endemic taxa were given according to the data of “Red Data Book of Turkish Plants” [8].

3. Results

In totally, 767 plant samples were collected and observed from 74 different locations by the field studies (Figure 1, Appendix 1). As a result of examination of these samples, 363 vascular plant taxa were identified, of which 39 were endemic, belonging to 63 families and 228 genera. A list is given in the Appendix 2 of the taxa determined.

Of the identified vascular plant taxa, 3 taxa belong to Pteridophyta and 360 to Magnoliophyta. In the Pteridophyta, there are 3 taxa under 3 genera belonging to 3 families. It is found 4 taxa under 2 genera belonging to 2 families in the Gymnospermae subdivision of Spermatophyta; In the Angiospermae subdivision of Spermatophyta, there are 356 taxa under 223 genera belonging to 58 families. It is found 312 taxa under 194 genera belonging to 47 families in the class Dicotyledonae, and 44 taxa under 29 genera belonging to 11 families in the class Monocotyledonae (Table 1).

Table 1. Distribution in the upper taxonomic categories of taxa determined in the flora of Beyağaç (Denizli) in terms of the numbers of family, genus, species and total taxa.

The upper taxonomic categories	The number of family	The number of genus	The number of species	The total number of taxa
Phanerogamae	63	228	362	363
Pteridophyta	3	3	3	3
Magnoliophyta	60	225	359	360
Gymnospermae	2	2	4	4
Angiospermae	58	223	355	356
Dicotyledonae	47	194	311	312
Monocotyledonae	11	29	44	44

39 of the 363 taxa identified herein are endemic to Turkey, and constituted 10.7% of the flora of Beyağaç (Denizli). The number of other non-endemic taxa is 324 (89.3%) (Figure 2). The threat categories of endemic taxa are given according to the Red Book of Plants of Turkey [19] (Table 2). It is found 21 taxa in LC (Least Concern) category, 4 taxa in NT (Near Threatened) category, 5 taxa in VU (Vulnerable)

category, 6 taxa in EN (Endangered) category and 2 taxa in CR (Critically Endangered) category. The threat category of 1 taxon could not be evaluated.

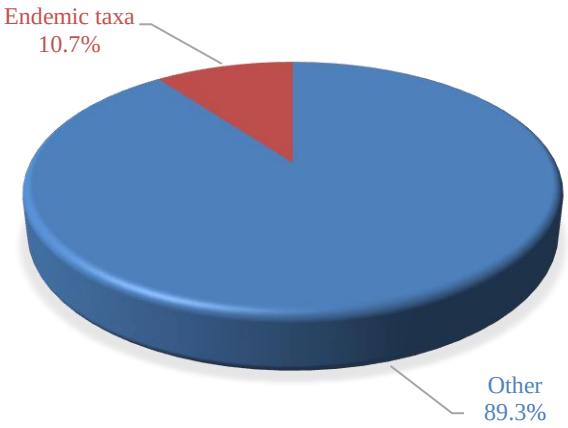

FIGURE 2. Endemism in the flora of Beyağaç (Denizli).

TABLE 2. Threat categories of the endemic taxa in the flora of Beyağaç (Denizli).

Abbreviations of threat categories: LC: Least Concern, NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered

No	Family	Taxon name	Threat category
1	Plumbaginaceae	*Acantholimon ulicinum* (Willd. ex Schult.) Boiss. var. *purpurascens* (Bokhari) Bokhari & J.R.Edm.	LC
2	Brassicaceae	*Alyssum caricum* T.R.Dudley & Hub.-Mor.	EN
3	Brassicaceae	*Alyssum hirsutum* M.Bieb. subsp. *caespitosum* (T.R.Dudley) Ančev, Kožuharov & Kuzmanov	NT
4	Brassicaceae	*Alyssum masmenaeum* Boiss.	LC
5	Brassicaceae	*Alyssum propinquum* Baumg.	LC
6	Fabaceae	*Astragalus tmoleus* Boiss. var. *tmoleus*	LC
7	Caryophyllaceae	*Bolanthus frankenioides* (Boiss.) Barkoudah var. *fasciculatus* (Boiss. & Heldr.) Barkoudah	LC
8	Caryophyllaceae	*Bolanthus thymoides* Hub.-Mor.	LC
9	Asteraceae	*Centaurea ensiformis* P.H.Davis	VU
10	Caprifoliaceae	*Cephalaria lycica* V.A.Matthews	NT
11	Lamiaceae	*Clinopodium troodi* (Post) Govaerts subsp. *vardaranum* (Leblebici) Govaerts	EN
12	Primulaceae	*Cyclamen alpinum* Dammann ex Spreng.	LC
13	Caryophyllaceae	*Dianthus eretmopetalus* Stapf	VU

TABLE 2 (CONTINUED).

No	Family	Taxon name	Threat category
14	Fabaceae	*Ebenus pisidica* Hub.-Mor. & Reese	CR
15	Apiaceae	*Eryngium thorifolium* Boiss.	LC
16	Brassicaceae	*Erysimum serpentinicum* Polatschek	CR
17	Euphorbiaceae	*Euphorbia anacampseros* Boiss. var. *anacampseros*	LC
18	Euphorbiaceae	*Euphorbia austroanatolica* Hub.-Mor. & M.S.Khan	LC
19	Apiaceae	*Ferulago sandrasica* Peşmen & Quézel	EN
20	Fabaceae	*Genista sandrasica* Hartvig & Strid	EN
21	Hypericaceae	*Hypericum aviculariifolium* Jaub. & Spach	LC
22	Plantaginaceae	*Linaria corifolia* Desf.	LC
23	Caryophyllaceae	*Minuartia recurva* (All.) Schinz & Thell. subsp. *carica* McNeill	VU
24	Asparagaceae	*Muscari racemosum* Mill.	VU
25	Asparagaceae	*Muscari sandrasicum* Karlén	EN
26	Lamiaceae	*Nepeta cadmea* Boiss.	LC
27	Brassicaceae	*Noccaea cariensis* (Carlström) Parolly, Nordt & Aytac	EN
28	Lamiaceae	*Origanum hypericifolium* O.Schwarz & P.H.Davis	LC
29	Asparagaceae	*Ornithogalum alpigenum* Stapf	NT
30	Crassulaceae	*Prometheum serpentinicum* (Werderm.) t Hart var. *serpentinicum*	LC
31	Caprifoliaceae	*Scabiosa polykratis* Rech.f.	LC
32	Crassulaceae	*Sedum lydium* Boiss.	LC
33	Asteraceae	*Senecio sandrasicus* P.H.Davis	LC
34	Caryophyllaceae	*Silene echinospermoides* Hub.-Mor.	LC
35	Lamiaceae	*Teucrium alyssifolium* Stapf	LC
36	Lamiaceae	*Teucrium sandrasicum* O. Schwarz	LC
37	Scrophulariaceae	*Verbascum cariense* Hub.-Mor.	NT
38	Scrophulariaceae	*Verbascum trapifolium* (Stapf) Hub.-Mor.	VU
39	Violaceae	*Viola heldreichiana* Boiss.	-

The phytogeographical spectrum of the flora of Beyağaç (Denizli) is as follows: Mediterranean elements 115 taxa (31.6%), Irano-Turanian elements 13 taxa (3.6%), Euro-Siberian elements 20 taxa (5.5%) and multi-regional and/or unknown origin 215 taxa (59.3%) (Figure 3).

The largest ten families in the flora of Beyağaç (Denizli) are as follows: Fabaceae 43 taxa (11.8%), Asteraceae 42 taxa (11.5%), Lamiaceae 28 taxa (7.7%), Caryophyllaceae 27 taxa (7.4%), Brassicaceae 24 taxa (6.6%), Poaceae 16 taxa (4.4%), Boraginaceae 14 taxa (3.8%), Apiaceae 13 taxa (3.6%), Asparagaceae 11 taxa (3.0%), and Plantaginaceae 10 taxa (2.7%). The largest ten families constitute 62.6% of the flora of Beyağaç (Denizli) (Figure 4).

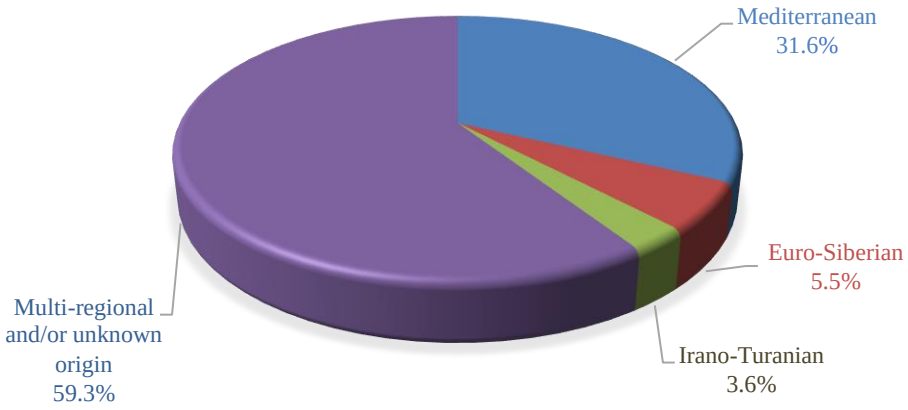

FIGURE 3. Phytogeographical spectrum for the flora of Beyağaç (Denizli).

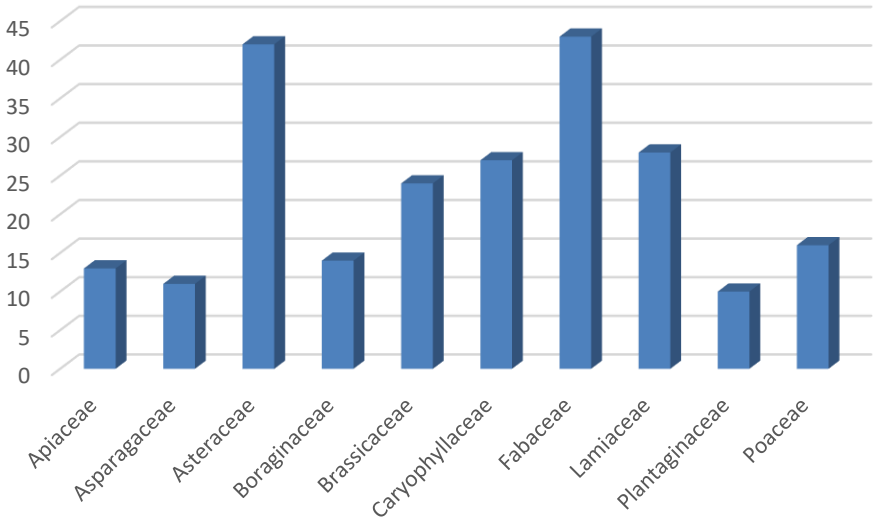

FIGURE 4. The largest ten families in the flora of Beyağaç (Denizli).

The largest ten genera in the flora of Beyağaç (Denizli) are as follows: *Alyssum* L. 8 taxa (2.2%), *Silene* L. 7 taxa (1.9%), *Medicago* L. 6 taxa (1.6%), *Salvia* L. 6 taxa (1.6%), *Cerastium* L. 5 taxa (1.4%), *Euphorbia* L. 5 taxa (1.4%), *Muscari* Mill. 5

taxa (1.4%), *Teucrium* L. 5 taxa (1.4%), *Veronica* L. 5 taxa (1.4%), and *Vicia* L. 5 taxa (1.4%) (Figure 5).

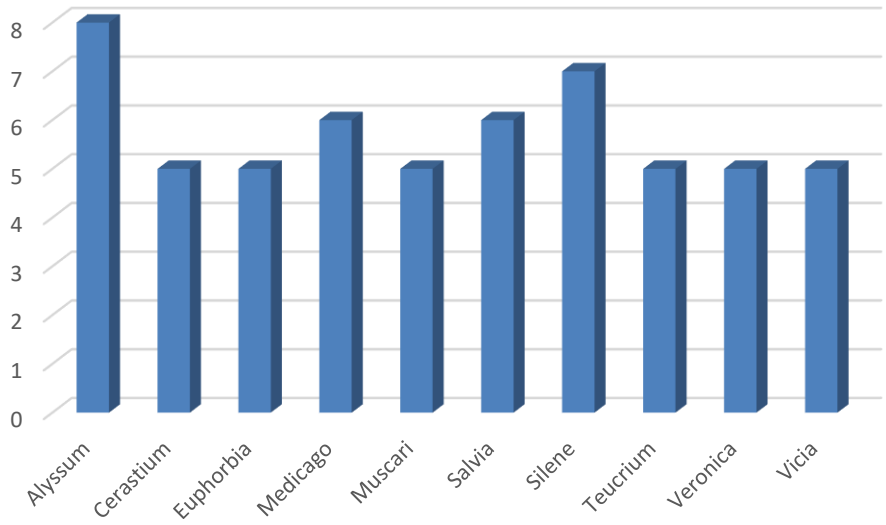

FIGURE 5. The largest ten genera in the flora of Beyağaç (Denizli).

4. DISCUSSION

The results of study revealed that compared to some other studies carried out in the region, the flora of Beyağaç (Denizli) have fewer plant diversity. Babadağ (Denizli) [17], Honaz Mountain (Denizli) [18,19,20] and Boncuk Mountains (Burdur-Muğla) [21] are the richest first three floras in the region, respectively (Table 3). Due to their having huge mass, big size, very different ecological conditions and habitat diversity, this is an expected situation that they contain more taxa. The flora of Beyağaç (Denizli) is in the tenth order.

TABLE 3. A comparison of the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the numbers of family, genus, species and total taxa.

Abbreviations of studies: Studies: 1: Flora of Beyağaç (Denizli) (Results of this study), 2: Flora of Mt Aydoğdu (Denizli/Turkey) [22], 3: Babadağ (Denizli)'ın Flora ve Vejetasyonu [17], 4: Bencik Dağı (Yatağan-Muğla) Florası [23], 5: Flora of Boncuk Mountains (Burdur-Muğla, Turkey) [21], 6: Denizli Acıpayam Bozdağ'ın Flora ve Vejetasyonu [24], 7: Flora of Çökelez Mountain (Denizli-Turkey) and its environs [25], 8: Honaz Dağı'nın Bitkileri I (The Flora of Honaz Dağı I) [18], Honaz Dağı'nın Bitkileri II (The Flora of Honaz Dağı II) [19], A Supplementary List to the Flora of Honaz Dağı [20], 9: Flora of Kurukümes Mountain (Milas-Muğla/Turkey) [26], 10: Sandras Dağı'nın (Muğla) Bitkisel Örtüsü ve Bazı Endemik Türleri Üzerinde Palinolojik, Sitolojik Araştırmalar [27], 11: Yılanlı Dağı (Muğla)'nın Florası [28], *Number not specified in the study.

Taxonomic categories	**Research areas**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Family	63	82	94	65	83	*	76	*	73	86	48
Genus	228	314	430	264	340	*	316	*	275	319	181
Species	362	*	*	407	*	*	*	*	522	*	338
Total Taxa	**363**	**586**	**1066**	**421**	**858**	**572**	**587**	**985**	**555**	**664**	**343**

Compared the phytogeographical spectrum of the flora of Beyağaç (Denizli) with those of some other studies carried out in the region, in all studies in the region, the Mediterranean phytogeographic region is in the first order, the Iran-Turanian phytogeographic region in the second order (except for the present study and Flora of Kurukümes Mountain [26]), and the Euro-Siberian phytogeographic region in the third order (Table 4). Since the study area is under the effect of the Mediterranean climate and is located within the borders of the Mediterranean phytogeographic region, it is an expected situation for the Mediterranean elements to be the first order in the phytogeographical spectrum. Secondly, considering all compared study areas to be close to the Irano-Turanian phytogeographic region, it is most likely for Iran-Turanian elements to be the second order.

TABLE 4. A comparison of the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the phytogeographical spectrum.

Phytogeographic regions	**Research areas**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Mediterranean	115 (31.6%)	180 (30.7%)	253 (23.7%)	171 (40.6%)	*	148 (25.8%)	153 (26.1%)	*	216 (38.9%)	175 (27.0%)	132 (38.4%)
Euro-Siberian	20 (5.5%)	24 (4.0%)	38 (3.6%)	8 (1.9%)	*	24 (4.1%)	30 (5.1%)	*	24 (4.3%)	7 (1.0%)	8 (2.3%)
Irano-Turanian	13 (3.6%)	43 (7.3%)	56 (5.3%)	18 (4.2%)	*	54 (9.4%)	46 (7.8%)	*	9 (1.6%)	21 (3.2%)	36 (10.5%)
Multiregional and/or unknown	215 (59.3%)	339 (58.0%)	719 (67.4%)	212 (50.3%)	*	346 (60.7%)	358 (61.0%)	*	306 (55.1%)	461 (68.8%)	167 (48.8%)

When we compared the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of endemism ratio (%), Boncuk Mountains (Burdur-Muğla) with 20.9% [21], Yılanlı Mountain (Muğla) with 18.6% [28] and Denizli Acıpayam Bozdağ [24] with 18.5% are in the first three order. Babadağ (Denizli) [17], Honaz Mountain (Denizli) [18,19,20] and Sandras Mountain (Muğla) [27] are in fourth, fifth and sixth order, respectively. The flora of Beyağaç (Denizli) is in seventh order with 10.7%. Aydoğdu Mountain (Denizli) [22] with 9.7%, Bencik Mountain (Muğla) [23] with 9.0%, Kurukümes Mountain (Muğla) [26] with 8.4% and Çökelez Mountain (Denizli) [25] with 5.6% are in eighth, ninth, tenth and eleventh order, respectively. The first three flora containing the largest number of endemic taxa; Boncuk Mountains (Burdur-Muğla) with 180 endemic taxa, Babadağ (Denizli) with 164 endemic taxa and Honaz Mountain (Denizli) with 135 endemic taxa. Denizli Acıpayam Bozdağ with 106 endemic taxa, Sandras Mountain (Muğla) with 76 endemic taxa, Yılanlı Mountain (Muğla) with 64 endemic taxa, Aydoğdu Mountain (Denizli) with 57 endemic taxa, Kurukümes Mountain (Muğla) with 47 endemic taxa, Beyağaç (Denizli) with 39 endemic taxa, Bencik Mountain (Muğla) with 38 endemic taxa, and Çökelez Mountain (Denizli) with 33 endemic taxa are in fourth, fifth, sixth, seventh, eighth, ninth, tenth and eleventh order, respectively (Table 5).

TABLE 5. A comparison of the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the numbers of total taxa and endemic taxa.

Endemism	**Research areas**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
The total number of taxa	363	586	1066	421	858	572	587	985	555	664	343
The number of endemic taxa (%)	39 (10.7%)	57 (9.7%)	164 (15.3%)	38 (9.0%)	180 (20.9%)	106 (18.5%)	33 (5.6%)	135 (13.7%)	47 (8.4%)	76 (11.4%)	64 (18.6%)

Compared the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the largest ten families (Table 6), the family Fabaceae is in the first order in the floras of Beyağaç (Denizli), Babadağ (Denizli), Boncuk Mountains (Burdur-Muğla), Denizli Acıpayam Bozdağ, Honaz Mountain (Denizli) and Yılanlı Mountain (Muğla). It is in the second order in the floras of Aydoğdu Mountain (Denizli), Bencik Mountain (Muğla), Çökelez Mountain (Denizli) and Kurukümes Mountain (Muğla). The family Asteraceae is in the first order in floras of Aydoğdu Mountain (Denizli), Bencik Mountain (Muğla), Çökelez Mountain (Denizli), Kurukümes Mountain (Muğla) and Sandras Mountain (Muğla); in the second order in the floras of Babadağ (Denizli), Denizli Acıpayam Bozdağ, Honaz Mountain (Denizli) and Boncuk Mountains (Burdur-Muğla); in the third order in the flora of

Yılanlı Mountain (Muğla). The family Lamiaceae is in the third order in the flora of Beyağaç (Denizli), Bencik Mountain (Muğla), Denizli Acıpayam Bozdağ, Sandras Mountain (Muğla) and Kurukümes Mountain (Muğla). The family Poaceae is in the second order in the flora of Boncuk Mountains (Burdur-Muğla) and in the third order in the floras of Babadağ (Denizli) and Çökelez Mountain (Denizli). The family Brassicaceae is in the third order in the floras of Aydoğdu Mountain (Denizli) and Honaz Mountain (Denizli). The family Caryophyllaceae is in the second order in the flora of Sandras Mountain (Muğla). Except for the floras of Sandras Mountain (Muğla) (Asteraceae only, in the first order) and Yılanlı Mountain (Muğla) (Fabaceae only, in the first order), Asteraceae and Fabaceae families are in the first two order in all floras compared. Indeed, it is an expected situation that the families Asteraceae and Fabaceae are in the first two order in the all floras, because of them to be the largest families of the Flora of Turkey in terms of the number of taxa. Depending on the family Lamiaceae including many taxa of mesophytic and Mediterranean origin, because the compared floras are also mostly located in the Mediterranean region, it can also be considered as a possible situation that Lamiaceae comes the first rank in the floras of Beyağaç (Denizli), Bencik Mountain (Muğla), Denizli Acıpayam Bozdağ and Kurukümes Mountain (Muğla). Because of Poaceae to be one of the largest families in the flora of Turkey, it is a possible situation that it is in the first three order in the floras of Boncuk Mountains (Burdur-Muğla), Babadağ (Denizli), Çökelez Mountain (Denizli) and Yılanlı Mountain (Muğla).

TABLE 6. A comparison of the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the largest ten families.

Families	**Research areas**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Apiaceae	13 (3.6%)	23 (3.9%)	38 (3.6%)	17 (4.0%)	35 (4.0%)	-	20 (3.4%)	44 (4.4%)	17 (3.0%)	25 (3.7%)	-
Asparagaceae	11 (3.0%)	-	-	-	-	-	-	-	-	-	-
Asteraceae	42 (11.5%)	76 (12.9%)	123 (11.5%)	57 (13.5%)	80 (9.3%)	64 (11.1%)	87 (14.8%)	106 (10.7%)	73 (13.1%)	73 (10.9%)	33 (9.6%)
Boraginaceae	14 (3.8%)	-	30 (2.8%)	-	26 (3.0%)	21 (3.6%)	19 (3.2%)	36 (3.6%)	-	-	18 (5.2%)
Brassicaceae	24 (6.6%)	43 (7.3%)	64 (6.0%)	22 (5.2%)	58 (6. % 7)	35 (6.1%)	26 (4.4%)	70 (7.1%)	33 (5.9%)	36 (5.4%)	10 (2.9%)
Caryophyllaceae	27 (7.4%)	24 (4.0%)	71 (6.7%)	17 (4.0%)	55 (6.4%)	32 (5.5%)	21 (3.5%)	60 (6.0%)	21 (3.7%)	48 (7.2%)	13 (3.7%)
Euphorbiaceae	-	-	-	-	-	-	-		-	16 (2.4%)	-
Fabaceae	43 (11.8%)	65 (11.0%)	137 (12.9%)	39 (9.2%)	99 (11.5%)	70 (12.2%)	70 (11.9%)	109 (11.0%)	60 (10.8%)	39 (5.8%)	44 (12.8%)

TABLE 6 (CONTINUED).

Families	Research areas										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Lamiaceae	28 (7.7%)	28 (4.7%)	71 (6.7%)	37 (8.7%)	74 (8.6%)	49 (8.5%)	32 (5.4%)	55 (5.5%)	41 (7.3%)	47 (7.0%)	37 (10.7%)
Liliaceae	-	27 (4.6%)	49 (4.6%)	23 (5.4%)	32 (3.7%)	23 (4.0%)	18 (3.0%)	31 (3.1%)	26 (4.6%)	45 (6.7%)	22 (6.4%)
Orchidaceae	-	-	-	-	-	-	-	-	18 (3.2%)	16 (2.4%)	-
Plantaginaceae	10 (2.7%)	-	-	-	-	-	-	-	-	-	-
Poaceae	16 (4.4%)	41 (6.9%)	79 (7.4%)	27 (6.4%)	81 (9.4%)	30 (5.2%)	47 (8.0%)	53 (5.3%)	34 (6.1%)	-	26 (7.5%)
Ranunculaceae	-	-	23 (2.2%)	-	16 (1.8%)	-	-	25 (2.5%)	-	-	-
Rosaceae	-	23 (3.9%)	42 (3.9%)	13 (3.0%)	23 (2.6%)	19 (3.3%)	19 (3.2%)	21 (2.1%)	-	-	17 (4.9%)
Scrophulariaceae	-	-	10 (0.9%)	11 (2.6%)	29 (3.3%)	19 (3.3%)	-	42 (4.2%)	17 (3.0%)	32 (4.8%)	10 (2.9%)

Compared the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the largest ten genera (Table 7), *Allium* L. in Sandras Mountain (Muğla); *Alyssum* L. in Beyağaç (Denizli), Denizli Acıpayam Bozdağ and Honaz Mountain (Denizli); *Anthemis* L. in Çökelez Mountain (Denizli); *Astragalus* L. in Babadağ (Denizli), Boncuk Mountains (Burdur-Muğla), Denizli Acıpayam Bozdağ and Honaz Mountain (Denizli); *Bromus* L. in Çökelez Mountain (Denizli); *Centaurea* L. in Bencik Mountain (Muğla) and Yılanlı Mountain (Muğla); *Euphorbia* L. in Sandras Mountain (Muğla); *Galium* L. in Babadağ (Denizli), Boncuk Mountains (Burdur-Muğla) and Denizli Acıpayam Bozdağ; *Lathyrus* L. in Çökelez Mountain (Denizli); *Medicago* L. in Beyağaç (Denizli) and Çökelez Mountain (Denizli); *Ornithogalum* L. in Aydoğdu Mountain (Denizli); *Salvia* L. in Beyağaç (Denizli); *Scorzonera* L. in Yılanlı Mountain (Muğla); *Silene* L. in Beyağaç (Denizli), Bencik Mountain (Muğla), Boncuk Mountains (Burdur-Muğla), Kurukümes Mountain (Muğla) and Sandras Mountain (Muğla); *Trifolium* L. in Aydoğdu Mountain (Denizli), Babadağ (Denizli), Bencik Mountain (Muğla), Denizli Acıpayam Bozdağ, Çökelez Mountain (Denizli), Honaz Mountain (Denizli), Kurukümes Mountain (Muğla) and Yılanlı Mountain (Muğla); *Trigonella* L. in Yılanlı Mountain (Muğla); *Veronica* L. in Aydoğdu Mountain (Denizli) and *Vicia* L. in Bencik Mountain (Muğla) and Kurukümes Mountain (Muğla) are among the largest three genera. *Trifolium* L. is in the first order in the floras of Aydoğdu Mountain (Denizli), Bencik Mountain (Muğla), Çökelez Mountain (Denizli), Honaz Mountain (Denizli), Kurukümes Mountain (Muğla) and Yılanlı Mountain (Muğla), but *Alyssum* L. in the first order in Beyağaç (Denizli). *Allium* L. is in the first order in the flora of Sandras Mountain (Muğla), wheras *Astragalus* L. is in the first order

in the floras of Babadağ (Denizli), Boncuk Mountains (Burdur-Muğla) and Denizli Acıpayam Bozdağ.

TABLE 7. A comparison of the flora of Beyağaç (Denizli) with some other studies carried out in the region in terms of the largest ten genera.

Genera	**Research areas**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Allium L.	-	-	17 (1.5%)	-	7 (0.8%)	-	-	-	-	18 (2.7%)	-
Alyssum L.	8 (2.2%)	7 (1.1%)	13 (1.2%)	-	15 (1.7%)	8 (1.3%)	-	19 (1.9%)	-	11 (1.6%)	-
Anthemis L.	-	7 (1.1%)	12 (1.1%)	-	6 (0.6%)	-	9 (1.5%)	-	8 (1.4%)	-	-
Astragalus L.	-	6 (1.0%)	21 (1.9%)	-	28 (3.2%)	15 (2.6%)	-	19 (1.9%)	-	-	-
Bromus L.	-	-	-	5 (1.1%)	-	-	9 (1.5%)	-	-	-	-
Centaurea L.	-	8 (1.3%)	13 (1.2%)	6 (1.4%)	12 (1.3%)	-	7 (1.2%)	13 (1.3%)	-	10 (1.5%)	6 (1.7%)
Cerastium L.	5 (1.4%)	-	-	-	-	-	-	-	-	-	-
Crepis L.	-	-	-	-	-	-	6 (1.0%)	-	-	-	-
Euphorbia L.	5 (1.4%)	-	-	-	-	-	-	-	-	16 (2.4%)	-
Galium L.	-	7 (1.1%)	20 (1.8%)	-	16 (1.8%)	9 (1.5%)	6 (1.0%)	13 (1.3%)	-	-	-
Geranium L.	-	-	-	-	-	-	-	-	7 (1.2%)	-	-
Lathyrus L.	-	-	-	-	-	-	9 (1.5%)	-	-	-	-
Medicago L.	6 (1.6%)	6 (1.0%)	-	-	-	-	9 (1.5%)	-	7 (1.2%)	-	-
Muscari Mill.	5 (1.4%)										
Orchis L.	-	-	-	-	-	-	-	-	7 (1.2%)	-	-
Ornithogalum L.	-	9 (1.5%)	-	-	-	-	-	-		8 (1.2%)	-
Ranunculus L.	-	7 (1.1%)	13 (1.2%)	5 (1.1%)	8 (0.9%)	-	-	14 (1.4%)	8 (1.4%)	11 (1.6%)	-
Rumex L.	-	-	-	-	-	-	7 (1.2%)	-	-	-	-
Salvia L.	6 (1.6%)	-	-	-	-	-	-	-	-	-	-
Scorzonera L.	-	-	-	-	-	-	-	-	-	-	6 (1.7%)
Sedum L.	-	-	9 (0.8%)	-	10 (1.1%)	7 (1.2%)	-	10 (1.0%)	-	-	-
Silene L.	7 (1.9%)	8 (1.3%)	14 (1.3%)	6 (1.4%)	16 (1.8%)	7 (1.2%)	-	18 (1.8%)	12 (2.1%)	14 (2.1%)	5 (1.4%)

TABLE 7 (CONTINUED).

Genera	Research areas										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Teucrium L.	5 (1.4%)	-	-	-	-	-	-	-	-	-	-
Trifolium L.	-	13 (2.2%)	18 (1.6%)	8 (1.9%)	9 (1.0%)	8 (1.3%)	13 (2.2%)	20 (2.0%)	18 (3.2%)	7 (1.0%)	10 (2.9%)
Trigonella L.	-	-	-	-	-	-	-	-	-	-	6 (1.7%)
Verbascum L.	-	7 (1.1%)	-	-	-	-	-	-	7 (1.2%)	11 (1.6%)	-
Veronica L.	5 (1.4%)	9 (1.5%)	-	-	-	-	6 (1.0%)	-	-	8 (1.2%)	-
Vicia L.	5 (1.4%)	-	-	6 (1.4%)	-	-	-	-	11 (1.9%)	-	-

5. CONCLUSION

Consequently, it would be said that the research area has an important plant diversity. The area is home to many endemic and rare plant species. The endemic species *Ebenus pisidica* and *Erysimum serpentinicum* with "Critically Endangered" (CR) category have a narrow population in this area. The data obtained in this study will contribute to future multidisciplinary studies.

Acknowledgement. This study was supported by Scientific Research Projects Coordination Unit of Pamukkale University (PAUBAP) (Project No: 2017FEBE034 and Project No: 2017FEBE035). We thank Scientific Research Projects Coordination Unit of Pamukkale University for their financial support.

Author Contribution Statement MÇ-specimen collection and identification, data analysis and manuscript writing. OÇ-data analysis and manuscript writing.

Declaration of Competing Interests The authors declare no conflict of interest.

REFERENCES

[1] Davis, P.H., Mill, R., Tan, K. (Editors), Flora of Turkey and the East Aegean Islands, Vol. 10, Edinburgh University Press, Edinburgh, 1988.
[2] Güner, A., Özhatay, N., Ekim, T., Başer, K.H.C. (Editors), Flora of Turkey and the East Aegean Islands, Vol. 11, Edinburgh University Press, Edinburgh, 2000.
[3] Özhatay, N., Diversity of bulbous monocots in Turkey with special reference. Chromosome numbers, *Pure and Applied Chemistry*, 74 (4) (2002), 547–555. https://doi.org/10.1351/pac200274040547.
[4] Ekim, T., Bitkiler, In: Türkiye'nin Biyolojik Zenginlikleri, Türkiye Çevre Sorunları

Vakfı Yayını, Ankara, 1990, 69–92.
[5] Boissier, P.E., Flora Orientalis: sive, Enumeratio plantarum in Oriente a Graecia et Aegypto ad Indiae fines hucusque observatarum, 5 vols.+Supplement, Basileae: H. Georg, 1867–1888.
[6] Davis, P.H. (Editor), Flora of Turkey and the East Aegean Islands, Vol. l–9, Edinburgh University Press, Edinburgh, 1965–1985.
[7] Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M.T. (Editors), Türkiye Bitkileri Listesi (Damarlı Bitkiler), Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını, İstanbul, 2012.
[8] Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., Adıgüzel, N., Türkiye Bitkileri Kırmızı Kitabı, Türkiye Tabiatını Koruma Derneği ve Van 100. Yıl Üniversitesi, Ankara, 2000.
[9] Kurt, B., Sandras Dağı. In: Eken, G., Bozdoğan, M., İsfendiyaroğlu, S., Kılıç, D.T., Lise, Y. (Editörler), Türkiye'nin Önemli Doğa Alanları, Cilt 1, Doğa Derneği, Ankara, 2006, 250–253.
[10] Altıoğlu, Y., Sandras Dağı (Muğla) Serpantin Substratı Üzerinde Yayılış Gösteren Bazı Endemik Bitkiler Üzerine Ekofizyolojik Çalışmalar, Ege Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, İzmir, 2009.
[11] Brady, K.U., Kruckeberg, A.R., Bradshaw Jr, H.D., Evolutionary ecology of plant adaptation to serpentine soils, *Annual Review of Ecology, Evolution, and Systematics*, 36 (1) (2005), 243–266. https://doi.org/10.1146/annurev.ecolsys.35.021103.105730.
[12] Iturralde, R.B., The influence of ultramafic soils on plants in Cuba, *South African Journal of Science*, 97 (11) (2001), 510–512.
[13] Doğan, M., Sandıras Dağı'nda (Muğla) Buzullaşma ve Buzul Şekilleri, *Ege Coğrafya Dergisi*, 20 (1) (2011), 29–52.
[14] Anonymous, The International Plant Names Index 2017: (http://www.ipni.org/), (2020a), (Accessed date: 01.09.2020).
[15] Anonymous, The Plant List, A working of all plant species 2016: (http://www.theplantlist.org/),(2020b), (Accessed date: 01.09.2020).
[16] Anonymous, Bizim Bitkiler: https://www.bizimbitkiler.org.tr/list.html), (2020c), (Accessed date: 01.09.2020).
[17] Gemici, Y., Oluk, S., Babadağ (Denizli)'ın Flora ve Vejetasyonu, TÜBİTAK, Temel Bilimler Araştırma Grubu, Proje No: TBAG-1387 (195T022), İzmir, 1999.
[18] Baytop, A., Tuzlacı, E., Honaz Dağı'nın Bitkileri I (The Flora of Honaz Dağı I), *İstanbul Üniversitesi Eczacılık Fakültesi Mecmuası (Journal of Faculty of Pharmacy Istanbul University)*, 12 (1976), 30–74.
[19] Tuzlacı, E., Honaz Dağı'nın Bitkileri II (The Flora of Honaz Dağı II), *İstanbul Üniversitesi Eczacılık Fakültesi Mecmuası (Journal of Faculty of Pharmacy Istanbul University)*, 13 (1977), 47–61.
[20] Tuzlacı, E., A Supplementary List to the Flora of Honaz Dağı (Honaz Dağı Florasına Ek Bir Liste), *İstanbul Üniversitesi Eczacılık Fakültesi Mecmuası (Journal of Faculty of Pharmacy Istanbul University)*, 19 (1983), 41–46.
[21] Çetin, E., Seçmen, Ö., Flora of Boncuk Mountains (Burdur-Muğla, Turkey), *International Journal of Botany*, 4 (2) (2008), 130–150. https://doi.org/10.3923/ijb.2008.130.150.

[22] Semiz, G., Çelik, A., Flora of Mt Aydoğdu (Denizli/Turkey), *Natura Croatica*, 14 (3) (2005), 185–212.
[23] Aytepe, H.A., Varol, Ö., Bencik Dağı (Yatağan-Muğla) Florası, *Ekoloji*, 16 (63) (2007), 41–61.
[24] Bekat, L., Denizli Acıpayam Bozdağ'ın Flora ve Vejetasyonu. Ege Üniversitesi Araştırma Fonu, Proje No: 1988/013, İzmir, 1992.
[25] Çiçek, M., Çelik, A., Flora of Çökelez Mountain (Denizli-Turkey) and its environs, *Bulletin of Pure and Applied Sciences*, 23 (2) (2004), 127–145.
[26] Arık, Ü.Ö., Görk, G., Flora of Kurukümes Mountain (Milas-Muğla/Turkey), *Biological Diversity and Conservation*, 9 (3) (2016), 149–167. https://doi.org/10.13140/RG.2.2.27206.88643.
[27] Özhatay, E., Sandras Dağı'nın (Muğla) Bitkisel Örtüsü ve Bazı Endemik Türleri Üzerinde Palinolojik, Sitolojik Araştırmalar. TÜBİTAK, Matematik, Fiziki ve Biyolojik Bilimler Araştırma Grubu, Proje No: TBAG-306, İstanbul, 1981.
[28] Varol, Ö., Doğru, A., Kaya, E., Yılanlı Dağı (Muğla)'nın Florası, *Ekoloji*, 13 (50) (2004), 23–36.

APPENDICES

APPENDIX 1. A list of the plant sampling localities in the flora of Beyağaç (Denizli)

Locality no	Locality	Altitude (m)	Date
1	**C2 Denizli:** Beyağaç	-	2017
2	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	795	13.07.2017
3	**C2 Denizli:** Beyağaç, above the chrome quarry, towards Eşen Pond	939	13.07.2017
4	**C2 Denizli:** Beyağaç, Eşen Pond, the edges of the pond	1013	13.07.2017
5	**C2 Denizli:** Beyağaç, Sandras Mountain, Kartal Lake road	1333	13.07.2017
6	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1351	13.07.2017
7	**C2 Muğla:** Köyceğiz, near Kartal Lake	1888	13.07.2017
8	**C2 Muğla:** Köyceğiz, Kartal Lake, the areas around the lake where the water is withdrawn and the stream sides	1900	13.07.2017
9	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1797	13.07.2017
10	**C2 Muğla:** Köycegiz, Sandras Mountain, Kartal Lake road	1756	13.07.2017
11	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1617	13.07.2017
12	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1415	16.09.2017
13	**C2 Denizli:** 9 km from Denizli to Beyağaç, under the *Pinus brutia* forest	933	08.04.2018
14	**C2 Denizli:** Beyağaç, Sazak entrance, field edges	902	08.04.2018
15	**C2 Denizli:** Beyağaç, Kapuz entrance	961	08.04.2018
16	**C2 Denizli:** Beyağaç, Serverler entrance	1114	08.04.2018
17	**C2 Denizli:** Beyağaç, Serverler entrance	1130	08.04.2018
18	**C2 Denizli:** Beyağaç, Kocabaşlar	1095	08.04.2018
19	**C2 Denizli:** Beyağaç, in the forest	757	08.04.2018
20	**C2 Denizli:** Beyağaç, in the forest	763	08.04.2018

21	**C2 Denizli:** Beyağaç, in the forest	763	08.04.2018
22	**C2 Denizli:** Beyağaç, in the forest	755	08.04.2018
23	**C2 Denizli:** Beyağaç, in the forest	754	08.04.2018
24	**C2 Denizli:** Beyağaç, in the forest	748	08.04.2018
25	**C2 Denizli:** Beyağaç, in the forest	742	08.04.2018
26	**C2 Denizli:** Beyağaç, above the chrome quarry, towards Eşen Pond	854	08.04.2018
27	**C2 Denizli:** Beyağaç, above the chrome quarry, towards Eşen Pond	882	08.04.2018
28	**C2 Denizli:** Beyağaç, above the chrome quarry, towards Eşen Pond	895	08.04.2018
29	**C2 Denizli:** Beyağaç, above the chrome quarry, towards Eşen Pond	925	08.04.2018
30	**C2 Denizli:** Beyağaç, between the chrome quarry and Eşen Pond	994	08.04.2018
31	**C2 Denizli:** Beyağaç, near Eşen Pond	998	08.04.2018
32	**C2 Denizli:** Beyağaç, near Eşen Pond	996	08.04.2018
33	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	840	21.04.2018
34	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	820	21.04.2018
35	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	852	21.04.2018
36	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	820	21.04.2018
37	**C2 Denizli:** Beyağaç, from Kozlar towards the chrome quarry, near the chrome quarry	790	21.04.2018
38	**C2 Denizli:** Beyağaç, in the forest	765	21.04.2018
39	**C2 Denizli:** Beyağaç, between Kızılcaağaç and Beyağaç	747	21.04.2018
40	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1195	21.04.2018
41	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1234	21.04.2018
42	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1267	21.04.2018
43	**C2 Denizli:** Beyağaç, Sandras Mountain, the right sides of the road before Kartal Lake-Karagöl junction	1237	21.04.2018
44	**C2 Denizli:** Beyağaç, Sandras Mountain, the right sides of the road before Kartal Lake-Karagöl junction	1199	21.04.2018
45	**C2 Denizli:** Beyağaç, Sandras Mountain, the right sides of the road before Kartal Lake-Karagöl junction	1148	21.04.2018
46	**C2 Denizli:** Beyağaç, Sandras Mountain, the right sides of the road before Kartal Lake-Karagöl junction	1245	21.04.2018
47	**C2 Denizli:** Beyağaç, Karagöl surroundings	1346	21.04.2018
48	**C2 Denizli:** Beyağaç, Karagöl, lakeside	1332	21.04.2018
49	**C2 Denizli:** Beyağaç, Karagöl, lakeside	1332	21.04.2018
50	**C2 Denizli:** Beyağaç, from Kartal Lake-Karagöl junction towards Karagöl	1398	21.04.2018
51	**C2 Denizli:** Beyağaç, from Kartal Lake-Karagöl junction towards Karagöl	1381	21.04.2018
52	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1197	21.04.2018
53	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1168	21.04.2018
54	**C2 Denizli:** Beyağaç, above Beyağaç, Sandras Mountain, Kartal Lake road	1020	21.04.2018

55	**C2 Denizli:** Beyağaç	-	18.05.2018
56	**C2 Denizli:** Beyağaç, Eşen Pond, the in-water and the areas around the pond where the water is drawn	1005	11.08.2018
57	**C2 Denizli:** Beyağaç, Eşen Pond, the in-water and the areas around the pond where the water is drawn	1006	11.08.2018
58	**C2 Denizli:** Beyağaç, near Eşen Pond, pastures	996	11.08.2018
59	**C2 Denizli:** Beyağaç, between the chrome quarry and Eşen Pond	970	11.08.2018
60	**C2 Denizli:** Beyağaç, the tops of the chrome quarry towards the Eşen Pond	915	11.08.2018
61	**C2 Denizli:** Beyağaç, Karagöl, the areas around the lake where the water is withdrawn	1329	11.08.2018
62	**C2 Denizli:** Beyağaç, Karagöl, the areas around the lake where the water is withdrawn	1328	11.08.2018
63	**C2 Denizli:** Beyağaç, Karagöl surroundings	1341	11.08.2018
64	**C2 Denizli:** Beyağaç, Karagöl surroundings	1375	11.08.2018
65	**C2 Denizli:** Beyağaç, Kartal Lake-Karagöl road junction	1337	11.08.2018
66	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1572	11.08.2018
67	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1743	11.08.2018
68	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1746	11.08.2018
69	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1749	11.08.2018
70	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1746	11.08.2018
71	**C2 Muğla:** Köyceğiz, Kartal Lake, the edges of the lake	1910	11.08.2018
72	**C2 Muğla:** Köyceğiz, Kartal Lake surroundings	1866	11.08.2018
73	**C2 Muğla:** Köyceğiz, Kartal Lake surroundings	1867	11.08.2018
74	**C2 Muğla:** Köyceğiz, Sandras Mountain, Kartal Lake road	1544	11.08.2018

APPENDIX 2. A list of the plant taxa determined in the flora of Beyağaç (Denizli)

Abbreviations: Obs.: Observation; Medit.: Mediterranean; Ir.-Tur.: Irano-Turanian; Euro-Sib.: Euro-Siberian; Phy. reg.: Phytogeographic region *: Endemic

No	Taxon name	Locality no	Collecter no	Phy. reg.
	PHANEROGAMAE			
	PTERIDOPHYTA			
	ASPLENIACEAE			
1	*Asplenium ceterach* L.	5; 46	Obs.; Obs.	
	DRYOPTERIDACEAE			
2	*Dryopteris filix-mas* (L.) Schott	1	2017-39, 2017-84	
	EQUISETACEAE			
3	*Equisetum fluviatile* L.	33	2018-211	
	MAGNOLIOPHYTA			
	GYMNOSPERMAE			
	CUPRESSACEAE			
4	*Juniperus excelsa* M.Bieb. subsp. *excelsa*	16; 57	Obs.; Obs.	
5	*Juniperus oxycedrus* L. subsp. *oxycedrus* var. *oxycedrus*	1; 16; 46	2017-46; Obs.; Obs.	
	PINACEAE			

6	*Pinus brutia* Ten. var. *brutia*	4; 13; 19; 25; 26; 27; 33; 36; 37; 39; 57	Obs.	Medit.
7	*Pinus nigra* J.F.Arnold subsp. *pallasiana* (Lamb.) Holmboe var. *pallasiana*	8; 42; 44; 63; 66; 69	Obs.	
	ANGIOSPERMAE			
	DICOTYLEDONAE			
	ACANTHACEAE			
8	*Acanthus hirsutus* Boiss. subsp. *hirsutus*	1	2017-109	
	AMARANTHACEAE			
9	*Chenopodium album* L. subsp. *album* var. *album*	4	2017-274	
	APIACEAE			
10	*Anethum graveolens* L.	2	Obs.	
11	*Apium nodiflorum* (L.) Lag.	33	Obs.	
12	*Bifora testiculata* (L.) Spreng.	34	Obs.	
13	*Bupleurum rotundifolium* L.	34	2018-261	
14	*Eryngium campestre* L. var. *campestre*	2	2017-247	
15	**Eryngium thorifolium* Boiss.	1; 3; 13; 29	2017-55; 2017-269; Obs.; Obs.	Medit.
16	**Ferulago sandrasica* Peşmen & Quézel	1; 6; 9; 50; 63	2017-51; 2017-293; Obs.; 2018-323; Obs.	Medit.
17	*Foeniculum vulgare* Mill.	2	2017-251	
18	*Pastinaca sativa* L. subsp. *urens* (Req. ex Gren. & Godr.) Celak.	14	2018-97, 2018-109	
19	*Pimpinella tragium* Vill. subsp. *lithophila* (Schischk.) Tutin	67	2018-547	
20	*Scandix pecten-veneris* L.	14	2018-92	
21	*Torilis arvensis* (Huds.) Link subsp. *arvensis*	55	2018-386, 2018-423	
22	*Turgenia latifolia* (L.) Hoffm.	1	2017-118	
	APOCYNACEAE			
23	*Vinca herbacea* Waldst. & Kit.	41; 43	2018-289; 2018-296	
	ARALIACEAE			
24	*Hedera helix* L.	15	2018-129	
	ASTERACEAE			
25	*Anthemis arvensis* L.	1; 37	2017-37; 2018-274	Euro-Sib.
26	*Anthemis cretica* L. subsp. *tenuiloba* (DC.) Grierson	13; 15; 17; 18; 34; 40; 42; 44	2018-72; 2018-142; 2018-158; 2018-168; 2018-238, 2018-240; 2018-284; 2018-292; 2018-301	
27	*Bellis perennis* L.	31; 32; 34	2018-205; Obs.; 2018-224	Euro-Sib.
28	*Carduus pycnocephalus* L. subsp. *arabicus* (Jacq. ex Murray) Nyman	15	Obs.	Medit.
29	*Carthamus dentatus* (Forssk.) Vahl	59	2018-538	
30	*Carthamus lanatus* L.	2	Obs.	

31	**Centaurea ensiformis* P.H.Davis	1; 7; 8; 9; 73	2017-81; Obs.; Obs.; 2017-327; Obs.	
32	*Centaurea solstitialis* L. subsp. *solstitialis*	2; 58	Obs.; Obs.	
33	*Centaurea urvillei* DC. subsp. *urvillei*	1; 27; 50; 55	2017-60, 2017-116; Obs.; 2018-326; 2018-406	Medit.
34	*Centaurea virgata* Lam.	1; 5; 9; 66	2017-41; 2017-283; Obs.; Obs.	Ir.-Tur.
35	*Chondrilla juncea* L.	64	Obs.	
36	*Cichorium intybus* L.	1; 2; 58	2017-82; 2017-233; 2018-533	
37	*Cirsium vulgare* (Savi) Ten.	1; 6; 58	2017-45; Obs.; 2018-534	
38	*Cnicus benedictus* L.	2	2017-254	
39	*Crepis capillaris* (L.) Wallr.	19	2018-176	
40	*Crepis foetida* L. subsp. *foetida*	2; 14; 34; 55	2017-241; 2018-87; 2018-248; 2018-404, 2018-405	
41	*Crepis sancta* (L.) Bornm. subsp. *nemausensis* (P.Fourn.) Babc.	23	2018-188	
42	*Cyanus segetum* Hill	55	2018-378	
43	*Cyanus thirkei* (Sch.Bip.) Holub	1; 9; 17; 43; 46	2017-49, 2017-78; 2017-326; 2018-161; Obs.; 2018-312	Medit.
44	*Cyanus triumfettii* (All.) Dostál ex Á.Löve & D.Löve subsp. *triumfettii*	23; 34; 46	2018-187; 2018-258; 2018-307	
45	*Doronicum orientale* Hoffm.	22	2018-184	
46	*Echinops sphaerocephalus* L. subsp. *sphaerocephalus*	59; 63	Obs.; Obs.	Euro-Sib.
47	*Echinops spinosissimus* Turra subsp. *spinosissimus*	2	2017-243	Medit.
48	*Filago pyramidata* L.	37; 54	2018-277; 2018-339	
49	*Helichrysum plicatum* DC. subsp. *plicatum*	7; 8	2017-310; Obs.	
50	*Inula anatolica* Boiss.	3	2017-270	
51	*Jurinea mollis* (L.) Rchb.	1	2017-112	Medit.
52	*Lactuca serriola* L.	2	Obs.	
53	*Leontodon asperrimus* (Willd.) Endl.	73	2018-563	Ir.-Tur.
54	*Leontodon hispidus* L. subsp. *hispidus*	58	2018-528	Euro-Sib.
55	*Matricaria chamomilla* L. var. *chamomilla*	1; 33; 55	2017-125; Obs.; 2018-402	
56	*Onopordum illyricum* L.	1	2017-137	Medit.
57	*Picnomon acarna* (L.) Cass.	1; 2; 58	2017-139; 2017-256; Obs.	Medit.
58	*Pilosella piloselloides* (Vill.) Soják subsp. *piloselloides*	1; 23; 55; 63	2017-59, 2017-73; 2018-190; 2018-412; Obs.	
59	*Pulicaria vulgaris* (L.) Gaertn.	2	2017-264	Euro-Sib.

60	*Scorzonera mollis* M.Bieb. subsp. *szowitzii* (DC.) D.F.Chamb.	68	2018-549	Ir.-Tur.
61	**Senecio sandrasicus* P.H.Davis	74	2018-564	Medit.
62	*Senecio vernalis* Waldst. & Kit.	1; 18; 19; 26; 27; 29; 34	2017-57; 2018-166; 2018-175; 2018-198; 2018-200; Obs.; Obs.	
63	*Sonchus asper* (L.) Hill subsp. *asper*	14; 15; 34	2018-94; Obs.; 2018-255	
64	*Taraxacum aleppicum* Dahlst.	17	Obs.	Medit.
65	*Taraxacum assemanii* Blanche ex Boiss.	8; 58	2017-324; 2018-535	Ir.-Tur.
66	*Tragopogon porrifolius* L. subsp. *longirostris* (Sch.Bip.) Greuter	1; 6; 15; 34; 35; 37	2017-102, 2017-103; 2017-295; 2018-147; 2018-230; 2018-266; 2018-272	
	BERBERIDACEAE			
67	*Berberis cretica* L.	1; 8	2017-43, 2017-72; 2017-318	Medit.
	BORAGINACEAE			
68	*Alkanna tubulosa* Boiss.	13; 35; 46; 55	2018-83; 2018-265; 2018-306; 2018-393	Medit.
69	*Anchusa azurea* Mill. var. *azurea*	34	2018-244	
70	*Anchusa hybrida* Ten.	15; 18; 55	2018-140; Obs.; 2018-394	Medit.
71	*Anchusa officinalis* L.	1; 55	2017-106; 2018-370	Euro-Sib.
72	*Buglossoides arvensis* (L.) I. M. Johnst subsp. *sibthorpiana* (Griseb.) R.Fern.	14	2018-100	
73	*Cynoglossum creticum* Mill.	34	2018-246, 2018-253	
74	*Echium italicum* L.	1	2017-100	Medit.
75	*Heliotropium hirsutissimum* Grauer	4; 57; 61; 62	2017-281; 2018-522; 2018-541; Obs.	Medit.
76	*Myosotis lithospermifolia* Hornem.	46	2018-305	
77	*Myosotis ramosissima* Rochel	15; 19; 55	2018-122; 2018-173; 2018-387	
78	*Nonea echioides* (L.) Roem. & Schult.	14	2018-111	Medit.
79	*Onosma frutescens* Lam.	15	2018-118	Medit.
80	*Onosma taurica* Willd. var. *taurica*	51	2018-328	
81	*Paracaryum lithospermifolium* (Lam.) Grande subsp. *cariense* (Boiss.) R.R.Mill var. *cariense*	1	2017-69, 2017-124	Medit.
	BRASSICACEAE			
82	*Aethionema arabicum* (L.) Andrz. ex DC.	13; 39	2018-75; 2018-280	
83	*Alyssum alyssoides* (L.) L.	13; 16; 17	2018-74; Obs.; 2018-164	
84	**Alyssum caricum* T.R.Dudley & Hub.-Mor.	3; 4; 57; 64	Obs.; 2017-277; 2018-523, 2018-524; Obs.	Medit.
85	*Alyssum corsicum* Duby	33; 58; 64	2018-221; 2018-531, 2018-532; Obs.	
86	*Alyssum desertorum* Stapf.	15	2018-116, 2018-123	

87	**Alyssum hirsutum* M.Bieb. subsp. *caespitosum* (T.R.Dudley) Ančev, Kožuharov & Kuzmanov	23; 50	2018-185; 2018-327	Ir.-Tur.
88	**Alyssum masmenaeum* Boiss.	2; 5; 7; 63; 66; 69; 70	2017-239; Obs.; 2017-307; Obs.; Obs.; 2018-551; 2018-557	
89	*Alyssum murale* Waldst. & Kit. subsp. *murale* var. *murale*	4; 15; 21; 53	Obs.; 2018-144; 2018-183; 2018-334	
90	**Alyssum propinquum* Baumg.	43	2018-295	
91	*Arabidopsis thaliana* (L.) Heynh.	47	2018-313	
92	*Arabis verna* (L.) R.Br.	15; 16; 17	2018-126; Obs.; 2018-162	Medit.
93	*Barbarea verna* (Mill.) Aschers.	49	2018-318	
94	*Capsella bursa-pastoris* (L.) Medik.	14; 34	2018-88; 2018-242	
95	*Clypeola jonthlaspi* L.	15; 16	Obs.; 2018-153	
96	*Draba verna* L.	15; 16; 21; 42; 48	2018-117; Obs.; Obs.; Obs.; 2018-316	
97	*Eruca vesicaria* (L.) Cav.	15	2018-113	
98	**Erysimum serpentinicum* Polatschek	44	2018-300	Medit.
99	*Hirschfeldia incana* (L.) Lagr.-Foss.	1	2017-98	
100	*Iberis carnosa* Willd.	8	Obs.	Medit.
101	*Isatis tinctoria* L. subsp. corymbosa (Boiss.) P.H.Davis	55	2018-373	
102	*Microthlaspi perfoliatum* (L.) F.K.Mey.	14; 17	2018-105; 2018-163	
103	**Noccaea cariensis* (Carlström) Parolly, Nordt & Aytac	1; 41; 43	2017-42; 2018-288; 2018-299	Medit.
104	*Sinapis arvensis* L.	13; 14; 15	2018-82, 2018-85; 2018-102; Obs.	
105	*Sisymbrium irio* L.	1; 15	2017-36, 2017-44; 2018-133	
	CAMPANULACEAE			
106	*Campanula erinus* L.	15	2018-148	
107	*Campanula glomerata* L. subsp. *hispida* (Witasek) Hayek	6	2017-294	Euro-Sib.
108	*Campanula lyrata* Lam. subsp. *lyrata*	1	2017-68	
109	*Campanula stricta* L. var. *libanotica* (A.DC.) Boiss.	7; 8; 9; 69; 73	2017-314; Obs.; 2017-328; 2018-554; Obs.	
110	*Legousia pentagonia* (L.) Thell.	1; 55	2017-120; 2018-396, 2018-420	Medit.
	CAPRIFOLIACEAE			
111	**Cephalaria lycica* V.A.Matthews	10; 68; 69	Obs.; 2018-550; Obs.	Medit.
112	*Pterocephalus plumosus* (L.) Coulter	15; 55	2018-114; 2018-372	
113	*Scabiosa columbaria* L.	2	2017-234	
114	**Scabiosa polykratis* Rech.f.	7; 69; 72	2017-313; 2018-555; 2018-559	Medit.
115	*Valerianella coronata* (L.) DC.	21; 23	Obs.; 2018-189	

116	*Valerianella vesicaria* (L.) Moench	1; 34; 54; 55	2017-123; 2018-227, 2018-259; Obs.; 2018-383	
117	*Valeriana officinalis* L.	2; 21; 26; 29; 40	2017-265; 2018-181; 2018-195; Obs.; Obs.	
	CARYOPHYLLACEAE			
118	*Arenaria serpyllifolia* L. subsp. *serpyllifolia*	14	2018-99	
119	**Bolanthus frankenioides* (Boiss.) Barkoudah var. *fasciculatus* (Boiss. & Heldr.) Barkoudah	7	2017-305	Medit.
120	**Bolanthus thymoides* Hub.-Mor.	5	2017-287	Ir.-Tur.
121	*Bufonia tenuifolia* L.	64	Obs.	
122	*Cerastium arvense* L.	16; 24; 25; 26	2018-150; 2018-191; Obs.; 2018-194	
123	*Cerastium brachypetalum* Pers. subsp. *roeseri* (Boiss. & Heldr.) Nyman	15; 19	2018-135; 2018-172	Medit.
124	*Cerastium dichotomum* L. subsp. *dichotomum*	34	Obs.	
125	*Cerastium glomeratum* Thuill.	34	2018-235	
126	*Cerastium ligusticum* Viv.	40; 43; 50; 51; 64	2018-282; 2018-297; Obs.; 2018-331; Obs.	Medit.
127	**Dianthus eretmopetalus* Stapf	7; 8; 70; 73	2017-302; Obs.; 2018-556; 2018-562	Medit.
128	*Dianthus zonatus* Fenzl var. *zonatus*	1; 3; 6; 55; 59; 66; 67	2017-34, 2017-53, 2017-113; 2017-271; 2017-297; 2018-413; Obs.; Obs.; 2018-548	
129	*Holosteum umbellatum* L. var. *umbellatum*	17	2018-157	
130	*Minuartia hybrida* (Vill.) Schischk. subsp. *hybrida*	20	2018-177	
131	*Minuartia mesogitana* (Boiss.) Hand.-Mazz. subsp. *mesogitana*	46; 50	2018-310; 2018-322	Medit.
132	**Minuartia recurva* (All.) Schinz & Thell. subsp. *carica* McNeill	7	2017-304	Medit.
133	*Moenchia mantica* (L.) Bartl.	19; 20; 42	Obs.; 2018-179; 2018-291	
134	*Polycarpon tetraphyllum* (L.) L.	62	Obs.	
135	*Saponaria calabrica* Guss.	13; 27; 40	2018-76; 2018-201; 2018-283	Medit.
136	*Silene bupleuroides* L. subsp. *bupleuroides*	10	2017-331	
137	*Silene conica* L.	34	2018-237, 2018-239	
138	**Silene echinospermoides* Hub.-Mor.	5	2017-282	Medit.
139	*Silene italica* (L.) Pers. subsp. *italica*	34	2018-231	Medit.
140	*Silene macrodonta* Boiss.	59	Obs.	
141	*Silene supina* M.Bieb. subsp. *pruinosa* (Boiss.) Chowdhuri	8	2017-317, 2017-321	
142	*Silene vulgaris* (Moench) Garcke	1	2017-117	

143	*Stellaria holostea* L.	15	2018-127	
144	*Vaccaria hispanica* (Mill.) Rauschert	1	2017-132	
	CISTACEAE			
145	*Cistus creticus* L.	1; 19; 54	2017-92; Obs.; 2018-335	Medit.
146	*Cistus laurifolius* L.	1	2017-35	Medit.
147	*Fumana aciphylla* Boiss.	5	2017-290	Ir.-Tur.
148	*Fumana arabica* (L.) Spach	5; 39	Obs.; Obs.	
149	*Fumana procumbens* (Dunal) Gren. & Godr.	33	2018-209	
150	*Helianthemum salicifolium* (L.) Mill.	16	2018-155	
	CLEOMACEAE			
151	*Cleome iberica* DC.	2	2017-267	Medit.
	CONVOLVULACEAE			
152	*Convolvulus arvensis* L.	1; 34; 55	2017-105; 2018-254; 2018-422	
153	*Convolvulus compactus* Boiss.	5; 55	2017-292; 2018-399	
	CRASSULACEAE			
154	**Prometheum serpentinicum* (Werderm.) t Hart var. *serpentinicum*	9	2017-329	Medit.
155	*Sedum album* L.	16	Obs.	
156	**Sedum lydium* Boiss.	69	2018-552	Medit.
157	*Sedum pallidum* M.Bieb.	4	2017-276	Euro-Sib.
158	*Umbilicus rupestris* (Salisb.) Dandy	15	Obs.	
	ERICACEAE			
159	*Arbutus andrachne* L.	54	2018-337	
160	*Erica manipuliflora* Salisb.	6; 45; 46	2017-298; 2018-302; Obs.	Medit.
	EUPHORBIACEAE			
161	**Euphorbia anacampseros* Boiss. var. *anacampseros*	9; 46; 50	Obs.; 2018-304; 2018-324	
162	**Euphorbia austroanatolica* Hub.-Mor. & M.S.Khan	25; 43; 46	Obs.; Obs.; 2018-309	Medit.
163	*Euphorbia exigua* L. subsp. *exigua*	15; 34	2018-124, 2018-137; 2018-257	
164	*Euphorbia falcata* L. subsp. *falcata* var. *galilaea*	34	Obs.	
165	*Euphorbia helioscopia* L. subsp. *helioscopia*	14	2018-96	
	FABACEAE			
166	*Anagyris foetida* L.	15	2018-128	Medit.
167	*Anthyllis vulneraria* L. subsp. *boissieri* (Sagorski) Bornm.	35; 55	2018-267; 2018-410, 2018-426	
168	*Astragalus angustifolius* Lam. subsp. *pungens* (Willd.) Hayek	7; 8	Obs.; 2017-316	
169	*Astragalus anthylloides* Lam.	55	2018-411	Ir.-Tur.
170	**Astragalus tmoleus* Boiss. var. *tmoleus*	2	2017-245	Medit.

171	*Cercis siliquastrum* L. subsp. *siliquastrum*	37	2018-269	Medit.
172	*Colutea melanocalyx* Boiss. & Heldr. subsp. *davisiana* (Browicz) D.F.Chamb.	33	2018-210	Medit.
173	*Cytisopsis dorycniifolia* Jaub. & Spach	1; 7; 13; 23; 36; 39; 44; 46	2017-50; 2017-303; 2018-80; Obs.; Obs.; Obs.; Obs.; Obs.	
174	*Cytisus eriocarpus* Boiss.	13	2018-73	Medit.
175	*Cytisus hirsutus* L.	18; 34; 37	Obs.; 2018-228; 2018-271	
176	*Dorycnium pentaphyllum* Scop. subsp. *anatolicum* (Boiss.) Gams	55	2018-418	
177	**Ebenus pisidica* Hub.-Mor. & Reese	1; 8	2017-70; 2017-319	Medit.
178	*Genista acanthoclada* DC.	3; 4; 13; 33; 35; 39; 55	Obs.; 2017-279; Obs.; 2018-220; 2018-264; Obs.; 2018-414	Medit.
179	*Genista januensis* Viv. subsp. *januensis*	16	2018-151	Medit.
180	**Genista sandrasica* Hartvig & Strid	7	2017-301	Medit.
181	*Hippocrepis emerus* (L.) Lassen subsp. *emeroides* (Boiss. & Spruner) Lassen	13; 26	2018-71; 2018-193	
182	*Hymenocarpos circinnatus* (L.) Savi	34	2018-234	
183	*Lathyrus cicera* L.	15; 18; 37; 50	2018-146; Obs.; 2018-270; 2018-320	Medit.
184	*Lathyrus setifolius* L.	14	2018-101	Medit.
185	*Lotus corniculatus* L. var. *corniculatus*	2; 7; 58; 61; 63	2017-242, 2017-259; 2017-300; 2018-536; 2018-542; Obs.	
186	*Medicago lupulina* L.	15	2018-132	
187	*Medicago minima* (L.) Bartal. var. *minima*	19	2018-171	
188	*Medicago monspeliaca* (L.) Trautv.	33	2018-219	Medit.
189	*Medicago rigidula* (L.) All. var. *rigidula*	15	2018-134	
190	*Medicago sativa* L. subsp. *sativa*	2	2017-250	
191	*Medicago truncatula* Gaertn. var. *truncatula*	15	2018-119, 2018-120	Medit.
192	*Melilotus indicus* (L.) All.	35	2018-268	
193	*Onobrychis aequidentata* (Sibth. & Sm.) d Urv.	55	2018-416	Medit.
194	*Onobrychis viciifolia* Scop.	55	2018-382, 2018-415	
195	*Ononis spinosa* L. subsp. *leiosperma* (Boiss.) Sirj.	1; 2; 58	2017-87; 2017-236; 2018-530	
196	*Ononis viscosa* L. subsp. *breviflora* (DC.) Nyman	1	2017-91	Medit.
197	*Pisum sativum* L. subsp. *elatius* (M.Bieb.) Aschers. & Graebn. var. *elatius*	14; 15	Obs.; 2018-145	Medit.

198	*Robinia pseudoacacia* L.	58; 59	Obs.; Obs.	
199	*Spartium junceum* L.	1; 55	2017-115; 2018-391	Medit.
200	*Trifolium campestre* Schreb. subsp. *campestre* var. *campestre*	55	2018-425	
201	*Trifolium hirtum* All.	34	2018-229	Medit.
202	*Trifolium stellatum* L. var. *stellatum*	55	2018-424	
203	*Trigonella corniculata* L.	55	2018-397	
204	*Vicia narbonensis* L. var. *narbonensis*	18	Obs.	
205	*Vicia sativa* L. subsp. *incisa* (M.Bieb.) Arc. var. *incisa*	14; 19	2018-91; 2018-174	
206	*Vicia sativa* L. subsp. *sativa*	15	2018-138	
207	*Vicia tetrasperma* (L.) Schreb.	33	2018-215	
208	*Vicia villosa* Roth subsp. *eriocarpa* (Hausskn.) P.W.Ball	39	2018-281	
	FAGACEAE			
209	*Quercus coccifera* L.	1; 4; 13; 15; 16; 37; 46	2017-89; Obs.; Obs.; Obs.; Obs.; Obs.; Obs.	Medit.
210	*Quercus infectoria* Oliv. subsp. *infectoria*	43; 44; 46	Obs.; Obs.; 2018-308	Euro-Sib.
211	*Quercus pubescens* Willd. subsp. *pubescens*	1	2017-88	
	GENTIANACEAE			
212	*Centaurium tenuiflorum* (Hoffmanns. & Link) Fritsch subsp. *tenuiflorum*	2	2017-263	
	GERANIACEAE			
213	*Erodium acaule* (L.) Becherer & Thell.	33; 55	Obs.; 2018-380	Medit.
214	*Erodium ciconium* (L.) L Her.	13	2018-79	
215	*Erodium cicutarium* (L.) L Hér. subsp. *cicutarium*	14; 15; 17; 34	Obs.; Obs.; Obs.; 2018-250	
216	*Geranium dissectum* L.	14; 15	2018-107; 2018-125	
217	*Geranium rotundifolium* L.	14; 15; 33	2018-98, 2018-103; 2018-121; 2018-223	
218	*Geranium tuberosum* L.	25; 37	2018-192; 2018-273	Ir.-Tur.
219	*Pelargonium endlicherianum* Fenzl	5	2017-284	
	HYPERICACEAE			
220	**Hypericum aviculariifolium* Jaub. & Spach	70	2018-558	Medit.
221	*Hypericum perforatum* L. subsp. *perforatum*	2; 55	2017-249; 2018-401	
	LAMIACEAE			
222	*Ajuga chamaepitys* (L.) Schreb. subsp. *palaestina* (Boiss.) Bornm.	1; 2; 13; 15; 34	2017-122, 2017-133; 2017-268; 2018-77; 2018-139; 2018-245	Medit.
223	*Clinopodium acinos* (L.) Kuntze	54	2018-338	Euro-Sib.
224	**Clinopodium troodi* (Post) Govaerts subsp. *vardaranum* (Leblebici) Govaerts	1; 9; 69; 70	2017-61, 2017-75, 2017-138; 2017-330; 2018-553; Obs.	Medit.

225	*Cyclotrichium origanifolium* (Labill.) Manden & Scheng.	2	Obs.	Medit.
226	*Lamium amplexicaule* L. var. *amplexicaule*	14	2018-106	
227	*Mentha spicata* L. subsp. *spicata*	2	2017-261	
228	**Nepeta cadmea* Boiss.	15	Obs.	Medit.
229	**Origanum hypericifolium* O.Schwarz & P.H.Davis	12; 63	2017-479; Obs.	Medit.
230	*Origanum onites* L.	1; 18	2017-111, 2017-114; Obs.	
231	*Phlomis armeniaca* Willd.	7; 8	Obs.; 2017-320	Ir.-Tur.
232	*Salvia argentea* L.	68	Obs.	Medit.
233	*Salvia candidissima* Vahl subsp. *occidentalis* Hedge	55	2018-409	
234	*Salvia sclarea* L.	1; 53; 55	2017-129; 2018-333; 2018-398	
235	*Salvia tomentosa* Mill.	1	2017-94	Medit.
236	*Salvia verbenaca* L.	2; 15	2017-240; Obs.	Medit.
237	*Salvia virgata* Jacq.	1	2017-52, 2017-79	Ir.-Tur.
238	*Scutellaria orientalis* L. subsp. *pinnatifida* J.R.Edm.	2; 13; 16; 21; 30; 33; 34; 39; 46; 50	2017-260; Obs.; Obs.; Obs.; Obs.; 2018-222; 2018-260; 2018-279; Obs.; Obs.	
239	*Sideritis libanotica* Labill. subsp. *linearis* (Benth.) Bornm.	8; 60	2017-322; Obs.	Medit.
240	*Stachys annua* (L.) L. subsp. *annua* var. *annua*	1; 55	2017-121; 2018-381	
241	*Stachys cretica* L. subsp. *cretica*	1; 2; 55	2017-101, 2017-140; 2017-235; 2018-389	
242	*Stachys germanica* L. subsp. *heldreichii* (Boiss.) Hayek	2	Obs.	Medit.
243	**Teucrium alyssifolium* Stapf	1; 6; 66	2017-58, 2017-77; 2017-296; Obs.	Medit.
244	*Teucrium chamaedrys* L. subsp. *chamaedrys*	1	2017-93	
245	*Teucrium polium* L. subsp. *polium*	1; 3; 5; 65; 72	2017-47; 2017-272; 2017-288; Obs.; 2018-560	
246	**Teucrium sandrasicum* O. Schwarz	11; 13; 64	2017-332; Obs.; Obs.	Medit.
247	*Teucrium scordium* L. subsp. *scordium*	57	2018-525	Euro-Sib.
248	*Thymus zygioides* Griseb.	5; 7; 55	2017-285; 2017-306; 2018-384, 2018-417	Medit.
249	*Ziziphora capitata* L.	55	2018-379	
	LENTIBULARIACEAE			
250	*Pinguicula crystallina* Sm.	1; 8	2017-71; 2017-323	Medit.
	LINACEAE			
251	*Linum bienne* Mill.	1	2017-110	
252	*Linum usitatissimum* L.	55	2018-419	
	LYTHRACEAE			

253	*Lythrum tribracteatum* Salzm. ex Ten.	56	2018-519	
	MALVACEAE			
254	*Malva sylvestris* L.	55	2018-403	
	OLEACEAE			
255	*Phillyrea latifolia* L.	5	2017-286	Medit.
	OROBANCHACEAE			
256	*Orobanche lutea* Baumg.	1; 43; 55	2017-136; 2018-298; 2018-390, 2018-421	
257	*Orobanche ramosa* L.	1; 14; 34	2017-80; 2018-93; 2018-256	
258	*Parentucellia latifolia* (L.) Caruel subsp. *latifolia*	42	2018-290	Medit.
	PAPAVERACEAE			
259	*Fumaria parviflora* Lam.	15; 55	2018-112; 2018-395	
260	*Fumaria vaillantii* Loisel.	14	2018-89	
261	*Glaucium flavum* Crantz	1; 55	2017-135; 2018-376, 2018-392	
262	*Hypecoum pendulum* L.	55	2018-385	
263	*Papaver argemone* L. subsp. *argemone*	1; 13; 37	2017-104; 2018-78; Obs.	
264	*Papaver dubium* L. subsp. *laevigatum* (M.Bieb.) Kadereit	2	2017-253	
265	*Papaver rhoeas* L.	1; 14; 34; 55	2017-107, 2017-131, 2017-134; 2018-90; 2018-233, 2018-241, 2018-249; 2018-407	
266	*Papaver virchowii* Asch. & Sint. ex Boiss.	1	2017-130	
	PLANTAGINACEAE			
267	*Linaria chalepensis* (L.) Mill. var. *chalepensis*	34	2018-236, 2018-262	Medit.
268	**Linaria corifolia* Desf.	55	2018-374	Ir.-Tur.
269	*Linaria genistifolia* (L.) Mill. subsp. *linifolia* (Boiss.) P.H.Davis	13	Obs.	
270	*Plantago albicans* L.	7	2017-309	Medit.
271	*Plantago lanceolata* L.	1; 14; 34; 62	2017-99; 2018-108; 2018-251; 2018-545	
272	*Veronica arvensis* L.	16	2018-156	Euro-Sib.
273	*Veronica chamaedrys* L.	48	2018-314	Euro-Sib.
274	*Veronica hederifolia* L.	16	2018-154	
275	*Veronica praecox* All.	16	2018-149	
276	*Veronica serpyllifolia* L.	1; 56	2017-66; 2018-520	
	PLATANACEAE			
277	*Platanus orientalis* L.	37	Obs.	
	PLUMBAGINACEAE			
278	**Acantholimon ulicinum* (Willd. ex Schult.) Boiss. var. *purpurascens* (Bokhari) Bokhari & J.R.Edm.	7	2017-308	Medit.
	POLYGALACEAE			

279	*Polygala anatolica* Boiss. & Heldr.	36; 50	Obs.; 2018-319, 2018-321	
280	*Polygala supina* Schreb. subsp. *supina*	8	2017-315	
	POLYGONACEAE			
281	*Polygonum salebrosum* Coode & Cullen	5; 7; 62	2017-289; 2017-312; 2018-546	Medit.
282	*Rumex crispus* L.	4	2017-280	
	PRIMULACEAE			
283	*Anagallis arvensis* L. var. *caerulea* (L.) Gouan	58	Obs.	
284	**Cyclamen alpinum* Dammann ex Spreng.	16	Obs.	Medit.
	RANUNCULACEAE			
285	*Adonis aestivalis* L. subsp. *aestivalis*	34	2018-243	
286	*Adonis flammea* Jacq.	1	2017-108	
287	*Anemone ranunculoides* L. subsp. *ranunculoides*	43; 46	2018-293; Obs.	Euro-Sib.
288	*Ceratocephala falcata* (L.) Pers.	17	2018-159	
289	*Delphinium peregrinum* L.	2	2017-237	Medit.
290	*Nigella arvensis* L. var. *involucrata* Boiss.	59	2018-539	
291	*Ranunculus arvensis* L.	14; 34	2018-95; 2018-232	
292	*Ranunculus repens* L.	33	2018-216	
293	*Ranunculus trichophyllus* Chaix ex Vill.	32; 56	2018-206; Obs.	
	RESEDACEAE			
294	*Reseda lutea* L. var. *nutans* Boiss.	1; 18	2017-126; 2018-167	
	ROSACEAE			
295	*Crataegus monogyna* Jacq. var. *monogyna*	1; 15; 33	2017-86; 2018-131; 2018-217	
296	*Pyrus elaeagnifolia* Pall. subsp. *elaeagnifolia*	18; 58	Obs.; 2018-527	
297	*Rosa canina* L.	1; 8; 18; 55	2017-95; 2017-325; Obs.; 2018-377	
298	*Rubus sanctus* Schreb.	2	2017-255	
299	*Sanguisorba minor* L. subsp. *lasiocarpa* (Boiss. & Hausskn.) Nordborg	1; 33; 34; 62	2017-56; Obs.; 2018-247; Obs.	
	RUBIACEAE			
300	*Asperula stricta* Boiss. subsp. *stricta*	7	2017-311	Medit.
301	*Crucianella latifolia* L.	2; 15	2017-246; Obs.	Medit.
302	*Cruciata taurica* (Pall. ex Willd.) Ehrend.	1; 3; 18; 26; 40; 41; 51	2017-48; 2017-273; 2018-169; 2018-196; 2018-285; Obs.; 2018-329	Ir.-Tur.
303	*Galium album* Mill. subsp. *amani* Ehrend. & Schönb.-Tem.	13; 15	2018-84; 2018-136	
304	*Galium aparine* L.	1; 13; 14; 18	2017-83; 2018-86; Obs.; 2018-165	

305	*Galium odoratum* (L.) Scop.	15	2018-130	Euro-Sib.
306	*Galium verum* L. subsp. *verum*	2	2017-244	Euro-Sib.
307	*Rubia tenuifolia* d'Urv. subsp. *tenuifolia*	74	2018-565	Medit.
308	*Sherardia arvensis* L.	34	2018-226	Medit.
	SANTALACEAE			
309	*Thesium bergeri* Zucc.	1; 33; 34	2017-38; 2018-218; 2018-225	Medit.
	SAXIFRAGACEAE			
310	*Saxifraga cymbalaria* L.	26; 29	2018-197; Obs.	
	SCROPHULARIACEAE			
311	*Scrophularia canina* L. subsp. *bicolor* (Sm.) Greuter	15; 37	2018-143; 2018-276	Medit.
312	**Verbascum cariense* Hub.-Mor.	1; 36; 51; 52	2017-67, 2017-74; Obs.; 2018-332; Obs.	Medit.
313	*Verbascum lasianthum* Boiss. ex Benth.	1; 4	2017-64; 2017-278	
314	*Verbascum sinuatum* L. subsp. *sinuatum* var. *sinuatum*	59	2018-537	Medit.
315	**Verbascum trapifolium* (Stapf) Hub.-Mor.	74	2018-566	Medit.
	TAMARICACEAE			
316	*Tamarix parviflora* DC.	1; 4; 56	2017-65; 2017-275; 2018-518	Medit.
	THYMELAEACEAE			
317	*Daphne sericea* Vahl subsp. *sericea*	15; 16; 54	2018-141; Obs.; 2018-336	Medit.
	VIOLACEAE			
318	**Viola heldreichiana* Boiss.	21; 30	2018-180; Obs.	Medit.
319	*Viola kitaibeliana* Roem. & Schult.	46; 50	Obs.; 2018-325	
	MONOCOTYLEDONAE			
	AMARYLLIDACEAE			
320	*Allium hirtovaginatum* Kunth	73	2018-561	Medit.
321	*Allium scorodoprasum* L. subsp. *rotundum* (L.) Stearn	2	2017-238	
322	*Allium stamineum* Boiss.	2	2017-252	Medit.
	ARACEAE			
323	*Dracunculus vulgaris* Schott	14; 18	Obs.; Obs.	Medit.
	ASPARAGACEAE			
324	*Asparagus aphyllus* L. subsp. *aphyllus*	1	2017-90	
325	*Muscari armeniacum* Leichtlin ex Baker	23	Obs.	
326	*Muscari comosum* (L.) Mill.	1; 40	2017-63; 2018-287	Medit.
327	*Muscari neglectum* Guss. ex Ten.	15; 21	2018-115; Obs.	
328	**Muscari racemosum* Mill.	1; 5; 13; 26; 27; 29	2017-40, 2017-76; 2017-291; 2018-81; Obs.; 2018-199; 2018-203	Medit.
329	**Muscari sandrasicum* Karlén	16; 48; 49	Obs.; 2018-315; Obs.	Medit.

330	**Ornithogalum alpigenum* Stapf	46	2018-311	Medit.
331	*Ornithogalum narbonense* L.	55	2018-375	Medit.
332	*Ornithogalum nutans* L.	17	2018-160	Medit.
333	*Ornithogalum umbellatum* L.	1; 7	2017-62; 2017-299	
334	*Prospero autumnale* (L.) Speta	61; 62	2018-544; Obs.	Medit.
	CYPERACEAE			
335	*Carex hirta* L.	33	2018-214	Euro-Sib.
336	*Eleocharis palustris* (L.) Roem. & Schult. subsp. *palustris*	1; 61; 71	2017-85; Obs.; Obs.	
337	*Scirpoides holoschoenus* (L.) Soják subsp. *holoschoenus*	2; 57; 61	2017-262; 2018-521; 2018-543	
	IRIDACEAE			
338	*Gladiolus illyricus* W.D.J.Koch	1; 55	2017-54; 2018-408	Medit.
	JUNCACEAE			
339	*Juncus effusus* L. subsp. *effusus*	33	2018-212	
	LILIACEAE			
340	*Tulipa armena* Boiss.	23	2018-186	
341	*Tulipa sylvestris* L. var. *sylvestris*	23; 28; 43; 49; 51	Obs.; 2018-202; 2018-294; 2018-317; 2018-330	
	ORCHIDACEAE			
342	*Cephalanthera epipactoides* Fisch. & C.A.Mey.	19; 20; 33; 38	Obs.; 2018-178; 2018-208; 2018-278	Medit.
343	*Cephalanthera rubra* (L.) Rich.	33	2018-207	
344	*Limodorum abortivum* (L.) Sw. var. *abortivum*	19; 35	Obs.; 2018-263	
345	*Orchis anatolica* Boiss.	16; 20; 28; 30	2018-152; Obs.; Obs.; 2018-204	Medit.
	POACEAE			
346	*Aegilops geniculata* Roth	1; 2	2017-128; Obs.	Medit.
347	*Aegilops triuncialis* L. subsp. *triuncialis*	55	2018-371	
348	*Avena barbata* Pott ex Link subsp. *barbata*	1	2017-96	Medit.
349	*Bromus racemosus* L.	2	2017-257	Euro-Sib.
350	*Bromus sterilis* L.	14	2018-110	
351	*Bromus tectorum* L.	1; 34	2017-127; 2018-252	
352	*Calamagrostis epigeios* (L.) Roth	2	2017-258	Euro-Sib.
353	*Crypsis alopecuroides* (Piller & Mitterp.) Schrad.	61	2018-540	
354	*Cynodon dactylon* (L.) Pers. var. *dactylon*	58	2018-529	
355	*Echinaria capitata* (L.) Desf.	1; 37	2017-119; 2018-275	
356	*Hordeum murinum* L. subsp. *murinum*	1	2017-97	
357	*Micropyrum tenellum* (L.) Link	33	2018-213	Medit.
358	*Phragmites australis* (Cav.) Trin. ex Steud.	57	Obs.	Euro-Sib.
359	*Poa bulbosa* L.	19; 40	2018-170; 2018-286	
360	*Poa pratensis* L.	14	2018-104	

361	*Setaria viridis* (L.) P.Beauv.	59	Obs.
	POTAMOGETONACEAE		
362	*Zannichellia palustris* L. subsp. *palustris*	56	2018-517
	TYPHACEAE		
363	*Typha angustifolia* L.	2	2017-266

INSTRUCTIONS TO CONTRIBUTORS

Communications Faculty of Sciences University of Ankara Series C Biology (Commun. Fac. Sci. Univ. Ank. Ser. C-Biology.) is a single-blind peer reviewed open access journal which has been published biannually since 1948 by Ankara University, accepts original research articles written in English in the field of Biology. Review articles written by eminent scientists can also be invited by the Editor.

The publication costs for Communications Faculty of Sciences University of Ankara Series C-Biology are covered by the journal, so authors do not need to pay an article-processing and submission charges. The PDF copies of accepted papers are free of charges and can be downloaded from the website. Hard copies of the paper, if required, are due to be charged for the amount of which is determined by the administration each year.

All manuscripts should be submitted via our online submission system https://dergipark.org.tr/en/journal/2987/submission/step/manuscript/new. Manuscripts should be submitted in the PDF form used in the peer-review process together with THE COVER LETTER and the TEX file (Source File). In the cover letter the authors should suggest the most appropriate Field Editor for the manuscript and potential four reviewers with full names, universities and institutional email addresses. Proposed reviewers must be experienced researchers in your area of research and at least two of them should be from different countries. In addition, proposed reviewers must not be co-auhors, advisors, students, etc. of the authors. In the cover letter, the author may enter the name of anyone who he/she would prefer not to review the manuscript, with detailed explanation of the reason. Note that the editorial office may not use these nominations, but this may help to speed up the selection of appropriate reviewers.

Manuscripts should be typeset using a word processor editor. Authors should prepare the article using the COMMUNICATIONS style before submission to the editor via our submission system. A template of manuscript can be downloaded in doc form from the link http://dergipark.org.tr/download/journal-file/ 13889 (or can be reviewed in pdf form). The title page should contain the title of the paper, full names of the authors, affiliations addresses and e-mail addresses of all authors. Authors are also required to submit their Open Researcher and Contributor ID (ORCID)'s which can be obtained from http://orcid.org as their URL address in the format http://orcid.org/xxxx-xxxx-xxxx-xxxx. Please indicate the corresponding author. The abstract should state briefly the purpose of the research. The length of the Abstract should be between 50 to 5000 characters. At least 3 keywords are required. Formulas should be numbered consecutively in the parentheses ( ). All tables must have numbers (TABLE 1) consecutively in accordance with their appearance in the text and a legend above the table. Please submit tables as editable text not as images. All figures must have numbers (FIGURE 1) consecutively in accordance with their appearance in the text and a caption (not on the figure itself) below the figure. Required final resolution is 300 dpi for coloured images (RGB), 300–600 dpi for black and white images and 600–1200 dpi for line art. Pictures and line drawings should be saved as TIFF files. Authors Contribution Statement, Declaration of Competing Interests and Acknowledgements should be given at the end of the article before the references. The following format for the references should be used. Authors are urged to use the Communication.csl style (https://dergipark.org.tr/en/download/journal-file/ 18514) in Mendeley Desktop or Zotero automated bibliography. If manual entry is prefered for bibliography, then all citations must be listed in the references part and vice versa. The Editor may seek the advice of two, or three referees, depending on the response of the referees, chosen in consultation with appropriate members of the Editorial Board, from among experts in the field of specialization of the paper. The reviewing process is conducted in strict confidence and the identity of a referee is not disclosed to the authors at any point since we use a single-blind peer review process..

Declarations/Ethics:
With the submission of the manuscript authors declare that:

- All authors of the submitted research paper have directly participated in the planning, execution, or analysis of study;
- All authors of the paper have read and approved the final version submitted;
- The contents of the manuscript have not been submitted, copyrighted or published elsewhere and the visual-graphical materials such as photograph, drawing, picture, and document within the article do not have any copyright issue;
- The contents of the manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the Journal is under consideration.
- The article is clean in terms of plagiarism, and the legal and ethical responsibility of the article belongs to the author(s). Author(s) also accept that the manuscript may go through plagiarism check using IThenticate software;
- The objectivity and transparency in research, and the principles of ethical and professional conduct have been followed. Authors have also declared that they have no potential conflict of interest (financial or non-financial), and their research does not involve any human participants and/or animals.

Research papers published in **Communications Faculty of Sciences University of Ankara** are archived in the Library of Ankara University (Volume 1-67) and in Dergipark immediately following publication with no embargo.

Editor in Chief
http://communications.science.ankara.edu.tr
Ankara University, Faculty of Sciences
06100, Besevler - ANKARA TURKEY